

Spatial patterns of bacterial signature biomarkers in marine sediments of the Gulf of Mexico

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Abstract

Sediment cores near a cold methane seep and gas hydrates in the Gulf of Mexico were analyzed for bacterial membrane phospholipid fatty acids (PLFA) and isoprenoid quinones. Nearby non-seep marine sediments were also analyzed for the purpose of comparison. Our goal was to use these biomarkers as proxies for microbial community structure and redox indicators. Total PLFA varied from core to core and were significantly less abundant in non-seep marine sediments than those near the cold seep or gas hydrates. In general, saturated straight chain, terminal branched and mid-chain branched, and monounsaturated fatty acids contributed >98% of the total PLFA. The branched fatty acids (i.e., *a*C15:0, *i*C15:0, Me10C16:0) could be largely attributed to sulfate-reducing bacteria and the monounsaturated fatty acid (i.e., 16:1 ω 7c and 18:1 ω 7c) could be attributed to sulfide-oxidizing bacteria (*Beggiatoa*/*Thioploca*). Principal component analysis of PLFA data revealed that microbial communities were consistent with the spatial distributions of gas hydrates and *Beggiatoa* mats and with the variation in geochemical conditions. Isoprenoid quinone profiles indicated that aerobic respiration was the dominant metabolic process in the top-most sediment layers while the anaerobic respiration dominated the deeper intervals in the marine sediments. Significant amount of cyclopropyl fatty acids at all sites indicated the stress adaptation of the bacteria in the extreme environment. The consistency between the distribution of PLFA and respiratory quinones and the variation in geochemical conditions suggested that analyses of PLFA and quinone could help us to locate the existence of the coupled sulfide-oxidization and sulfate-reduction processes. © 2006 Elsevier B.V. All rights reserved.

Keywords: Gulf of Mexico; Gas hydrate; Phospholipid fatty acids; Respiratory quinones; Biomarker; *Trans/cis* ratio; Multivariate statistics

1. Introduction

Gas hydrate is an ice-like crystalline structure, in which hydrocarbon and non-hydrocarbon gases are held within rigid cages of water molecules (Jeffrey and McMullan,

1967). Gas hydrate occurs worldwide, onshore, in polar regions and offshore at water depths under proper pressure–temperature conditions (Milkov and Sassen, 2002). The hydrate-bounded methane is a huge sink of greenhouse gas and the stability of methane hydrates plays a potentially significant role in globe warming, stability of ocean bottom sedimentary layer and carbon cycling in the marine environments (e.g., Kvenvolden, 1988a,b; Jiang et al., 2006). A cold seep is an area of the ocean floor where

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seepage of methane and other hydrocarbon-rich fluids occurs; however, a cold seep is not necessary accompanied by gas hydrate. The anaerobic oxidation of methane (AOM) at both gas hydrate (e.g., Boetius et al., 2000; Zhang et al., 2002, 2005) and seep (e.g., Schouten et al., 2001) sites by methanotrophy-sulfate reduction-based communities has been demonstrated to be a significant mechanism in converting methane to CO_2 (e.g., Boetius et al., 2000). However the microbial ecological structure, the spatial distribution of the density of biomass, and the connection of microorganisms to the local geochemical cycling have not been well-delineated.

Phospholipids are found in all bacterial cellular membranes, have a rapid turnover in living cells and are rapidly hydrolyzed upon cell death. They are found in reasonably constant amount in bacterial cells (White et al., 1979). Analysis of cellular membrane phospholipids fatty acids (PLFA) represents a quantitative and sensitive method for determining the interactions between members of consortia in terms of biomass, community structure, nutritional status, and metabolic

activities (Ringelberg et al., 1997). The measurement of microbial PLFA has been proved to be valid in estimating biomass (Ringelberg et al., 1997) and assessing changes in community composition (Taylor and Parkes, 1983; Guckert et al., 1985, 1991; Vainshtein et al., 1992; Guezennec and Fiala-Medioni, 1996; Elvert et al., 2003; Zhang et al., 2005), nutritional status (Guckert et al., 1986; Kieft et al., 1994), metabolic activity (e.g., Kaneda, 1991), and environmental stress (DeLong and Yayanos, 1985; Guckert et al., 1986; Kamimura et al., 1993; Yano et al., 1998; Fang et al., 2004).

PLFA can serve as fingerprints of special microbial groups and can be used as a taxonomy signature for bacteria classification (e.g., Taylor and Parkes, 1983; Kaneda, 1991; Vainshtein et al., 1992; Koga et al., 1993). They also carry information of the community structure (e.g., Volkman et al., 1980; Guezennec and Fiala-Medioni, 1996; Zhang et al., 2002). Fatty acids are valuable phenotypic properties for characterizing pure cultures (e.g., Guckert et al., 1991; Vainshtein et al., 1992). However, they often represent multi-sources in the environmental samples.

