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Phospholipid fatty acid profiles at depositional and non-depositional sites in the North Sea

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ABSTRACT: The distribution of ester-linked phospholipid fatty acid (PLFA) in sediments of 12 sampling stations located in the German Bight, Skagerrak/northern Kattegat, Frisian Front, Oyster Ground, eastern North Sea and the Dogger Bank area was studied to compare the *in situ* microbial community structure of depositional and non-depositional sites. A total of 36 fatty acids in the range of C_{12} to C_{24} were determined. They consisted of saturated, branched, monounsaturated, polyunsaturated and hydroxy fatty acids, and variation was revealed in the relative proportions (mol %) of these fatty acids. The distribution of specific fatty acids was significantly different between depositional and non-depositional sites. Additionally, we could discriminate communities of sites which are intermediate in sedimentation regime. Highest total PLFA content was found at depositional sites. The PLFA profiles of all sites were dominated by bacteria, with highest bacterial contribution to the sedimentary PLFA pool at depositional sites. A high proportion of aerobic prokaryotes (up to 40 %) at depositional sites indicated the occurrence of aerobic microniches in the corresponding anoxic sediments. Bacterial groups being a component of the degradation pathways of complex macromolecules (e.g. *Cytophaga* and *Actinomycetes*) were found in significant higher abundances at depositional sites compared to the other sites. At depositional sites, sulphate-reducing bacteria (SRB) (e.g. *Desulfobacter* spp.) were more abundant compared to the other stations. The relative contribution of microeukaryotes to the sedimentary PLFA pool was significantly higher at depositional sites. A nutritionally limited benthic system at non-depositional sites in the offshore is indicated by a microbial community exposed to higher physiological stress than those of the organically enriched sediments of the depositional sites.

KEY WORDS: Microbial communities · Phospholipid fatty acids · North Sea · Sediment · *Actinomycetes* · *Cytophaga* · Sulphate-reducing bacteria

INTRODUCTION

Shelf seas such as the North Sea are responsible for $\frac{1}{5}$ to $\frac{1}{3}$ of the global marine primary production, while they make up less than $\frac{1}{10}$ of the Earth's surface. However, the organic carbon mineralisation in shelf seas is characterised by an efficiency of 95 % or more. Only recently has it been shown that the benthic realm of depositional areas plays a decisive role in the process of organic matter metabolism (Boon et al. 1999). Due to the quantitative input of organic material and the prevailing anoxic conditions in sediments of depositional areas, the benthic mineralisation processes differ significantly from those of more shallow and turbulent non-depositional sites (Osinga et al. 1996 and literature therein). Since the microbial communities play a decisive role in mediating essential biochemical cycles of carbon, nitrogen, sulphur, hydrogen and oxygen, it is important to detect changes in microbial community structures. In this context, relatively little is known about the role of depositional areas in influencing the diversity of benthic microbial communities that are

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actively involved in the decomposition processes of deposited organic matter and how they compare to non-depositional areas. To gain insight into these questions, we studied the structure of microbial communities at different depositional and non-depositional sites in sediments of the North Sea.

The determination of the microbial community structure *in situ* is one of the most difficult problems in microbial ecology. In nature, microorganisms rarely exist as monocultures, but live in assemblages with other microbes. Classical techniques of microbial community analyses are based on selective enrichment culture (Brock 1975). However, studies have shown that subculturing microbes on artificial media often reveals only 0.01 to 10% of the total microbes present (Vestal & White 1989). During the last decade, microbial ecologists sought to develop ways to measure microbial community structure *in situ* to overcome these problems. Promising techniques comprise molecular biological methods (DeLong et al. 1993, Amann et al. 1995) and biochemical markers (reviewed by White et al. 1996). The analysis of ester-linked phospholipid fatty acids (PLFA) is one acknowledged biochemical approach to microbial community characterisation that has been successful in a wide variety of applications (e.g. Baird & White 1985, Dowling et al. 1986, Nichols et al. 1986, Findlay et al. 1990, Mancuso et al. 1990, Rajendran et al. 1992, Findlay & Dobbs 1993, Frostegård et al. 1993, Zelles & Bai 1994). PLFAs are structural components of all biological membranes. These compounds have no storage function and thus represent a relatively consistent fraction of cell mass. They also degrade quickly upon an organism's death, and current extraction and derivatisation methods permit recovery of PLFAs from living organisms (White 1988). Extraction and subsequent analysis by gas chromatography and mass spectrometry provide precise resolution, sensitive detection and accurate quantification of a broad array of PLFAs. Each analysis yields a profile composed of numerous PLFAs defined on the basis of compound structure and the quantity of each compound present in the sample. Certain PLFAs isolated from prokaryotic and eukaryotic cell membrane can serve as unique signatures for certain functional groups of microorganisms (specific biomarker fatty acids; reviewed by Findlay & Dobbs 1993). However, it must be kept in mind that *Archaea* are not represented in the analyses (their membrane lipids employ ether rather than ester bonds; Tornabene & Langworthy 1979). Furthermore, the signature lipid biomarker analysis cannot detect every species of microorganisms in environmental samples as many share overlapping PLFA patterns. Nevertheless, comparison of total community PLFA profiles accurately mirrors shifts in community composition and provides an excellent way to correlate community composition to specific metabolic properties and environmental conditions (White et al. 1996), which was the task of the present study. Validations of PLFA in the determination of microbial community structure has been extensively reviewed (White 1988, Findlay & Dobbs 1993).

PLFA data and the knowledge about microbial community structure in North Sea sediments are scarce (Boon et al. 1999). With the present study we provide a data set allowing for the relation of microbial community structure to environmental conditions at acknowledged depositional (German Bight, Skagerrak/north-

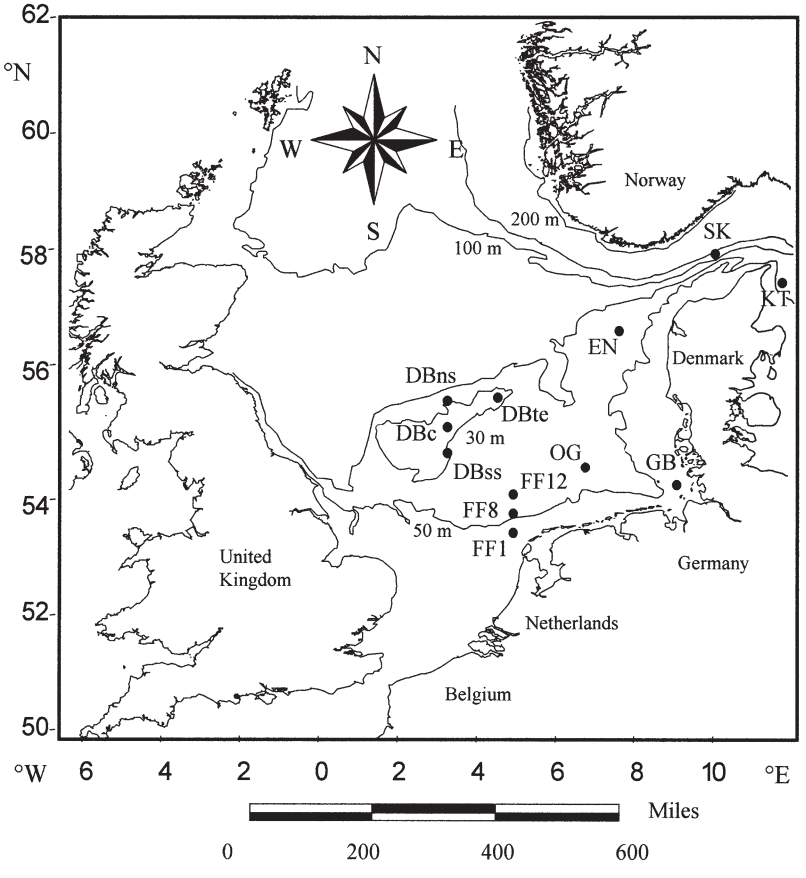

Fig. 1. Study area with our 12 sampling sites in the German Bight (GB), eastern Oyster Ground (OG), Dogger Bank (DB), Frisian Front (FF), eastern North Sea (EN), Skagerrak (SK) and Kattegat (KT). ns: northern slope; te: tail end; c: central; ss: southern slope. 1 mile = 1.6 km

