SUMMARY OF RESEARCH RESULTS ON BACTERIAL DEGRADATION OF TRIFLUOROACETATE (TFA), OCTOBER, 1993 - OCTOBER, 1995

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ABSTRACT

Addition of 2-^{14}C-trifluoroacetate (TFA) to methanogenic freshwater sediments resulted in its sequential defluorination to 2-^{14}C-acetate, followed by the formation of $^{14}CH_4$. Sulfate-reducing freshwater and saltmarsh sediments also defluorinated 2-^{14}C-TFA to 2-^{14}C-acetate, which was subsequently oxidized to $^{14}CO_2$. Degradation of ^{14}C-TFA was observed in these sediments in experiments conducted from September 1993 to June 1994. However, subsequent experiments with these materials have not shown ^{14}C-TFA degradation. It is not known why the sediments lost the ability to degrade ^{14}C-TFA and several experiments were undertaken to reestablish their ability to biodegrade ^{14}C-TFA. These experiments included addition of various electron donors and acceptors, as well as pre-incubation of sediments with unlabeled, fluorinated acetates. In addition, several other soils and sediments were screened for their ability to degrade ^{14}C-TFA, including aerobically and anaerobically incubated soils from a northeastern hardwood forest which had received prior exposure to TFA. We did not detect significant ^{14}C-TFA biodegradation in any of the sediment systems studied, regardless of whether or not they were manipulated with amendments of electron donors, acceptors, or pre-incubated with fluorinated acetates. Methane-oxidizing soils were also unable to degrade ^{14}C-TFA. Additionally, bacterial isolates which utilize acetate, as well as known dehalogenating bacteria, were assayed for the ability to degrade ^{14}C-TFA. None of the pure cultures of methanogens, sulfate-reducing bacteria, or selected dehalogenating bacteria were able to degrade ^{14}C-TFA. The presence of labeled or unlabeled TFA did not appear to inhibit the growth of any of these bacteria. We conclude that TFA is a molecule which is generally refractory to microbial degradation.

INTRODUCTION

Recently, much concern about trifluoroacetate (TFA) has arisen because of findings that this compound may accumulate in the biosphere (Tromp and others, 1995). TFA is a predicted product of the tropospheric photolysis and hydrolysis of certain hydrochlorofluorocarbons (HCFCs) and hydrofluorocarbons (HFCs) (Franklin, 1993; Wallington and others, 1994). For example, HFC-134a (CF_3CFH_2) undergoes a reaction sequence which forms a trifluoroacetyl compound, which is then hydrolyzed in clouds to form TFA (Franklin, 1993). The TFA produced from these reactions is predicted to be deposited on the Earth's surface through wet or dry deposition. TFA has not been shown to be acutely toxic to humans (Rusch, 1994), although it may be phytotoxic (Thompson, 1994). However, monofluoroacetate (MFA) is a tricarboxylic acid cycle inhibitor and is known to be acutely toxic to humans, as well as most life forms (Calver and King, 1986).

TFA is thought to be highly resistant to degradation in the biosphere, leading to concern that TFA could accumulate in ground waters and the oceans (Chumley, 1992; Franklin, 1993; Tromp and others, 1995). Tromp and others (1995), in a modeling study, have indicated that TFA could accumulate to > 0.62 μM in evapotranspirative environments. Although this conclusion may represent extreme conditions, it does seem possible in light of earlier work that suggests this compound is not readily biodegradable. For example, Hirsch and Alexander (1960) observed that isolates of *Nocardia* and *Pseudomonas* sp. were not able to degrade TFA or monofluoroacetate (MFA). However, recent work by Visscher and others (1994) has shown that TFA can be biodegraded in both anoxic and oxic sediments. These workers observed that TFA was metabolized by bacteria in both freshwater and marine sediments (Visscher and others, 1994). Sediments were assayed for TFA-degrading activity under methanogenic, sulfate-reducing, nitrate-reducing and aerobic conditions. Methanogenic, sulfate-reducing and aerobic slurries of both freshwater and marine sediments were able to degrade TFA. Under anaerobic conditions TFA was reductively defluorinated to acetate, which was then cleaved to CH_4 and CO_2 under methanogenic conditions, or oxidized to CO_2 under sulfate-reducing conditions. When oxygen served as the electron acceptor, TFA was decarboxylated to form fluoroform (CHF_3) and CO_2. The absolute concentration of TFA employed appeared to be a critical factor controlling biodegradation, with little or no activity observed at concentrations >1.9 μM, and high activity and degradation efficiencies occurring at sub-micromolar levels (Visscher and others, 1994). These results suggest that TFA may be consumed in a wide variety of ecosystems and hence may not accumulate in the biosphere.

In light of the attempts to predict the fate of TFA in the environment, the objective of the studies reported here was to determine if TFA biodegradation was common in a variety of soils and sediments, and to delineate the mechanisms of TFA degradation in nature. We have found that, in contrast of our earlier results, TFA appears widely recalcitrant to biodegradation.

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MATERIALS AND METHODS

Soil And Sediment Experiments

Sediments (upper 10 cm) were collected from four locations: an estuarine saltmarsh in South San Francisco Bay (Oremland and others, 1982); the freshwater Searsville Lake (Smith and Oremland, 1983); a heavily-used yacht harbor in the San Francisco Marina, and an estuarine saltmarsh in Bolinas Bay, Calif. Sediments were stored in completely filled mason jars at 4 °C and, unless indicated otherwise, were used within 2 h of collection. Soil samples from a sub-surface zone of bacterial methane oxidation (80 cm deep) of a peatland soil in the Sacramento Delta were collected by augering (Oremland and Culbertson, 1992), placed in sealed mason jars, and stored at 4 ° C. Soil and water were also taken from areas within two ground-water seeps in a northeastern hardwood forest (Hubbard Brook Experimental Forest). One seep had received prior exposure to TFA for approximately one year (Yavitt and Fahey, 1993; S. Tartowski, oral comm.) the other was located hydrologically upgradient and had not been exposed to TFA. These samples were collected in sealed mason jars, shipped at ambient temperatures and stored at 4 °C upon receipt in the laboratory.

Soils and sediments from the estuarine sites, hardwood forest, and Searsville Lake were homogenized in a Waring blender (under N_2 for anaerobic incubations) with an equal volume of artificial bay water (ABW) (Oremland and Polcin, 1982) for estuarine slurries or with lakewater for freshwater lake slurries. Forest soils were homogenized with and equal volume of water collected from the site, 1.15 M phosphate buffer or mineral salts medium (Egli and others, 1988) as indicated. Sacramento Delta soils were either weighed in 10 g portions and used without liquid addition, or slurried with tapwater. Portions of the homogenate (20 mL) were dispensed into 60-mL serum bottles and crimp-sealed (black butyl rubber stoppers) under air, N_2, O_2 plus CH_4, or H_2 as indicated in the text. Additional oxygen was injected (1-5 mL) into aerobic bottles to insure aerobic conditions during incubation. Similarly, methane was injected (1-5 mL) in the case of methanotrophic incubations. Inhibitors or substrates were added either prior to sealing, or through the stoppers before addition of ^{14}C-TFA. Various electron donors were added (final concentration in parentheses) to determine whether each might elicit TFA degradation: sodium acetate (10 mM), trimethylamine hydrochloride (TMA; 10 mM), anhydrous D-glucose (5 mM), sodium pyruvate (5 mM), propionic acid (5 mM), disodium oxalate (5 mM), sodium lactate (5 mM), and sodium benzoate (5 mM). In some cases, ammonium chloride (0.185 mM) was added to 20 mL sediment slurries to determine if the addition of this nitrogen source elicited TFA degradation. Also, various electron acceptors were added to selected sediment slurries to study their effect on TFA degradation. These included (final concentration): sodium sulfate (20 mM in saltmarsh sediments; 5 mM in freshwater slurries), sodium nitrate (20 mM), Fe(III)-nitrilotriacetic acid (NTA; 10 mM), FeOOH (amorphous; ca. 0.1 mmole/L) and MnO_2 (20 mmol/L). Inhibitors were added to some slurries to block the activity of selected populations of bacteria. 2-Bromoethanesulfonate (BES; 5 mM) was used to inhibit methanogens, and

sodium molybdate (2.5 mM) to inhibit sulfate-reducing bacteria (Oremland and Capone, 1988). Heat-killed controls were autoclaved (250 kPa and 121 °C for 1 h) and then cooled and sealed under the appropriate atmosphere. An aqueous solution of 2-^{14}C-TFA (0.2 to 1 μCi/0.1 mL, 3.7 to 18.5 nmole/0.1 mL, as indicated in text; Amersham Inc., Arlington Heights, Illinois; specific activity = 54 mCi/mmol; purity 99.6 percent) was injected through the stopper and the slurries were incubated in the dark at 21-28 °C, either statically or with shaking (200 rpm on a rotary platform shaker). Unless stated otherwise, all experiments were performed in triplicate. In selected experiments, 1-^{14}C-TFA (0.5 to 1 μCi/0.1 mL, 9.4 to 18.9 nmole/0.1 mL, as indicated in text; Amersham Inc., Arlington Heights, Illinois; specific activity = 53 mCi/mmol; purity 98.9 percent) was added to follow the fate of the carboxylic acid group of TFA. All sediment slurries were monitored at periodic intervals for the production of CH_4, CO_2, N_2O, $^{14}CH_4$, $^{14}CO_2$ and liquid-phase products as described below.

Pre-Incubation Of Sediments With Fluorinated Acetates

To determine whether TFA-degradation could be induced, 250 mL of San Francisco saltmarsh sediment slurries were pre-incubated with 1 μM TFA, difluoroacetate (DFA) or MFA in 500 mL Erlenmeyer flasks. The flasks were sealed under N_2 with recessed butyl rubber stoppers and incubated on a platform shaker at 28 °C for approximately one month. The flasks were then subsampled by removing 10 mL of the slurry through the stopper with a syringe fitted with an 18 gauge needle. The subsample was placed in a N_2-sparged 20-mL serum bottle, crimp-sealed with butyl rubber stoppers, and injected with 0.5 μCi (0.46 μM) 2-^{14}C-TFA. The bottles were incubated statically at ca. 20 °C and the headspace was periodically sampled for analysis of the production of CH_4, CO_2, $^{14}CH_4$, $^{14}CO_2$ as described below.

Inhibitory Effect Of Fluorinated Acetates On Methanogenesis In Sediment Slurries

The inhibition of methanogenesis in San Francisco saltmarsh sediment slurries by TFA, DFA and MFA was studied in sealed 60-mL serum bottles containing 20 mL slurry and varying concentrations (0-1 mM) of the fluorinated compound. The slurries were incubated at 28 °C on a platform shaker. Methane production was measured in headspace samples taken at periodic intervals and analyzed as described below. Inhibition was measured as a decrease in the rate of methane production, relative to uninhibited controls, in samples containing fluorinated acetates.

Analyses Of Gaseous And Liquid-Phase Products

Gaseous-phase products were monitored in each bottle during the incubation by removing 0.25 mL sample from the bottle headspaces and analyzing for CH_4, CO_2, N_2O,

$^{14}CH_4$, $^{14}CO_2$ and $^{14}CHF_3$. Methane was measured by gas chromatography with flame ionization detection (Oremland and Polcin, 1982), and CO_2 by thermal conductivity detector/gas chromatography (GC301 gas chromatograph, HNU Systems Inc., Newton MA; oven temperature = 70 °C; 12 m Poropak column; He carrier flow = 20 mL/min). Radioactive gases were determined by gas chromatography with gas-proportional counting (Culbertson and others, 1981). In some of the samples, Σ $^{14}CO_2$ (liquid phase plus gas phase) was determined at the end of the incubation by injection of 1.0 mL of 6 N HCl followed by shaking, prior to analysis of the headspace. Positive pressures generated by the evolution of CO_2 in the acidified samples were measured by deflection of a wetted, ground-glass syringe, and used to calculate the internal pressures for final computation of quantity of gas produced. Liquid-phase products, such as acetate, ^{14}C-DFA, ^{14}C-MFA, and ^{14}C-acetate were determined by withdrawing ca. 1 mL of liquid then removing the sediment by micro-centrifugation (6,000 × g for 5 min) followed by sterile filtration (0.2 μm). The supernatant was assayed by high-performance liquid chromatography (Culbertson and others, 1988) and emerging fractions were collected in 30 sec intervals
(~ 0.3 mL) for determination of radioactivity by liquid scintillation counting (Ecolume scintillation cocktail, ICN, Costa Mesa, CA; Beckmann Model LS 6000SC scintillation counter).

Experiments With Bacterial Cultures

Several bacterial cultures were assayed for their ability to degrade TFA during growth phase. The isolates were chosen either by their ability to use acetate, hence possible substrate interchangeability with TFA, or because they have known dehalogenating ability. The cultures were grown in Balch tubes (10 mL liquid volume in 20 mL tubes) or serum bottles (20 mL liquid volume in 60 mL bottles) and amended with 0.5 to 1 μCi (0.46 to 0.93 μM) 2-^{14}C-TFA. An aceticlastic methanogen, *Methanosarcina mazei* strain S-6, was grown at 28 °C in MS medium (Boone and others, 1989) with acetate, TMA, methanol or H_2 plus CO_2 as substrates. The cultures were incubated statically at 37 °C. Methane was measured as an indicator of growth. *Geobacter metallireducens*, a strict anaerobe which couples metal reduction to the oxidation of organic compounds (Lovley and others, 1993), was grown on acetate in a defined medium (Lovley and Phillips, 1988) and incubated statically at ca. 20 °C. The growth of this organism was qualitatively ascertained by the disappearance of an orange-colored ferric iron precipitate, indicating reduction of Fe(III) to Fe(II), which forms blue-green or black oxides. *Desulfobacter curvatus* (Widdel, 1987) was grown statically at 28 °C on acetate in media described in Widdel and Pfennig (1984) and monitored for growth by measuring acetate utilization. The anaerobic, sulfur-reducing, acetate oxidizing *Desulfuromonas acetoxidans* was grown on acetate at ca. 20 °C statically in basal salts medium (Pfennig and Biebl, 1976). Acetate-utilization was followed as an indicator of growth. *Desulfomonile tiedjei*, a sulfate-reducing bacterium capable of chlorophenol dehalogenation, was grown statically at 28 °C on pyruvate in basal media (DeWeerd and

others, 1990) and growth was measured by absorbance at 680 nm. All cultures were monitored for purity by light microscopy.

Each bacterial culture was grown in the presence of 0 to 1 mM unlabeled TFA to determine the minimum inhibitory concentration of TFA. Balch tubes containing media, substrate, and TFA were inoculated with the organism and observed for growth. Subsequently, the culture that had not been exposed to TFA was transferred to triplicate tubes of media and substrate. These cultures then received 0.4 µCi (0.75) of 2-^{14}C-TFA and sampled for gaseous and liquid phase products as described above. Each culture was maintained in the presence of 0.1 mM TFA for 1 to 2 months, and subsequently assayed for its ability to degrade 2-^{14}C-TFA.

RESULTS AND DISCUSSION

Experiments With Estuarine Sediments

Inhibition by fluorinated acetates. Anoxic, estuarine sediment slurries from the San Francisco saltmarsh were amended with varying amounts (0 to 100 μM) of unlabeled TFA, DFA, and MFA to determine if these compounds inhibited methanogenesis. Fig. 1 shows that methane production was not significantly different from the unamended control over the range of concentrations studied for all compounds.

Degradation of TFA under methanogenic and sulfate-reducing conditions. Anoxic estuarine sediments collected in April, 1994 from the San Francisco saltmarsh were not able to defluorinate 0.2 μCi 2-^{14}C-TFA (fig. 2). However, saltmarsh sediments collected one month later, and slurried under sulfate-reducing conditions, were able to oxidize 2 μCi 2-^{14}C-TFA to $^{14}CO_2$ (fig. 3a). Liquid samples from these slurries showed that TFA was reductively defluorinated to ^{14}C-acetate (fig. 3b) which was metabolized to $^{14}CO_2$. The percent recovery of $^{14}CO_2$ from degradation of 2-^{14}C-TFA was 36 percent. Sediment slurries collected prior to December 1994 and amended with sulfate were able to degrade ^{14}C-TFA (see fig. 3 and 4; and Visscher and others, 1994). However, sediments collected after December 1994 were not able to degrade ^{14}C-TFA under methanogenic or sulfate-reducing conditions (fig. 5-20).