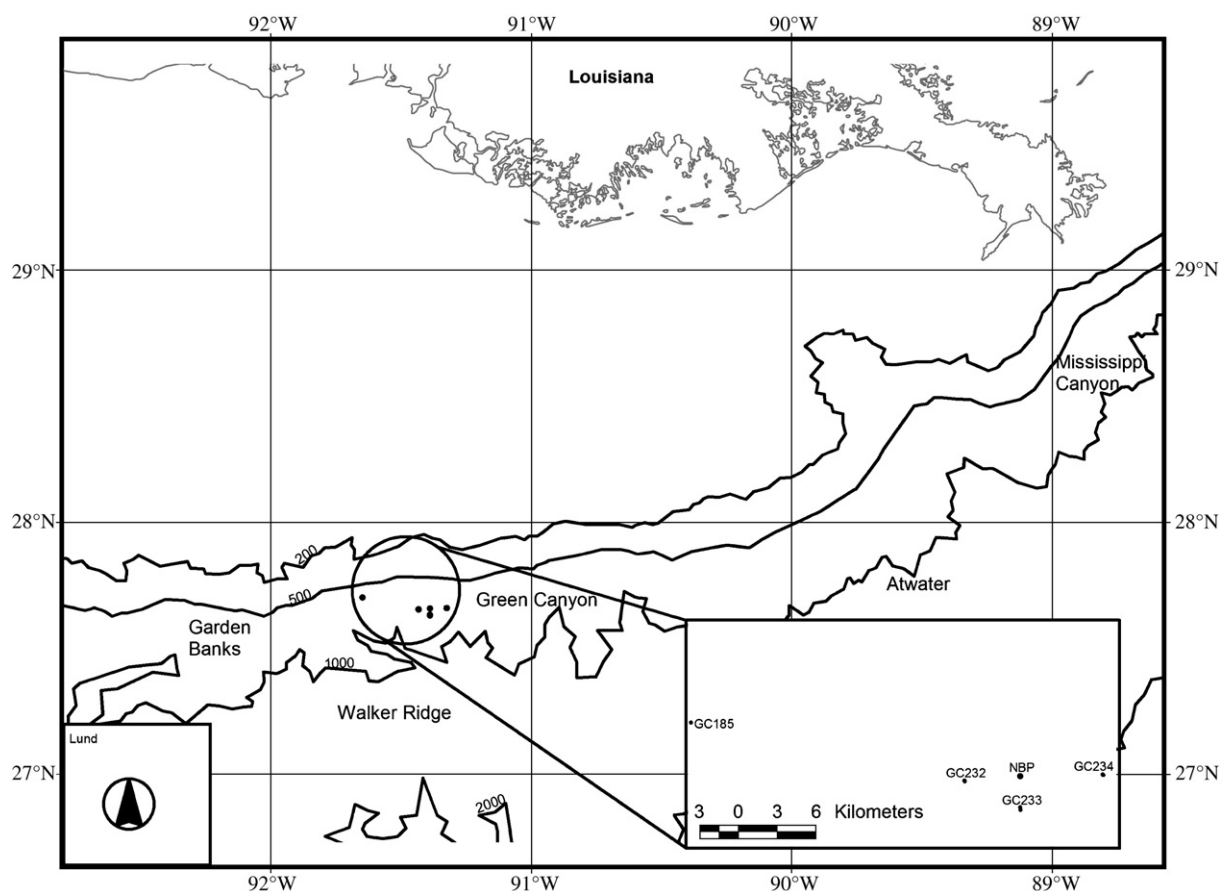


Fig. 1. Geographic locations of cores collected from gas hydrates (GC232, GC234), cold seep (GC233), and non-seep marine sediment (NBP).

Statistical methods can be used to understand the ecological structure using lipid profiles. For example, hierarchical cluster analysis (HCA) and principal component analysis (PCA) have been used in classification of the PLFA profiles of a deciduous forest biome (White et al., 1996). HCA grouped different levels of the forest biome by their similarities in profiles, and PCA identified different fatty acid groups, which represented Gram-positive, Gram-negative, and sulfate-reducing bacteria (White et al., 1996). Statistical analyses of PLFA are also employed to reveal the correlations between the pollution factor and specific groups of organisms (e.g., Pennanen et al., 1996) and changes in community structure in space and time (e.g., Smoot and Findlay, 2001).

Previous studies have reported on the PLFA from gas hydrate-related microbial communities (Fang et al., 1993; Zhang et al., 2002; Elvert et al., 2003; Zhang et al., 2005; Orcutt et al., 2005; Niemann et al., 2005). Those studies examined a limited number of samples, which may hinder the characterization of the PLFA profiles statistically. The molecular DNA techniques can reveal the exact species but are often not used to tell the abundance of biomass in a particular geochemical site. On the other hand, PLFA are often used to delineate the general distribution of microorganisms with different metabolic capabilities. In this study, we analyzed PLFA and isoprenoid quinone profiles from gas hydrate sites, cold seep and background sediment using a large number of samples from five spatially separated sediment cores.

2. Materials and methods

2.1. Study sites and sample collection

Sediment samples were collected from a variety of locations in the Gulf of Mexico (Fig. 1) in 2002 using the submersible *Johnson Sea-Link* aboard the R/V Seward Johnson. These sites were located in the Green Canyon (GC) area (27°2' to 27°55'N and 91°33' to 91°40'W). Our sampling locations included two gas hydrate sites (GC232 and GC234) and a hydrocarbon cold seep site (GC233). Cores were collected during several dives at these locations. A box core (NBP 2B) was collected approximately 2.6 km north of a brine pool at GC233 during the 2002 cruise. It represented a control site without hydrocarbon or methane release. The water depth ranged from 530 m to 716 m at these locations (Table 1). Cores at GC234 (D4424C3 and D4426C4) contained unclassified red bacterial mats on the surface of sediments. Bacterial mats were not visually observed in cores from GC233 (D4425C1) and GC232 (D4428C2). Because cores were collected

away from the chemosynthetic communities, no invertebrate remains were observed in the samples.

2.2. Total lipid extraction, column chromatography and methylation

Core samples were cut into 2-cm intervals and lyophilized. About 10 g (dry weight) of each sample were weighed into a glass centrifuge bottle. A modified Bligh and Dyer (1959) single-phase solvent system (White et al., 1979) was used for lipid extraction. Total lipids were extracted overnight in 37.5-ml chloroform/methanol/potassium phosphate buffer (1:2:0.9 by volume at pH 7.4) and then separated into neutral-, glycol- and polar lipids by silicic acid column chromatography (Guckert et al., 1985). The polar lipid fraction (eluted by methanol) with ester-linked fatty acids were prepared for gas chromatography–mass spectrometry analysis by a mild alkaline transesterification (Guckert et al., 1985). The resulting fatty acid methyl esters were identified and quantified as described elsewhere (e.g., Ringelberg et al., 1997).

2.3. Isoprenoid quinones

The neutral lipid fraction was analyzed for the content of major respiratory quinones by high performance liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry as described by Geyer et al. (2004). Ubiquinones (UQ-n),

Table 1
Push cores collected during Cruise 2002 in the Green Canyon (GC) Leasing Block of the Gulf of Mexico

Site	Dive	Core #	Location and description of core	Methane ¹	HC ²	Water depth (m)
GC232	4428	2	Hydrate mound, no mat	+	++	620
GC233	4425	1	210 ft away from the brine pool ³ , no mat, on hard white surface	++	+	716
GC233	4425	2	Control site, 2.6 km north of the brine pool (NBP2B), no mat	–	–	716
GC234	4424	3	Hydrate mound, red/orange mat	+	++	585
GC234	4426	4	Hydrate mound red/orange mat	+	++	586

¹Methane flux: “++”, High; “+”, relatively low; “–”, unknown.

²Non-methane hydrocarbon (HC) flux: “++”, High; “+”, relatively low; “–”, unknown.

³Sometimes referred to as cold methane seep.