ern Kattegat) and non-depositional sites (stations on the Dogger Bank, southern Frisian Front and eastern North Sea) in the North Sea. Additionally, we sampled sites which were of intermediate character in respect to sedimentation rates, silt and organic carbon content (eastern Oyster Ground, southern slope of the Dogger Bank, central and northern Frisian Front) (see Fig. 1).

MATERIALS AND METHODS

Study sites. Sediment samples were collected at 12 stations in the North Sea and in the Skagerrak/northern Kattegat (Fig. 1). Our sampling stations visited with RV 'Senckenberg' were located in the German Bight, the eastern Oyster Ground and in the Dogger Bank area (sampled in May and September 1999), the eastern North Sea and Skagerrak/northern Kattegat (sampled in May and September 2000). Due to limited ship time, the Frisian Front was sampled only in autumn 1999 with RV 'Pelagia'. The sample sites were chosen to reflect gradients in sedimentation rates (Cadée 1984, DeHaas & VanWeering 1997, DeHaas et al. 1997, Puls et al. 1997) and in organic matter supply (Eisma & Kalf 1987, Kröncke & Knust 1995, DeHaas & VanWeering 1997). The co-ordinates and some characteristics of the sites are given in Table 1 and Fig. 2. Thus, in accordance with the above-cited literature, our sampling stations could be classified either as depositional, non-depositional or intermediate sites.

Sampling design. Undisturbed sediment samples were obtained using an 0.1 m^2 USNEL box corer. Any disturbance of the sediment water interface and contamination by water from the upper water column was prevented by a closing lid on the upper end of the box corer. At each station, 3 cores were collected to decrease bias due to patchiness. Each core was subsampled using 5 PVC tubes (40 cm long, 10 cm in diameter). All tube subsamples of the different cores (15 in all per station) were sliced at a depth of 2 cm. All subsamples of the same station were pooled and mixed to prevent selective sampling. Mixed samples were frozen at –20°C.

Probe measurements. Temperature and salinity were measured in the overlying bottom water retained in the box cores using a Hydrobios temperature/salinity probe. Redox potential (Eh) was measured in the surficial sediment in each of the different box cores immediately after sampling. We used a GAT Ionode IH30 (Gamma Analysen Technik) together with a Portamess 651-2 Microprocessor (Knick).

Silt content. The silt content at each station was analysed as described in Stoeck et al. (2000) using the laser particle sizer Analysette 22 Economy (Fritsch). An aliquot of a freeze-dried sediment sample was placed into the laser particle sizer and was automatically homogenised by a stirrer and ultrasonication. For this study, we measured the percentage of different grain size fractions and the silt content (wt %) of the sediment, which we define as the grain size fraction < 63 µm.

Organic carbon and nitrogen. Analyses of sediment organic carbon and total nitrogen were determined on freeze-dried samples that had been finely powdered and homogenised. A 10 to 30 mg split was combusted at 1010°C in a Heraeus C/N analyser following acidification of the samples with concentrated HCl within silver sample cups to remove inorganic carbonates (Hedges & Stern 1984).

Chlorophyll *a*. Chlorophyll *a* (chl *a*) was extracted from 5 g sediment with 5 ml 90 % acetone. After incu-

Table 1. General characteristics of the 12 sample stations under study. Bottom water temperature, salinity and organic carbon (C_{org}) are given for May (M) and September (S). -----: no data available

Station	Latitude	Longitude	Depth (m)	Bottom water temperature (°C) M/S	Salinity (‰) M/S	C_{org} (% in sediment) M/S	Depositional character[a]
German Bight (GB)	54° 02′ N	8° 02′ E	25	11.6/18.5	32.6/33.2	1.53/1.48	Depositional
Kattegat (KT)	57° 38′ N	10° 58′ E	28	11.8/15.8	22.0/23.0	1.34/1.46	Depositional
Skagerrak (SK)	57° 49′ N	9° 10′ E	140	10.0/10.8	32.5/35.8	0.65/0.80	Depositional
Dogger Bank southern slope (DBss)	54° 37′ N	3° 00′ E	34	11.7/14.9	35.9/36.5	0.24/0.20	Intermediate
Oyster Ground (OG)	54° 25′ N	6° 15′ E	37	11.4/17.9	34.6/34.9	0.29/0.37	Intermediate
Frisian Front 8, central (FF8)	53° 48′ N	4° 30′ E	41	-----/13.3	-----/34.8	-----/0.38	Intermediate
Frisian Front 12, north (FF12)	54° 00′ N	4° 30′ E	44	-----/12.8	-----/34.6	-----/0.20	Intermediate
Dogger Bank tail end (DBte)	55° 29′ N	4° 10′ E	29	10.6/15.0	35.3/35.7	0.13/0.08	Non-depositional
Dogger Bank central (DBc)	55° 01′ N	3° 00′ E	23	12.6/16.7	36.3/35.4	0.11/0.10	Non-depositional
Dogger Bank northern slope (DBns)	55° 33′ N	3° 00′ E	42	10.2/12.2	36.2/36.3	0.12/0.13	Non-depositional
Eastern North Sea (EN)	56° 30′ N	7° 21′ E	35	10.2/15.3	35.8/34.8	0.06/0.02	Non-depositional
Frisian Front 1, south (FF1)	53° 27′ N	4° 30′ E	28	-----/13.9	-----/34.9	-----/0.08	Non-depositional

[a]Characterisation is based on Cadée (1984), Eisma (1987), DeHaas & VanWeering (1997), Dauwe et al. (1998)