Inhibition of TFA degradation under nitrate-reducing conditions. Earlier work in our laboratory (Visscher and others, 1994) did not detect ^{14}C-TFA degradation in slurries containing nitrate. These experiments were repeated with sediments containing with 10 mM nitrate and varying amounts of 2-^{14}C-TFA (fig. 4). $^{14}CH_4$ was produced in slurries with 1 μCi and 1.6 μCi 2-^{14}C-TFA (Fig. 4c) and $^{14}CO_2$ was produced in all slurries, except the slurry with the lowest amount of TFA (0.16 μCi). Slurries amended with 1 μCi had the highest percent recovery of 2-^{14}C-TFA as both $^{14}CO_2$ (127 ± 38 percent; fig. 4a) and $^{14}CH_4$ (3.1 ± 0.5 percent; fig. 4c). The liquid phase of slurries amended with 1 μCi 2-^{14}C-TFA were assayed for soluble radiolabeled products. Figure 4(d) shows that TFA was sequentially reduced to DFA, MFA and acetate. While TFA was degraded in these nitrate-amended slurries, a prolonged incubation was required before gaseous and liquid-phase degradation products of TFA were observed. Figure 4(b) shows that production of nitrous oxide ceased after 7 days, after which TFA degradation commenced. It is likely that TFA degradation in this experiment was inhibited by nitrogen oxides produced during bacterial nitrate-respiration. Methanogenesis is inhibited by nitrogen oxides (Balderston and Payne, 1976), and this may account for the absence of both methanogenesis and TFA degradation that we observed while nitrous oxide was present in our samples. In these experiments 30± percent of the 1 μCi 2-^{14}C-TFA was converted to $^{14}CH_4$ and 75± percent to $^{14}CO_2$. The large quantity of $^{14}CO_2$ implies oxidation of the methyl group by an unidentified electron acceptor, possibly Fe(III), Mn(IV), or SO_4^{2-} formed by reaction of reduced forms with nitrogen oxides.

Degradation of TFA in sediments amended with other electron acceptors. Slurries of sediments collected in May, 1995 were amended with 20 mmol/L MnO_2 to elicit TFA degradation by manganese-reducing bacteria, or 10 mmol/L Fe(III)NTA to elicit TFA degradation by iron-reducing bacteria. Figure 5 shows that neither $^{14}CH_4$ nor $^{14}CO_2$ were produced from ^{14}C-TFA. Liquid phase products were not detected under any of these conditions. Sulfate was added to slurries on several occasions but did not elicit TFA degradation (fig. 5, 8-14, 17, 18)

Degradation of 2-^{14}C-TFA vs. 1-^{14}C-TFA. In selected experiments the position of the radiolabeled carbon was varied using 1-^{14}C-TFA or 2-^{14}C-TFA. This was done to determine if the carboxyl group of 1-^{14}C-TFA was oxidized to carbon dioxide under methanogenic or sulfate-reducing conditions (fig. 12-14). However, neither 1-^{14}C-TFA or 2-^{14}C-TFA were degraded in the sediments studied.

Degradation of TFA in anaerobic sediments plus hydrogen. An experiment was designed to determine the effect of hydrogen gas addition to the saltmarsh slurries with, and without added sulfate. Figure 14 shows that, while the samples amended with hydrogen gas stimulated CH_4 production compared to samples incubated under nitrogen, 2-^{14}C-TFA was not degraded to gaseous products under either hydrogen or nitrogen atmospheres.

Degradation of TFA using various TFA concentrations. The concentration of ^{14}C-TFA was varied in several experiments to observe whether the concentration of TFA was a critical factor in the diminished TFA degradation at higher concentrations of TFA (for example, Visscher and others, 1994; and fig. 4). The experiment shown in figure 4 used a range of 2-^{14}C-TFA concentrations from 0.15 µM to 1.5 µM. Generally, more $^{14}CH_4$ and $^{14}CO_2$ was produced in 0.9 µM 2-^{14}C-TFA amended sediments than those with 1.5 µM 2-^{14}C-TFA. Another experiment was conducted with sediments collected in June, 1995 with varying amounts (0.5 to 1 µCi) of 2-^{14}C-TFA (fig. 15). No radiolabeled gas phase or liquid phase products were observed. The concentration of 2-^{14}C-TFA did not appear to inhibit methane production in these sediments.

Degradation of TFA in sediments amended with various electron donors. Experiments were designed to determine if various electron donors would stimulate TFA degradation in saltmarsh slurries. These included amendment with lactate, pyruvate, glucose, oxalate, propionate, and benzoate. While all additions stimulated methane production in the sediments, none of the additions stimulated production of $^{14}CH_4$ or $^{14}CO_2$ (fig. 16). Other electron donors were added to experiments throughout the course of this study. TMA (fig. 5 and 18) and acetate (fig. 19 and 20) did not stimulate degradation of 2-^{14}C-TFA, although these amendments enhanced CH_4 and CO_2 production over unamended controls.

Degradation of TFA in nitrogen-amended sediments. Sediment slurries were amended with ammonium chloride to determine if the sediments were nitrogen limited, which

might cause loss of TFA-degradation activity. Slurries amended with 0.1 mM NH_4Cl, with and without 10 mM sulfate, did not degrade 2-^{14}C-TFA (fig. 19).

Degradation of TFA in sediments pre-incubated with fluorinated acetates. To determine if TFA degradation could be induced, sediments were pre-incubated with unlabeled fluorinated acetates prior to the addition of 2-^{14}C-TFA. The pre-incubation of sediments with fluorinated acetates did not elicit degradation of 2-^{14}C-TFA and no radiolabeled products were detected in either the gas or liquid phase (fig. 20).

Degradation of TFA in sediments collected from Bolinas Bay, Calif. In an effort to see whether estuarine sediments from a site other than the San Francisco saltmarsh could degrade TFA, sediments were collected from a mudflat in Bolinas Bay, near Stinson Beach , Calif. These sediments were collected from two sites (south and north locations) of the mudflats in May, 1995. The sediments collected from both sites were slurried with or without sulfate or TMA. Fig.s 21 and 22 show that, while the TMA-amended slurries from both sites produced greater quantities of methane than sediments without TMA, none of the slurries produced $^{14}CH_4$ or $^{14}CO_2$. Liquid samples from these sediments did not show ^{14}C-labeled intermediates (data not shown).

Experiments With Freshwater Sediments

Sediments collected from Searsville Lake in October of 1993 were methanogenic and degraded 1.85 µCi 2-^{14}C-TFA to $^{14}CH_4$, via ^{14}C-labeled intermediates (fig. 23). In this same experiment, slurries with both inhibitors (molybdate and BES) did not degrade 2-^{14}C-TFA, while those with molybdate produced ^{14}C-intermediates and $^{14}CH_4$ (data shown in Visscher et al., 1994). Sediments collected three months later were also able to degrade ^{14}C-TFA to $^{14}CH_4$ and $^{14}CO_2$ under methanogenic and sulfate-reducing conditions, respectively, but not under nitrate-reducing conditions (Visscher and others, 1994).

However, slurries from sediments collected in January, 1995 did not produce $^{14}CH_4$ or $^{14}CO_2$ (fig. 24) from either 1-^{14}C-TFA or 2-^{14}C-TFA. These sediments were re-assayed in May of 1995. Once again, sediments did not produce gaseous or liquid-phase degradation products from 2-^{14}C-TFA under methanogenic, sulfate-reducing, or nitrate-respiring conditions (fig. 25). A final experiment was made in August, 1995 with fresh material. No activity was detected under aerobic, methanogenic or sulfate-reducing conditions (fig. 26).

Experiments With Sacramento Delta Peat Soil

Soil samples, collected in April, 1994, were slurried anaerobically and amended with 20 mM nitrate and either 4 µCi or 10.2 µCi 2-^{14}C-TFA. Soils given 4 µCi 2-^{14}C-TFA converted 0.51 ± 0.05 percent of the 2-^{14}C-TFA to $^{14}CO_2$, while those amended

with the higher concentration degraded 0.42 ± 0.01 percent to $^{14}CO_2$ (fig. 27). Autoclaved controls did not degrade 2-^{14}C-TFA. Liquid-phase samples were not analyzed for soluble intermediates. Slurries from this same sediment were again made in May, 1994 under aerobic conditions. None of these slurries produced $^{14}CO_2$, even after acidification of the samples (data not shown).

Soils were again collected from the same site in August, 1995 and assayed for TFA degradation under methanotrophic conditions. Sediments were amended with O_2 and CH_4 and 2-^{14}C-TFA. Figure 28 shows that TFA was not degraded to $^{14}CO_2$ in this experiment.

Experiments With Forest Soil

Soils from the Hubbard Brook Experimental Forest were collected in June and July of 1995. Studies concerning the fate and transport of unlabeled TFA were ongoing at the site since May, 1994. After four months these researchers observed a 100 percent loss of TFA in the system (S. Tartowski, oral comm.). Several soil slurry experiments were done with these soils to determine if the TFA loss was due to microbial degradation. Figures 29-32 show that none of the soil slurries were able to significantly degrade ^{14}C-TFA to $^{14}CH_4$ or liquid phase products, after anaerobic or aerobic incubations. However, we did observe trace (0.1 nCi/0.25 mL, <1 percent) amounts of $^{14}CO_2$ during gas phase sampling of the slurries but it was no longer detectable after 24 h of incubation. Slurries incubated aerobically with CH_4 demonstrated methane-oxidation activity (fig. 33). However, $^{14}CO_2$ was not produced from 2-^{14}C-TFA (fig. 33b).

Slurries were amended with various electron acceptors (sulfate, nitrate, FeOOH) to determine if these elicited reducing conditions favored by microorganisms capable of degrading TFA. However, 2-^{14}C-TFA did not decrease under any of these conditions (fig. 34-36) (liquid phase data not shown). $^{14}CO_2$ was again observed in trace amounts but did not accumulate to significant levels. TFA degradation was also studied in forest soil slurries amended with acetate. Figure 36 shows that anaerobic, acetate-amended slurries did not produce either $^{14}CO_2$ or $^{14}CH_4$ from 2-^{14}C-TFA.

Liquid phase samples taken at the end of each experiment described did not reveal the presence of radiolabeled intermediates (data not shown). Additionally, ^{14}C-TFA levels did not decrease significantly with time (data not shown).

In light of the acidic conditions of the forest soils (pH ca. 4.5), an experiment was designed to buffer the slurries at neutral pH to determine if this condition would enhance TFA-degradation. The anaerobic, phosphate-buffered slurries had a pH of ca. 6.5-7. However, they did not degrade 2-^{14}C-TFA to gaseous products (fig. 35) or liquid-phase products (data not shown).

Experiments With Small Cores From Estuarine Sediments

Sediment sub-cores were taken to determine if undisturbed soil or sediment could degrade 2-^{14}C-TFA. Figures 37-40 show that none of the samples degraded 2-^{14}C-TFA to gaseous products. In one of the core experiments, sulfate, TMA, or oxygen were added to the samples to determine if these elicited TFA degradation. However, none of the samples with these additions degraded 2-^{14}C-TFA to gaseous products (fig. 39). None of these experiments were assayed for liquid-phase radiolabeled products.

Experiments With Bacterial Cultures

Several pure cultures of bacteria were studied for their ability to degrade TFA during growth on acetate. Cultures were first assayed for inhibition by non-radiolabeled TFA during growth on acetate, statically at 28 °C. Figure 41 shows that the growth of *Methanosarcina mazei* S-6 and *Desulfobacter curvatus* were not inhibited by TFA concentrations from 0-1 mM. None of the bacteria studied exhibited inhibition by unlabeled TFA during growth on acetate. Subsequent to the inhibition studies, each bacterial culture was studied for the ability to degrade 2-^{14}C-TFA. Figure 42 shows that 2-^{14}C-TFA was not degraded by growing, acetate-fed cultures of *M. mazei* S-6. These results are representative of all cultures studied because none of the cultures were able to degrade 2-^{14}C-TFA.

CONCLUSIONS

TFA was shown to be degraded in experiments conducted by our laboratory with saltmarsh and freshwater sediments collected from October 1993 to June 1994. In their 1994 publication, Visscher and others reported some of these findings. These authors showed that degradation of ^{14}C-TFA was the result of bacterial activity and that it occurred under both oxic and anoxic conditions. In addition, these workers showed that ^{14}C-TFA was reductively defluorinated under methanogenic conditions to acetate, which was then metabolized by methanogens to methane.

Our results, described here, show that ^{14}C-TFA is also reductively defluorinated under sulfate-reducing conditions (fig. 3). Visscher and others (1994) and our results show that ^{14}C-TFA was sequentially defluorinated to DFA, MFA and acetate (fig. 3b), and the methyl group of the acetate was then oxidized to carbon dioxide (fig. 3a). It is not known whether methanogens and/or sulfate-reducing bacteria directly reductively defluorinate TFA, however it appears that both are involved in the metabolism of the resulting acetate.

Our further attempts to explore the microbial ecology of TFA degradation have been unsuccessful due to the inability of sediments collected from several sites, including those with prior TFA-degradation activity, to degrade ^{14}C-TFA. Although we were able to show repeated degradation of ^{14}C-TFA to $^{14}CH_4$, $^{14}CO_2$, and liquid phase products prior to December, 1994, experiments conducted since that time have not shown TFA degradation.

Since the loss of TFA-degrading activity, a variety of redox conditions have been studied including methanogenic, sulfate-reducing, nitrate-respiring, iron-reducing, manganese-reducing, and aerobic conditions. Due to our prior observations that TFA was degraded in repeated experiments under methanogenic and sulfate-reducing conditions, we tested these conditions with monthly samples from the South San Francisco Bay saltmarsh. In addition, these conditions were tested with other saltmarsh sites and the freshwater site. All samples from these sites, slurried and incubated anaerobically, were methanogenic (fig. 5-20) but did not degrade TFA. It seems likely that we would have encountered TFA degradation in these samples, similar to that observed in samples taken prior to December 1994, if the organisms responsible were from the general methanogenic or sulfate-reducer populations. These results suggest that the organisms responsible for TFA degradation were specialized members of these populations.

In addition, various electron donors have been studied for their ability to elicit TFA-degrading microorganisms in anaerobic sediment slurries. These experiments were conducted to determine if bacterial cometabolism of these substrates elicited TFA degradation. None of these conditions elicited TFA degradation in the saltmarsh or freshwater sediments. Although all of these sediments produced methane, none of the electron donor additions elicited TFA degradation.

Visscher and others, 1994, showed that TFA degradation could be inhibited at concentrations greater than 1.85 μM. Our results also show slurries amended with 0.9 μM 2-^{14}C-TFA yielded three times the amount of $^{14}CH_4$ than slurries with 1.5 μM (fig. 4). Our subsequent experiments used amounts of TFA well within the range of non-inhibiting concentrations (0.15 to 1.7 μM); therefore the lack of TFA degradation in these studies was not a result of inhibition by TFA.

To determine if specific, inducible enzymes were involved in TFA degradation, pre-incubation with fluorinated acetates was used as a means to induce TFA degradation in the soils and sediments. However this experiment was unsuccessful since none of the pre-incubated sediments were able to degrade TFA to gas phase products. These results indicate that prior exposure of the sediments to TFA, or fluorinated analogs of TFA, does not elicit TFA degradation by sediment microflora. However, these sediments were pre-exposed for a relatively short time (1 month) and it remains possible that longer exposure times may elicit TFA degradation.

While we did observe a small amount of $^{14}CO_2$ production in the northeastern hardwood forest soils, the $^{14}CO_2$ was transient and did not accumulate. These results may be due to a very low conversion of ^{14}C-TFA to $^{14}CO_2$, which then disappeared from the gas phase, possibly by partitioning into organic material. Production of $^{14}CO_2$ in these soils seemed to occur mostly under anaerobic conditions but was not enhanced by addition of acetate, nitrate, sulfate, or ferric iron. The inability of our efforts to consistently reproduce $^{14}CO_2$ production, or observe $^{14}CO_2$ accumulation, does not allow us to make conclusions about TFA degradation in these soils. It does remain a possible explanation for the loss of TFA observed in the seep, but cannot be proved by our results.

The fact that saltmarsh and freshwater sediments capable of TFA degradation became inactive suggests that this activity is not a normal occurrence in these ecosystems and is the result of either a rare circumstance favoring TFA-degrading microorganisms, or is a routine condition that was lost because of unusually high precipitation occurring during the winter months of 1994-95. However, the latter explanation seems improbable due to the fact that repeated sampling during our studies encompassed a sufficient time period for observation of conditions similar to those prior to this precipitation. Thus, we conclude that our previous results were the result of specialized, and maybe somewhat rare, microorganisms and that TFA is generally recalcitrant to biological degradation.