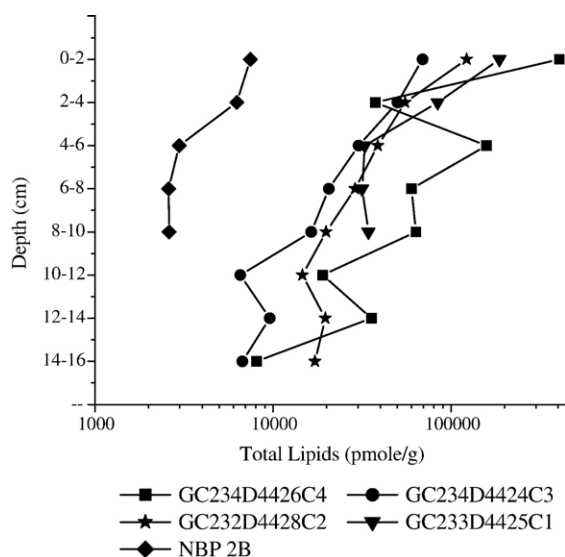


Fig. 2. Total phospholipid fatty acids (PLFA) in background marine sediment (NBP 2B, diamond), cold methane seep (GC233), and gas hydrate (GC232, GC234) sites.

menaquinones (MK-n) and demethylmenaquinone (DMK) were quantified in the sediment core samples and reported as mol-percentage. UQ-n_s are formed by *Proteobacteria* of the alpha, beta and gamma groups during growth with high-potential terminal electron acceptors like oxygen and nitrate, whereas MK-n_s are common in anaerobically growing *Proteobacteria*, all Gram-positive bacteria as well as archaea (Collins and Jones, 1981; Hedrick and White, 1986; Hiraishi, 1999).

2.4. Statistical analysis

Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS 10.0) developed by SPSS Inc, Chicago. For HCA, each PLFA profile of absolute concentration was treated as a multivariate and calculation was performed without further standardization. Nearest neighbor was used for clustering by cal-

culating the distance between variables. Clustering was made by calculating the cosine similarity of variables and reported in a dendrogram. For PCA, principal components were extracted by calculating the correlation matrix and the principal components with eigenvalues great than 1. Oblique promax with Kaiser Normalization was applied for the rotation of factor matrix to maximize the differences among factors extracted. PCA was also conducted by using PLFA as variables to extract dominant fatty acids. For comparison, the profiles of relative abundance (percentage) were also used for both PCA and HCA, which yield the same results as those calculations with the absolute concentration profiles (data not shown).

3. Results

3.1. Microbial biomass represented by PLFA

About 90 different PLFA were identified in all samples but only those of greater than 1 mol% were listed in the Appendix. PLFA concentrations ranged from 2.6 pico-mole/g (pmol/g) at the 8–10 cm interval of NBP 2B to 407.0 pmol/g at the 0–2 cm interval of GC234D4426C4. Overall, PLFA in all intervals of the NBP 2B core were more than ten times lower than those in cold seep and gas hydrate-containing cores (Fig. 2).

Total PLFA and PLFA of the top and bottom intervals of the cores were summarized, together with the concentrations of sulfate and dissolved sulfide (Table 2). The top and bottom intervals represented the range of PLFA, which generally decreased with depth. Specially, PLFA decreased dramatically from 0–2 cm to 2–4 cm in core GC234D4426C4, increased slightly below 2–4 cm and decreased slightly to the bottom. The decreasing trends were less dramatic in other cores (Fig. 2).

Changes in PLFA appeared to correlate with changes in sulfate and H₂S (data not shown). Sulfate concentration in gas hydrate and cold seep sites decreased from

Table 2
Sulfate, sulfide, and overview of PLFA in different cores

Site	Sulfate ¹ (mM)	Dissolved Sulfide ¹ (μM)	Total PLFA (nmol/g)	PLFA of top interval ² (nmol/g)	PLFA of bottom interval ³ (nmol/g)
GC232D4428C2	0–26	207–11051	316.9	122.4	17.2
GC234D4424C3	0–8	3083–10653	209.6	69.3	6.7
GC234D4426C4	0–24	<500–5632	789.7	407.0	8.1
GC233D4425C1	18–20	1440–2595	370.4	187.3	34.4
NBP 2B	21–27	4–8	21.9	7.5	2.6

¹The concentrations of sulfate and dissolved sulfide were from Formolo (unpublished data).

²0–2 cm.

³14–16 cm depth for the gas-hydrate sites and 8–10 cm depth for the background and cold-seep sites.

the top interval to deeper intervals accompanied by an increase in dissolved sulfide. The control site NBP 2B showed the highest concentrations of sulfate, which was close to the average of seawater. NBP 2B also contained the lowest concentrations of dissolved sulfide (less than 8 μM). These results implied that the enhanced biomass at hydrate or hydrocarbon seep sites was related to sulfate reduction.

3.2. PLFA profiles in different cores

To assess differences in the microbial community structure among different sites, the PLFA were classified into saturated straight chain — (NSats), monounsaturated- (Monos), branched- (BrSat), cyclopropyl- (Cy) and polyunsaturated (PUFA)- fatty acids (Fig. 3), and were compared among different sites.

