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Heterotrophic microbial activity in lake sediments: effects of organic electron donors

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Abstract Allochthonous and autochthonous organic matter deposited in benthic sediments are mineralized by microbial communities, resulting in release of nutrients to the water column. Lakes with different trophic states may have sediments with different carbon and nutrient concentration with consequently different microbial communities. Microbial diversity of surface sediments of three subtropical lakes of different trophic state was investigated by measuring catabolic response to a wide variety of carbon-substrates. Basal carbon dioxide and methane production rates were highest in Lake Apopka (hypereutrophic), followed by Lake Annie (oligo-mesotrophic) and Lake Okeechobee (eutrophic) sediments. The oligo-mesotrophic Lake Annie showed the highest metabolic quotient (qCO_2; proportion of basal respiration per unit of microbial biomass, 0.008 ± 0.001) indicating inefficient use of energy. The low qCO_2 found in Lake Apopka sediment indicated higher efficiency in using energy. Lake Okeechobee sediments had intermediary values of qCO_2 (M9 0.005 ± 0.001; M17 0.006 ± 0.0003; KR 0.004 ± 0.001) as compared with other lakes (lake Apopka 0.004 ± 0.14). Lake Apopka's sediment catabolic diversity was higher than that observed in other sediments. Addition of organic electron donors to sediment samples from all lakes stimulated heterotrophic activity; however, the extent of the response varied greatly and was related to microbial biomass. The hypereutrophic Lake Apopka sediments had the highest respiration per unit of microbial biomass with the addition of electron donors indicating that these sediments respired most of the C added. These results showed that sediments with different biogeochemical properties had microbial communities with distinct catabolic responses to addition of the C sources.

Keywords Electron donor · Microbial activity · Nutrients · Organic carbon · Sediment

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Introduction

Organic matter deposition is an important source of carbon (C) in lake sediments. Organic compounds and associated nutrients supplied to sediments are mineralized through heterotrophic decomposition (Capone and Kiene 1988; Megonigal et al. 2004). The composition and activities of microbial communities are regulated by the quality and availability of carbon. In high depositional environments such as eutrophic or deep thermally stratified lakes, organic content in sediments is often high, such that oxygen (O_2) is rapidly consumed, and is depleted within several millimeters below the sediment water interface (Jørgensen 1983). In these systems, facultative and obligate anaerobic

communities dominate. Complete oxidation of a broad range of organic compounds in these systems can occur through the sequential activity of different groups of anaerobic bacteria (Capone and Kiene 1988).

In anaerobic environments, cellulolytic bacteria hydrolyze organic polymers through extracellular enzyme production and further break down monomers to alcohols, fatty acids, and hydrogen (H_2) through fermentation. Alcohols and fatty acids are then further degraded by syntrophic bacteria (secondary fermenters) into acetate, H_2, and carbon dioxide (CO_2), which can be used as substrate by methanogens (Zinder 1993; Conrad 1999). The structure and function of anaerobic microbial communities are therefore strongly affected by competition for fermentation products such as H_2, CO_2 and acetate. Microorganisms derive energy by transferring electrons from an external source or donor to an external electron sink or terminal electron acceptor. Range of organic electron donors vary from monomers that support fermentation to simple compounds such as acetate and methane (CH_4). Fermenting, syntrophic, methanogenic bacteria and most other anaerobic microorganisms (e.g., sulfate and iron reducers) are sensitive to the concentrations of substrates and their activities can be inhibited by accumulation of their end products. Consequently they are dependent on the end product consumption by other organisms (Stams 1994; Megonigal et al. 2004).

Measuring catabolic response profile has been widely applied in soil studies as a method to characterize the microbial functional diversity (Degens and Harris 1997; Lu et al. 2000; Degens et al. 2000). Substrate induced respiration (SIR) is often dependent on the size of the microbial biomass pool; however, response of microbial communities is also related to the catabolic diversity of soil microorganisms (Degens 1998; Wright and Reddy 2001, 2007). A greater catabolic response to a substrate in one system as compared with another indicates that the microbial community is more functionally adept in utilizing that substrate and may indicate previous exposure to the associated C-sources (Degens and Harris 1997; Degens 1998; Baldock et al. 2004).

The varied metabolic response of microbial communities in lake sediments may also be related to the physico-chemical characteristics of lakes (the source and chemical composition of particulate matter and biogeochemical processes in the sediment and the water column). Accumulation and retention of particulate matter and nutrients in sediments depends on lake morphometry, water renewal, nutrient loading, edaphic characteristics of the drainage basin, among other factors (Boström et al. 1988). Eutrophic and hypereutrophic lakes typically receive high external loads of nutrients and display high primary productivity and nutrient concentrations in the water column. Consequently, sediments from eutrophic and hypereutrophic lakes are expected to have high concentrations of organic matter and associated nutrients. Net accumulation rates of organic matter and nutrients increase with trophic state of lakes (Binford and Brenner 1986; Deevey et al. 1986). In contrast, small oligotrophic lakes are expected to exhibit a relatively high proportion of allochthonous carbon input to sediments (Gu et al. 1996).

Lake depth can also affect the quality of organic material reaching the sediment. In deep lakes, sedimenting organic matter undergoes intense decomposition in the water column due to the prolonged period of settling. Consequently, low amounts of labile organic carbon reach the sediment in deep lakes (Suess 1980; Meyers 1997). In shallow lakes, the supply of labile organic C and nutrients can be higher in sediments than in deep lakes, while deep lakes often can have more refractory organic matter (Suess 1980).

Sediments receiving organic C of varying lability can support different microbial communities. These communities can display distinct catabolic responses as the mineralization rates of a microbial community are dependent upon the metabolic capacity for a given substrate (Torien and Cavari 1982). The objectives of this study were: (1) to evaluate the short-term potential catabolic response of microbial communities in sediments of three subtropical lakes characterized with different trophic states, and (2) to examine linkages between these microbial potential catabolic responses and the physico-chemical nature of the lakes. The central hypothesis was that sediments with higher C availability would have higher catabolic diversity.

Methods

Study sites

Three subtropical Florida lakes (USA) were selected for this study based on water quality variables and

Table 1 Characteristics of sampled sites in the three different lakes with sampling date, location, sediment type and water quality parameters (measured at 1 m)

Parameters	Lake				
	Annie[a]	Okeechobee[b]			Apopka[b]
	Central	M9	M17	KR	West
Sampling date	June/2005	July/2005			May/2005
Sediment type	Mud/Clay	Mud	Peat	Sand	Organic
Water column depth (m)	20	4.0	2.5	3.1	2.0
Secchi (m)	2.0	0.08	0.15	0.5	0.3
Latitude	27°12′27″	26°58′17.6″	26°45′24.4″	27°58′11.5″	28°38′01″
Longitude	81°21′44″	80°45′38.4″	80°46′36.8″	81°00′51.8″	81°39′36″
Temperature (°C)	30.2	29.5	28.8	30.8	26.6
Electrical Conductivity (μS cm^{-1})	42	385	320	143	443
pH	5.1	7.8	7.6	6.0	7.6
Dissolved oxygen (mg l^{-1})	6.4	6.5	6.3	1.8	8.7
Dissolved organic carbon (mg l^{-1})*	13.8	14.5	17.9	19.8	31.1
Total phosphorus (μg l^{-1})*	33	256	263	146	70
Dissolved reactive phosphorus (μg l^{-1})*	7	90	113	62	11
Total nitrogen (μg l^{-1})*	1,807	3,439	3,362	2,957	11,149
Ammonium—NH_4–N (μg l^{-1})*	182	103	60	84	120