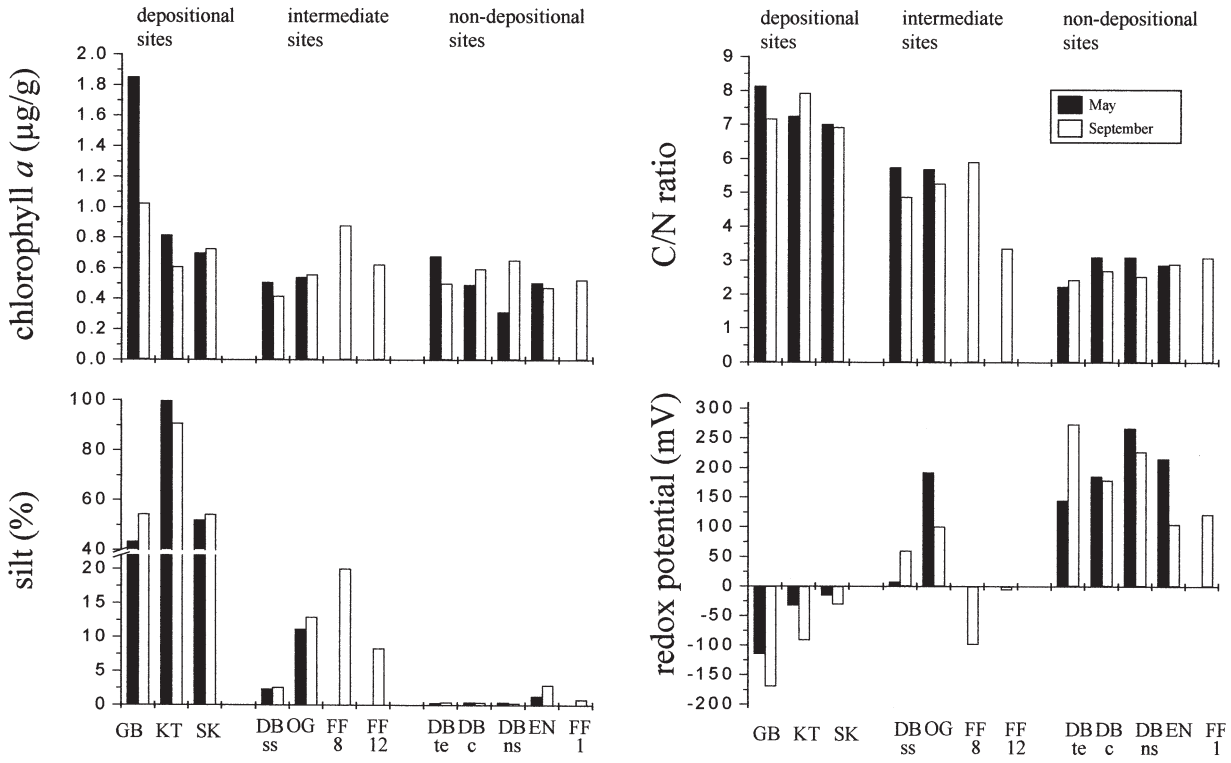

Fig. 2. Relevant sediment characteristics of our sampling areas. Chlorophyll *a* is expressed per dry weight sediment. We considered the grain size fraction <63 µm as % silt. For abbreviations of the sampling stations see Fig. 1

bation of the suspension for 1 h at 4°C in darkness, it was mixed for 1 min, followed by an ultrasonication in a water bath for 3 min at medium power. To remove particles, the suspension was centrifuged at 1745 × *g* at 0°C for 25 min. Chl *a* was analysed in the supernatant by high performance liquid chromatography (HPLC) as described by Wallerstein & Liebezeit (1999). For quantification of chl *a*, we used a standard (Sigma). Chl *a* contents were expressed as µg per dry weight of sediment.

PLFA analyses. PLFA analyses were performed according to Frostegård et al. (1993) and Palojärvi et al. (1997), with slight modifications. Lipid extractions and fractionations were carried out from 8 g (samples of Stns GB, KT and SK) or 16 g (samples of all other stations) of freeze-dried sediment.

The lipids were separated into neutral, glyco- and phospholipids on columns containing silicic acid (Varian Bond Elut SILICA Si 500 mg) by eluting with chloroform, acetone and methanol, respectively. Known amounts of methyl nonadecanoate (19:0; Sigma) were added to the phospholipid fractions as an internal standard. Following a mild, alkaline hydrolysis of phospholipids, the resulting fatty acid methyl esters (FAMEs), dissolved in isooctane, were separated, quantified and identified by gas chromatography-mass spectrometry (GC-MS). The GC-MS measurements were carried out with a Hewlett Packard 5890 series II gas chromatograph, equipped with a 50 m long non-polar phenylmethyl silicone capillary column (HP-5; 0.2 mm, 0.33 µm), combined with a Hewlett Packard 5971 series mass selective detector. Helium was used as a carrier gas with a flow rate of 0.9 ml min^{-1}. The injector had a temperature of 280°C. The time-temperature program for the oven was as following: initial temperature 90°C for 2 min, increase 30°C min^{-1} until 160°C, increase 3°C min^{-1} until 280°C, final temperature 280°C for 10 min. All together, 36 PLFAs were identified from the samples. The identification and the response factors of different PLFA compounds were based on FAME standards (Sigma, Supelco, Nu-Chek-Prep, Larodan Fine Chemicals AB). The individual PLFA values were transformed to mol %.

Fatty acid nomenclature. The nomenclature used to describe fatty acids is as follows. The number before the colon indicates the number of carbon atoms in the fatty acid. The number after the colon indicates the degree of unsaturation (= the number of carbon-carbon double bonds). The position of the first double bond is indicated by the number of carbon units from the methyl (or aliphatic; 'ω') end of the molecule of the monounsaturated fatty acid (MUFA). In the polyunsat-

urated fatty acid (PUFA), 'ω' is followed by the position of the first double bond from the terminal methyl end of the molecule. When the exact position of the double bond was unknown, the 'ω' was omitted. The suffixes 'c' and 't' indicate *cis* and *trans* geometry. The prefixes 'i' and 'a' refer to iso and anteiso branching; 'br' indicates unknown methyl branching position. Other methyl branching is indicated by the position of the additional methyl carbon from the carboxyl end followed by 'Me' (i.e. 10Me18:0). The number before prefix 'OH' indicates the position of a hydroxy group from the carboxyl end (i.e. 3-OH14:0). Cyclopropane fatty acids are designated by the prefix 'cy'.

Statistics. A multivariate statistical technique, canonical correspondence analysis (CCA), was applied to evaluate relative similarities in the PLFA community pattern among samples. This ordination technique, developed for community analysis (Ter Braak 1986), differs from other 2-step ordination techniques, since CCA allows for a direct analysis of the effect of specific environmental variables because ordination axes are constrained to be linear combinations of environmental variables (Ter Braak & Verdonschot 1995). We used a Monte Carlo permutation test (Ter Braak & Smilauer 1998) to check the statistical validity of the association between environmental variables and variance in the community pattern (Software: CANOCO, Microcomputer Power).

Since the aim of our study was to compare the microbial community structures of sites with different sedimentation rates, the stations were grouped into 3 areas (depositional, non-depositional and intermediate sites), to detect the differences in the PLFA composition between them. Significant differences between the groups were tested with ANOVA. The location of the differences was determined with a post hoc Tukey's HSD for unequal N (Spjotvoll/Stoline). We tested for unequal variances with Bartlett's test before performing ANOVA (Sokal & Rohlf 1995). Intra-group similarities and inter-group dissimilarities were calculated with a SIMPER analyses based on Bray-Curtis coefficients (Software: Primer 5.2 (Primer-E) and Statistica 5.1 (Statsoft, Clarke & Warwick 1994).