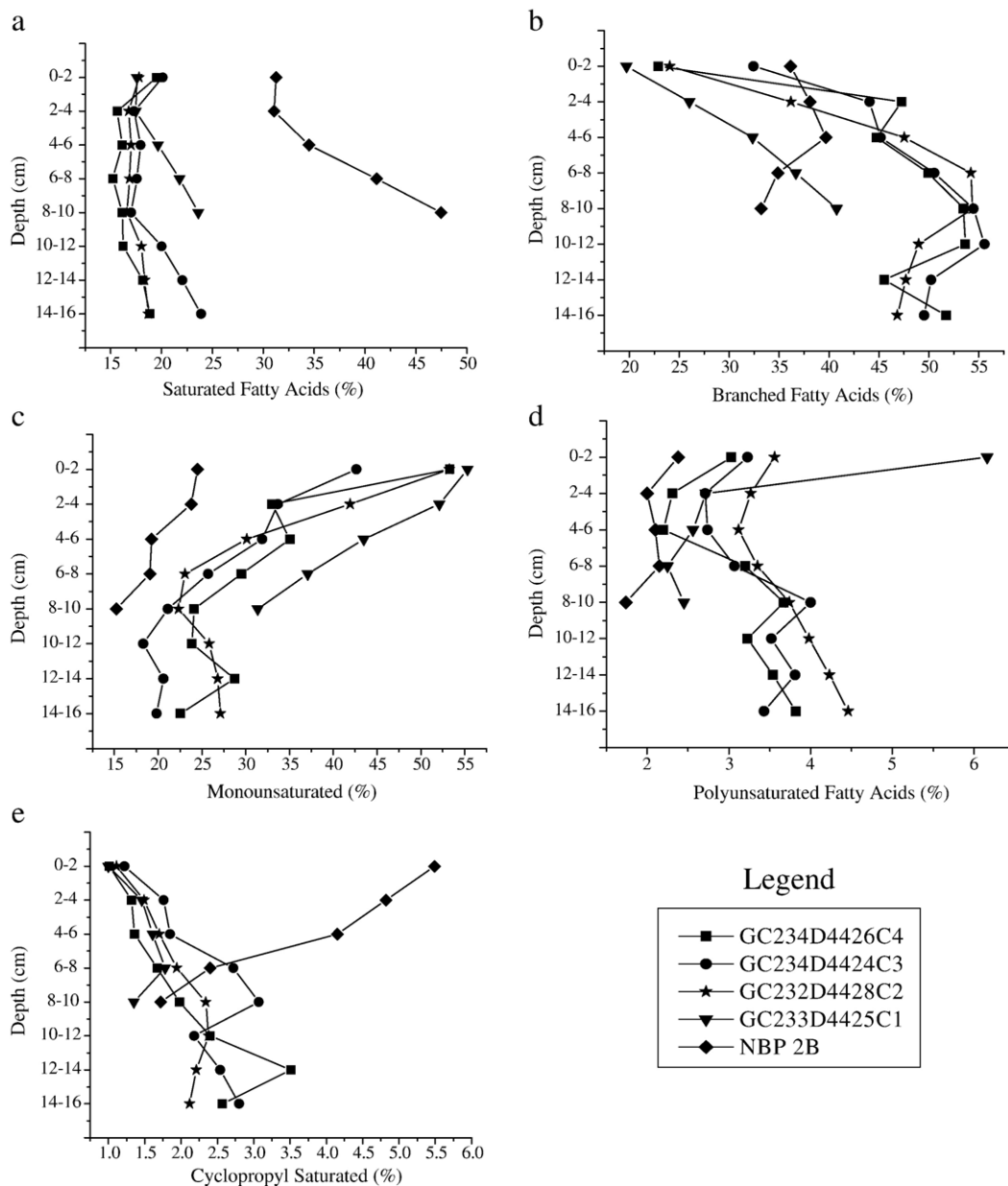


Fig. 3. Distribution of saturated, branched (sum of terminal and mid-chain branched), monounsaturated, polyunsaturated, and cyclopropyl phospholipid fatty acids in sediments from different sites in the Gulf of Mexico.

Core NBP 2B was dominated by NSats — (31.0–47.5%), BrSat (33.2–39.7%) and Monos (15.2–25.8%). The NSats increased consistently with depth while other groups decreased with depth (Fig. 3a–e); the BrSat (sum of mid-chain and terminal branched) first increased to 4–6 cm and then decreased with depth (Fig. 3b); the Cys consistently decreased with depth, which showed an opposite trend against those in the hydrate- or hydrocarbon seep cores (Fig. 3e).

Core GC233D4425C1 at the cold seep site had relatively low percentages of NSats (17.6–23.6%), which increased slightly from the 0–2 cm interval to the 8–10 cm interval. The BrSat increased from 19.7% at 0–2 cm to 40.8% at 8–10 cm, which appeared to be at the expense of Monos that decreased from 55.4% to 31.4% from top to bottom (Fig. 3b, c). The relative percentages of Monos were higher at the cold seep site than any other sites at all depths. The top interval of the cold seep also yielded the highest PUFA (6.2%), which, however, decreased to lower values (2.7–2.4%) below 2–4 cm than in other hydrate cores (Fig. 3d).

Cores at the gas hydrate sites (GC234D4424C3, GC234D4426C4, and GC232D4428C2) showed similar percentages and trends from the 0–2 cm interval to the 14–16 cm interval (Fig. 3). Their NSats were consistently low (15.2–23.9%). Monos were high (46.5 to 53.3%) at 0–2 cm and decreased to 19.8 to 27.1% at deeper depth. Their BrSats, however, increased from 22.9 to 32.4% at 0–2 cm to the highest values at 8–10 cm (48.9 to 53.5%).

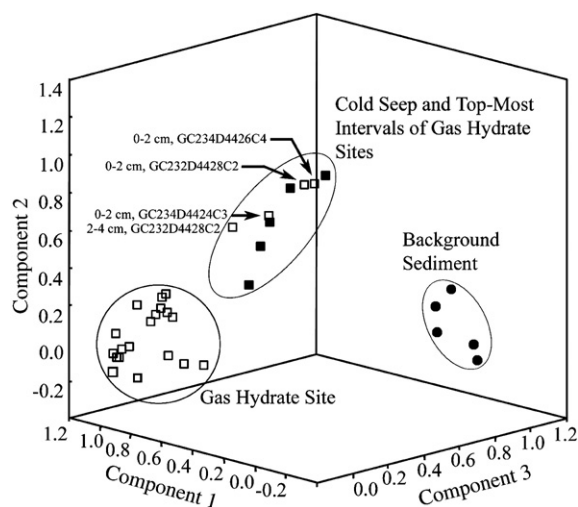


Fig. 4. Three-dimensional plot of the three principal components extracted by principal component analysis (PCA) of PLFA within individual samples. Empty square: all gas hydrate sites (GC232, Core 3 and 4 of GC234); solid square: cold seep site GC233; solid circle: background site NBP 2B.

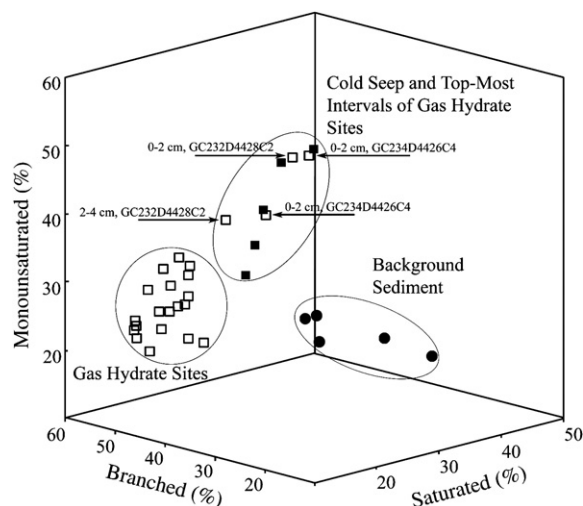


Fig. 5. Three-dimensional plot of the summed branched-, monounsaturated- and saturated fatty acids. It gives almost the same patterns as plotted by three principal components extracted by PCA in Fig. 4. Empty square: all gas hydrate sites (GC232, core 3 and 4 of GC234); solid square: cold seep site GC233; solid circle: background site NBP 2B.

3.3. Quinone profiles in different cores

Quinones were extremely low at NBP 2B. For example, UQ-ns were less than 4.0 pmol/g and MK-ns less than 11.8 pmol/g through the whole core. Quinones were significantly enhanced at the cold seep and hydrate sites, which had similar UQ-n contents but different MK-n contents. For example the UQ-ns were 51.7 pmol/g at GC233 and 55.0–57.3 pmol/g at GC232 and GC234. MK-ns were about 3–7 times lower at GC233 (66.6 pmol/g) than at GC234 (150.4–437.0 pmol/g) or GC232 (172.4 pmol/g). Among the UQ-ns detected at the cold seep or hydrate sites, UQ-8 was the most abundant and showed sharp decrease with depth; MK-4 and MK-6 were the most abundant of MK-ns and showed high concentrations around 4–6 cm (data not shown).