* Mean concentration in the water column

[a] Average depth: 0.5, 1, 2, 5, 10, 20 (m)

[b] Average depth: 0.5, 1, 2 (m)

trophic status (Table 1; Fig. 1). Lake Annie (Fig. 1a), a small (0.37 km^2) oligo-mesotrophic lake, is located in south-central Florida (Highlands County) at the northern end of the Archbold Biological Station. Lake Annie is characterized by pristine water quality with little surface water input (most is ground water), and low anthropogenic impact due to the absence of development around the lake (Layne 1979). This lake has no natural surface streams but two shallow man made ditches allow surface water to flow into the lake and contribute to water and nutrient inputs during high rainfall periods (Battoe 1985). Benthic sediments vary from organic to sand in the littoral zone (Layne 1979). Lake Okeechobee (Fig. 1b) is a large (1,800 km^2) shallow lake located in south Florida. It is considered to be a eutrophic lake that has experienced cultural eutrophication over the past 50 years (Engstrom et al. 2006). Benthic sediments include mud (representing 44% of the total lake surface area), sand and rock (28%), littoral, dominated by macrophyte growth (19%), and peat (9%) that refers to partially decomposed plant tissues (Fisher et al. 2001). Lake Apopka (Fig. 1c) is also a shallow lake with 125 km^2 surface area, located in central Florida. Once a clear-water macrophyte-dominated lake, since 1947 Lake Apopka has transitioned to a turbid, algal-dominated lake (Clugston 1963) considered hypereutrophic. This shift may have been caused by nutrient input from several sources, including agricultural drainage from adjacent vegetable farms (Schelske et al. 2000). Benthic sediments are characterized by unconsolidated material mainly consisting of algal deposits (Reddy and Graetz 1991).

Field sampling

Triplicate intact sediment cores were collected using a piston corer (Fisher et al. 1992) or by SCUBA divers. The topmost 10 cm of sediment was collected from one central site in Lake Annie on June 25, 2005 and a western site in Lake Apopka on May 28, 2005 (Fig. 1a, c; Table 1). Cores were collected at three sites in Lake Okeechobee on July 16, 2005: M17 = peat, M9 = mud, and KR = sand (Fig. 1b; Table 1). Although there was a difference in the days

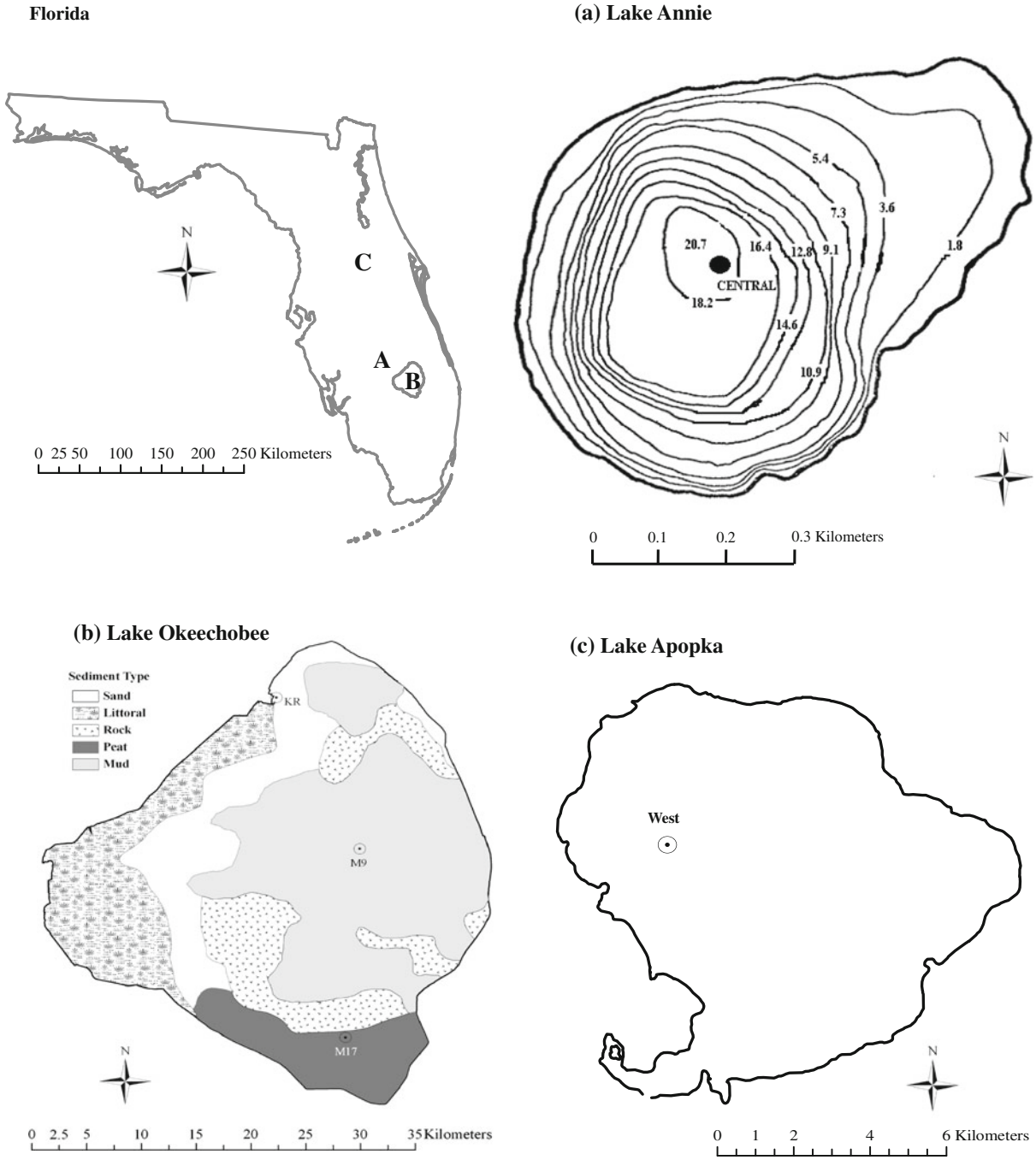

Fig. 1 Map of the three subtropical lakes with sampled sites and their locations in Florida State: **a** Lake Annie (with water column depth in meters, modified from Layne 1979), **b** Lake Okeechobee with different sediment types, and **c** Lake Apopka

the samples were collected, all lakes were sampled within the same season (summer time) when temperatures were high. So, the seasonality effect that can occur in subtropical lakes would not interfere in the outcome of the experiments. Sediment samples (0–10 cm) were placed in sterile plastic sampling bags, sealed, and kept on ice. All measured sediment variables are reported on a dry weight basis (dw).

Water samples were collected from various depths (Table 1) at each site using a Van Dorn bottle and several chemical parameters were measured to characterize the lakes in relation to their trophic status. Water transparency was determined using a Secchi disk. Water temperature (°C), electrical conductivity (EC), pH, and dissolved oxygen (DO) were measured with a YSI 556 Multi-Probe Sensor (YSI Environmental, Yellow Springs, OH, USA) at different depths (Table 1). Water column nutrient concentrations were measured using U.S. EPA methods (EPA 1993). Total Kjeldahl nitrogen (TKN) was measured by digestion with concentrated sulfuric acid (H_2SO_4) and Kjeldahl salt catalyst, and determined colorimetrically (Method—351.2). Total phosphorus (TP) was digested with 11 N H_2SO_4 and potassium persulfate (Method—365.1). Water samples were filtered through a 0.45 μm membrane filter and filtrate was analyzed for dissolved reactive phosphorus (DRP) (Method—365.1), ammonium-N (NH_4–N) (Method—351.2), and dissolved organic carbon (DOC) (automated Shimadzu (Shimadzu Corp., Kyoto, Japan) TOC 5050 analyzer (Method—415.1).