RESULTS

Environmental variables

The environmental characteristics of the study sites are shown in Table 1 and Fig. 2. The water depth at the sample stations varied between 23 m in the shallow area of the Dogger Bank (central North Sea) and 140 m in the Skagerrak. The silt content at depositional sites ranged from 43 up to 99% (Fig. 2). Non-depositional sites had a silt content of <1%, while at intermediate sites the silt content ranged from 2 up to 20%. Differences between all 3 areas were significant (1-factor ANOVA, $p < 0.01$). The organic carbon content varied between 0.65 and 1.53% at depositional sites, between 0.02 and 0.13% at non-depositional sites and between 0.2 and 0.38% at intermediate sites (Fig. 2). Differences were significant between all 3 areas (1-factor ANOVA, $p < 0.01$). Also, Eh values showed significant variations between the areas (Fig. 2). Only negative Eh values up to –170 mV were measured in the surficial sediment of the depositional sites. While both negative and positive values occurred at intermediate sites, only positive values (up to 267 mV) were measured at stations of non-depositional sites. Highest chl *a* values were found in the sediments at depositional sites (up to 1.8 µg g^{-1}). No obvious differences were found when comparing non-depositional sites with intermediate sites (Fig. 2). Moreover, temporal differences in chl *a* content were only pronounced at depositional sites with higher values in May. Carbon to nitrogen ratios in the sediment, calculated with both compounds expressed on a weight basis (w/w), decreased from depositional sites towards intermediate sites to non-depositional sites (Fig. 2). C/N ratios at depositional sites were extremely low. Although similar values have been reported from other marine sediments (Burdige 1991, P. J. Müller unpubl.) we cannot exclude that we might be dealing with an analytical problem due to an extremely low organic matter content in the corresponding sediments (Table 1).

PLFA composition

A total of 36 different fatty acids was identified. The total PLFA concentrations (nmol g^{-1}), indicative of microbial biomass, are summarised in Fig. 3. Highest PLFA biomass was found at depositional sites, significantly decreasing towards intermediate and non-depositional sites (1-factor ANOVA, $p < 0.05$). In most cases, the values in September were exceeding those measured in May. PLFA biomass at intermediate sites is only slightly higher than in sediments of non-depositional sites. The total number as well as the relative proportion of single fatty acids present in the sediments of the study sites showed distinct variations (Table 2). For the purpose of comparing differences in PLFA composition among sites, we have presented the distribution of the PLFAs at the study sites as mol % in Table 2.

MUFA, PUFA, branched (br), cyclopropyl (cy), saturated and hydroxy (OH) fatty acids in the range of C_{12} to C_{24} were identified (Table 2). The fatty acids, which accounted for a major percentage of the total PLFA at all stations, were 14:0, i15:0, a15:0, 16:1ω7c, 16:0 and

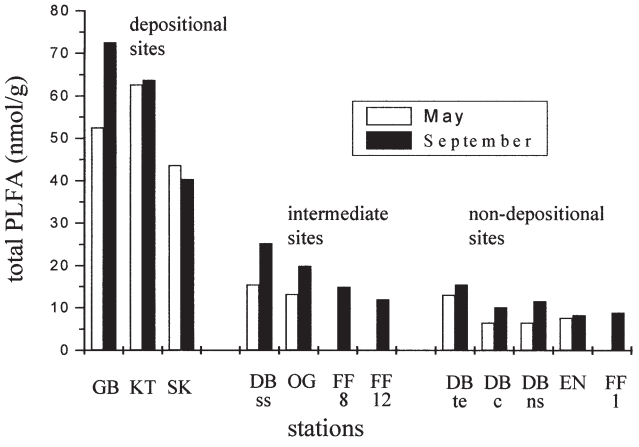

Fig. 3. Absolute concentrations of total phospholipid fatty acids (PLFAs) in nmol g^{-1} expressed per dry weight sediment at our sampling stations. For abbreviations of the sampling stations see Table 1

18:1ω7. The CCA of all PLFA patterns clearly separated depositional sites from non-depositional sites based on redox state, silt content, C/N ratios and chl *a* content (Fig. 4). CCA accounted on average for 79.35% of the variance in the PLFA profile with respect to canonical and environmental axes, the values of the canonical eigenvalues being 0.064 for Axis 1 and 0.009 for Axis 2. Axis 1 of the CCA reveals a gradient from the PLFA profiles of non-depositional to depositional sites and reflects a redox and a C/N gradient, followed by a silt gradient. Sites with low silt content (particularly non-depositional sites) have high redox potentials and low C/N ratios (Table 3).

Conversely, depositional sites reflect the higher silt content, higher C/N ratios and lower Eh values in the sediments. The CCA plot furthermore shows that the intermediate sites are indeed located between depositional and non-depositional sites with respect to their specific PLFA composition. However, at Stn FF8 (central Frisian Front) the environmental conditions (chl *a*, redox potential, silt content, C/N ratio) as well as the PLFA profile seem to be more similar to the depositional sites, while Stn FF12 at the northern Front tends to be closer to the group of non-depositional sites. The correlation with Axis 2 did not reveal a distinct environmental gradient.

To determine the similarity in the composition of PLFA in sediments of the study sites, a similarity analysis was performed. The results are shown in Fig. 5. This calculation also discriminated depositional, intermediate and non-depositional sites and identified 3 clusters. A SIMPER analysis (Table 4) revealed that the similarity of stations each belonging to the same cluster is >90% for all 3 groups. Highest dissimilarity was found between the depositional cluster and the non-depositional cluster (20.15%). The intermediate cluster differs more from the depositional cluster (16.64%) than from the non-depositional group (8.73%).

To detect differences in the relative proportions of PLFAs of stations grouped in these 3 clusters, an ANOVA followed by Tukey's honestly significant post hoc test was performed. The results are shown in Table 5. They illustrate the differences in community structure of the 3 clusters. Of the 36 fatty acids identified and tested, 14 fatty acids (12:0, 13:0, 3-OH 12:0, 3-OH 14:0, 16:1ω5, 17:1, 10Me18:0, cy 19:0 and all PLFAs ≥ C_{20}) were significantly more abundant in the cluster of depositional sites compared to both other clusters. Seven additional fatty acids were present in significantly higher quantities at depositional sites, but only when compared with non-depositional sites (i14:0, br17:0, 10Me16:0, cy17:0, 17:0, 18:1_b and 18:0). Four fatty acids were significantly more abundant in the intermediate cluster only compared to the non-depositional cluster (i14:0, 16:1ω5, a17:0 and 18:1ω9c).

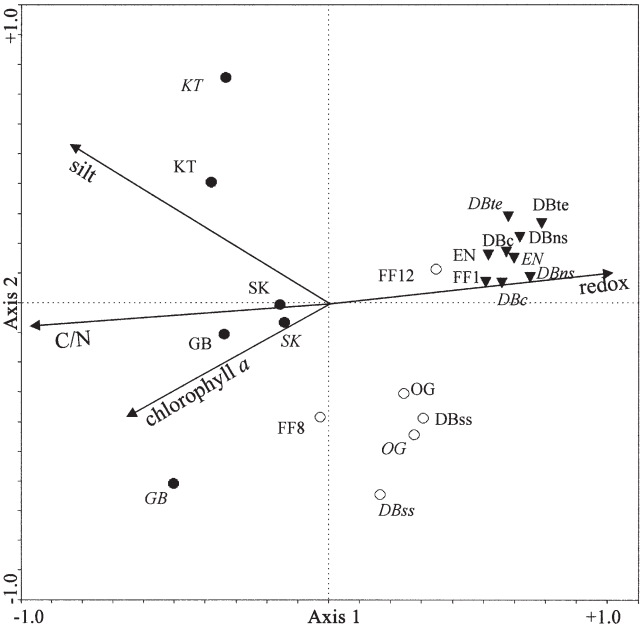

Fig. 4. Canonical correspondence analysis (CCA) biplot of sites based on phospholipid fatty acid (PLFA) profiles (expressed in mol %) in May and in September as presented in Table 2 and significant environmental variables (chlorophyll a, silt content, C/N ratio and redox potential). The profile of 1 site comprises 36 different fatty acids. Values of sediment characteristics are shown in Fig. 1. Arrows indicate the direction and relative importance (lengths) of the 4 environmental variables. The 1.0 scale refers to the environmental variables. Intraset correlations of environmental variables with the first 2 axes of CCA are shown in Table 3. To aid interpretation of the plot, depositional sites (●), intermediate sites (○) and non-depositional sites (▼). Labels are marked in italics for September samples