3.4. Hierarchical cluster analysis and principle component analysis

In using HCA and PCA, we treated every PLFA profile as an integrated data array in calculating the similarities of all intervals. HCA gave results consistent with that of PCA. The three largest factors in PCA accounted for 84.0%, 8.7% and 6.0% of the squared loading variances. They accounted for 98.7% of all variances and therefore well represented the whole array of PLFA profiles. The projection of the three largest

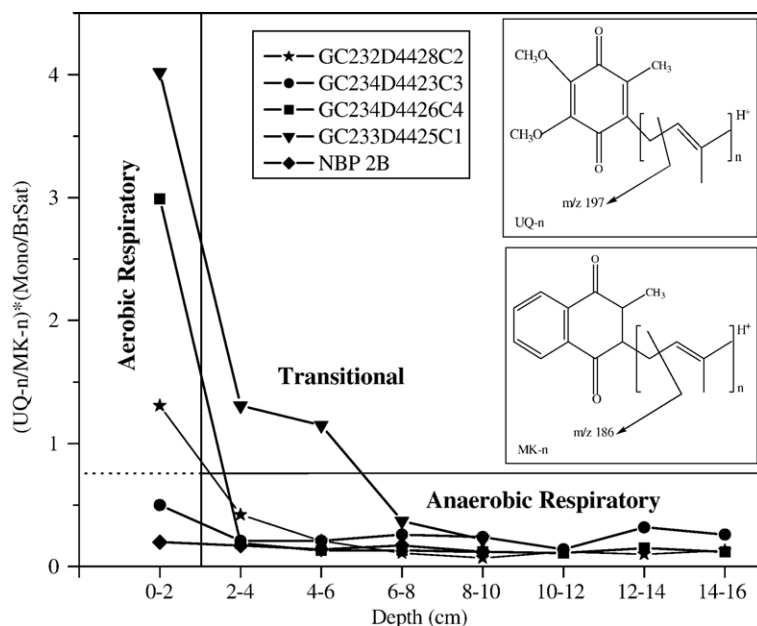


Fig. 6. Depth profiles of the ratio of respiratory quinones within monounsaturated and branched saturated fatty acids indicating the rapid shift from aerobic to anaerobic microbial respiration processes in the sediment cores. Insert: General structure of respiratory ubiquinone (UQ-n) and menaquinone (MK-n) species and their fragment ions monitored in mass spectrometry analysis.

principal components grouped the PLFA profiles into three distinct clusters (Fig. 4). All NBP 2B samples were grouped in one cluster (C1). The cold seep profile plus all 0–2 cm layers of gas hydrate sites were grouped into a second cluster (C2), and all other samples from gas hydrate cores (except top layers) were grouped in the third cluster (C3). We designated C1 as the background-marine community, C2 as the cold-seep community, and C3 as the gas-hydrate community. Monos, BrSat and NSat fatty acids were plotted as three dimensions (Fig. 5), which showed high similarity to the PCA results and suggested that these types of fatty acids were predominant in each sample. The dendrogram exported from HCA yielded the same result as PCA. All the gas hydrate columns without top-most layers were hierarchically clustered in one group with at least 96% similarity; all the cold seep layers plus the top-most layers of sediment at the gas hydrates were clustered in one group with at least 96% similarity. These two clusters had a 94% similarity to each other and together had only a 74% similarity to NBP 2B. When single fatty acids were used as the variables for PCA, brC15:0, C16ω7c and C18:1ω7c were the three highly scored fatty acids in the first component; C12:0 and C16:0 and C18:1ω7c were the three highly scored fatty acids in the second component; and aC15:0, br15:0 and 10MeC16:0 were the highly scored fatty acids in the third components.

PCA of two component analysis of quinone profiles yielded results consistent with the results of PLFA (data not shown). The quinones with the highest contribution to the observed clustering were MK-6 and UQ-8, followed by MK-4.

The ratio of (UQ/MK)*(Mono/BrSat) estimated the ratio of oxygen/nitrate respiration to anaerobic respiration with respect to *Beggiatoa/Thioploca* (Monos) and sulfate-reducing bacteria (BrSat)-dominated communities (Fig. 6). The top-most layers of the sediment indicated the dominance of aerobic respiratory type; the 2–4 cm and 4–6 cm layers of sediments, particularly in GC233D4425C1, indicated a transition between aerobic and anaerobic respiratory types; the deeper intervals (below 6 cm) at the gas hydrate and cold seep sites indicated the dominance of anaerobic respiration. The control site NBP 2B didn't show such a change in respiratory types due to its low content of PLFA and trace amount of quinones.

4. Discussion

4.1. Lipid biomarkers and microbial consortia

PLFA are a reliable estimation of bacterial biomass and activity (White et al., 1979). The significantly low total PLFA of NBP 2B (Fig. 2) implied that microbial biomass in the non-seep marine sediments was

considerably lower than the hydrate-seep sites. Changes in PLFA in each core (Figs. 2 and 3) and differences among clusters C1, C2, and C3 revealed by PCA (Fig. 4) and HCA (data not shown) indicated that the microbial biomass and the compositions of the PLFA profiles are sensitive signatures for microbial communities in different environments. The classification of all top intervals of hydrate sites into cluster C2 suggested that all of the hydrate sites might contain *Beggiatoa/Thioploca* mats, which have signature PLFA (Zhang et al., 2005).