Sediment properties

Sediment samples were transported on ice and stored in the dark at 4°C. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density (g dry wt cm^{-3} wet) was determined at 70°C for 72 h, and pH was determined on wet sediments (1:2 sediment-to-water ratio). Sediment samples were ground in a ball mill and passed through a # 40 mesh sieve. Organic matter content (LOI-loss on ignition) was determined by weight loss at 550°C. Total P was analyzed by ignition method, followed by acid digestion (6 M HCl) and measured colorimetrically (Anderson 1976; Method—365.1, EPA 1993). Total carbon (TC) and total nitrogen (TN) were determined using a Flash EA-1121 NC soil analyzer (Thermo Electron Corporation, Waltham, MA, USA).

Extractable nitrogen (N) and phosphorus (P)

Sediment samples were extracted with 0.5 M K_2SO_4 for extractable N and with 0.5 M $NaHCO_3$ (pH = 8.5) for extractable P, using a 1:50 dry sediment-to-solution ratio (Mulvaney 1996; Ivanoff et al. 1998). Extracts from N samples were centrifuged at 10,000×*g* for 10 min and filtered through Whatman # 42 filter paper. For N analysis, 5 ml of the extracts were subjected to Kjeldahl nitrogen digestion and analyzed for total Kjeldahl-N colorimetrically (Method—351.2, EPA 1993). Digested samples represent total labile nitrogen (TLN). Undigested N extracts were analyzed for ammonium (NH_4–N) (Method— 351.2, EPA 1993), and represent extractable ammonium (Ext–NH_4–N). The difference between TLN and Ext-NH_4-N represents extractable labile organic nitrogen (LON). Extracts from P samples were centrifuged at 10,000×*g* for 10 min and filtered through a 0.45 μm membrane filter, and analyzed for dissolved reactive P or digested for TP (with sulfuric acid and potassium persulfate). Solutions were analyzed by colorimetry, determined by reaction with molybdate (Method 365.1, EPA 1993). Undigested P extracts represent labile inorganic P (LIP), and digested samples represent total labile P (TLP). The difference between TLP and LIP represents labile organic phosphorus (LOP).

Extractable and microbial biomass carbon (C)

Microbial biomass carbon (MBC) was measured with the chloroform fumigation-extraction method (Vance et al. 1987). Briefly, sediment samples were split in two: one sample was treated with alcohol-free chloroform (0.5 ml) to lyse microbial cells, placed in a vacuum desiccator, and incubated for 24 h. The duplicate sample was left untreated. Both sets were extracted with 0.5 M K_2SO_4 centrifuged at 10,000×*g* for 10 min and filtered through Whatman # 42 filter paper. Carbon extracts were acidified (pH < 2) and analyzed in an automated Shimadzu TOC 5050 analyzer (Method—415.1, EPA 1993). Microbial biomass C was calculated by the difference between treated and non-treated samples. Extracts from the untreated samples represent labile organic carbon (LOC).

Electron donors

Basic catabolic response was characterized by measuring CO_2 and CH_4 production rates from sediment samples by addition of different electron donors. Eight different simple organic compounds (electron donors) were added separately to each sediment

sample. These compounds represent model C sources commonly present in lake sediments. The C compounds used in the study ranged from amino acids (alanine and arginine), carboxylic acids (acetate, formate, butyrate, and propionate), polysaccharide (glucose), and complex polymeric C (lake suspended solids, lake-SS). Wet sediment (based on 0.5 g of dry weight) was added to incubation bottles, sealed with rubber stoppers and aluminum crimp seals, and purged with N_2 gas to remove oxygen from the headspace. Alanine, arginine, acetate, formate, butyrate, propionate, and glucose were added from anaerobic sterile stock solutions to sediments on a C-equivalent basis, reaching a final concentration of 42 mM C (504 μg of C g^{-1} on a dry weight basis) (Degens 1998). All stock solutions were adjusted to pH 7.0 (±0.2) using either HCl or NaOH at the time of preparation to avoid any substrate-pH effects on microbial communities. The electron donor substrates were added above background thresholds for each lake.

Lake-SS was obtained by centrifugation (10,000×*g* for 30 min) of water samples collected at approximately 50 cm depth in the water column of each lake. At that depth, lake suspended material would have labile carbon. However, in case of Lake Annie, a deeper lake, the suspended material at 50 cm depth was expected to be different from the suspended material that actually reaches the sediment. In deep lakes, due to organic matter decomposition and C-utilization through the water column, the suspended material that reaches the lake sediments is relatively more recalcitrant than that found at shallow depths of the lakes. The intent of this experiment was to verify the effect of addition of labile carbon present in the lake suspended material on CO_2 and CH_4 production rates.

Lake-SS was characterized for LOI, TC, and TN, as described previously, and was added on the same C-equivalent basis (504 μg of C g^{-1} on a dry weight basis) as other electron donors. A sample from each lake-SS was incubated to account for CO_2 and CH_4 production of the material. Values obtained were subtracted from CO_2 and CH_4 production rates of the lake-SS treatment for each lake. Sediments from each site were also incubated with no substrate addition (control) to obtain values of basal anaerobic CO_2 and CH_4 production rates. Samples were incubated in the dark under anaerobic conditions at 30°C. Gas samples were taken at 1, 2, 4, 7, 10, and 14 days and quantified for CO_2 and CH_4. Gas samples from Lake Annie and Lake Okeechobee sediments were also taken at 20 days of incubation due to low CH_4 production detected in some treatments at 14 days of the experiment. Headspace CO_2 was measured by gas chromatography using a Shimadzu GC-8A thermal conductivity detector at 25°C, and Poropak N column at 20°C (Supelco Inc., Bellefonte, PA) with He as a carrier gas. Headspace CH_4 was analyzed on a Shimadzu gas chromatograph-8A fitted with flame ionization detector (110°C), N_2 as the carrier gas and a 0.3 cm by 2 m Carboxyn 1000 column (Supelco Inc., Bellefonte, PA) at 160°C. Prior to measurements of both CO_2 and CH_4, headspace pressure was determined with a digital pressure indicator (DPI 705, Druck, New Fairfield, CT). Concentrations of CO_2 and CH_4 produced in samples were determined by comparison with standard gas concentrations and production rates were calculated by linear regression ($r^2 > 0.95$). Final production rates were determined after removing the lag phase (the time between substrate addition and quantifiable gas production) in each site.

Statistical analysis

All statistical analyses were conducted with standardized values of anaerobic CO_2 and CH_4 production rates by microbial biomass carbon of each sediment sample (CO_2 or CH_4 production divided by MBC). One-way analysis of variance (ANOVA) with pairwise multiple comparisons Tukey's HSD test was used to assess the effect of different electron donor additions on CO_2 and CH_4 production. One-way ANOVA was performed separately on each site. Principal Component Analysis (PCA) was performed to determine major patterns of CO_2 and CH_4 production rates with the addition different electron donors. All statistical analyses were conducted with Statistica 7.1 (StatSoft 2006, Tulsa, OK) software.