Table 2. Percentages and total number (total N) of phospholipid fatty acids (PLFAs) in the surficial sediments of our sampling stations in May (M) and September (S). For abbreviations of the sampling stations see Table 1. Depositional sites are marked with $^{+}$, intermediate sites are in italics

Fatty acid	Sampling stations																				
	GB^{+} (M)	GB^{+} (S)	KT^{+} (M)	KT^{+} (S)	SK^{+} (M)	SK^{+} (S)	*DBss* (M)	*DBss* (S)	*OG* (M)	*OG* (S)	*FF8* (S)	*FF12* (S)	DBte (M)	DBte (S)	DBc (M)	DBc (S)	DBns (M)	DBns (S)	EN (M)	EN (S)	FF1 (S)
12:0	0.56	0.60	0.54	0.62	0.28	0.38	0.00	0.36	0.22	0.00	0.00	0.00	0.13	0.17	0.00	0.35	0.00	0.00	0.26	0.00	0.00
13:0	0.24	0.27	0.23	0.27	0.14	0.17	0.00	0.14	0.00	0.00	0.00	0.00	0.18	0.13	0.00	0.00	0.00	0.00	0.12	0.00	0.00
3-OH12:0	0.08	0.09	0.07	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
i14:0	2.90	4.17	2.97	3.52	2.02	2.31	1.61	1.96	2.29	2.65	2.57	2.54	1.88	1.38	0.85	1.44	1.75	1.43	1.73	0.97	2.04
14:0	8.44	8.50	8.03	7.85	7.17	7.83	7.85	7.19	9.02	7.92	8.42	8.34	3.12	4.66	7.24	6.48	10.30	5.73	7.13	4.01	5.56
i15:0	5.78	7.13	7.68	8.03	5.64	5.38	6.50	6.51	7.40	8.21	9.02	10.27	4.04	4.59	3.94	4.68	5.54	5.29	6.34	6.29	7.39
a15:0	9.50	9.77	11.61	11.95	9.29	8.94	11.01	12.40	11.16	12.66	12.28	13.39	11.48	9.81	5.83	8.05	7.88	8.32	12.32	11.37	13.24
15:0	2.37	2.52	2.03	2.39	1.91	2.19	2.48	2.25	2.27	2.46	2.31	2.21	1.29	1.98	3.10	2.32	2.01	1.62	2.12	1.73	2.46
3-OH14:0	0.00	0.14	0.11	0.13	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
i16:0	1.70	2.08	1.87	1.98	1.43	1.47	1.36	1.23	1.60	1.82	2.18	2.11	1.57	1.10	1.19	1.16	1.33	1.36	1.17	1.39	1.96
16:1ω9	1.67	2.14	2.74	2.82	2.80	2.84	1.60	1.90	1.97	1.84	1.92	2.44	3.39	2.62	1.50	1.62	2.20	3.23	1.63	2.39	1.46
16:1ω7c	14.69	14.62	18.25	15.50	18.63	18.16	20.10	21.45	15.79	15.41	12.95	13.04	29.73	24.66	20.07	23.88	19.68	22.42	22.31	20.50	16.38
16:1ω7t	2.71	2.17	1.83	1.72	1.43	1.33	1.65	2.00	1.38	1.85	0.87	0.82	2.06	1.80	1.45	2.22	1.04	1.35	1.88	1.50	1.49
16:1ω5	2.41	2.67	4.01	3.40	3.00	3.27	1.61	1.77	2.61	2.57	2.28	2.35	1.93	1.75	0.95	1.10	1.49	1.99	1.52	1.63	1.52
16:0	17.35	15.22	16.70	15.89	16.37	16.08	22.92	19.59	22.12	22.13	24.58	21.90	18.56	20.16	31.71	26.58	26.01	22.98	21.23	21.79	23.48
br17:0	1.26	1.04	2.08	1.61	1.20	0.84	1.58	1.64	1.13	1.26	1.43	1.87	2.65	3.83	1.11	1.87	1.99	3.98	1.65	3.89	2.95
10Me16:0	0.72	0.87	0.97	0.94	0.87	0.92	0.61	0.64	0.66	0.56	0.60	0.84	0.72	0.56	0.36	0.29	0.42	0.34	0.63	0.83	0.43
i17:0	1.49	1.50	1.32	1.33	1.18	1.25	1.02	0.91	1.19	1.18	1.46	1.51	0.54	0.83	1.68	0.79	1.14	1.20	0.81	1.25	1.24
a17:0	1.37	1.33	1.36	1.44	0.95	1.00	1.28	1.20	1.22	1.30	1.43	1.43	0.50	0.78	0.75	0.67	0.76	0.70	1.06	1.19	1.51
17:1	1.02	1.28	0.22	1.22	1.34	1.49	0.72	0.85	0.80	0.81	0.60	0.69	0.57	0.99	0.00	0.68	0.57	0.70	0.71	0.90	0.61
cy17:0	0.56	0.77	0.73	0.81	0.83	0.86	0.43	0.48	0.53	0.63	0.00	0.49	0.75	0.60	0.57	0.40	0.00	0.00	0.34	0.00	0.00
17:0	1.84	1.78	1.32	1.48	1.37	1.71	1.33	1.21	1.37	1.59	1.62	1.38	0.99	1.03	1.58	1.32	0.97	1.06	1.12	1.32	1.63
10Me17:0	0.24	0.12	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18:1ω9c	2.63	2.84	2.15	2.99	3.92	4.05	3.30	2.94	2.96	2.63	3.09	3.05	2.68	3.99	4.33	3.21	4.29	4.66	2.73	4.41	3.52
18:1ω7	6.67	6.15	3.52	6.02	7.32	7.09	7.65	8.31	6.14	6.04	5.57	5.48	8.84	10.19	7.41	7.55	7.50	8.99	8.46	9.68	7.95
18:1$_{a}$‡	0.76	0.75	0.79	0.39	0.38	0.30	0.39	0.48	0.30	0.47	0.17	0.19	0.41	0.47	0.38	0.46	0.24	0.40	0.41	0.45	0.51
18:1$_{b}$‡	1.62	2.07	0.75	1.14	1.06	1.16	0.59	0.43	1.29	0.87	0.75	0.69	0.27	0.31	0.58	0.53	0.78	0.20	0.19	0.23	0.22
18:0	4.24	3.07	3.49	2.16	3.96	3.66	2.33	1.97	3.79	2.73	3.22	2.56	1.59	1.27	3.45	2.23	2.12	2.05	1.99	2.29	2.23
10Me18:0	0.27	0.34	0.26	0.24	0.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cy19:0	0.22	0.22	0.25	0.22	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:4	0.59	0.58	0.55	0.51	1.02	1.30	0.00	0.08	0.46	0.07	0.17	0.00	0.14	0.15	0.00	0.00	0.00	0.00	0.03	0.00	0.00
20:1	3.37	2.20	0.88	0.45	3.48	2.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:0	0.43	0.38	0.22	0.34	0.22	0.23	0.00	0.14	0.33	0.32	0.41	0.32	0.00	0.09	0.00	0.00	0.00	0.00	0.12	0.00	0.20
22:6	0.00	0.35	0.12	0.22	0.28	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:0	0.16	0.14	0.15	0.16	0.10	0.11	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
24:0	0.16	0.14	0.17	0.14	0.11	0.12	0.00	0.00	0.00	0.00	0.11	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total N	34	36	36	35	34	33	24	27	26	25	25	25	27	29	22	25	22	22	27	22	23