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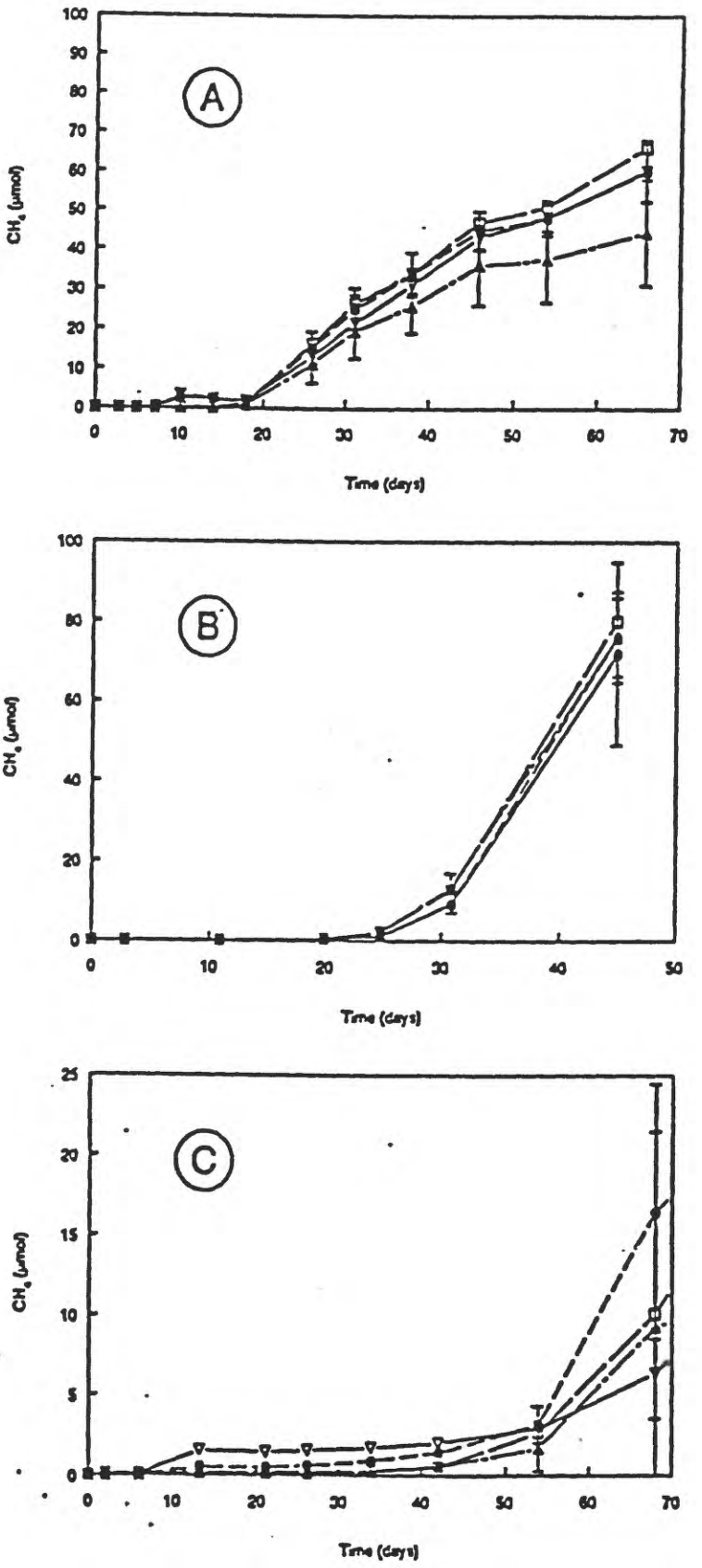


Figure 1. Methanogenesis in anaerobic South San Francisco Bay saltmarsh slurries, from January through March 1994, with varying (A) TFA additions, (B) DFA additions, and (C) MFA additions. Symbols: (▽) CH_4 for unamended samples; (●) slurries amended with 0.1 µM TFA, DFA, or MFA; (▲) slurries amended with 1 µM fluorinated acetates; and (□) amended with 100 µM. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

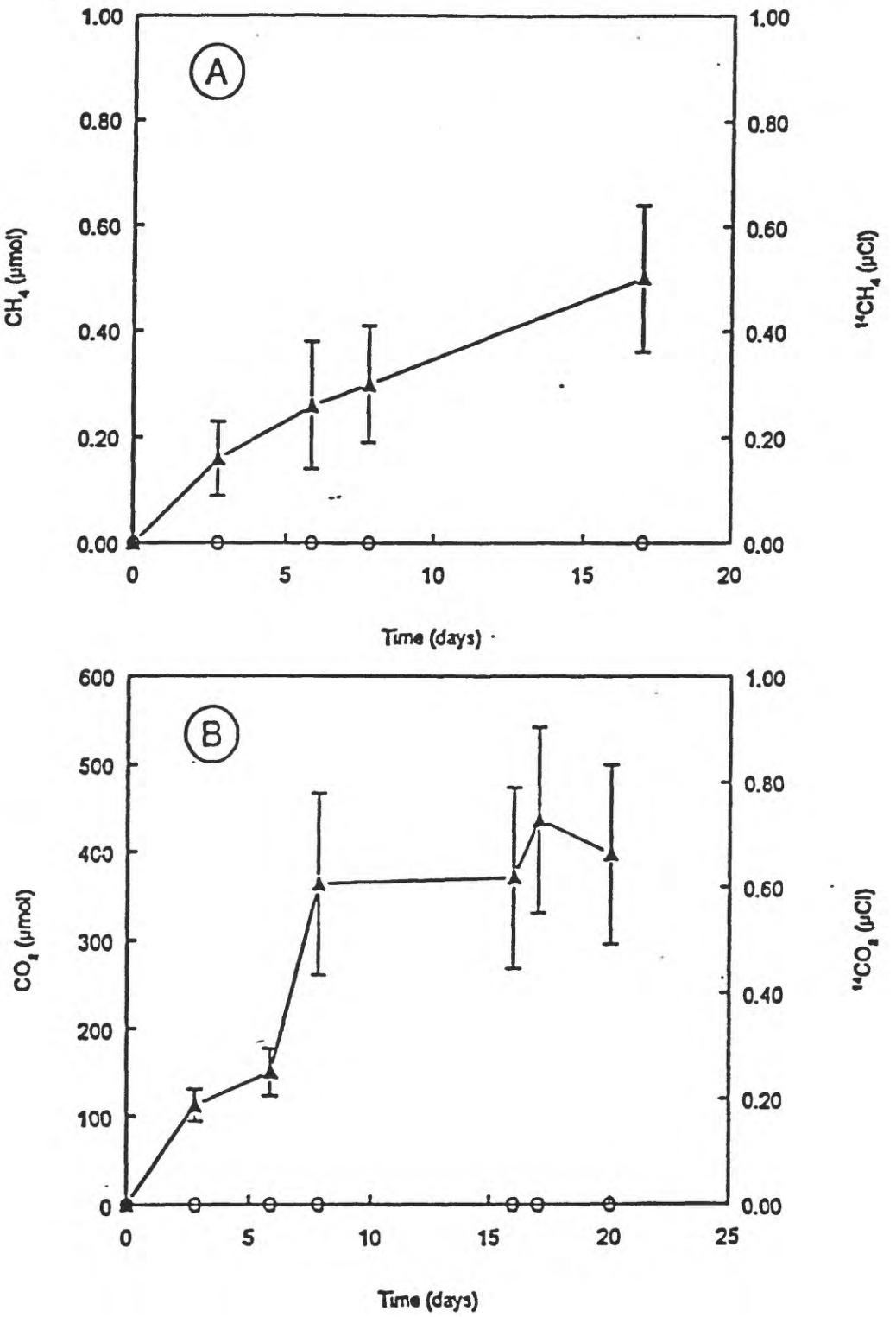


Figure 2. Methanogenesis and CO_2 production in anaerobic South San Francisco Bay saltmarsh slurries from 4/12/94, amended with 20 mM sulfate and 0.2 µCi (0.185 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$. (B) CO_2 and $^{14}CO_2$. Symbols: (▲) CH_4 and CO_2; (O) $^{14}CH_4$ and $^{14}CO_2$. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

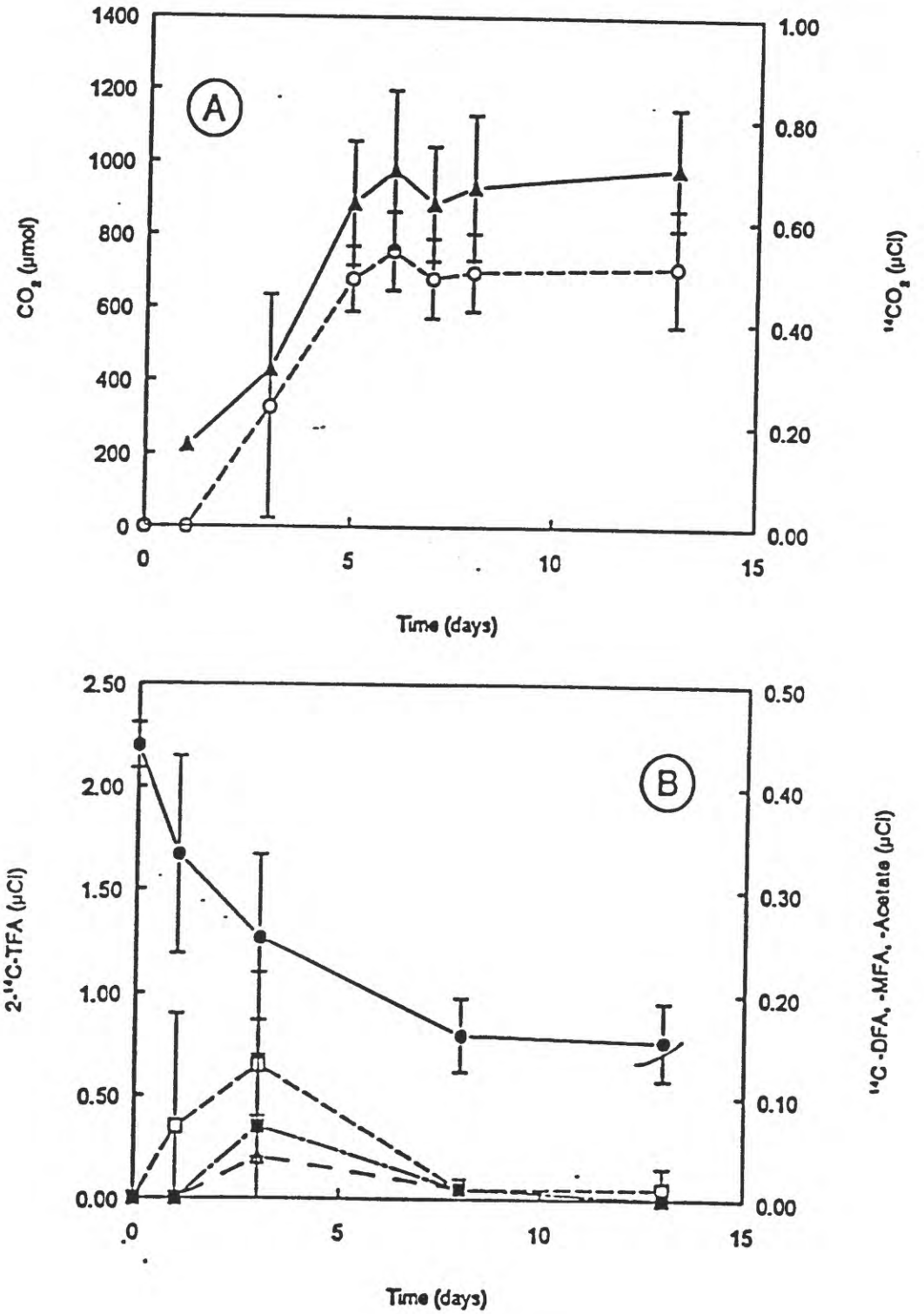


Figure 3. Degradation of 2 µCi (1.85 µM) 2-^{14}C-TFA in anaerobic South San Francisco Bay saltmarsh slurries from 5/11/94 amended with 20 mM sulfate. (A) CO_2 and $^{14}CO_2$, (B) disappearance of 2-^{14}C-TFA and appearance of ^{14}C-liquid phase products. Symbols: (▲) CO_2; (○) $^{14}CO_2$; (●) ^{14}C-TFA; (□) ^{14}C-DFA; (■) ^{14}C-MFA; and (△) ^{14}C-acetate. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

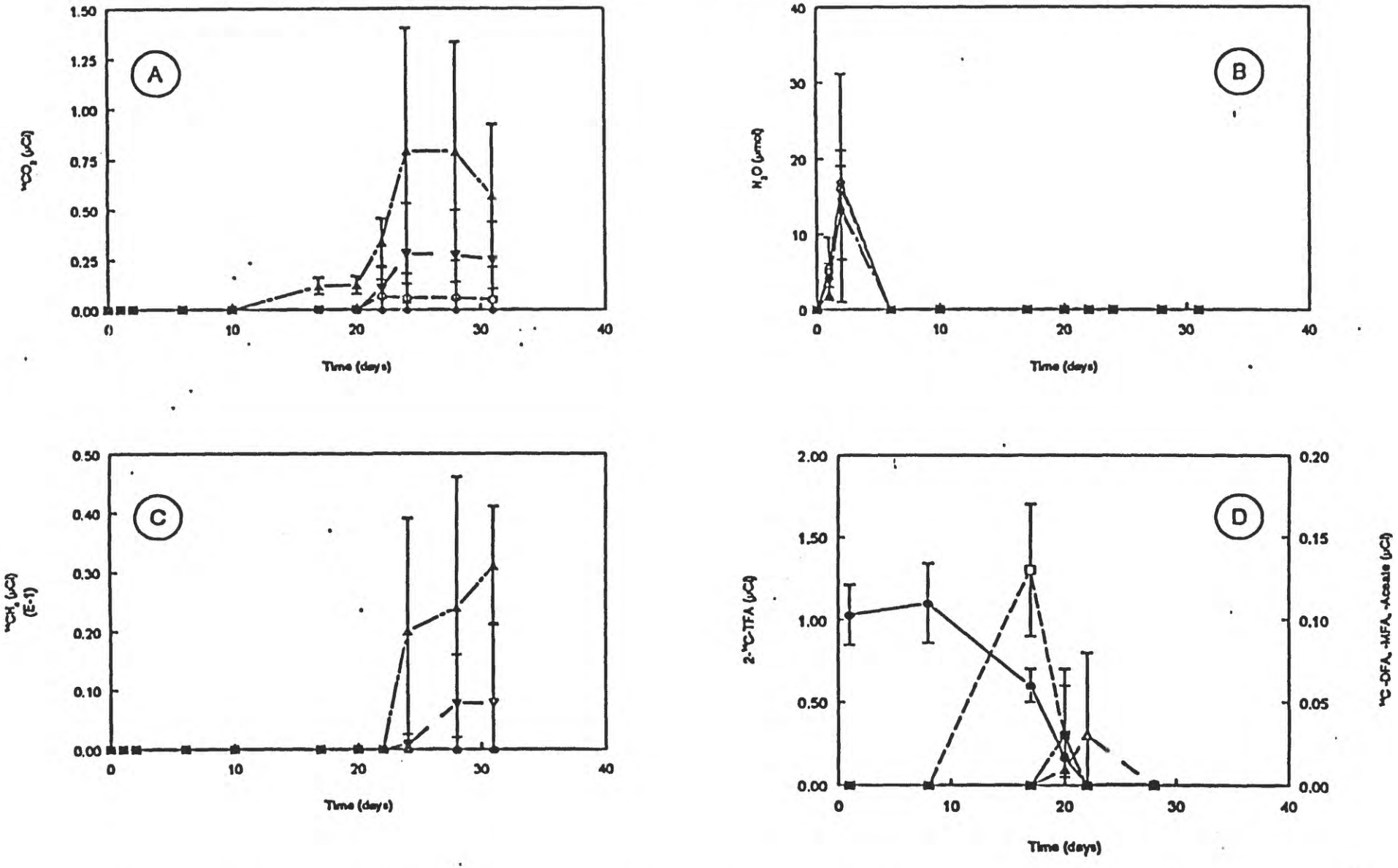


Figure 4. Degradation of TFA in anaerobic South San Francisco Bay saltmarsh slurries from 6/7/94, amended with varying concentrations of 2-^{14}C-TFA and 20 mM nitrate. (A) $^{14}CO_2$, (B) N_2O, (C) $^{14}CH_4$. Symbols: (◇) slurries amended with 0.16 µCi (0.15 µM) 2-^{14}C-TFA; (○) slurries with 0.4 µCi (0.37 µM); (▲) slurries with 1 µCi (0.93 µM); and (▽) slurries with 1.6 µCi (1.5 µM). (D) Disappearance of 2-^{14}C-TFA and production of ^{14}C-liquid phase products in 1 µCi amended slurries, (●) ^{14}C-TFA; (□) ^{14}C-DFA; (■) ^{14}C-MFA; and (△) ^{14}C-acetate. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