Results

Sediment properties

Sediment pH ranged from 5.9 to 7.8 across all locations (Table 2). Lake Annie sediment pH (5.9) was lower

than other lakes. Sediment pH values in both Lake Okeechobee (M17: 7.7, M9: 7.8 and KR: 7.6) and Lake Apopka (7.5) were circum-neutral to alkaline (Table 2). Surface sediment bulk densities were lowest in Lake Apopka (0.019 g cm^{-3}), followed by Lake Annie (0.052 g cm^{-3}), and Lake Okeechobee sites M9 (0.137 g cm^{-3}), M17 (0.143 g cm^{-3}), and KR (1.183 g cm^{-3}), respectively (Table 2). Sediment organic matter content was highest at Lake Okeechobee site M17 (86.6%), reflecting high peat content. Next in order were Lake Apopka (64.9%), Lake Annie (55.6%), followed by site M9 (37.5%), and sandy KR (4.6%) in Lake Okeechobee (Table 2). Total C was highest in peat zone sediments of Lake Okeechobee (482 g kg^{-1}), followed by sediments from Lake Apopka (288 g kg^{-1}) and Lake Annie (272 g kg^{-1}). Total N in sediments of Lake Apopka (27.3 g kg^{-1}) and peat zone (27.7 g kg^{-1}) sediments of Lake Okeechobee exhibited similar values (Table 2). Lake Annie (1,427 mg kg^{-1}) sediments exhibited higher TP concentrations than Lake Okeechobee (M9: 1,018 g kg^{-1}, M17: 207 mg kg^{-1} and KR: 366 g kg^{-1}) and Lake Apopka (1,185 mg kg^{-1}) sediments (Table 2).

Labile organic C and MBC were highest in Lake Apopka sediments (4,029 and 42,618 mg kg^{-1}, respectively) (Table 2). Lake Apopka sediments also had the highest concentrations of LON (859 mg kg^{-1}) and Ext–NH_4–N (386 mg kg^{-1}) (Table 2). Lake Annie sediments, however, had the highest concentrations of LIP (124 mg kg^{-1}) and LOP (71 mg kg^{-1}) (Table 2). Sediment LOC:TLN ratio was similar in all lake sediments (Table 2). Lake Annie and sites M9 and KR in Lake Okeechobee exhibited low LOC:TLP and TLN:TLP ratios.

Electron donors

Dry suspended material content of the three lakes (lake-SS) was characterized as: 34% C and 2.9% N from Lake Annie; 16% C and 1.5% N from Lake Okeechobee; and 34% C and 3.7% N from Lake Apopka. Addition of electron donors to sediment microcosms stimulated heterotrophic microbial activity in most samples (Tables 3, 4). All sediments showed a rapid response to addition of electron donors. Basal CO_2 and CH_4 production rates were highest in Lake Apopka sediments (217 mg CO_2–C kg^{-1} day^{-1} and 80 mg CH_4–C kg^{-1} day^{-1}) (Tables 3, 4). Sediments from Lake Okeechobee had the longest lag phase in CH_4 production (Data not presented). Addition of different electron donors produced a different response in each lake sediment (Table 3). Results from one-way ANOVAs were significantly different ($P < 0.05$, Table 5). Lake Annie sediments had the highest CO_2 production rates with both amino acids (alanine: 217 mg CO_2–C kg^{-1} day^{-1} and arginine: 212 mg CO_2-C kg^{-1} day^{-1}) and formate (209 mg CO_2–C kg^{-1} day^{-1}) addition. Lake Okeechobee sediments had the highest CO_2 production rates with both amino acids (alanine—M9: 62 CO_2-C kg^{-1} day^{-1}, M17: 75 CO_2-C kg^{-1} day^{-1} and KR: 18 CO_2–C kg^{-1} day^{-1}; arginine—M9: 50 CO_2–C kg^{-1} day^{-1}, M17: 86 CO_2–C kg^{-1} day^{-1} and KR: 10 CO_2–C kg^{-1} day^{-1}) and glucose (M9: 66 CO_2–C kg^{-1} day^{-1}, M17: 104 CO_2–C kg^{-1} day^{-1} and KR: 13 CO_2–C kg^{-1} day^{-1}) addition. Lake Apopka sediments had the highest CO_2 production rates with alanine (874 CO_2–C kg^{-1} day^{-1}) and formate (773 CO_2–C kg^{-1} day^{-1}) (Table 3).

Higher CH_4 production rates in Lake Annie sediments were detected with the addition of both amino acids (alanine: 120 mg CH_4–C kg^{-1} day^{-1} and arginine: 159 mg CH_4–C kg^{-1} day^{-1}) and acetate (155 mg CH_4–C kg^{-1} day^{-1}) (Table 4). In Lake Okeechobee mud (site M9) sediments, higher CH_4 production rates were detected in alanine (45 mg CH_4–C kg^{-1} day^{-1}), butyrate (41 mg CH_4–C kg^{-1} day^{-1}), and glucose (52 mg CH_4–C kg^{-1} day^{-1}) treatments. In Lake Okeechobee sand (site KR) sediments, higher CH_4 production rates were detected in alanine (12 mg CH_4–C kg^{-1} day^{-1}) and glucose (13 mg CH_4–C kg^{-1} day^{-1}) treatments. In the peat zone sediments (site M17) of Lake Okeechobee, addition of the two amino acids (alanine: 10 mg CH_4–C kg^{-1} day^{-1} and arginine: 11 mg CH_4–C kg^{-1} day^{-1}), acetate (11 mg CH_4–C kg^{-1} day^{-1}), and butyrate (8 mg CH_4–C kg^{-1} day^{-1}) produced higher CH_4 than other substrates. In Lake Apopka sediments, highest CH_4 production rates were detected with the addition of alanine (563 mg CH_4–C kg^{-1} day^{-1}), glucose (374 mg CH_4–C kg^{-1} day^{-1}), and lake-SS (355 mg CH_4–C kg^{-1} day^{-1}) (Table 4). The magnitude of CO_2 and CH_4 production following addition of different electron donors was related to microbial biomass at each site. There was a strong significant positive correlation (Pearson's correlation) between MBC and CO_2 ($r = 0.91$), and MBC and CH_4 ($r = 0.86$) production rates (Fig. 2a, b).

Table 2 Sediment biogeochemical properties of Lake Annie, Lake Okeechobee, and Lake Apopka (mean ± SD)

Variables	Lake				
	Annie	Okeechobee			Apopka
	Central	M9	M17	KR	West
pH	5.9 ± 0.01	7.8 ± 0.07	7.7 ± 0.02	7.6 ± 0.2	7.5 ± 0.06
BD (g cm^{-3})	0.052 ± 0.002	0.137 ± 0.06	0.143 ± 0.018	1.183 ± 0.29	0.019 ± 0.003
LOI (%)	55.6 ± 1.0	37.5 ± 0.7	86.6 ± 2.0	4.6 ± 4.3	64.9 ± 1.8
Carbon					
TC (g kg^{-1})	272 ± 6.2	193 ± 2.2	482 ± 8.9	25 ± 24.0	288 ± 9.2
LOC (mg kg^{-1})	946 ± 142	279 ± 8	894 ± 87	76 ± 19	4,029 ± 719
MBC (mg kg^{-1})	12,116 ± 487	3,910 ± 157	4,081 ± 157	666 ± 231	42,618 ± 6,423
qCO_2	0.008 ± 0.001	0.005 ± 0.001	0.006 ± 0.00	0.004 ± 0.001	0.004 ± 0.001
Nitrogen					
TN (g kg^{-1})	20.3 ± 0.9	12.6 ± 0.3	27.7 ± 0.4	1.5 ± 1.5	27.3 ± 1.2
Ext–NH_4–N (mg kg^{-1})	226 ± 96	48 ± 4	27 ± 1	8 ± 4	386 ± 32
LON (mg kg^{-1})	147 ± 23	83 ± 7	141 ± 14	17 ± 1	859 ± 89
Phosphorus					
TP (mg kg^{-1})	1,427 ± 34	1,018 ± 48	207 ± 12	366 ± 78	1,185 ± 74
LIP (mg kg^{-1})	124 ± 9	99 ± 6	4.8 ± 2	6.5 ± 2	1.8 ± 0.6
LOP (mg kg^{-1})	71 ± 19	8.3 ± 1.6	4.2 ± 1.1	1.2 ± 1.5	32.3 ± 6.5
Ratios					
LOC:TLN	3	2	5	3	3
LOC:TLP	5	3	102	10	119
TLN:TLP	2	1	19	3	37