‡Double bond position not determined

Table 3. Intraset correlations of environmental variables with the first 2 axes of canonical correspondence analysis (CCA). The environmental variables were standardised to unit variance after log-transformation

Axis variable	Correlation coefficients 1	2
Silt	−0.84	0.52
Redox	0.91	0.11
C/N	−0.97	−0.08
Chlorophyll *a*	−0.64	−0.38

Between-cluster differences in selected fatty acids can be used as specific biomarkers and are shown in Fig. 6. In addition to the marker fatty acids 24:0, 10Me18:0 and 16:1ω5, the relative contribution of PUFA, cyclopropyl fatty acids and saturated fatty acids $>C_{20}$ were significantly higher at depositional sites compared to intermediate and non-depositional sites ($p < 0.01$). Also, the relative contribution of 10Me16:0 decreased from depositional sites towards non-depositional sites. At non-depositional sites, MUFAs and trans fatty acids were higher compared to intermediate and non-depositional sites.

DISCUSSION AND CONCLUSIONS

Most of the 36 PLFAs that we identified in sediments of the North Sea and the Skagerrak/northern Kattegat are considered to be of bacterial origin, because fatty acids in the range of C_{12} to C_{19} are known to be characteristic of bacteria (Lechevalier 1977). Several studies have revealed that branched, monounsaturated, cyclopropyl fatty acids and also certain saturated fatty acids present in sediments are definitely contributed by *in situ* bacterial production (Perry et al. 1979, Gillan & Hogg 1984, White et al. 1984). Furthermore, we detected other fatty acids, which are known to be present either in both prokaryotes and microeukaryotes or only in microeukaryotes. By contrast, palmitic acid (16:0) is ubiquitously present in most organisms (Mancuso et al. 1990) and high percentages were measured in all sediment samples. However, since our PLFA profiles strongly suggest that bacteria are the major contributors to the sedimentary PLFA pool, palmitic acid is assumed to be predominantly of bacterial origin.

The relative abundance of phospholipid ester-linked fatty acids with more than 19 carbon atoms (Table 2) indicates that other microbial groups as e.g. heterotrophic microeukaryotes and microalgae are only of minor

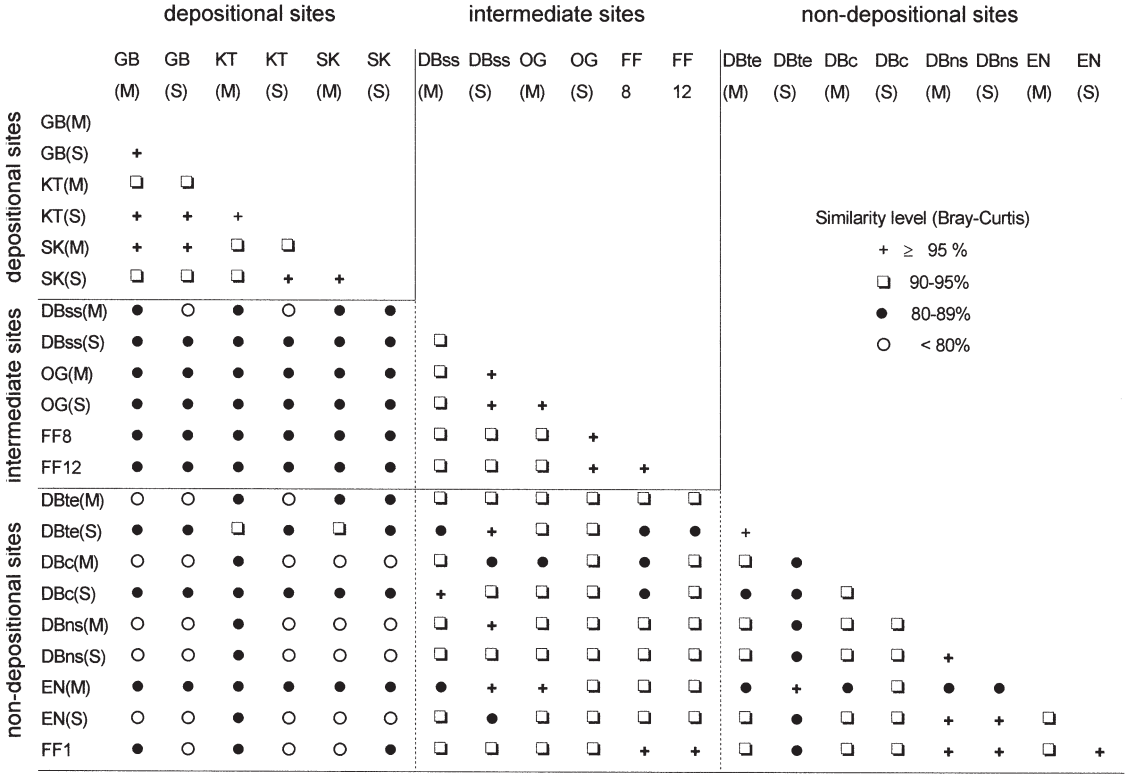

Fig. 5. Similarity matrix of the phospholipid fatty acid (PLFA) composition in sediments collected at our 12 sampling stations in May (M) and September (S). The calculation is based on a Bray-Curtis similarity and 36 PLFAs (presence/absence). For PLFAs see Table 2. To aid interpretation, absolute similarity levels were classified and categories were expressed as symbols

Table 4. Average similarity (Bray-Curtis) of sites within a cluster and dissimilarity (Bray-Curtis) between clusters. The cluster 'depositional sites' includes Stns GB, KT and SK; the cluster 'intermediate sites' includes Stns DBss, OG, FF8 and FF12; and the cluster 'non-depositional sites' includes Stns DBte, DBc, DBns, EN and FF1

Cluster	Intra-cluster similarity (%)	Clusters	Inter-cluster dissimilarity (%)
Depositional sites	93.58	Intermediate-depositional	16.64
Intermediate sites	93.62	Intermediate-non-depositional	8.73
Non-depositional sites	91.62	Depositional-non-depositional	20.15

importance in a quantitative respect for our study sites. Thus, the total PLFA pattern with its maximum values at depositional sites (Fig. 3) mainly mirrors the bacterial abundance. Our results are in agreement with the findings of Stoeck & Kröncke (2001), that bacterial biomass in depositional areas like the German Bight is higher compared to the central North Sea and the Oyster Ground as measured by epifluorescence microscopy. Hondeveld et al. (1995) also found higher bacterial abundances in sediments of the German Bight and the Skagerrak area than in the Oyster Ground and the Dogger Bank. These findings are discussed in detail by Hondeveld et al. (1995) and by Stoeck & Kröncke (2001).