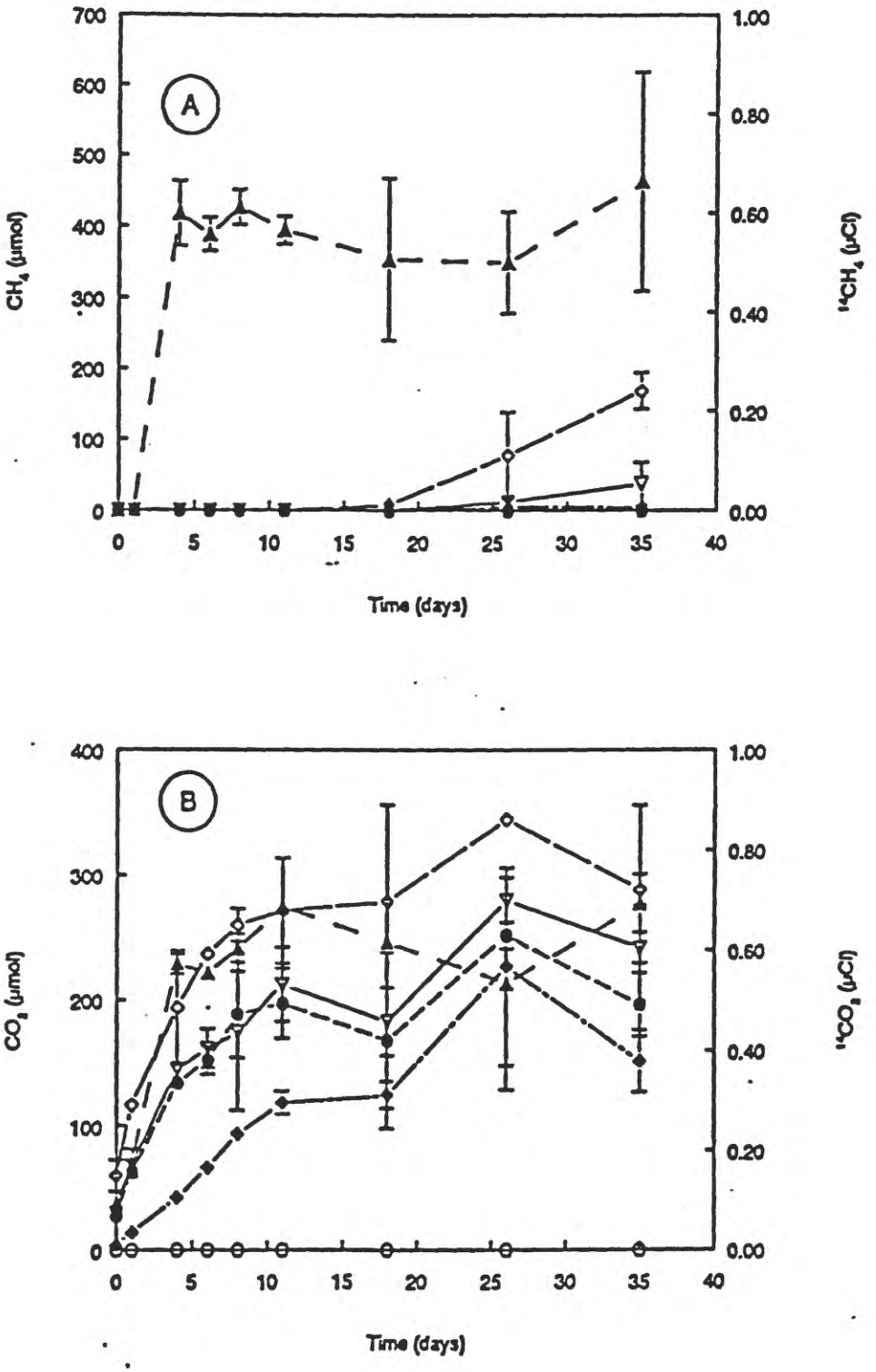


Figure 5. South San Francisco Bay saltmarsh sediments amended with 20 mM sulfate, 20 mM MnO_2, 10 mM Fe(III)NTA, or 10 mM TMA. Sediments collected and slurried anaerobically on 5/3/95. All received 0.4 µCi (0.37 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$, (B) CO_2 and $^{14}CO_2$. Symbols: (▽) unamended slurries; (●) sulfate-amended slurries; (◆) Mn(IV)-amended slurries; (▲) TMA-amended slurries; and (◇) slurries amended with Fe(III)NTA; (○) show $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

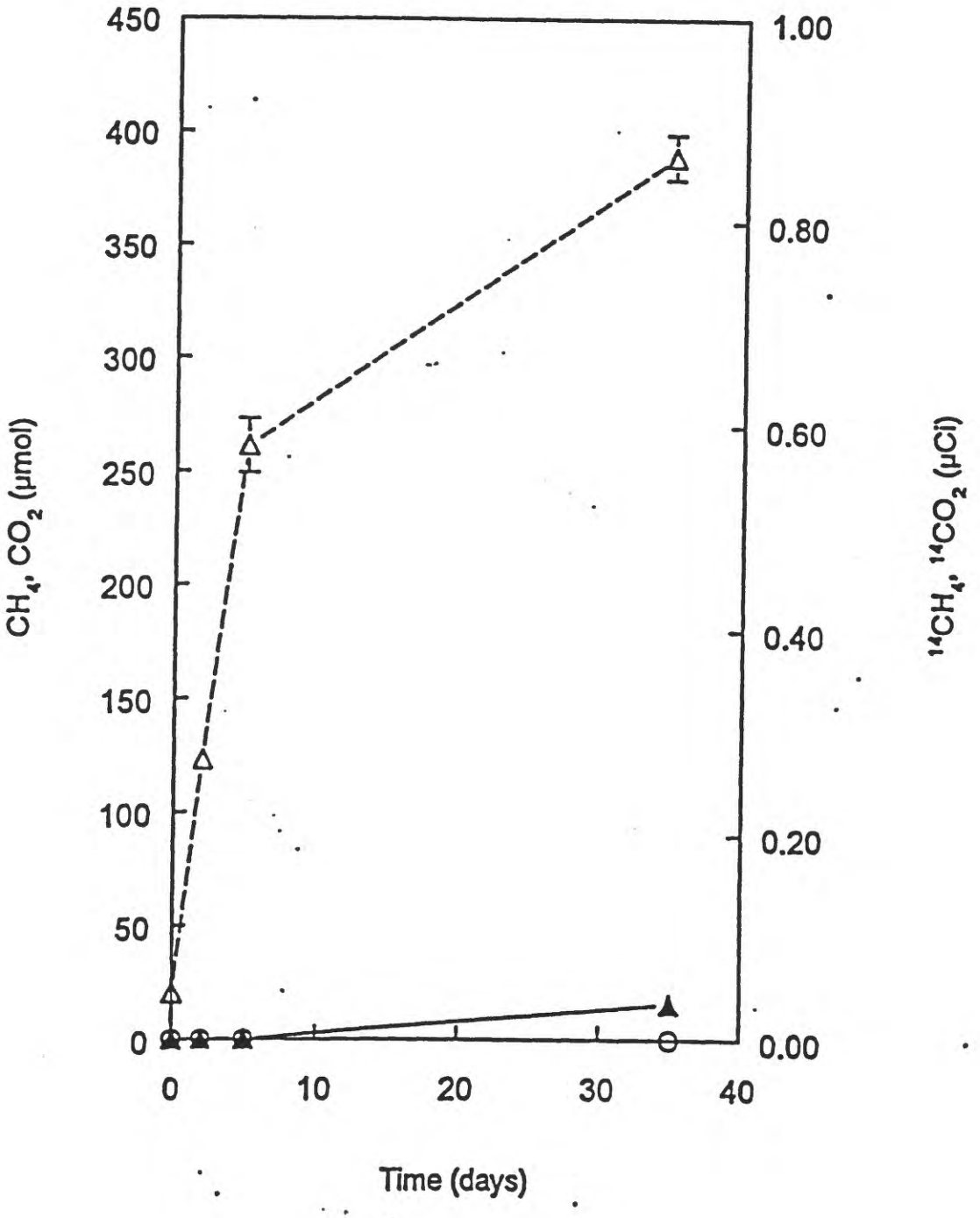


Figure 6. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 5/17/95. All slurries received 0.8 µCi (0.74 µM) 2-^{14}C-TFA. Symbols: (▲) CH_4; (△) CO_2; (O) $^{14}CH_4$ and $^{14}CO_2$. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

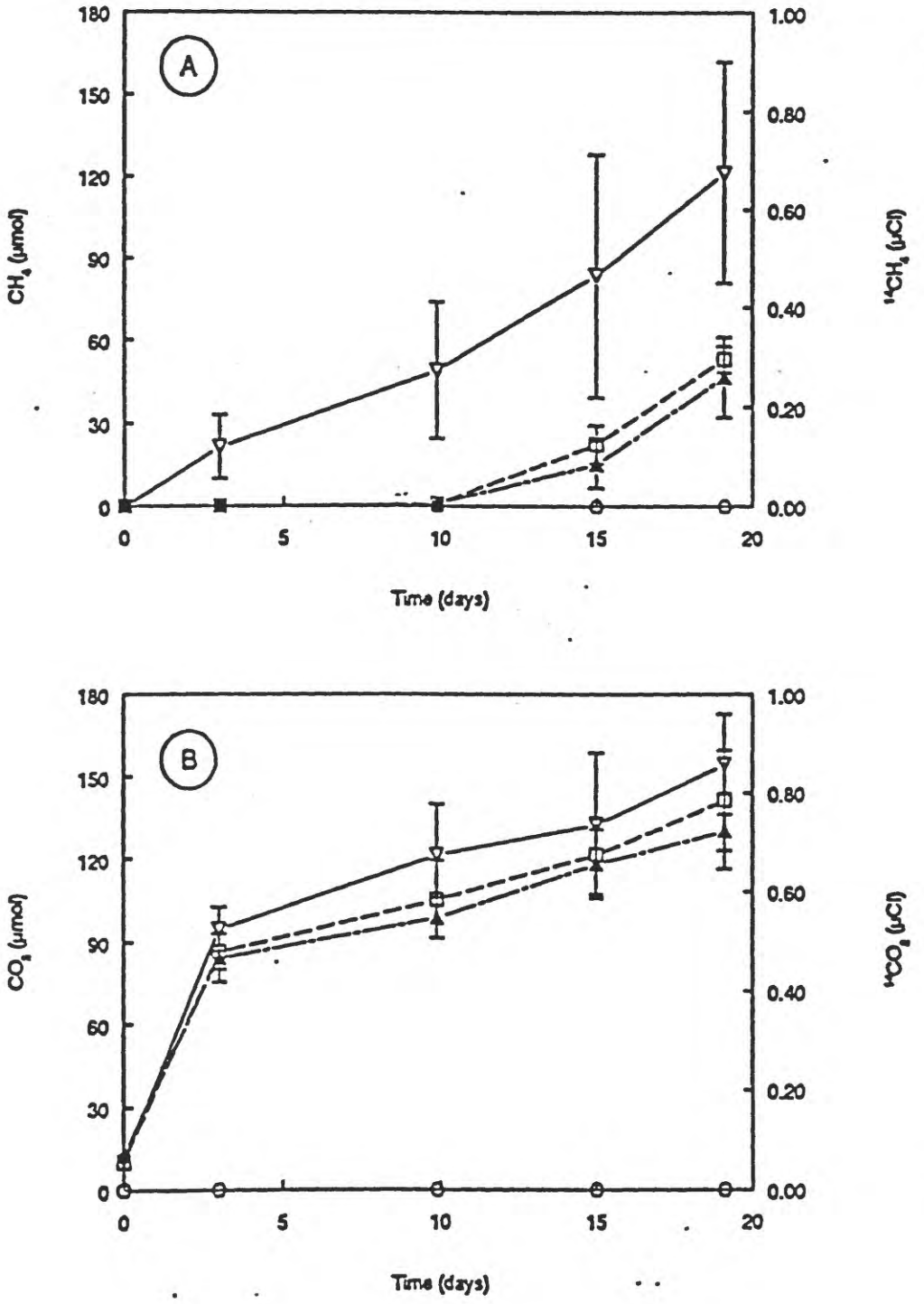


Figure 7. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 7/13/95. All received 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in sediments slurried with artificial bay water (ABW); (□) ABW and water collected from the site; (▲) slurries with site water only; (○) $^{14}CH_4$ and $^{14}CO_2$ for slurries with ABW, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

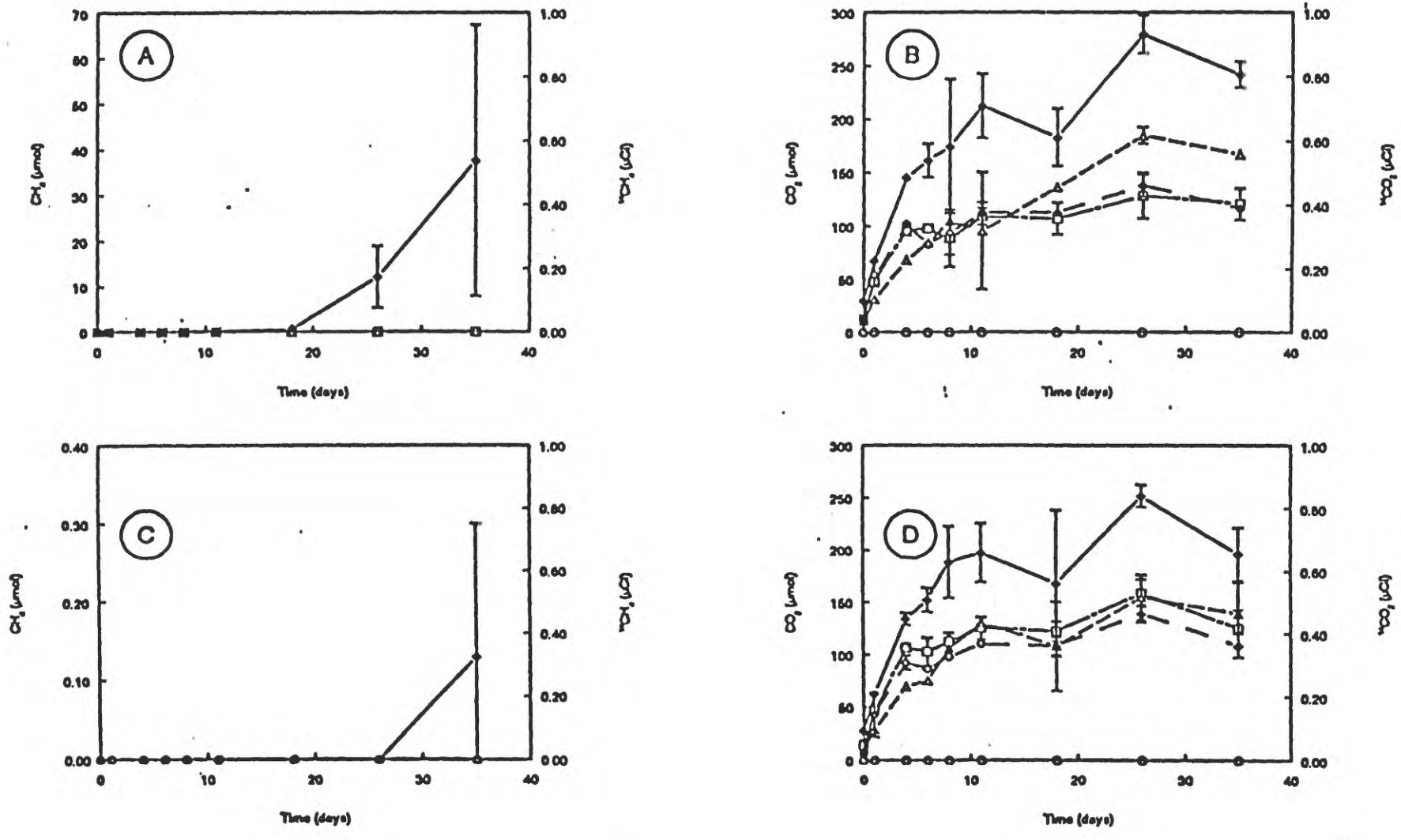


Figure 8. South San Francisco Bay saltmarsh sediments collected on 5/3/95 at various depths. Each depth slurried anaerobically with and without 20 mM sulfate. All received 0.4 µCi (0.37 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$ without sulfate, (B) CO_2 and $^{14}CO_2$, without sulfate; (C) CH_4 and $^{14}CH_4$ with sulfate, (D) CO_2 and $^{14}CO_2$, with sulfate. Symbols: (◆) slurries from depth 0-4.5 cm; (△) 4.5-9 cm; (□) 9-12.5 cm; (◇) 25-30 cm; (O) show $^{14}CH_4$ and $^{14}CO_2$ for 0-4.5 cm unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

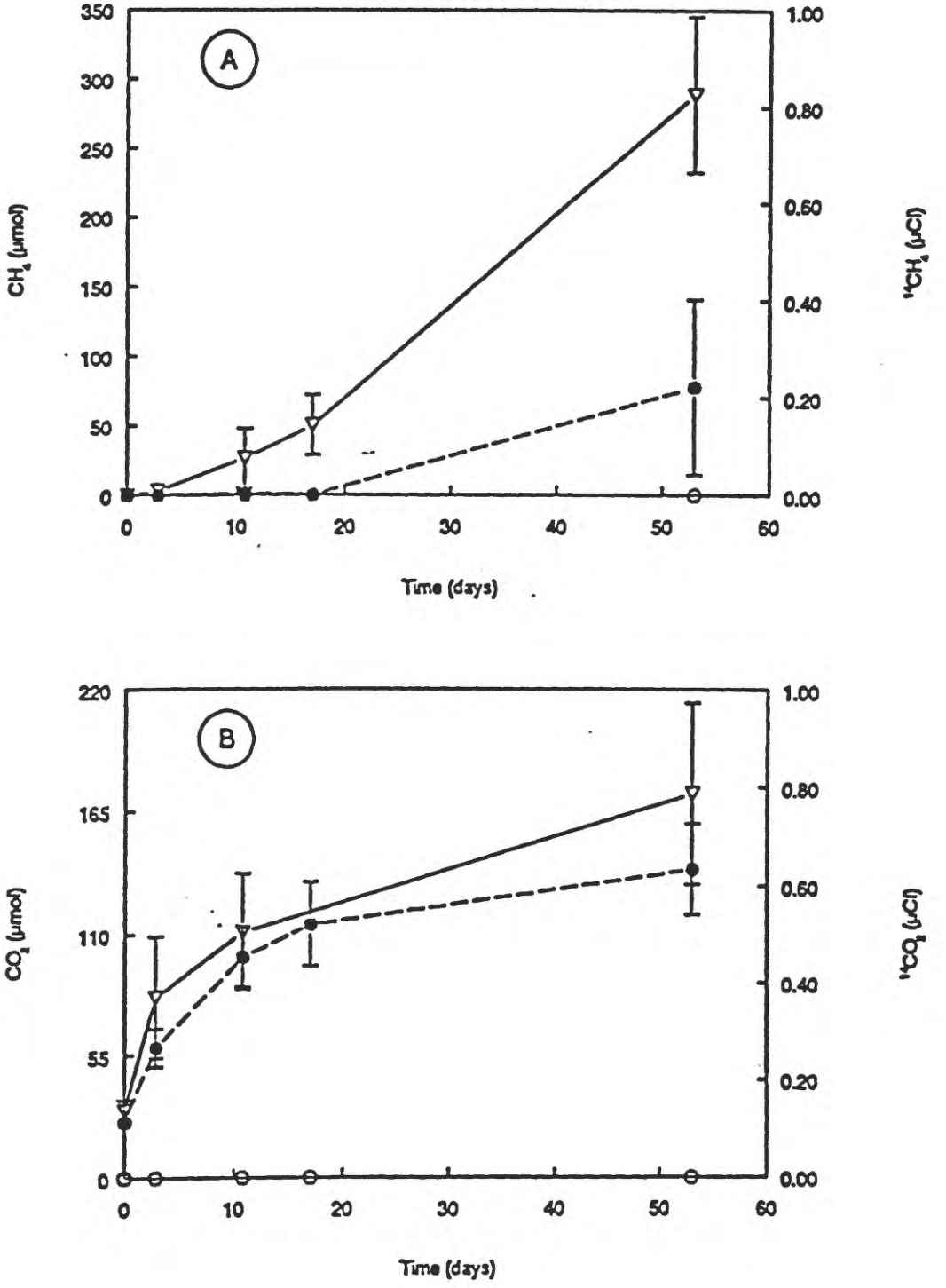


Figure 9. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 8/3/95. All received 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (●) slurries amended with 20 mM sulfate; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for both conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