BD bulk density, *LOI* loss on ignition, *TC* total carbon, *LOC* labile organic carbon, *MBC* microbial biomass carbon, qCO_2 metabolic quotient, *TN* total nitrogen, *TLN* total labile nitrogen, *Ext–NH_4–N* extractable ammonium, *LON* labile organic nitrogen, *TP* total phosphorus, *LIP* labile inorganic phosphorus, *LOP* labile organic phosphorus, *TLP* total labile phosphorus

Table 3 Sediment anaerobic respiration (mg CO_2–C kg^{-1} day^{-1}) with the addition of different carbon sources

Treatment	Anaerobic respiration (mg CO_2–C kg^{-1} day^{-1})				
	Lake				
	Annie	Okeechobee			Apopka
	Central	M9	M17	KR	West
Basal	100 ± 9a	26 ± 1a	21 ± 4ae	3 ± 2ac	217 ± 23a
Amino acids					
Alanine	217 ± 9b	62 ± 7bcd	75 ± 10bcd	18 ± 4bc	874 ± 109b
Arginine	212 ± 10b	50 ± 6bc	86 ± 8bcf	10 ± 4abc	623 ± 350ab
Carboxylic acids					
Acetate	135 ± 16a	33 ± 1a	50 ± 10bde	5 ± 5ac	500 ± 259ab
Butyrate	87 ± 3ac	30 ± 2a	33 ± 6ade	4 ± 3ac	307 ± 183ab
Formate	209 ± 22b	28 ± 2a	51 ± 4bde	3 ± 3ac	773 ± 324b
Propionate	55 ± 13c	32 ± 1a	42 ± 11ade	4 ± 3ac	629 ± 152ab
Polysaccharide					
Glucose	96 ± 12a	66 ± 5bd	104 ± 25cef	13 ± 3bc	559 ± 129ab
Natural carbon					
Lake-SS	101 ± 12a	29 ± 1a	33 ± 3ade	4 ± 2ac	418 ± 160ab

Tukey's test was conducted within sites and different letters indicate significant statistical differences at $P < 0.05$ (mean ± SD)

Table 4 Sediment methane production rates (mg CH_4–C kg^{-1} day^{-1}) with the addition of different carbon sources

Treatment	Methanogenesis (mg CH_4–C kg^{-1} day^{-1})				
	Lake				
	Annie	Okeechobee			Apopka
	Central	M9	M17	KR	West
Basal	37 ± 8a	0.09 ± 0.0a	0.16 ± 0.02a	0.04 ± 0.01a	80 ± 7a
Amino acids					
Alanine	120 ± 15b	45 ± 4be	10 ± 4b	12 ± 5b	563 ± 20b
Arginine	159 ± 7c	19 ± 3cdf	11 ± 3b	2 ± 2a	220 ± 24ace
Carboxylic acids					
Acetate	155 ± 15c	26 ± 1cd	11 ± 1b	5 ± 9a	102 ± 23acd
Butyrate	85 ± 4d	41 ± 3b	8 ± 1b	5 ± 2a	192 ± 16acd
Formate	36 ± 15a	5 ± 2a	0.3 ± 0.08a	3 ± 4a	44 ± 8acd
Propionate	3 ± 1e	13 ± 1ce	0.8 ± 0.4a	0.4 ± 0.5a	48 ± 10acd
Polysaccharide					
Glucose	44 ± 8a	52 ± 5bf	0.7 ± 0.4a	13 ± 5b	374 ± 22ce
Natural carbon					
Lake-SS	47 ± 8a	8 ± 2ae	0.0 ± 0.0a	2 ± 1a	355 ± 14ce

Tukey's test was conducted within sites and different letters indicate significant statistical differences at $P < 0.05$ (mean ± SD)

Principal Component Analyses (PCA) of the data on the effect of electron donor additions on CO_2 and CH_4 production rates indicated that 41% of the data variability was explained by Axis 1 while Axis 2 explained 20% (Fig. 3a). Anaerobic respiration with the additions of acetate, butyrate, formate, and lake-

Table 5 One-way ANOVA statistics of the effect of the different carbon sources addition to sediment CO_2 and CH_4 production rates

ANOVA	Lake				
	Annie	Okeechobee			Apopka
	Central	M9	M17	KR	West
Anaerobic respiration (mg CO_2–C kg^{-1} day^{-1})					
n	27	27	27	27	27
df	8	8	8	8	8
F	63.39	33.83	27.36	9.80	3.68
P	<0.00001	<0.00001	<0.00001	0.00003	0.0103
Methanogenesis (mg CH_4–C kg^{-1} day^{-1})					
n	27	27	27	27	27
df	8	8	8	8	8
F	91.14	124.30	43.94	4.32	24.34
P	<0.00001	<0.00001	<0.00001	0.00471	<0.00001

SS were the variables selected by Axis 1. Basal anaerobic CO_2 production was selected by Axis 2. The position of sites in relation to variable loadings in PCA-1 showed that sediments from each lake and site are separated into different groups (Fig. 3b). Lake Annie sediments were plotted in the position of basal CO_2 production (Fig. 3b). Lake Apopka sediments with lake-SS cluster (lake-SS, butyrate, acetate, formate, and propionate) opposite from Lake Annie. Lake Okeechobee site M17 was plotted close to Lake Apopka sediments, while the KR site was in the position with glucose and alanine additions. Lake Okeechobee mud zone (site M9) was not placed with any specific carbon addition (Fig. 3b).

The PCA-2 had 34% of the data variability explained by Axis 1 while Axis 2 explained 27% (Fig. 4a). Methane production rates with additions of alanine, butyrate, and glucose were the variables selected by Axis 1. Methane production rates from arginine, and basal production rate were selected by Axis 2. The position of the sites in relation to the variable loadings in PCA-2 showed a separation of sediments from each lake and site (Fig. 4b). Lake Annie sediment was placed with the basal production, arginine, and acetate cluster. Lake Okeechobee M9 site was plotted in the position of propionate and formate and close to the KR site that was positioned with glucose, alanine, and butyrate. Site M17 was plotted in opposite position of all C-sources. Lake Apopka sediments were placed with lake-SS (Fig. 4b).

Discussion

Addition of organic electron donors to sediments stimulated heterotrophic activity. Similar results have also been shown by other studies. Findlay et al. (2003) showed that the addition of different carbon sources, i.e., glucose, bovine serum albumin and natural leaf leachate to hyporheic biofilms enhanced microbial activities. Wang et al. (2007) showed that addition of electron donors (glucose, sucrose, potato starch, and sodium acetate) stimulated denitrification in Lake Taihu (China) sediments. In the study of benthic microbial response to the deposition of natural seston in Lake Erken (Sweden), Törnblon and Rydin (1998) showed that seston addition caused an immediate increase in bacterial production, activity, and total sediment metabolism.