The dominant fatty acids extracted by the PCA according to their scores are br-C15:0, C16:1 ω 7c, C18:1 ω 7c, C12:0, C16:0, *a*C15:0 and 10MeC16:0. However, not all the fatty acids in this list are signature fatty acids of three different microbial consortia because the plot of the three components extracted in this way failed to separate the *Beggiatoa/Thioploca*, SRB and the background groups of microbes. However, the three principal components extracted from the PLFA profiles indicated three major groups of fatty acids (Fig. 4), which accounted for more than 90% of total PLFA. Each of these three groups also behaved differently in each core (Fig. 3a, b and c). A three-dimensional projection by the sum of BrSat, NSat and Monos was drawn (Fig. 5), which was surprisingly similar to the result of PCA. Hence, the three components extracted in PCA may represent these major fatty acid groups. The relative abundances of these groups at different sites or different depths of the core may result from changes in microbial communities. NSats generally represent universal or non-specific lipids and the terminal branched fatty acids are generally the major cellular components in SRB in marine sediments (Parkes and Taylor, 1983; Findlay et al., 1990; Vainshtein et al., 1992; Zhang et al., 2002). The bacterial lipids extracted from the AOM community were significantly contributed by the syntrophic partners of the AOM consortium (e.g., Orphan et al., 2001). In gas hydrate or cold seep settings, C16:1 ω 7 and C18:1 ω 7 have widely been observed to be the most abundant fatty acids in *Beggiatoa/Thioploca* mats (McCaffrey et al., 1989; Jacq et al., 1989; Guezennec and Fiala-Medioni, 1996). Zhang et al. (2005) collected pure surface mat from the top of hydrate at GC234 site, which excluded the fatty acids from SRB demonstrated that C16:1 ω 7 and C18:1 ω 7 accounted for 83.0 mol% of all PLFA. These results suggest that 16:1 ω 7 and 18:1 ω 7 can be used as signature biomarkers for sulfur-oxidizing bacteria in H₂S-rich marine sediment.

Significantly high percentages of 10MeC16:0 in NBP 2B core (5.75–8.06%), seep core GC233 (2.76–4.88%), and gas hydrate cores (2.93–5.32%) support the

presence of SRB (Taylor and Parkes, 1983; Dowling et al., 1986; Vainshtein et al., 1992; Kuever et al., 2001) and specifically the family *Desulfobacteriaceae* (Matsui et al., 2004).

Mid-chain BrSats are also recognized as biomarkers for SRB in the marine environments (Taylor and Parkes, 1983; Vainshtein et al., 1992; Zhang et al., 2002). Some strains of *Desulfovibrio* contain high *i*C15:0 or *a*C15:0 or both (Dowling et al., 1986; Vainshtein et al., 1992; Dzierzewicz et al., 1996; Feio et al., 1998). The relative abundance of *a*C15:0 increased only slightly below the surface layer (0–2 cm) at NBP 2B but increased significantly at the gas hydrate/seep sites, which may suggest enhanced abundance of *Desulfovibrio* in the hydrocarbon-disturbed environments. This is different from the Hydrate Ridge, which was characterized by high content of C16:1 ω 5c which is potentially from *Desulfosarcina/Desulfococcus* species (Elvert et al., 2003; Orcutt et al., 2005).

The trends and relative contents of fatty acids from top to bottom of the sediment cores reflected the variation in cell density and community structure (Figs. 2 and 3). The microbial communities could be classified into three categories with different compositions: (1) the background site NBP 2B was dominated by microorganisms containing mostly saturated fatty acids; (2) the cold seep and the top intervals of the gas hydrate sites were dominated by biomass from *Beggiatoa/Thioploca* with increasing contribution of SRB at depths; and (3) the deeper parts of the gas hydrate sites were dominated by SRB with significant contribution from *Beggiatoa/Thioploca*.

4.2. Geochemical environments revealed by PLFA and quinones

The low biomass in NBP 2B reflected the oligotrophic environment. This is partially supported by the enhanced production of cyclopropyl FAs (Cys) at shallow sediment intervals of NBP 2B (Fig. 3e). The ability to either synthesize *trans*-monoenoic acids or to modify the more volatile *cis*-monoenoic acids to their Cy-derivatives is a survival mechanism that helps cells to maintain a functional membrane during starvation-induced lipid synthesis (Guckert et al., 1986; Kieft et al., 1994). The decreasing Cys with depth is likely caused by the decrease in biomass with depth. At cold seep and hydrate sites, the low Cys in surface sediments may indicate relatively higher concentrations of nutrients, whereas the increase in Cys with depth may be that microorganisms at depth remained active due to the downward transportation of food sources.

Beggiatoa and/or *Thioploca* colonize a typical interface where sources of reduced sulfur and dissolved oxygen both exist (Jannasch et al., 1989). As a result, the dissolved sulfide produced by SRB at depth may favor the blooming of *Beggiatoa/Thioploca* at the top (Sassen et al., 1993; Larkin et al., 1994; Larkin and Henk, 1996); in return, the *Beggiatoa/Thioploca* community may provide carbon substrates for the SRB community below. In this way, the *Beggiatoa/Thioploca* mats provide a geochemical interface with oxic environment above it and highly reduced environment below it. The potentially low activity of SRB (indicated by low hydrogen sulfide) in the NBP 2B core could be due to the absence of *Beggiatoa/Thioploca* mat that would exhaust oxygen to maintain an anaerobic environment below it (Nelson and Jannasch, 1983; Sassen et al., 1993; Larkin and Henk, 1996). Consequently, the lack of growth substrates, such as organic carbon derived from a *Beggiatoa/Thioploca* community may have constrained the growth of SRB at NBP 2B.

The spatial variation in respiratory quinones (UQ-ns and MK-ns) supported the view that the oxic environments existed at the top of cores and the anaerobic condition at the depths in the hydrate and seep cores. This is also consistent with the spatial relations of the *Beggiatoa/Thioploca* community and the SRB community. All gas hydrate sites were characterized by their high UQ-8 at the top-most layer and high MK-4 and MK-6 at depth where SRB flourish. Consistent with the lipids data, the lack of gas hydrate and consequently less SRB of the AOM community in GC233 were corroborated by its low content of MK-n quinone and no obvious trend of MK-4 and MK-6 through the core. Similarly, lacking of sulfide-oxidizing bacteria at the surface sediment and low activity of SRB at the depth explained the extremely low content of quinones in NBP 2B. Cai et al. (unpublished data) measured an oxygen penetration depth (OPD) (e.g., Wenzhöfer et al., 2001) of 0.4–0.5 cm in a mat-covered core at GC234, which was shallower than the OPD of mat-free sediment and indicative of the consumption of oxygen by microbes at this depth. The profile of (UQ/MK) * (Mono/BrSat) therefore revealed that the *Beggiatoa/Thioploca* community can serve as a geochemical barrier between the oxic environment above it and the reduced environment below (Fig. 6). Overall, the spatial distribution of biomarkers and the microbial consortia reflected the chemical environment where the organisms lived.

4.3. The source of PUFA and environmental stress

PUFA are considered rare or absent in prokaryotes. In this study, the PUFA in all gas hydrate sites and the background site show no obvious trend from the top to

the bottom; however, the abundance of PUFA in seep site and NBP 2B was significantly less than that in the gas hydrate sites. The PUFA of the top-most layer of cold seep GC233 was much higher than any other sites, which might indicate the presence of a nearby eukaryotic chemosynthetic community.