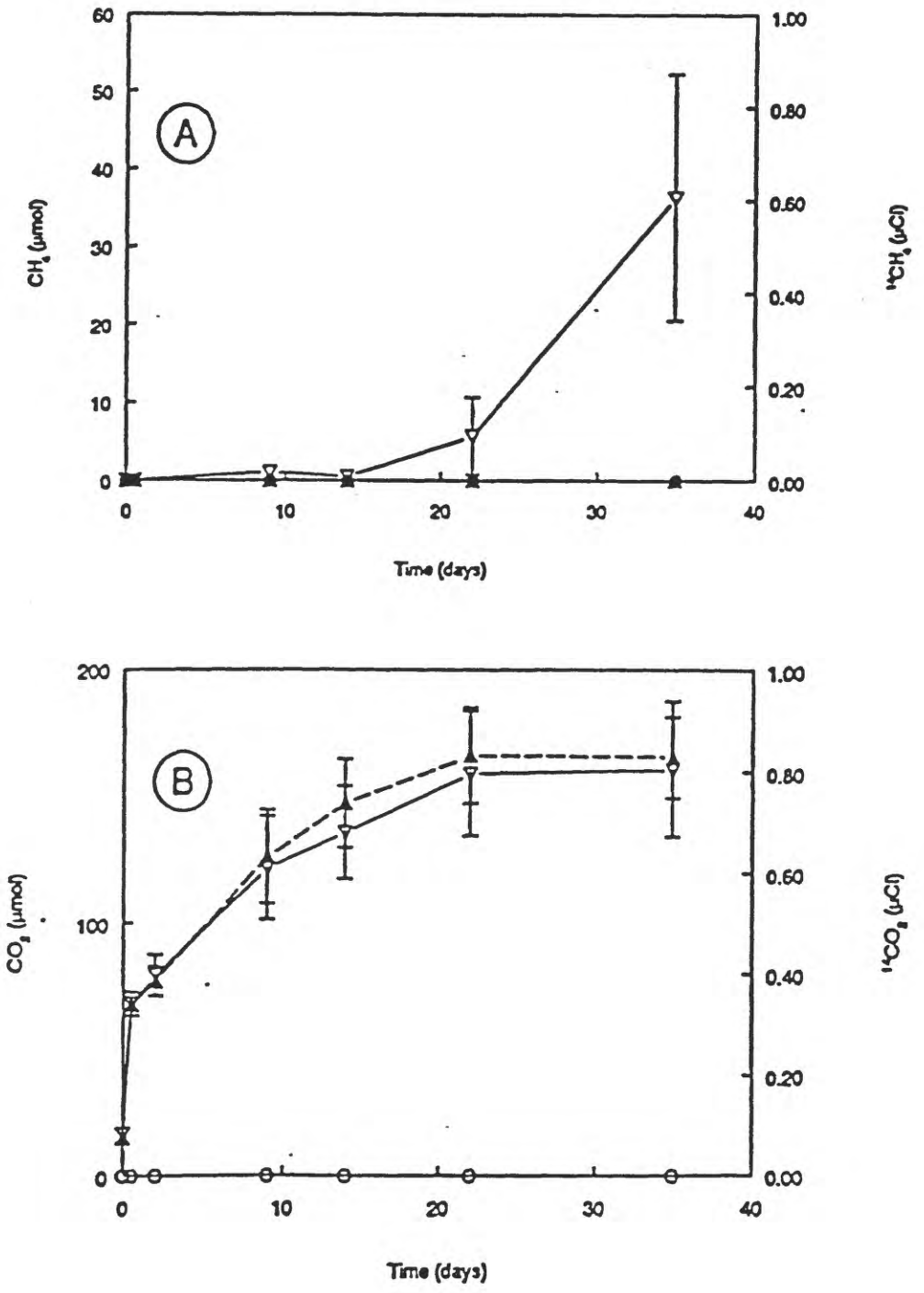


Figure 10. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 8/28/95 with 20 mM sulfate. All received 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (▲) sulfate-amended slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for both conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

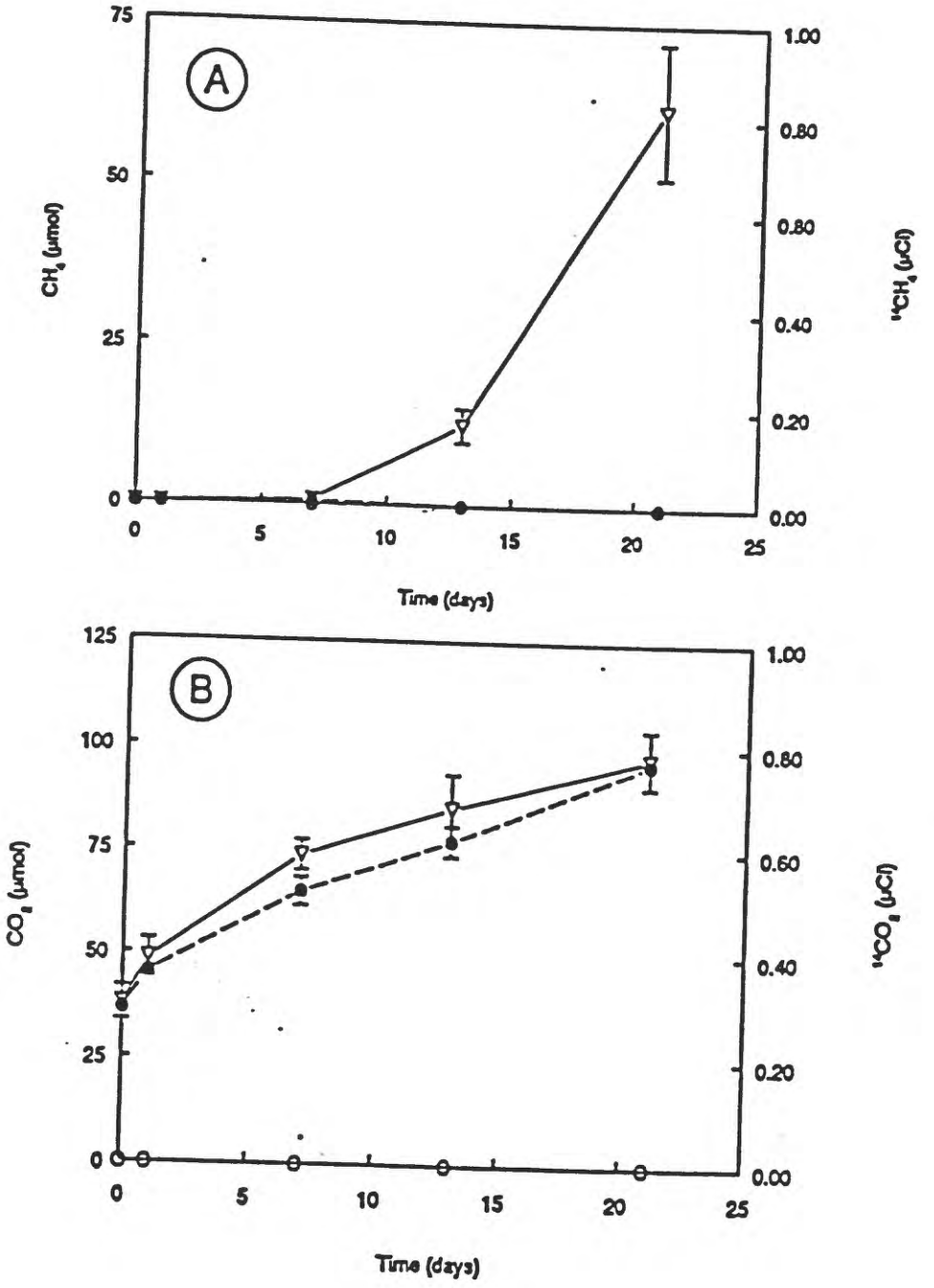


Figure 11. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 10/3/95 with 20 mM sulfate. All received 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (●) sulfate-amended slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for both conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

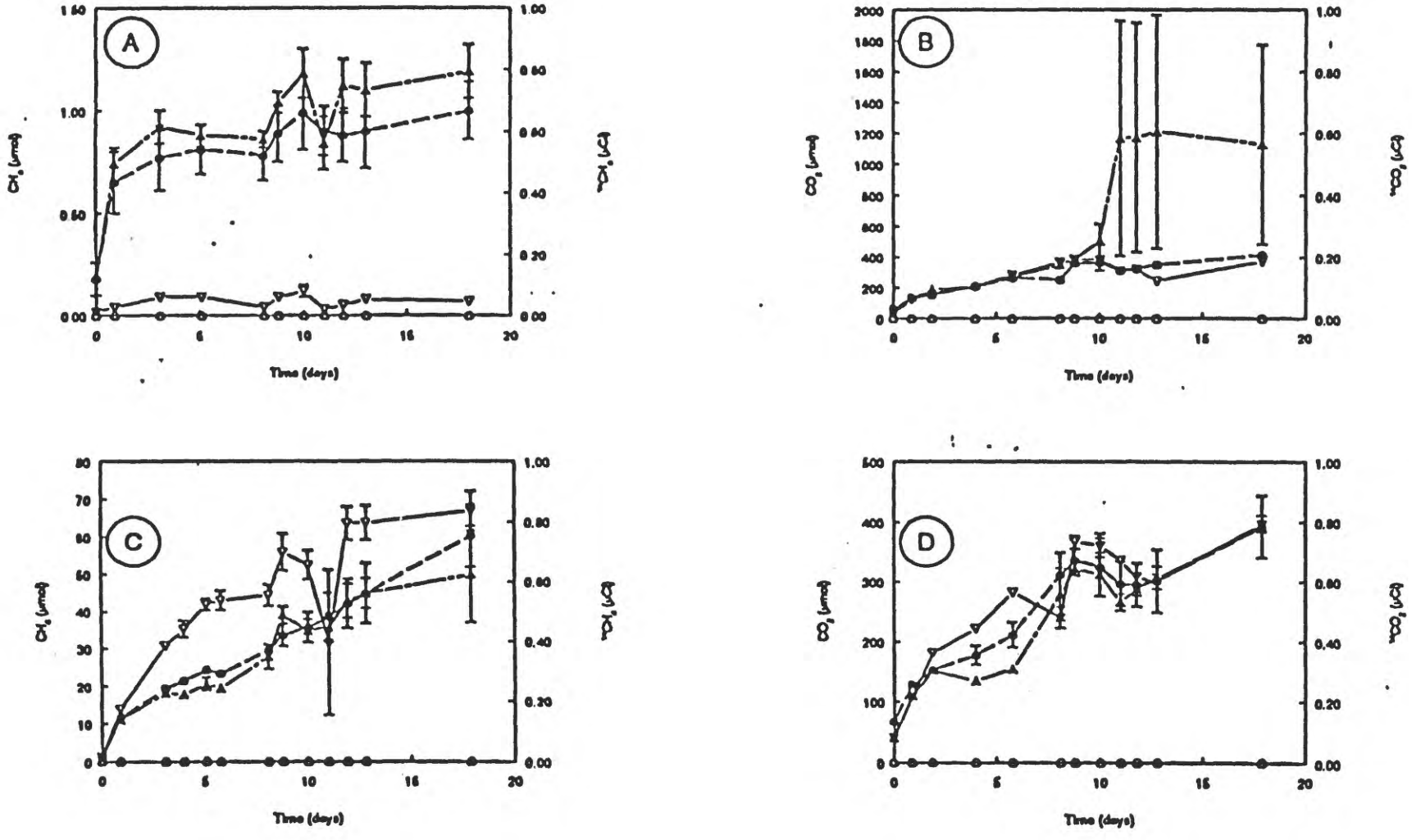


Figure 12. South San Francisco Bay saltmarsh sediments collected and slurried anaerobically on 12/9/94. (A) and (B) amended with 20 mM sulfate; (C) and (D) without sulfate. Symbols: (▽) CH_4 and CO_2 in slurries without ^{14}C-TFA; (●) slurries amended with 1 μCi (0.94 μM) 1-^{14}C-TFA; (▲) 0.8 μCi (0.74 μM) 2-^{14}C-TFA; (○) show $^{14}CH_4$ and $^{14}CO_2$ for slurries amended with 1 μCi (0.94 μM) 1-^{14}C-TFA, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

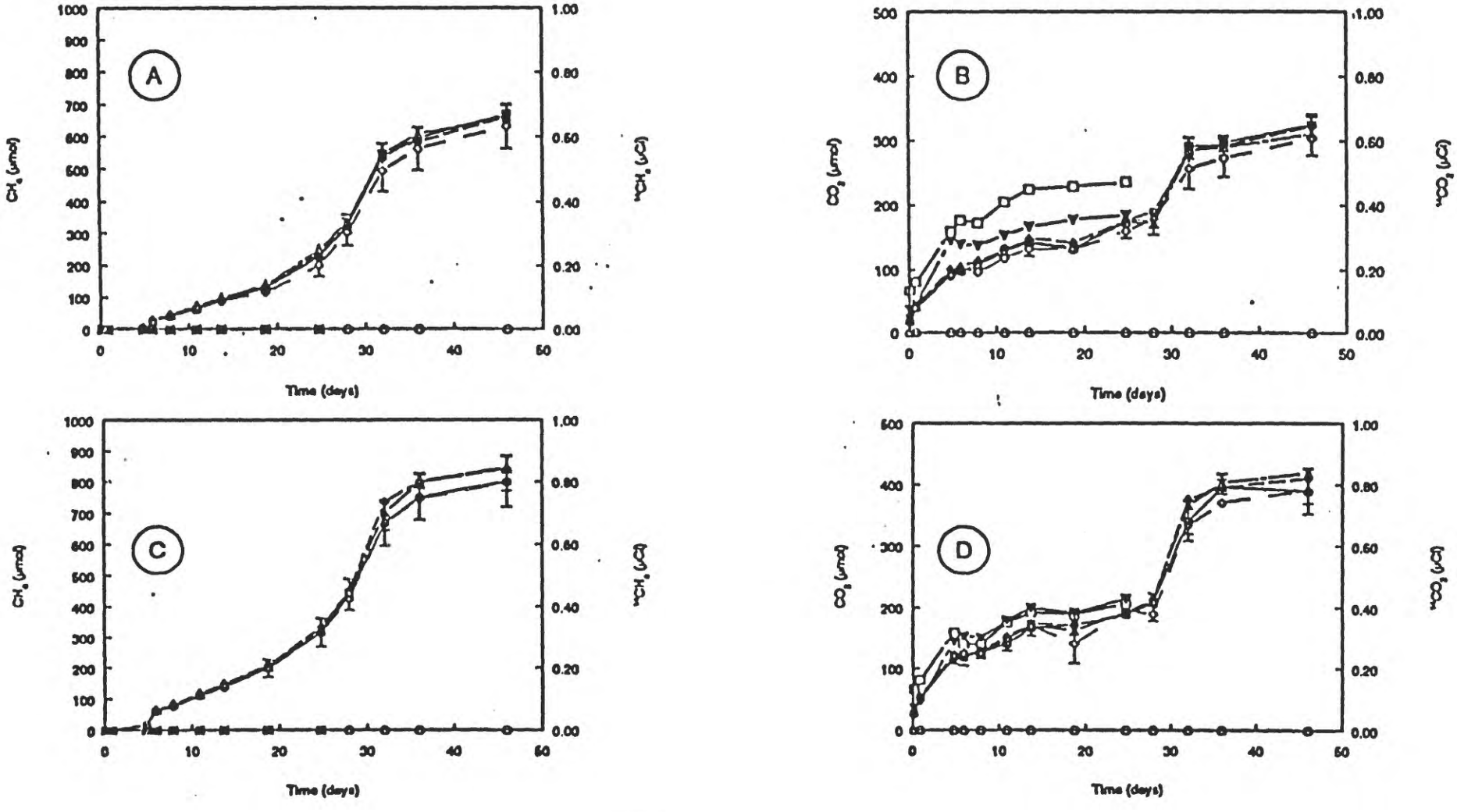


Figure 13. South San Francisco Bay saltmarsh sediments collected and slurried anaerobically on 12/28/94. (A) and (B) amended with 20 mM sulfate; (C) and (D) without sulfate. Symbols: (●) slurries amended with 1 µCi (0.94 µM) 1-^{14}C-TFA; (◆) slurries amended with 0.5 µCi (0.47 µM) 1-^{14}C-TFA; (▲) slurries amended with 0.76 µCi (0.70 µM) 2-^{14}C-TFA; (◇) slurries amended with 0.38 µCi (0.35 µM) 2-^{14}C-TFA; (□) autoclaved slurries amended with 1 µCi (0.94 µM) 1-^{14}C-TFA; (▼) autoclaved slurries amended with 0.76 µCi (0.70 µM) 2-^{14}C-TFA; (○) show $^{14}CH_4$ and $^{14}CO_2$ for slurries amended with 1 µCi (0.93 µM) 1-^{14}C-TFA, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