While the short-chain fatty acids which we identified in the range of C_{12} to C_{14} are of unspecified bacterial origin, the fatty acid 16:1ω5 is a typical marker for the abundance of bacteria of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum (Findlay et al. 1990, Findlay & Dobbs 1993, Frostegård et al. 1993). Since the application of molecular biological tools, members of the CFB phylum have been detected in an increasing number of different marine habitats (e.g. DeLong et al. 1993, Bowman et al. 1997, Llobet-Brossa et al. 1998, Rath et al. 1998, Eilers et al. 2000, Uphoff et al. 2001, Weller et al. 2000). Although members of the CFB group can adapt to rather low nutrient levels (Höfle 1983) as we observed at the non-depositional sites (Fig. 6, Table 1), they are specialised to the degradation of complex macromolecules (Coughlan & Mayer 1992, Reichenbach 1992) and occur commonly in habitats rich in organic matter (Reichenbach 1992). This is the case in the depositional areas under study. While the Skagerrak/northern Kattegat is assumed to be the ultimate sink for about ½ of the refractory organic carbon produced in the North Sea (DeHaas & VanWeering 1997, DeHaas et al. 1997), the rivers Elbe and Weser transport high loads of organic matter and complex macromolecules into the German Bight (Dippner 1993, 1998, Luff & Pohlmann 1995, Dauwe et al. 1998). Stoeck et al. (in press) studied carbon source utilisation profiles of benthic microbial communities in the North Sea and found a signifi-

Table 5. Significant differences in single fatty acids between depositional (GB, KT, SK), intermediate (DBss, OG, FF8, FF12) and non-depositional sites (DBte, DBc, DBns, EN, FF1) calculated with ANOVA. Location of significant differences was calculated with Tukey's HSD for unequal N (Spjotvoll/Stoline). Data are relative contribution of each fatty acid to the total phospholipid fatty acid pool and were pooled over both sampling periods. In each case, N = 21 and df = 2, 18 (effect, error). + = differences are significant between depositional and intermediate sites, ⋆ = differences are significant between depositional and non-depositional sites, ● = differences are significant between intermediate and non-depositional sites. For abbreviations of sampling sites, see Fig. 1

Dependent variables	Independent variables and location of significant differences (Tukey's HSD)			df	p
Fatty acid	Depositional	Intermediate	Non-depositional		
12:0	+⋆	+	⋆	17.23	<0.01
13:0	+⋆	+	⋆	12.75	<0.01
3-OH12:0	+⋆	+	⋆	10.31	<0.01
i14:0	⋆	●	⋆●	14.81	<0.01
3-OH14:0	+⋆	+	⋆	5.16	<0.05
16:1ω5	+⋆	+●	⋆●	28.18	<0.01
br17:0	⋆		⋆	6.30	<0.01
10Me16:0	⋆		⋆	4.49	<0.05
a17:0		●	●	6.88	<0.01
17:1	+⋆	+	⋆	12.52	<0.01
cy17:0	⋆		⋆	9.90	<0.01
17:0	⋆		⋆	4.28	<0.05
18:1ω9c		●	●	4.59	<0.05
$18:1_a$ ‡	+	+		3.58	<0.05
$18:1_b$ ‡	⋆		⋆	6.97	<0.01
18:0	⋆		⋆	6.38	<0.01
10Me18:0	+⋆	+	⋆	42.63	<0.01
cy19:0	+⋆	+	⋆	24.04	<0.01
20:4	+⋆	+	⋆	24.50	<0.01
20:1	+⋆	+	⋆	22.22	<0.01
20:0	+⋆	+	⋆	7.63	<0.01
22:6	+⋆	+	⋆	12.71	<0.01
22:0	+⋆	+	⋆	10.75	<0.01
24:0	+⋆	+	⋆	13.67	<0.01

‡Double bond position not determined

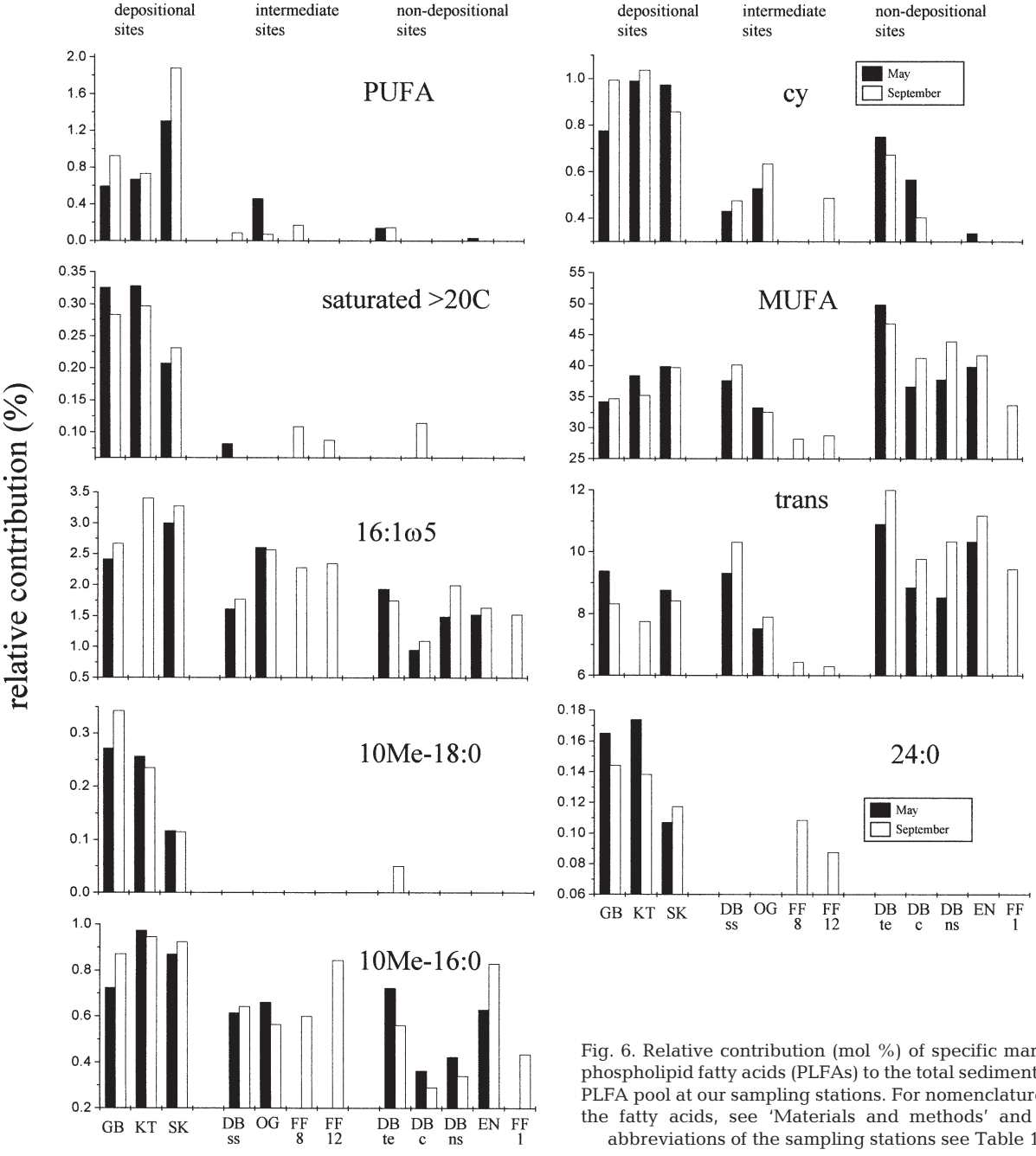

Fig. 6. Relative contribution (mol %) of specific marker phospholipid fatty acids (PLFAs) to the total sedimentary PLFA pool at our sampling stations. For nomenclature of the fatty acids, see 'Materials and methods' and for abbreviations of the sampling stations see Table 1

cantly higher rate of complex macromolecule degradation in German Bight sediments compared to the open sea.