The ratio of *trans* to *cis* PLFA indicates environmental stress that might be caused by high pressure, low temperature and low nutrient conditions (e.g., Guckert et al., 1985, 1986; Kieft et al., 1994; Yano et al., 1998; Fang et al., 2004, 2006). Only trace amount of *trans* monounsaturated fatty acids can be detected in the tidal zone of marine sediments or shallow *Beggiatoa/Thioploca* mats and the *trans/cis* ratio is generally less than 0.02 (Volkman et al., 1980; Jacq et al., 1989). However, this ratio increases to 0.09–0.30 (for 16:1ω7) at 530–660 m water depth in the Gulf of Mexico (Zhang et al., 2005 and this study), to around 0.30 at 780 m water depth at Hydrate Ridge (Elvert et al., 2003), and to about 0.50–0.73 (for C16:1ω5) at water depth of more than 4900 m (Guezennec and Fiala-Medioni, 1996). These observations suggest that hydrostatic pressure may be responsible for the increasing *trans/cis* in deep-sea microorganisms.

5. Conclusions

Bacterial PLFA profiles allowed us to delineate the spatial distribution of microbial communities using PCA analysis, which clearly separated the non-seep marine sediment (NBP) from gas hydrate (GC234 and GC232) or hydrocarbon seep (GC233) sites. The fatty acid biomarkers also indicated that the top layers of sediments of seep and hydrate sites were dwelled by *Beggiatoa*-type communities whereas the deeper sediments were dominated by sulfate-reducing bacteria. The ratio of UQ/MK indicated change from the oxygen/nitrate respiratory at the surface to anaerobic respiratory at depth, which is consistent with the function of the *Beggiatoa* mat serving as a redox interface between oxic and anoxic environments. High ratios of *trans/cis* fatty acid are consistent with enhanced turnover rate of *cis*-monoenoic fatty acids due to the environmental stress in the deep-sea sediments. The significant amount of PUFA indicated possible source of fatty acids from nearby chemosynthetic community. In summary, this study revealed the consistency between the spatial distribution of microbial membrane phospholipids fatty acids and respiratory quinones and the spatial distribution of different microbial consortia or metabolic processes. This consistency gives an integrated view that biomarkers can provide valuable information on microbial communities living in different chemical environments at cold seeps or gas hydrates.

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Appendix A

Simplified profiles of phospholipid fatty acids (PLFA) in different sediment cores in the Gulf of Mexico.

PLFA (mol%)	NBP 2B, Depth profile (cm)				
	0–2	2–4	4–6	6–8	8–10
C12:0	2.51	2.01	3.31	4.78	7.79
iC14:0	1.17	1.34	1.44	1.19	1.27
C14:0	7.95	7.61	8.45	10.43	12.89
iC15:0	5.56	5.18	4.97	4.13	4.12
aC15:0	6.47	8.00	9.06	8.21	8.28
C15:0	1.44	1.56	1.67	1.56	1.77
iC16:0	1.68	1.78	1.99	1.79	1.73
aC16:0	2.55	2.17	1.72	1.44	1.09
C16:1 ω 7c	6.94	5.65	4.54	3.70	2.84
C16:1a	3.11	2.46	1.83	1.35	1.05
C16:0	14.89	14.63	15.37	17.17	17.82
10MeC16:0	6.66	5.81	5.68	4.14	3.02
iC17:0	1.03	0.96	0.97	0.77	0.67
aC17:0	2.03	2.30	2.38	1.85	1.70
cyC17:0 a	1.20	1.23	0.97	0.49	0.45
cyC17:0 b	1.53	1.28	1.08	0.69	0.59
C17:0	0.81	0.97	1.00	0.98	1.07
C18:1 ω 9c	3.99	4.49	4.00	5.44	5.22
C18:1 ω 7c	6.99	6.22	4.38	3.37	2.77
C18:1	0.21	1.70	1.97	2.56	1.01
C18:0	2.73	3.07	3.41	4.60	4.53
cyC19:0	2.77	2.31	2.10	1.21	0.68
PLFA (mol%)	GC233D4425C1 (Brine pool), Depth profile (cm)				
	0–2	2–4	4–6	6–8	8–10
C12:0	0.62	0.89	1.49	1.74	2.23
iC14:0	0.96	1.29	1.80	1.97	2.14
C14:1a	0.76	0.79	1.31	1.65	1.22
C14:0	3.32	3.45	4.47	5.61	6.49
iC15:0	3.54	3.74	4.22	4.29	4.98

Appendix A (continued)