The extent of response to electron donor addition was related to microbial biomass (Tables 2, 3, 4; Fig. 2a, b). Generally, sediments responded rapidly to addition of most electron donors by increasing CO_2 production rates. Sediments from site KR in Lake Okeechobee with the lowest microbial biomass exhibited the longest lag phase before responding to electron donor addition. Microbial biomass was significantly related to rate of C source consumption in all lake sediments, suggesting that microbial activity is limited by C availability (Fig. 2a, b) (Lu et al. 2000).

Although the magnitude of response to electron donor additions was related to microbial biomass,

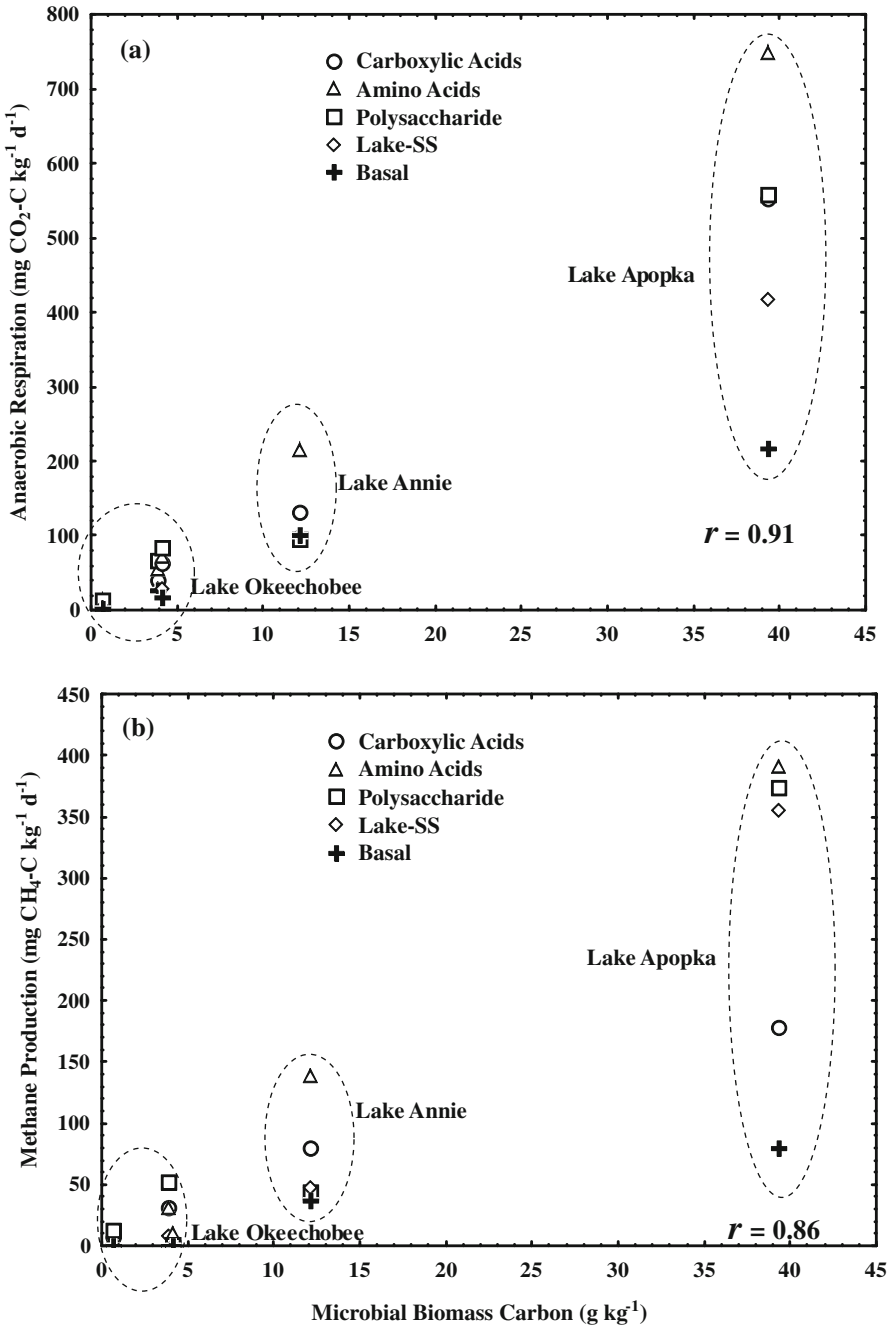

Fig. 2 Relationship between microbial biomass carbon and activity: **a** CO_2, and **b** CH_4 production rates, with the different groups of carbon sources added to sediments from different lakes

varied responses in sediments were probably related to catabolic diversity of microorganisms utilizing these substrates. Results of PCA 1 showed that Lake Apopka sediments had the highest respiration per unit of microbial biomass with most of the electron donor additions (propionate, lake-SS, butyrate, acetate, and, formate), indicating that these sediments respired most of the added C (Fig. 3a, b). This suggests higher microbial activity and catabolic diversity in these sediments as compared to sediments from the other lakes. Increased biogeochemical diversity can occur in environments with high organic matter content due to the presence of a wide range of organic compounds. Similar conclusions were made by Castro et al.