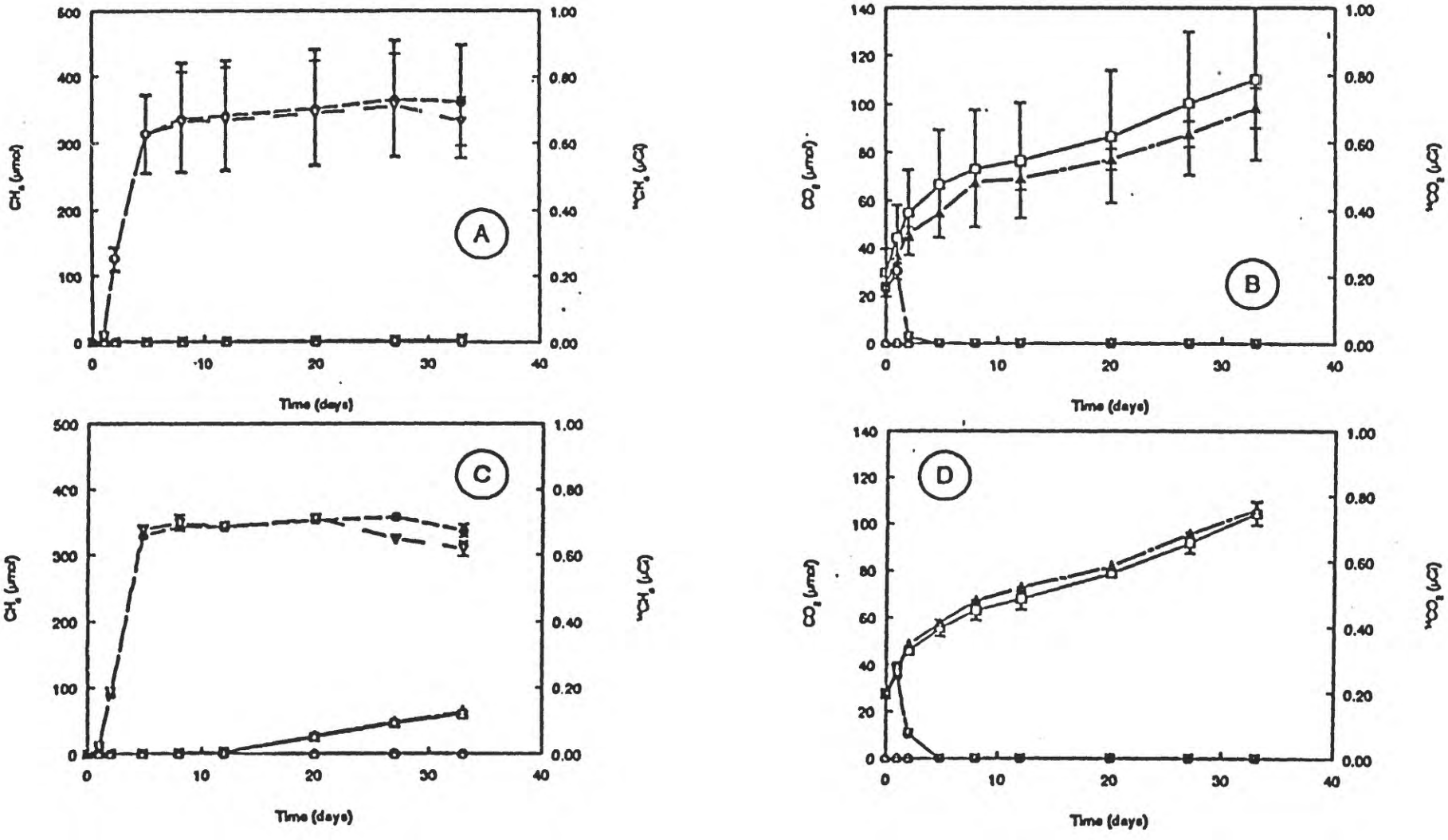


Figure 14. South San Francisco Bay saltmarsh sediments collected and slurried anaerobically on 1/24/95. (A) and (B) amended with 20 mM sulfate; (C) and (D) without sulfate. Symbols: (□) slurries amended with 0.5 μCi (0.47 μM) 1-^{14}C-TFA and N_2; (●) slurries amended with 0.5 μCi (0.47 μM) 1-^{14}C-TFA and H_2; (△) slurries amended with 0.5 μCi (0.46 μM) 2-^{14}C-TFA and N_2; (▽) slurries amended with 0.5 μCi (0.46 μM) 2-^{14}C-TFA and H_2; (○) show $^{14}CH_4$ and $^{14}CO_2$ for slurries amended with 0.5 μCi (0.47 μM) 1-^{14}C-TFA, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

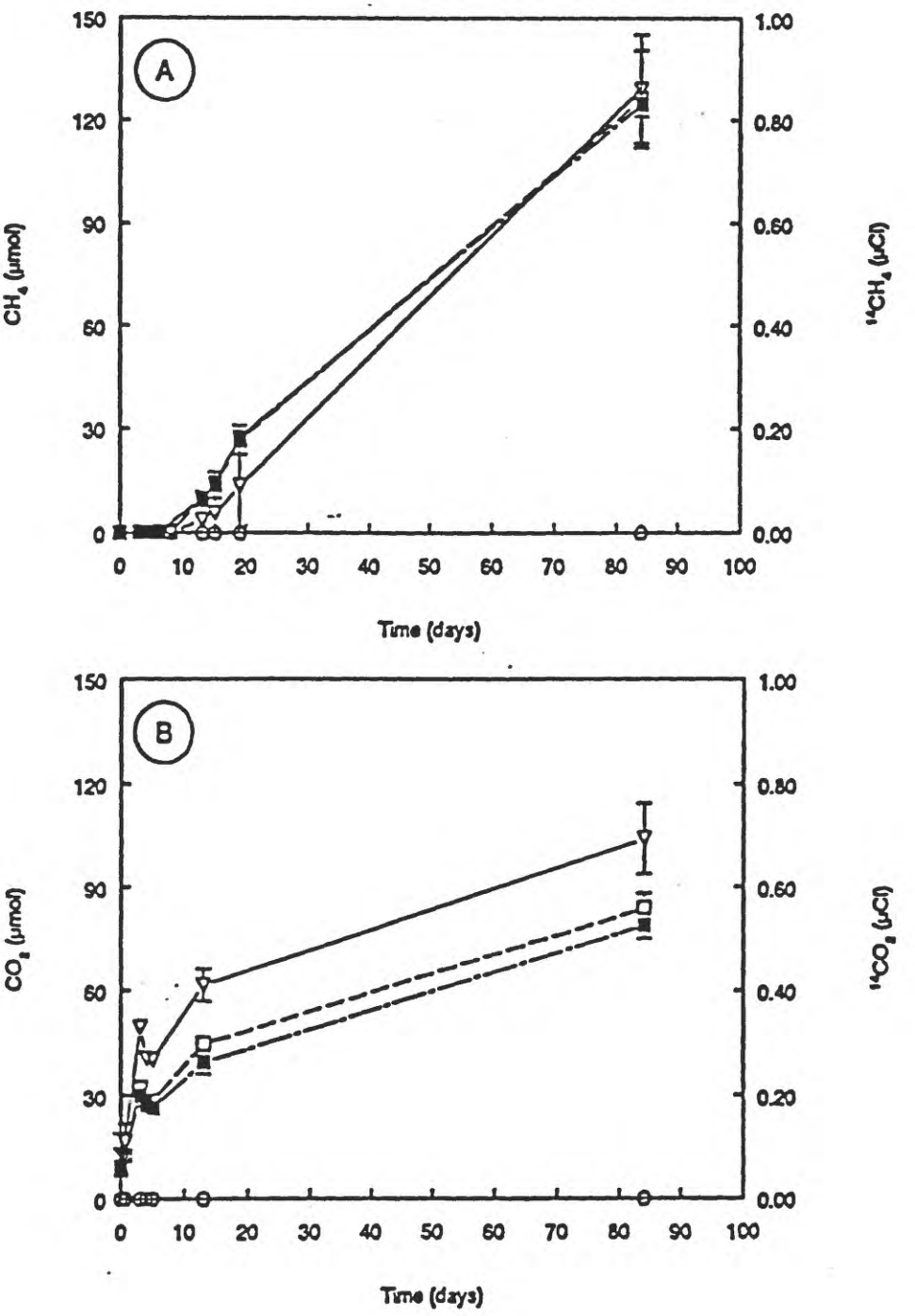


Figure 15. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 6/22/95. The slurries received varying 2-^{14}C-TFA concentrations. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (□) slurries amended with 0.5 µCi (0.46 µM) 2-^{14}C-TFA; (■) 1 µCi (0.93 µM) 2-^{14}C-TFA; (○) $^{14}CH_4$ and $^{14}CO_2$ for slurries amended with 1 µCi (0.93 µM) 2-^{14}C-TFA, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

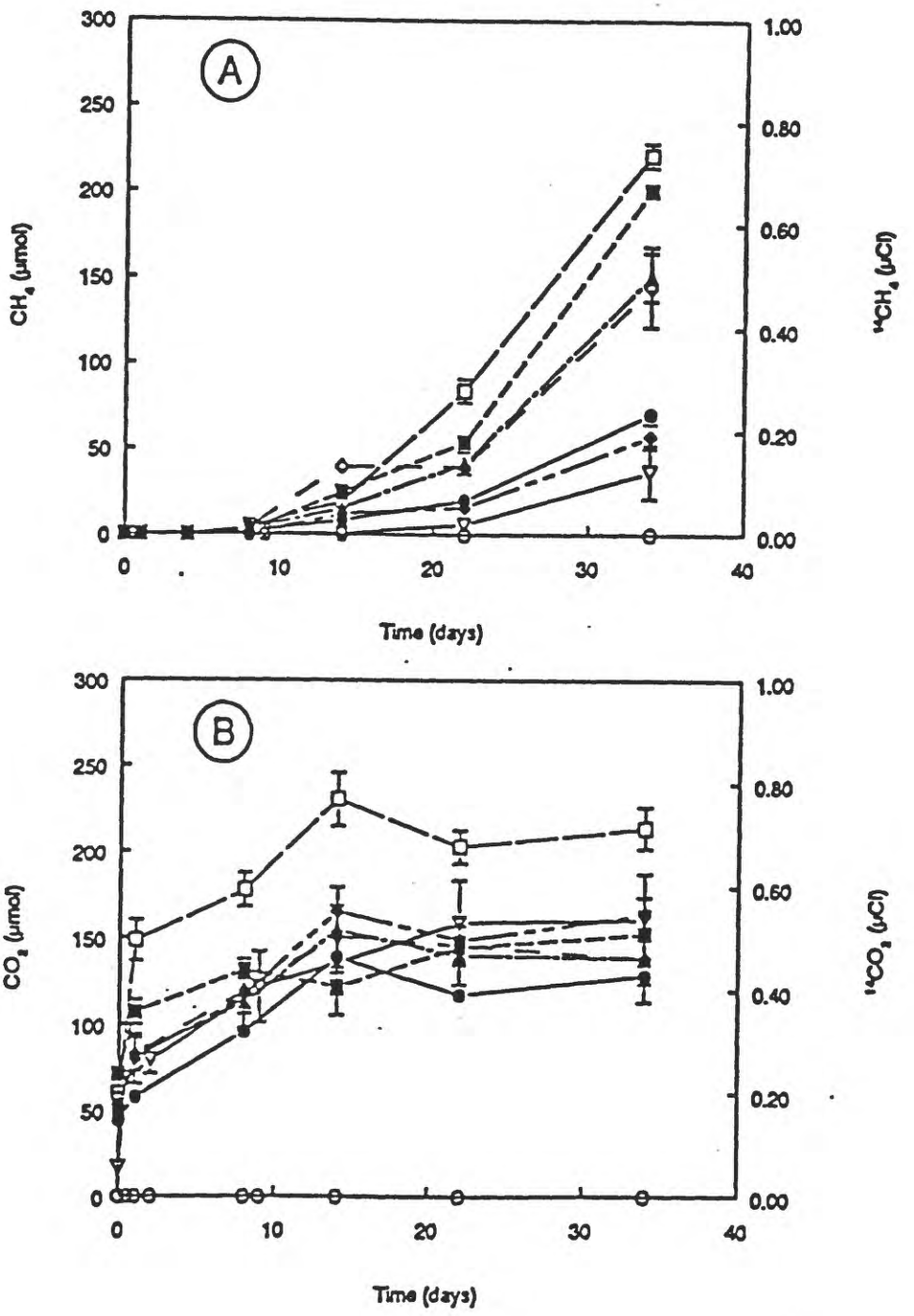


Figure 16. South San Francisco Bay saltmarsh sediment collected on 8/28/95 and slurried anaerobically with 5 mM of various electron donors on 8/29/95. All received 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (●) oxalate-amended slurries; (■) propionate; (▲) lactate; (◇) pyruvate; (□) glucose; (◆) benzoate; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

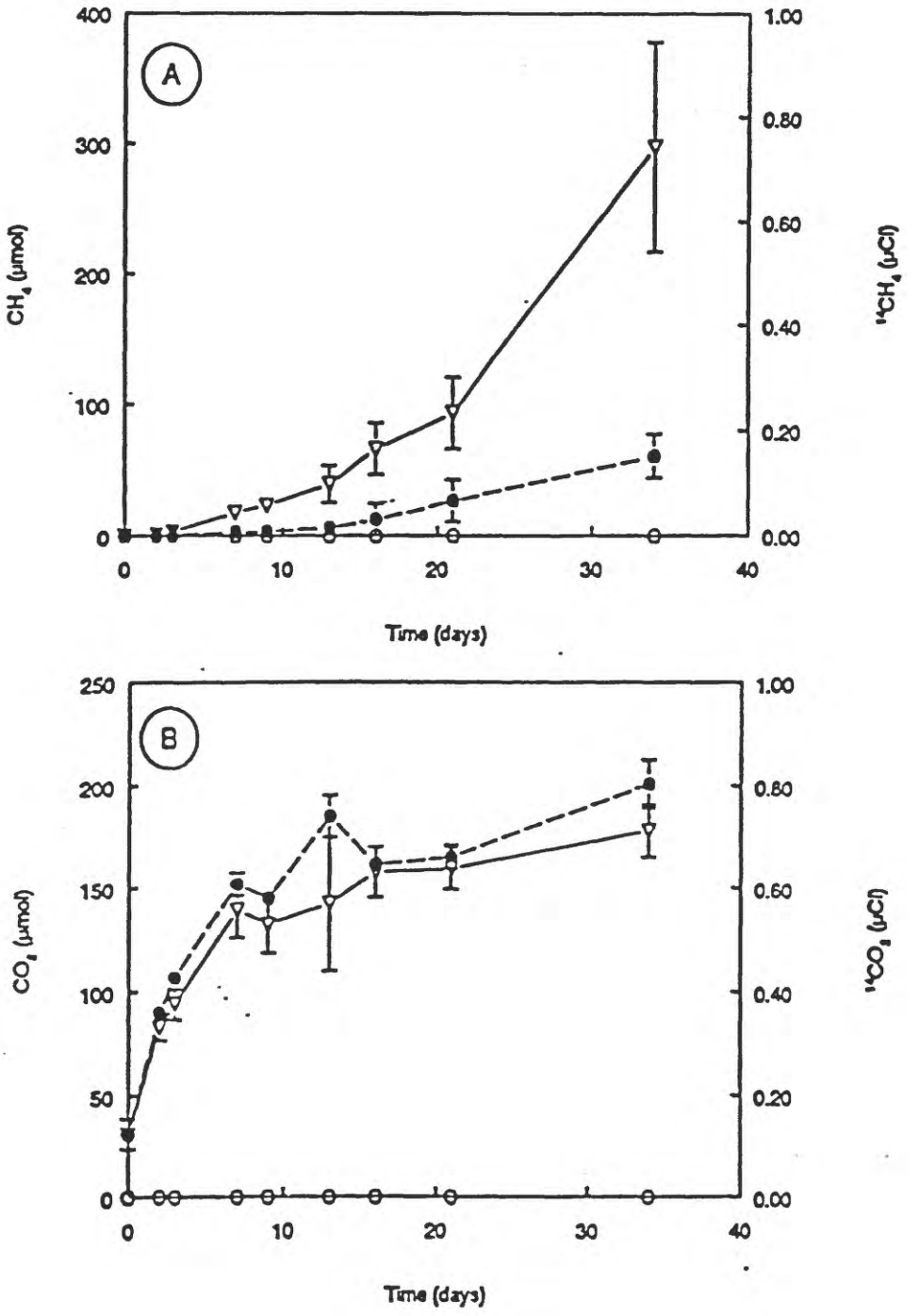


Figure 17. South San Francisco Bay saltmarsh sediment collected 6/93 and slurried anaerobically, with or without 20 mM sulfate on 5/23/95. All slurries received 0.8 µCi (0.74 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$, (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (●) sulfate-amended slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