PLFA (mol%)	GC233D4425C1 (Brine pool), Depth profile (cm)							
	0–2	2–4	4–6	6–8	8–10			
<i>a</i> C15:0	5.66	9.20	11.93	13.01	14.14			
C15:0	0.75	0.82	1.03	1.56	2.40			
brC15:0	0.32	0.54	0.92	1.55	2.40			
<i>i</i> C16:0	0.66	0.87	1.16	1.55	1.79			
<i>a</i> C16:0	1.12	1.04	0.94	1.15	1.68			
C16:1 ω 7c	23.30	22.12	17.33	12.87	9.51			
C16:1 ω 7t	4.14	4.14	3.26	2.87	2.05			
C16:1a	5.05	5.38	4.18	4.00	4.46			
C16:0	10.59	10.08	9.83	9.89	9.80			
10MeC16:0	2.06	2.49	2.72	2.79	2.53			
<i>a</i> C17:0	1.01	1.07	1.33	1.58	1.50			
C18:3 ω 3	0.25	0.81	1.03	1.18	1.17			
C18:1 ω 9c	2.66	2.24	1.85	1.65	1.82			
C18:1 ω 7c	15.43	14.20	12.77	10.97	8.31			
C18:1 ω 7t	1.27	1.11	0.75	0.61	0.60			
C18:0	1.55	1.31	1.59	1.69	1.38			
C20:4 ω 6	1.41	0.36	0.30	0.26	0.16			
C20:5 ω 3	2.39	0.31	0.24	0.23	0.39			
C20:1 ω 9c	1.05	0.15	0.18	0.21	0.19			
PLFA (mol%)	GC232D4428C2, Depth profile (cm)							
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16
C12:0	0.77	0.71	1.03	0.87	0.91	0.96	1.30	1.21
<i>i</i> C14:0	1.06	1.65	2.38	2.99	2.92	2.11	2.07	1.91
brC13:0	0.18	0.36	0.74	1.00	1.06	0.68	0.58	0.64
C14:0	3.59	3.66	4.96	5.89	5.97	5.53	5.48	5.32
brC14:0 a	0.26	0.56	0.89	1.15	1.31	1.04	0.94	0.85
brC14:0 b	0.25	0.60	1.08	1.50	1.70	1.52	1.44	1.57
<i>i</i> C15:0	3.66	5.14	5.69	4.88	4.53	4.37	4.63	4.83
<i>a</i> C15:0	7.54	13.17	18.10	22.03	22.85	19.02	18.50	17.40
C15:0	1.09	1.36	2.07	2.15	2.16	1.98	2.06	2.10
brC15:0	0.78	1.37	2.25	2.98	3.51	3.20	3.18	3.00
<i>i</i> C16:0	0.77	0.98	1.39	1.66	1.59	1.78	1.62	1.35
<i>a</i> C16:0	1.21	1.12	2.21	2.30	2.00	1.44	1.17	1.24
C16:1 ω 7c	20.59	16.46	10.42	6.61	6.42	7.67	8.23	7.73
C16:1 ω 7t	5.92	4.03	1.95	1.00	0.74	1.22	1.29	1.19
C16:1a	5.90	5.29	4.57	4.27	3.98	4.07	4.12	4.07
C16:0	9.98	8.45	6.69	5.83	5.58	7.15	6.98	7.39
10MeC16:0	1.91	2.29	2.46	2.53	2.24	1.70	2.05	2.04
<i>a</i> C17:0	1.22	1.36	1.44	1.60	1.50	1.72	1.78	1.84
cyC17:0 a	0.58	0.70	0.82	0.90	1.24	1.25	1.19	1.22
cyC17:0 b	0.42	0.68	0.77	0.95	0.98	1.00	0.84	0.74
C17:1	0.28	0.47	0.87	1.19	1.31	1.15	1.03	1.17
C18:3 ω 3	0.66	1.71	2.07	2.66	3.01	3.24	3.39	3.64
C18:1 ω 9c	2.76	2.10	1.26	0.90	0.76	0.90	0.91	1.18
C18:1 ω 7c	13.51	10.09	7.97	6.44	6.89	8.50	8.66	9.11
C18:1 ω 7t	1.30	0.90	0.44	0.31	0.25	0.28	0.40	0.35
C18:0	1.39	1.45	1.05	0.97	0.89	1.13	1.21	1.30
C20:5 ω 3	1.01	0.20	0.19	0.10	0.15	0.10	0.15	0.14
PLFA (mol%)	GC234D4424C3, Depth profile (cm)							
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16
<i>i</i> C14:0	1.33	1.92	2.09	2.68	3.07	3.65	3.15	3.60
C14:0	6.01	5.31	5.71	6.04	5.48	6.25	5.99	6.76

Appendix A (continued)

PLFA (mol%)	GC234D4424C3, Depth profile (cm)							
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16
brC14:1	0.33	0.22	0.23	0.31	0.33	0.43	0.28	0.25
iC15:0	4.50	5.23	4.90	5.35	5.92	6.29	6.27	6.07
aC15:0	12.10	18.97	19.42	22.50	24.01	23.08	18.91	18.66
C15:0	1.28	1.47	1.62	2.01	1.98	2.07	1.89	1.92
brC15:0	0.87	1.61	1.72	2.44	4.30	5.53	5.52	5.36
iC16:0	0.86	1.30	1.38	1.27	1.47	1.83	2.45	2.61
aC16:0	1.31	1.74	1.83	2.05	2.37	1.78	1.17	0.80
C16:1ω7c	16.87	10.58	8.86	6.38	4.75	4.44	4.79	4.89
C16:1ω7t	3.32	2.78	2.65	1.41	0.76	0.78	0.86	0.91
C16:1a	5.66	6.43	6.46	5.21	3.78	3.19	3.08	2.86
C16:0	9.87	7.55	7.38	6.51	6.35	7.95	9.61	10.71
10MeC16:0	2.86	2.50	2.96	2.99	2.12	2.15	1.89	1.95
aC17:0	1.03	1.22	1.30	1.26	1.43	1.50	1.60	1.45
cyC17:0 b	0.42	0.67	0.84	1.56	2.04	1.55	1.71	2.10
C17:1	0.46	0.92	1.25	1.81	2.42	2.68	2.69	3.09
C18:3ω3	1.07	1.92	1.92	2.40	3.20	2.94	2.61	2.98
C18:1ω9c	1.74	1.26	1.09	0.93	0.96	0.73	1.36	1.69
C18:1ω7c	11.13	8.38	8.44	7.22	5.92	4.68	4.75	4.24
C18:0	1.38	1.16	1.19	1.07	1.09	1.56	2.02	2.01
PLFA (mol%)	GC234D4426C4, Depth profile (cm)							
	0–2	4–6	6–8	8–10	10–12	12–14	14–16	14–16
C12:0	0.49	0.56	0.49	0.56	0.44	0.85	0.69	1.32
iC14:0	0.99	1.91	1.33	2.01	2.34	2.37	2.20	4.33
brC13:0	0.13	0.40	0.25	0.41	0.56	0.60	0.45	0.36
C14:0	4.02	4.18	4.02	4.37	4.82	4.64	5.09	5.60
iC15:0	3.63	4.47	3.98	4.60	5.72	6.34	6.13	7.93
aC15:0	8.02	22.96	23.57	24.44	23.30	22.93	17.55	17.80
C15:0	0.88	1.31	1.24	1.38	1.85	1.67	1.98	1.62
brC15:0	0.56	1.53	1.18	2.21	3.65	3.22	2.47	3.25
iC16:0	0.72	1.44	1.15	1.47	1.79	1.98	1.85	2.60
aC16:0	0.80	1.65	1.22	1.86	2.35	2.16	1.70	0.91
C16:1ω7c	23.20	10.90	10.48	8.00	5.83	5.27	6.71	4.48
C16:1ω7t	5.04	2.38	2.57	2.28	1.43	1.06	0.93	0.47
C16:1a	5.20	5.38	6.67	6.30	4.80	4.99	5.24	3.73
C16:0	11.75	7.27	7.61	6.85	6.65	6.68	7.84	7.45
10MeC16:0	1.64	1.95	1.45	2.04	2.19	1.87	1.95	2.62
aC17:0	1.08	1.47	1.34	1.39	1.46	1.52	1.49	1.35
cyC17:0 a	0.59	0.73	0.88	0.86	0.83	0.98	1.62	1.05
cyC17:0 b	0.35	0.47	0.38	0.66	0.97	1.31	1.72	1.36
C17:1	0.24	0.66	0.62	1.18	1.55	1.78	2.19	3.62
C18:3ω3	0.34	1.29	1.52	2.58	3.05	2.85	2.71	2.63
C18:1ω9c	2.07	1.63	1.54	1.16	1.00	0.93	1.46	0.74
C18:1ω7c	13.80	9.12	10.26	7.97	6.82	7.04	9.93	7.07
C18:0	1.49	1.17	1.33	1.06	1.00	1.14	1.15	1.28

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