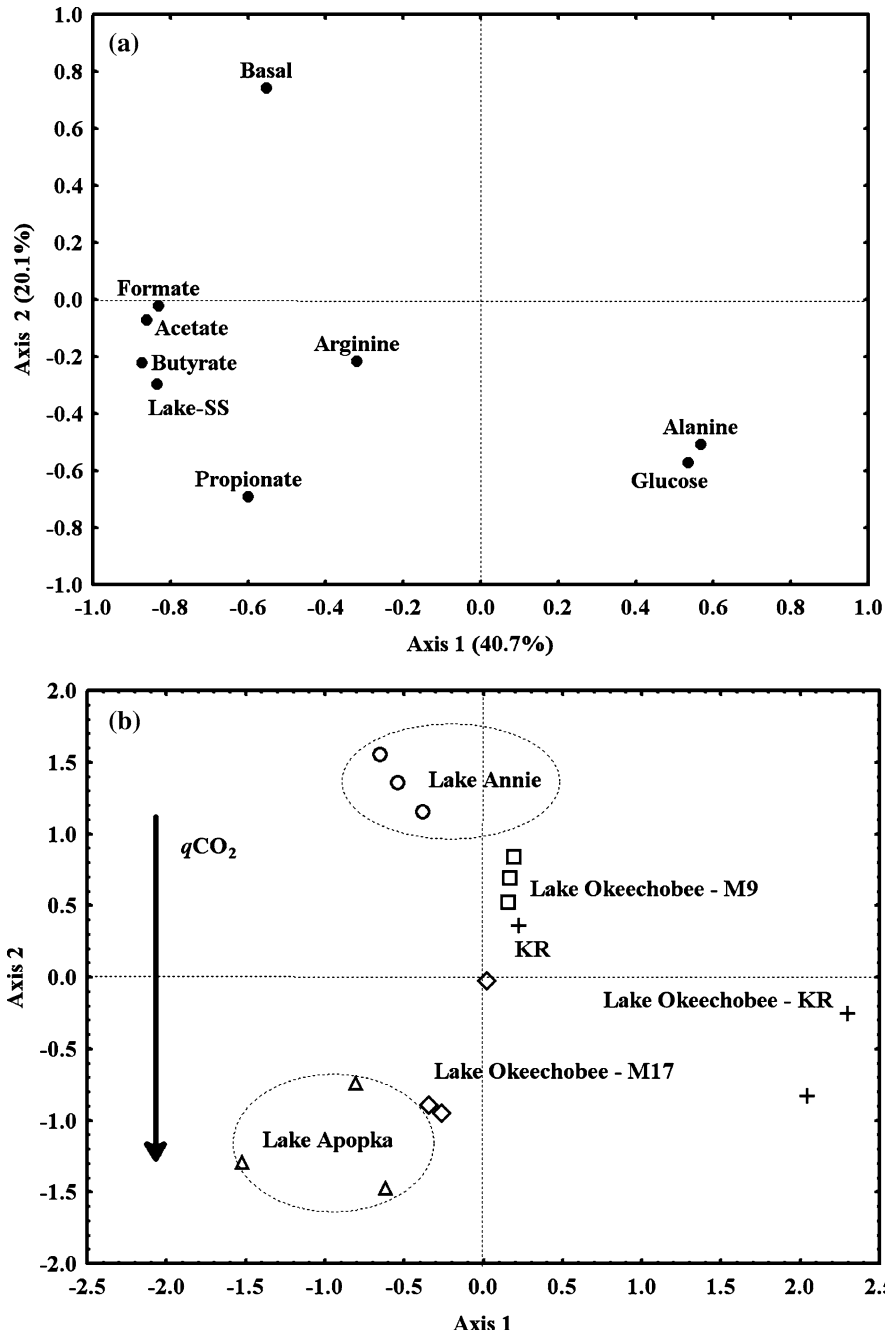

Fig. 3 Results of the Principal Component Analysis (PCA-1): **a** loadings of the effect of different carbon sources addition on sediment CO_2 production rates, and **b** the plot of the scores of the sites from Lake Annie (*circles*), Lake Okeechobee: M9 (*squares*), M17 (*diamonds*), KR (*crosses*), and Lake Apopka (*triangles*). CO_2 production rates were normalized by microbial biomass carbon

(2005), who reported that complete oxidizing species, capable of using a broader array of electron donors were dominant in eutrophic and transitional sites of the Everglades wetlands, while incomplete oxidizers, that are more efficient in utilizing low concentrations of fewer substrates, were present in oligotrophic regions. The authors concluded that eutrophic regions with high C availability select microbial communities dominated by generalists that are capable of utilizing a greater diversity of C substrates (Castro et al. 2005). Lake Apopka was formed as a result of high primary productivity in the water column and subsequent deposition of plankton biomass to benthic sediments (Gale and Reddy 1994). These sediments support high rates of microbial activity, as indicated by rapid utilization of a wide range of electron donors.

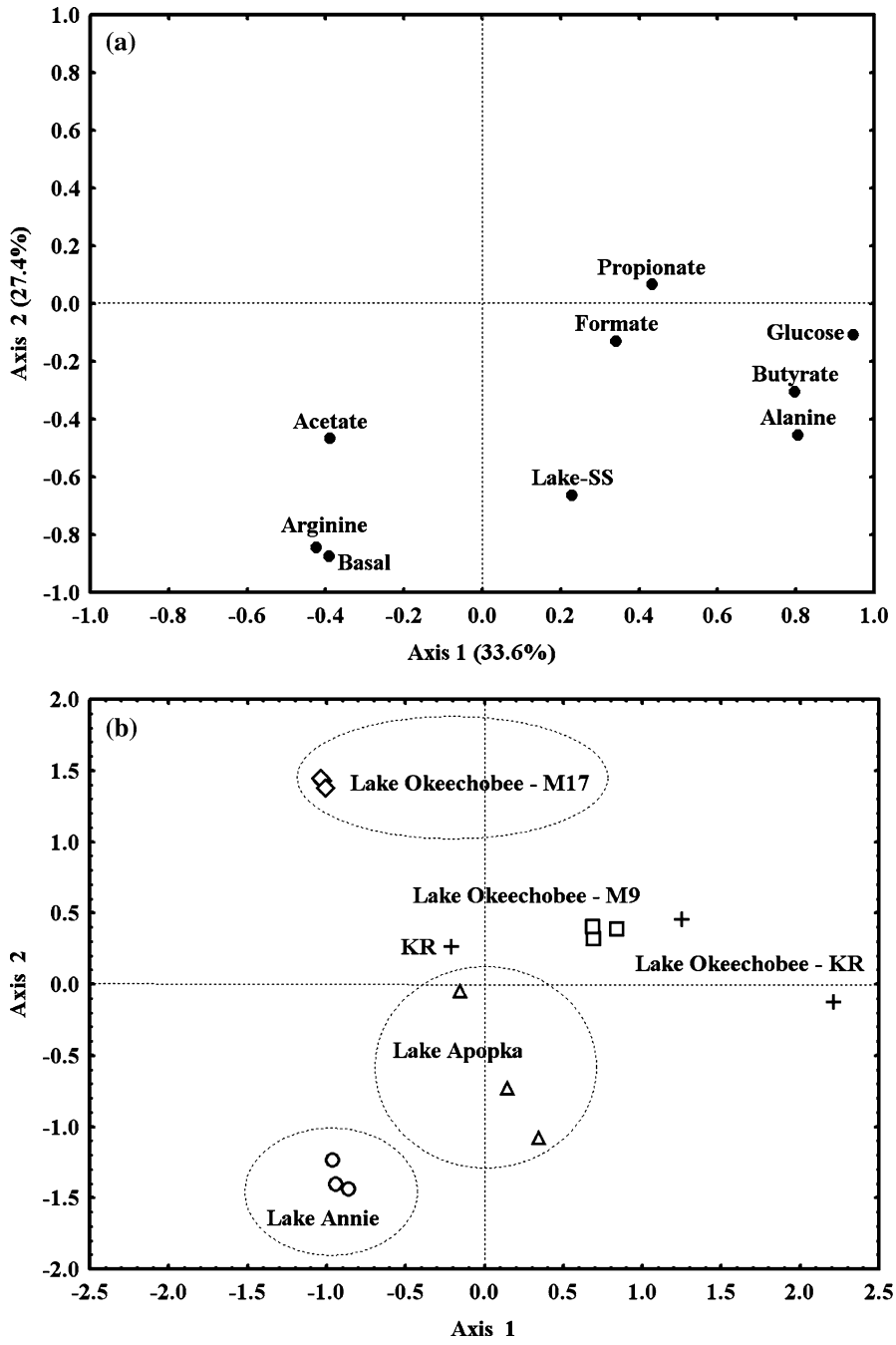

Fig. 4 Results of the principal component analysis (PCA-2): **a** loadings of the effect of different carbon sources addition on sediment CH_4 production rates, and **b** the plot of the scores of the sites from Lake Annie (*circles*), Lake Okeechobee: M9 (*squares*), M17 (*diamonds*), KR (*crosses*), and Lake Apopka (*triangles*). The CH_4 production rates were normalized by microbial biomass carbon

Alternatively, heterotrophic bacteria tend to respire most of the added C under conditions of P limitation. In controlled experiments with bacterioplankton in subarctic Lake Diktar Erik, Sweden, Jasson et al. (2006) showed that bacterioplankton respired large portions of added C under P limiting conditions and that a significant portion of the added C was also used to support the growth of microbial biomass.