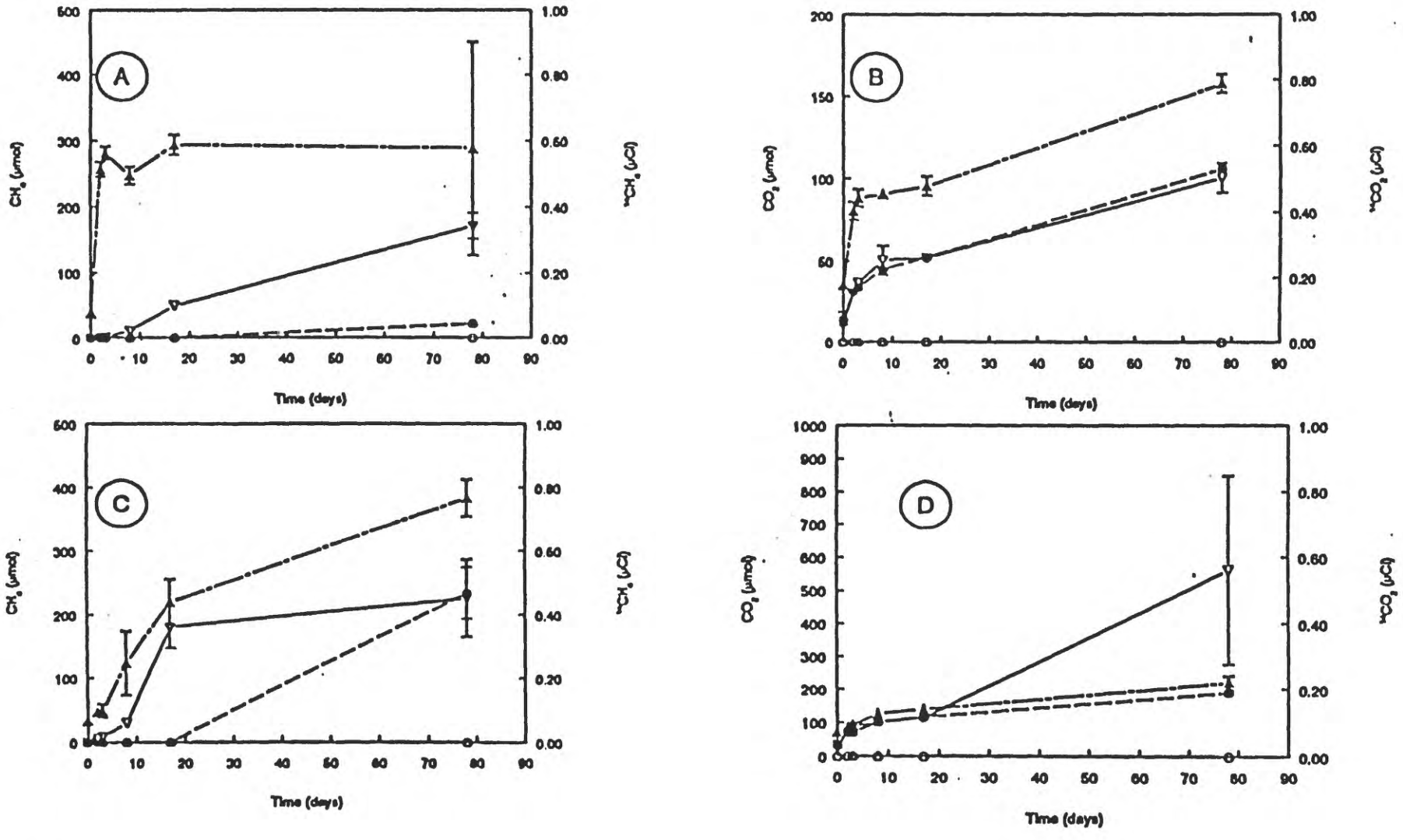


Figure 18. South San Francisco Bay saltmarsh sediment collected from various depths and slurried anaerobically, with 20 mM sulfate or 10 mM TMA, on 6/5/95. All slurries received 0.8 μCi (0.74 μM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$ for slurries from 0-5 cm depth; (B) CO_2 and $^{14}CO_2$ from 0-5 cm, (C) CH_4 and $^{14}CH_4$ for slurries from 5-10 cm depth; (D) CO_2 and $^{14}CO_2$ from 5-10 cm. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (●) sulfate-amended slurries; (▲) TMA-amended slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

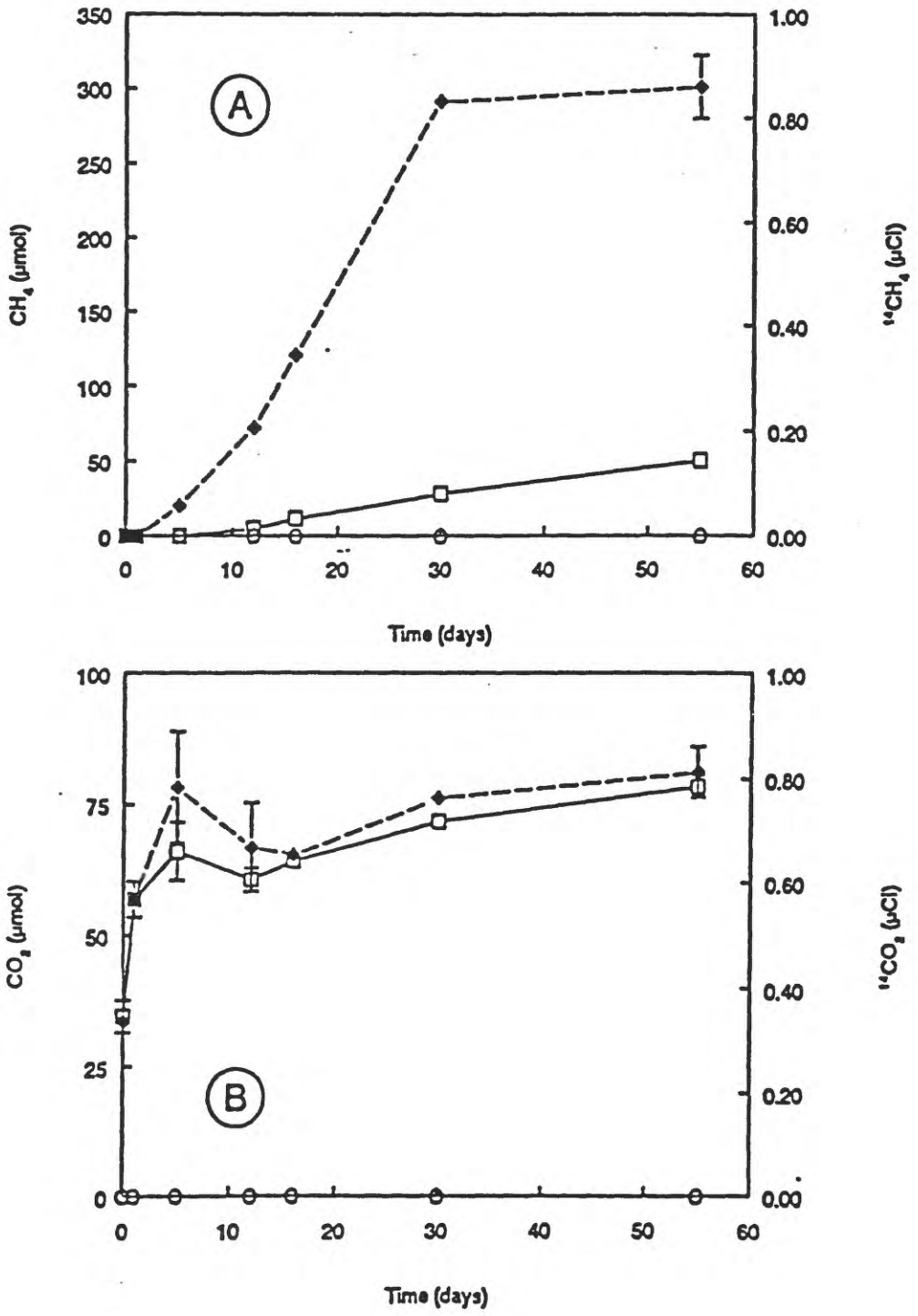


Figure 19. South San Francisco Bay saltmarsh sediment collected and slurried anaerobically on 8/9/95 with 0.1 mM NH_4Cl and 20 mM acetate. All received 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (□) CH_4 and CO_2 in slurries amended with NH_4Cl; (◆) slurries amended with NH_4Cl and acetate; (○) $^{14}CH_4$ and $^{14}CO_2$ for NH_4Cl-amended slurries, which are representative results for both conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

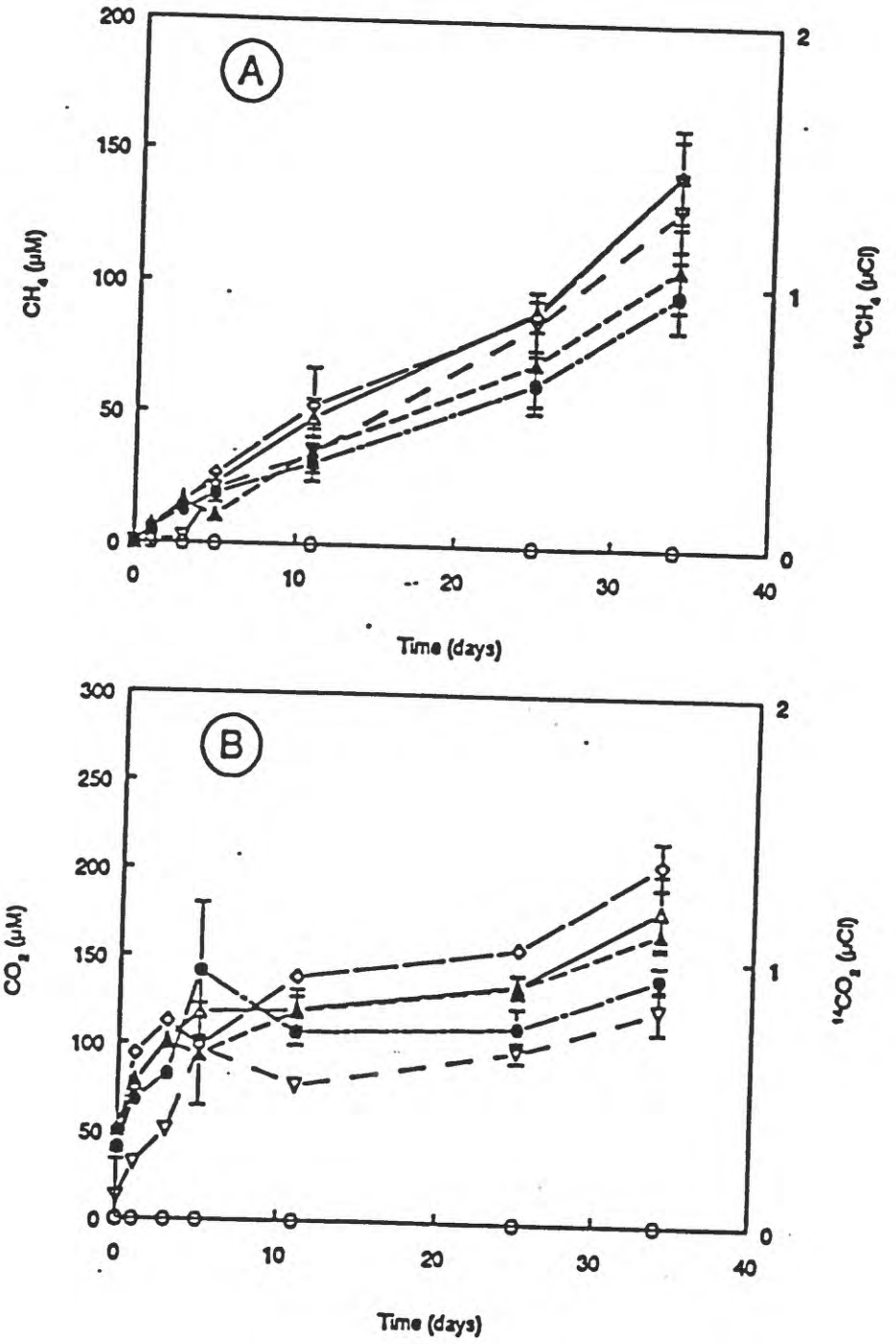


Figure 20. South San Francisco Bay saltmarsh sediment slurries pre-incubated with 1 µM TFA, DFA, MFA, acetate, or no addition. Sediments collected on 6/2/95, slurried anaerobically on 6/15/95, and incubated with additions until each was subsampled on 7/28/95 and amended with 0.91 µCi (0.84 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (△) CH_4 and CO_2 in slurries pre-incubated with TFA; (▲) DFA; (●) MFA; (▽) acetate; (◇) no addition; (○) $^{14}CH_4$ and $^{14}CO_2$ for slurries pre-incubated with TFA, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

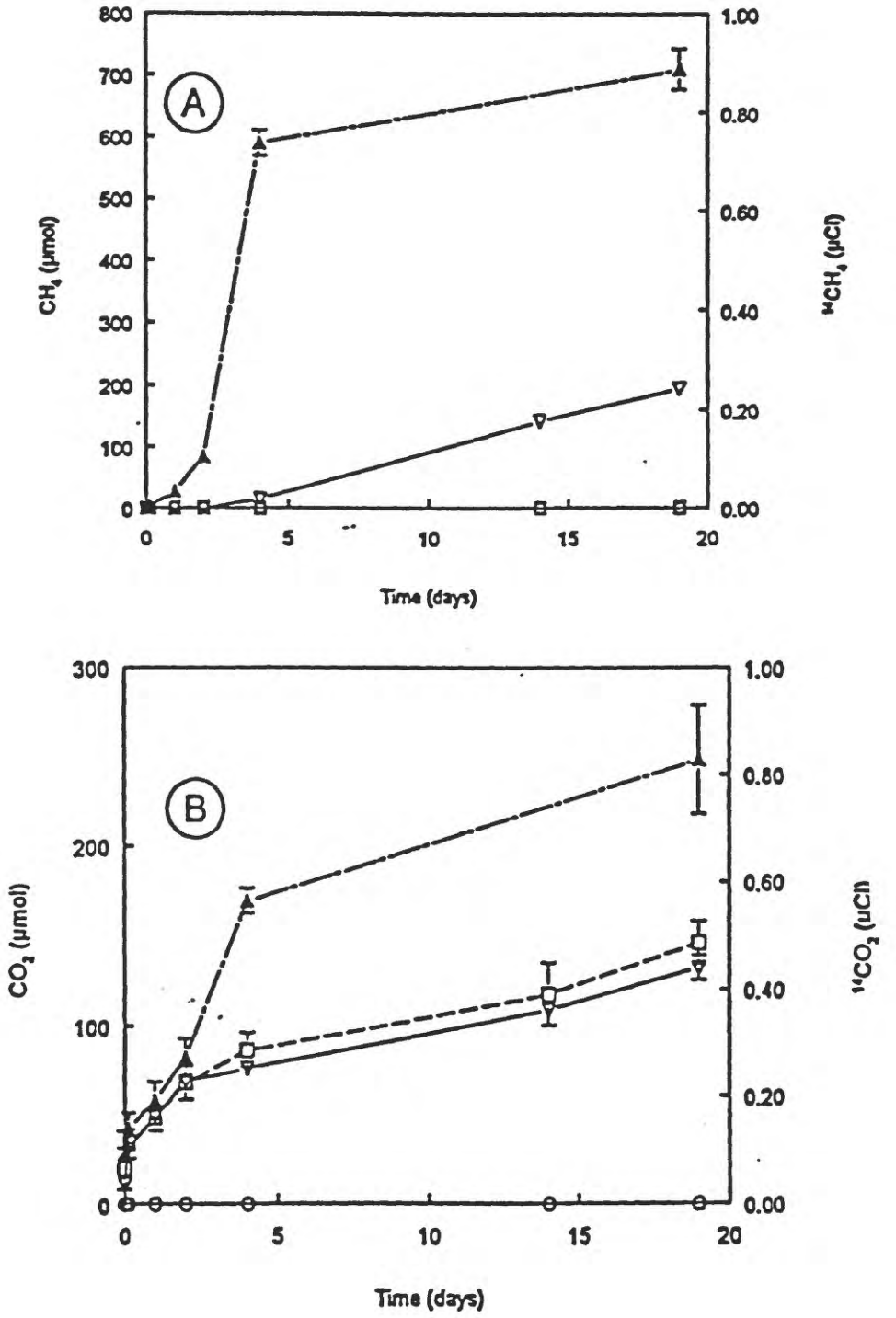


Figure 21. Bolinas Bay saltmarsh sediment from the south sampling location. Collected on 5/3/95 and slurried anaerobically on 5/26/95. All received 0.79 µCi (0.73 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (□) slurries amended with 20 mM sulfate; (▲) 10 mM TMA; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