The described organism-environment relationship is supported by another fatty acid marker. The PLFA 10Me18:0, which was found in low (up to 0.35%) but significant abundance at all depositional sites compared to intermediate and non-depositional sites, was suggested as a marker PLFA in *Actinomycetes* (Vestal & White 1989, Sundh et al. 1997). Although these Gram-positive bacteria mainly occur in the aerobic zone of soils (Ensign 1992), some studies have observed the distribution of *Actinomycetes* in marine sediments (Barcina et al. 1987, Do et al. 1991, Jensen et al. 1991). However, due to the terrestrial input at the depositional sites, we cannot exclude that these organisms might be of terrestrial origin. *Actinomycetes* are prolific producers of extracellular enzymes that degrade complex macromolecule substrates (McCarthy 1989). Thus, the significant abundance of *Actino-*

mycetes, together with the higher abundance of members of the CFB phylum at depositional sites compared to non-depositional sites, reflects the environmental conditions in these areas and the nature of organic matter available to the benthic communities.

Cyclopropyl fatty acids in the range of C_{17} to C_{19}, which were also found to be significantly higher at depositional sites compared to intermediate and non-depositional sites, have been suggested as specific bacterial signatures in sediments (Cranwell 1973, Perry et al. 1979, Baird & White 1985). However, while the results of Vestal & White (1989) indicated that cyclopropyl fatty acids are a good marker of anaerobic eubacteria, Parkes & Taylor (1983) suggested that they are more indicative of aerobic bacteria. From our point of view, we can neither confirm nor reject these hypotheses. Although our sediments in the depositional areas are characterised by very low Eh values, indicating anoxic conditions (Graf et al. 1983, Meyer-Reil 1983), we cannot exclude the formation of aerobic microniches in the top 2 cm of the sediment due to bioturbation of macrofauna (Kröncke & Rachor 1992, Dauwe et al. 1998) or to resuspension during a tidal cycle (Boon & Duineveld 1996). An indication for the occurrence of aerobic prokaryotes in these generally anoxic sediments is given by another marker fatty acid group. Monounsaturated PLFAs are acknowledged signatures for aerobic prokaryotes (Findlay et al. 1990, Rajendran et al. 1993). The relative contribution of MUFAs of up to 40% at depositional sites also indicates that a high proportion of aerobic organisms occurs in microniches of these anoxic sediments. Furthermore, cyclopropyl fatty acids in the range of C_{17} to C_{19} have been reported as major constituents in *Desulfobacter* spp. (Dowling et al. 1986).

Besides the cyclopropyl fatty acids, 10Me16:0 is also specific for the SRB (sulphate-reducing bacteria) genus *Desulfobacter* (Dowling et al. 1986, Smith et al. 1986, Mancuso et al. 1990, Rajendran et al. 1993). As also indicated by this fatty acid, *Desulfobacter* contributes more to the PLFA profiles of the depositional sites than to those of intermediate and non-depositional sites (Fig. 6). As observed by Findlay et al. (1990), the reduced availability of oxygen in sediments may cause the increase in SRB. However, the use of 10Me16:0 as a specific biomarker for *Desulfobacter* spp. should done with care (Boschker et al. 2001).

Fatty acids with 2 or more unsaturated bonds (PUFAs) are produced by microeucaryotes (Findlay et al. 1990, Rajendran et al. 1993, Sundh et al. 1997). Furthermore, saturated fatty acids with a chain length of more than 20 carbon atoms are also suggested to be a biomarker for eukaryotic organisms as microalgae, protozoa and diatoms (Vestal & White 1989). Reflecting the pattern of PUFAs (Fig. 6), these eukaryotic markers occurred in significant abundances at depositional sites compared to intermediate and non-depositional sites. However, low percentages of PUFAs (<2%) and saturated fatty acids with >20 carbon atoms (e.g. <0.18% of 24:0 and <0.35% of the summed abundance of fatty acids with >20 carbon atoms) compared to the summed abundance of MUFAs (up to >50%) supports the view that bacteria are the predominant contributor of fatty acids to the sediment (Fig. 6).

A lack of microeukaryotes in the central North Sea might be due to enhanced sediment reworking by the prevailing hydrographical regime in this area (Pingree & Griffith 1982, Lwiza et al. 1991). An enhanced sediment reworking on the Dogger Bank is indicated by the low sedimentary chl *a* (Fig. 2) and organic carbon (Table 1) values despite a high primary production in the water column of this frontal area (up to 700 mg C m^{-2} d^{-1}, Richardson et al. 1998). Only negligible amounts of algal material reach the sea floor at most parts of the Dogger Bank (Stoeck & Kröncke 2001). These benthic microeukaryotes seem to favour an environment which is subjected to less physical disturbance by currents and waves, resulting in greater stability and the formation of stable microniches. However, also at study sites which are not subjected to sediment reworking as our depositional sites, the relative abundance of microeukaryotes is still <0.35%. Low percentages of these fatty acids in coastal sediments have been attributed to the organic pollution and the prevailing anoxic conditions (Smith et al. 1985, Rajendran et al. 1991). Such conditions cause a loss of microeukaryotic biomass and an increase in bacterial biomass (Findlay et al. 1990). This fits well with our own findings, which are also in agreement with the PLFA patterns found in the anoxic sediments of different eutrophic bays (Rajendran et al. 1992).

The situation of a nutritionally limited system and sediment reworking processes in the central North Sea is also reflected in another variable measured by means of PLFA. While *cis* isomers of MUFAs are common microbial cellular components (Guckert et al. 1987), high abundances of *trans* isomers have been associated with strategies for survival during physiological stress in bacteria (Guckert et al. 1985, 1986, 1987, Nichols et al. 1986, Rajendran et al. 1992). Highest abundances of *trans* isomers have been detected at non-depositional sites compared to the sediments of the other sites (Fig. 6). Limited nutrient supply and a physically unstable habitat are supposed to be factors responsible for a stressed bacterial community (Stoeck & Kröncke 2001). Thus, in this offshore area, benthic microbial communities do not benefit from a high algal matter production in the water column.

Comparative summary of community composition

Summarising the results discussed above, we can conclude that the structures of microbial communities are significantly different between the PLFA patterns of sediments at depositional and non-depositional sites. Furthermore, we can discriminate a third group of communities of sites, which is a link between depositional and non-depositional areas with respect to the sedimentation regime which we called 'intermediate'.

The communities of all sites were dominated by bacteria. The bacterial proportion was lowest in the sediments of non-depositional sites and highest in the sediments of depositional sites. Bacterial groups being a component of the degradation pathways of complex macromolecules, e.g. *Cytophaga* and *Actinomycetes*, were found in significant abundances at depositional sites compared to the other sites. Additionally, SRB such as *Desulfobacter* spp. occurred in higher abundances at depositional sites. Although the abundance of microeukaryotes was significantly higher at depositional sites compared to non-depositional and intermediate sites, the relative contribution of these organisms to microbial biomass is low. A nutritionally limited benthic system in the sediments of the non-depositional sites in the offshore is indicated by a microbial community exposed to greater physiological stress than in the organically enriched sediments of the deposition areas. This is supported by total absolute PLFA biomass values at depositional sites, which exceed those of intermediate and non-depositional sites by far.

It is striking that the PLFA profile of the communities in spring and in autumn in most cases is similar on a 95% level. This indicates that the structure of microbial communities may be rather stable over time, while the metabolical profiles of microbial communities differed seasonally to a larger extent (Stoeck et al. in press).

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