Addition of some electron donors (i.e., butyrate and propionate) did not stimulate heterotrophic microbial respiration. With others the stimulation was not significantly different from basal activities (Tables 3, 4),

suggesting either absence of organisms that can utilize these substrates or assimilation of added C into microbial biomass (Bremer and van Kessel 1990; Degens 1998). Törnblon and Rydin (1998) found that after seston addition to sediment, bacterial biomass doubled indicating assimilation of C into microbial biomass. For forested soils, the partitioning between biomass-C incorporation and respiratory CO_2-C was determined to be substrate- rather than soil-dependent. van Hees et al. (2005) reported for forested soils that 60–90% of organic acid, 20–60% of monosaccharide, and 10–30% of amino acid is evolved as CO_2. Studies with different microorganisms reported that between 30 and 40% of glucose and up to 80% of formate of the C source supplied is immediately used for respiration and the remaining for biomass growth (Stouthamer 1976). King and Klug (1982) reported that the addition of glucose into microbial biomass was low (20%) in a eutrophic lake sediment (Wintergreen Lake). Results from this study suggest that a large proportion of added amino acids, glucose and formate were used through respiratory pathways rather than added into biomass (King and Klug 1982).

PCA results placed Lake Annie sediments with basal CO_2 production rates indicating that this site had the highest anaerobic respiration per microbial biomass (Fig. 3a, b). Lake Apopka was positioned on the opposite side, indicating the lowest basal anaerobic respiration per microbial biomass. The metabolic quotient (qCO_2; proportion of basal respiration per microbial biomass) has been used in soil studies to indicate ecological efficiency of the soil microbial community (Anderson and Domsch, 1990; Degens, 1998). This index is based on Odum's theory of ecosystem succession (1969), where during ecosystem succession towards maturity there is a trend of increasing efficiency in energy utilization concomitant with an increase in diversity. High qCO_2 indicates inefficient use of energy, while low qCO_2 indicates high efficiency and more carbon utilized for biomass production (Anderson and Domsch 1990; Anderson 2003; Francaviglia et al. 2004). Moreover, if the progression of lakes in time from less productive (oligotrophic) to more productive (eutrophic-hypereutrophic) can be viewed as a natural succession, higher qCO_2 should be detected in oligotrophic lakes. The trend of decreasing qCO_2 with increasing trophic state is clearly presented in Axis 2 of PCA-1 (Fig. 3a, b). Similar results were also reported by Smith and Prairie (2004) in a study of bacterioplankton in lakes of different trophic states, where they concluded that oligotrophy places high respiratory demands on bacterioplankton, with greater carbon flow to CO_2 rather than to biomass.

Basal CH_4 production rates were highest in sediments of hypereutrophic Lake Apopka. These results are in accordance with those observed in other studies that have shown higher CH_4 production rates in sediments from eutrophic lakes when compared with those from oligotrophic lakes (Falz et al. 1999; Nüsslein and Conrad 2000; Huttunen et al. 2003). Low rates of basal CH_4 production found in eutrophic Lake Okeechobee sediments may be explained by electron donor limitation, or by the presence of iron oxides which potentially can inhibit methanogenesis (Roden and Wetzel 2003). Presence of Fe has been previously documented in Lake Okeechobee sediments by Fisher et al. (2001). In a previous study (Torres 2007), basal CH_4 production was not detected, but was stimulated after the addition of acetate and/or H_2 in sediments of Lake Okeechobee. Although a lag phase for CH_4 production was observed in all sediments, CH_4 production was much delayed in sediments from sites M17 and KR in Lake Okeechobee (data not shown). Methanogens have the ability to use only a limited number of substrates, including H_2, CO_2, formate, acetate, methanol, and methylated amines (Oreland, 1988). The most important substrates for methanogens are H_2/CO_2 and acetate, and they often depend on other anaerobic bacteria for these substrates (Conrad 1999).

Other anaerobic bacteria (i.e., Fe and SO_4^{-2} reducers) can outcompete methanogens for H_2/CO_2 and acetate due to higher substrate affinities and higher energy and growth yields (Lovley and Klug 1983; Lovley and Phillips 1986; Bond and Lovley 2002); however, both processes can coexist (Holmer and Kristensen 1994; Roy et al. 1997; Roden and Wetzel 2003; Wand et al. 2006). Coexistence occurs because of spatial variation in the abundance of terminal electron acceptors or because the supply of electron donors is non-limiting (Roy et al. 1997; Megonigal et al. 2004). The lag phase observed for CH_4 production in all sediments can be explained by two mechanisms: (1) methanogenic activity stimulated in the presence of substrates produced by fermentative activity, and (2) methanogens becoming active after

other electron acceptors (Fe(III), SO_4^{-2}) were consumed and depleted in sediment microcosms.

Most methanogenic species use H_2/CO_2 and a fewer number of species can use acetate (Garcia et al. 2000). In lake sediments the dominance of acetoclastic (acetate utilizers) versus hydrogenotrophic (H_2 utilizers) methanogenesis has been reported to be related to sediment properties (i.e., pH and temperature). In acidic Lake Grosse Fuchskuhle (Germany), with high humic content, acetate utilizers (*Methanosarcinaceae*) were the only detected methanogens (Casper et al. 2003). Phelps and Zeikus (1984) reported that acetoclastic methanogenesis was the major pathway for CH_4 production in a mildly acidic (pH 6.2) lake (Knaack Lake, Wisconsin). The increase in pH to neutral values enhanced total CH_4 production from H_2/CO_2, but did not affect the CH_4 produced from acetate (Phelps and Zeikus 1984). In mesotrophic Lake Rotsee (Switerland) sediments, it was reported that in these cold sediments acetate is the main CH_4 precursor (Falz et al. 1999). Other studies have shown that CH_4 production at low temperature (4°C) in sediments was mainly from acetate, however, an increase in temperature (20–25°C) lead to an increase in contribution of CH_4 production from H_2/CO_2 (Schulz and Conrad 1996; Schulz, et al. 1997; Nüsslein and Conrad 2000; Glissmann et al. 2004).

Lake Annie sediments are acidic and probably maintain fairly constant low temperatures. Thermal stratification of the water column was detected during sampling in this lake with a temperature of 17.3°C below 14 m water column depth (Torres 2007). Sediment temperature is probably much lower in this deep (20 m) lake. Sediment acidic pH and low temperatures as well as the high CH_4 production rate with addition of acetate and the placement of Lake Annie with acetate cluster in the PCA-2 suggest that acetoclastic methanogenesis may be an important pathway for CH_4 production in these sediments (Table 5; Fig. 4a, b). Lake Okeechobee and Lake Apopka had high temperatures at the sediment–water surface (26.3–30.7°C), and both lakes had circumneutral to alkaline sediment pH (Table 2), creating good conditions for hydrogenotrophic methanogenesis. In Lake Okeechobee sediments it has been determined that hydrogenotrophic methanogenesis is the main pathway of CH_4 production (Torres 2007). Our results suggest that phytoplankton deposition is an important source of C to methanogenic activity in Lake Apopka sediments as evidenced by high rates of CH_4 production with addition of lake-SS (Table 3; Fig. 4a, b).

In conclusion, this study has shown that sediments with different biogeochemical properties have microbial communities that exhibit distinct catabolic responses to a range of C-sources. The hypereutrophic lake with higher C availability in its sediments had the highest catabolic diversity, where the microbial communities were able to efficiently use a broader range of substrates. The oligo-mesotrophic lake sediment microorganisms had lowest efficiency in use of energy. An increasing efficiency in use of C sources by the sediment microbial community with increasing trophic conditions was detected. This study establishes a close linkage between physical and chemical properties of lake sediments and associated microbial activities.

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