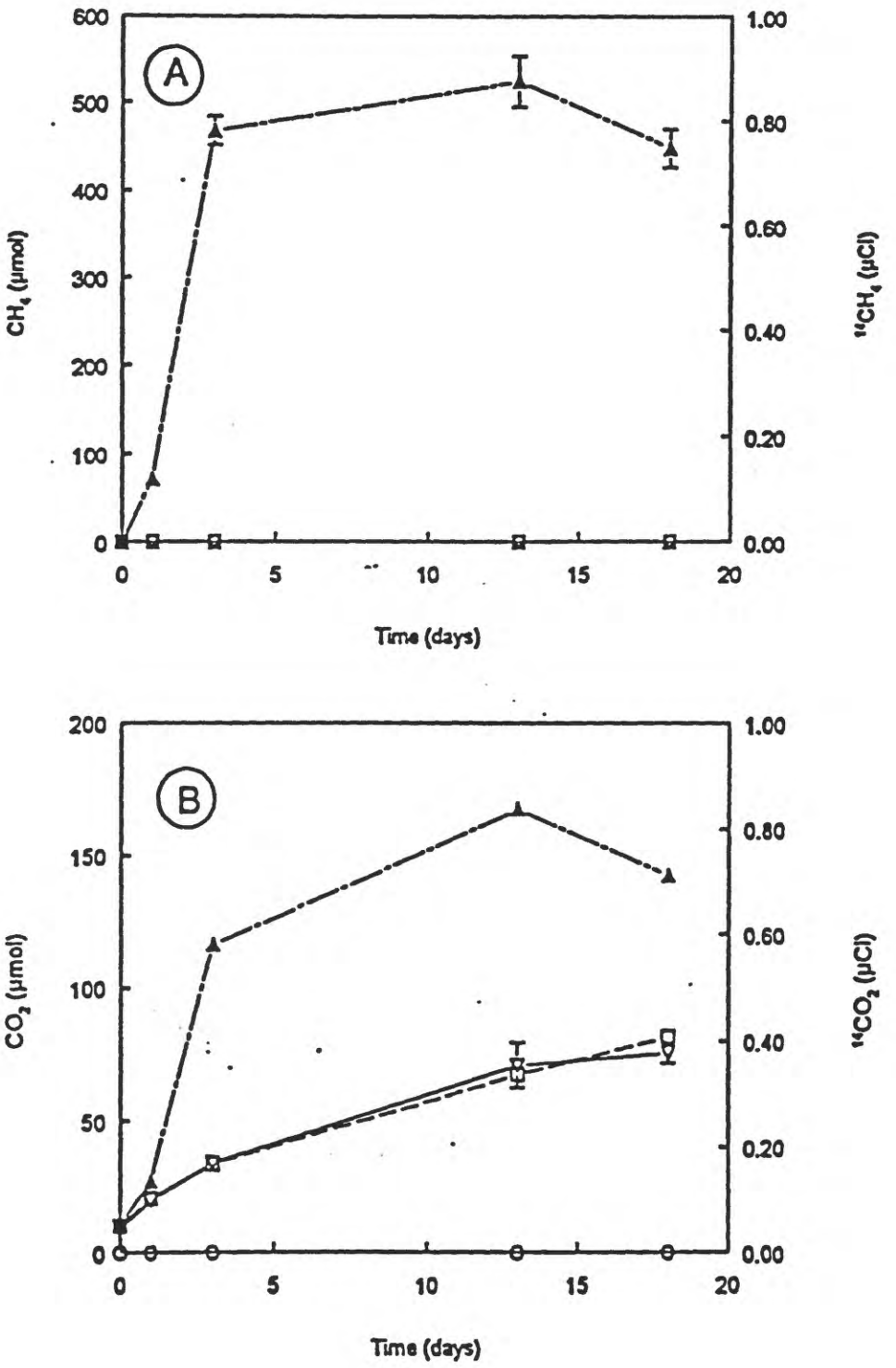


Figure 22. Bolinas Bay saltmarsh sediment from the north sampling location. Collected on 5/3/95 and slurried anaerobically on 5/26/95. All received 0.79 µCi (0.73 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (□) slurries amended with 20 mM sulfate; (▲) 10 mM TMA; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

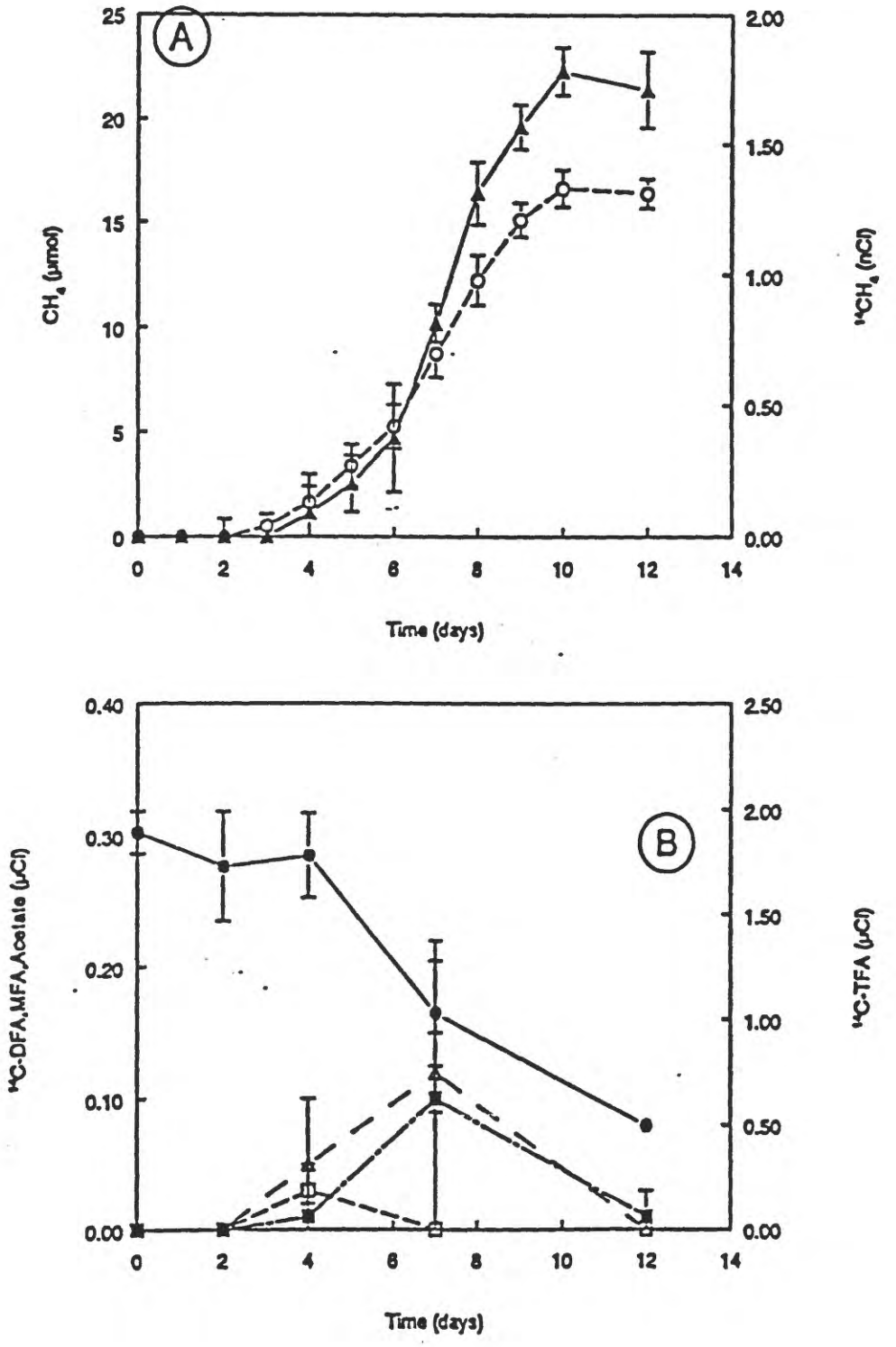


Figure 23. Degradation of 1.85 µCi (1.71 µM) 2-^{14}C-TFA in Searsville lake freshwater sediment collected and slurried anaerobically on 10/6/93. (A) CH_4 and $^{14}CH_4$, (B) disappearance of 2-^{14}C-TFA and appearance of ^{14}C-liquid phase products. Symbols: (▲) CH_4; (○) $^{14}CH_4$; (●) ^{14}C-TFA; (□) ^{14}C-DFA; (■) ^{14}C-MFA; and (△) ^{14}C-acetate. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

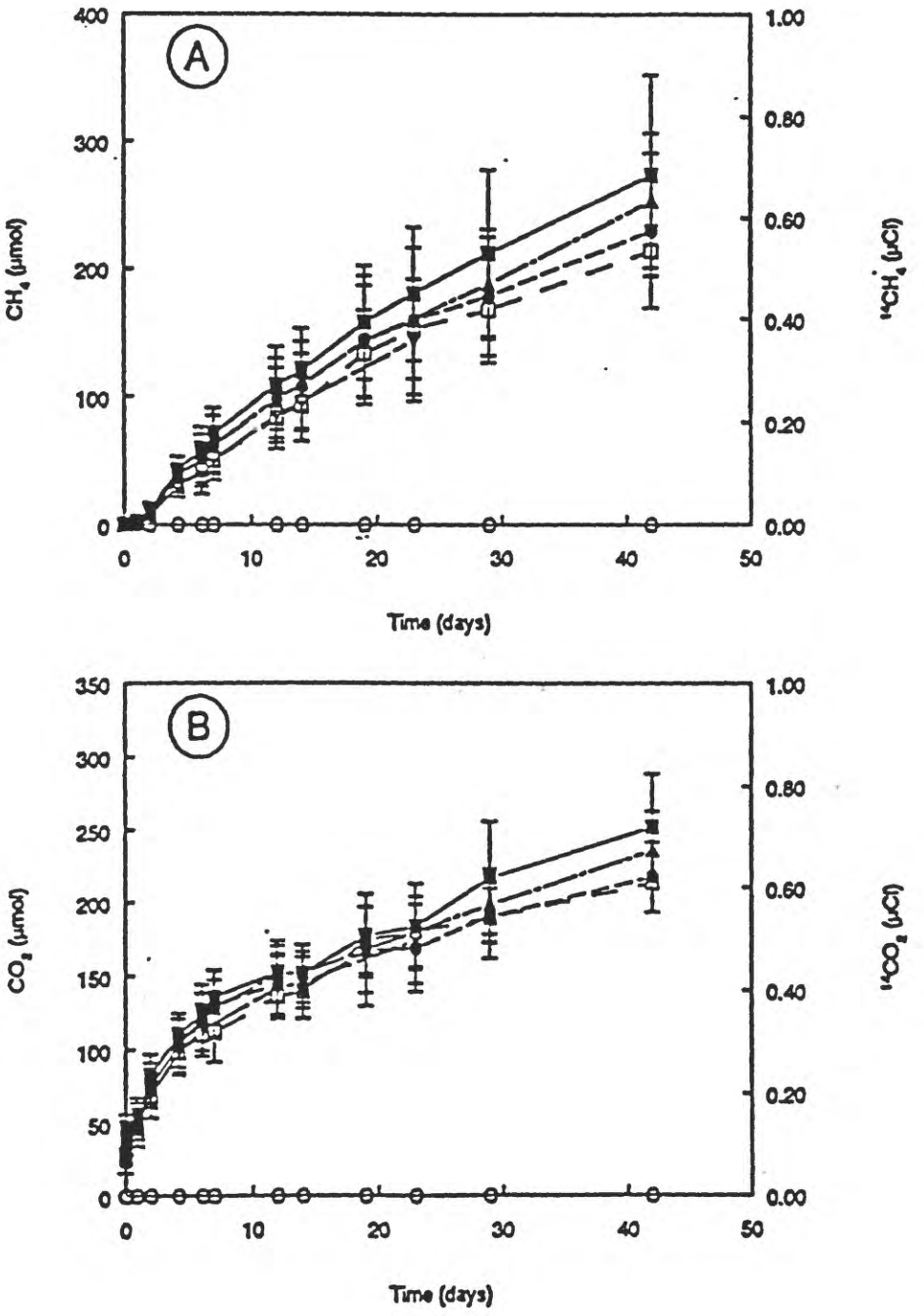


Figure 24. Searsville Lake freshwater sediments collected and slurried anaerobically on 1/18/95 and amended with varying concentrations of 1-^{14}C-TFA and 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in slurries without ^{14}C-TFA; (■) slurries amended with 1 µCi (0.94 µM) 1-^{14}C-TFA; (●) 0.5 µCi (0.47 µM) 1-^{14}C-TFA; (▲) 0.76 µCi (0.70 µM) 2-^{14}C-TFA; (□) 0.38 µCi (0.35 µM) 2-^{14}C-TFA; (○) show $^{14}CH_4$ and $^{14}CO_2$ for slurries amended with 1 µCi (0.94 µM) 1-^{14}C-TFA, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

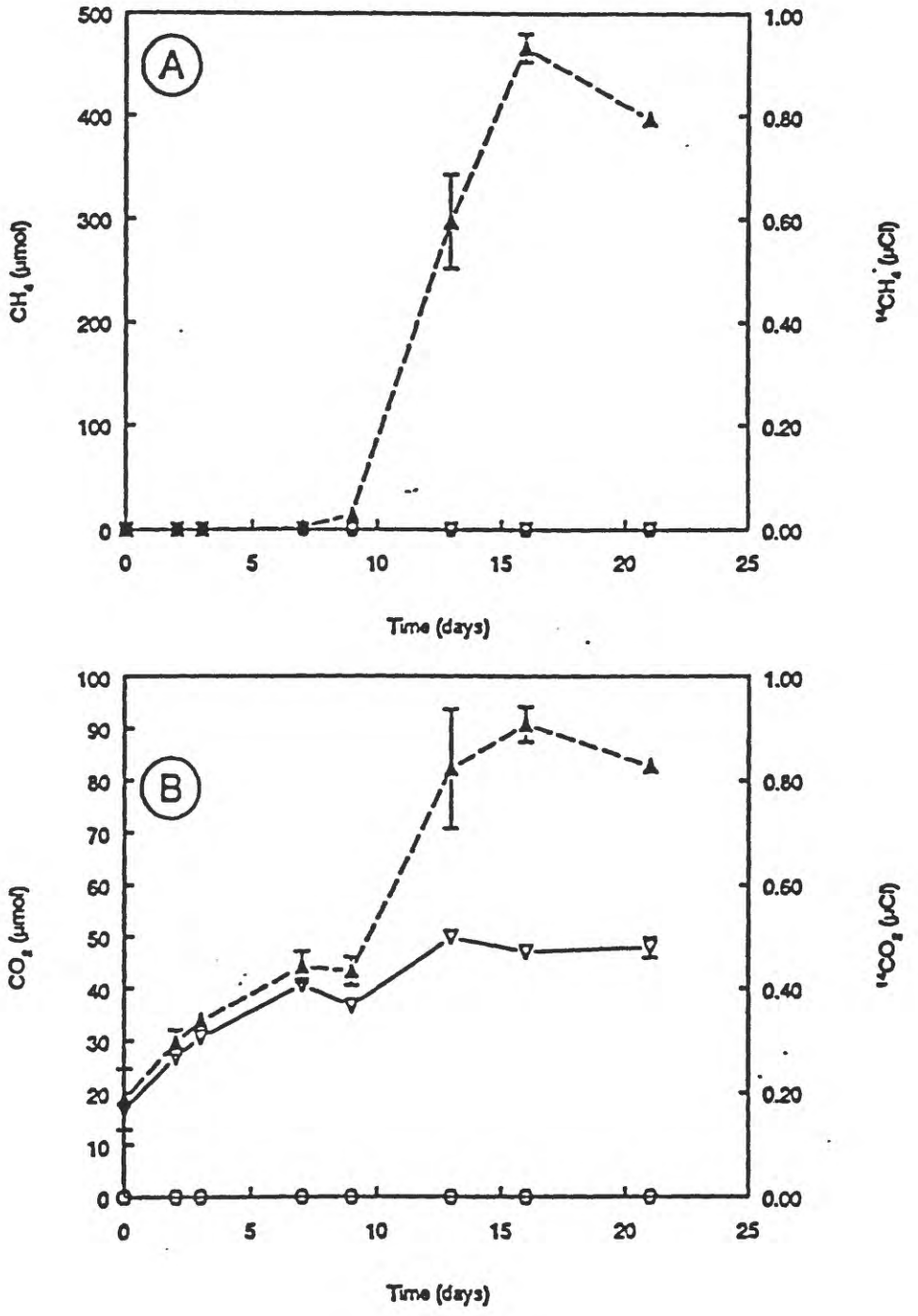


Figure 25. Searsville Lake sediment collected on 10/93 and slurried anaerobically on 5/23/95. All received 0.79 µCi (0.73 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (▲) 10 mM TMA; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

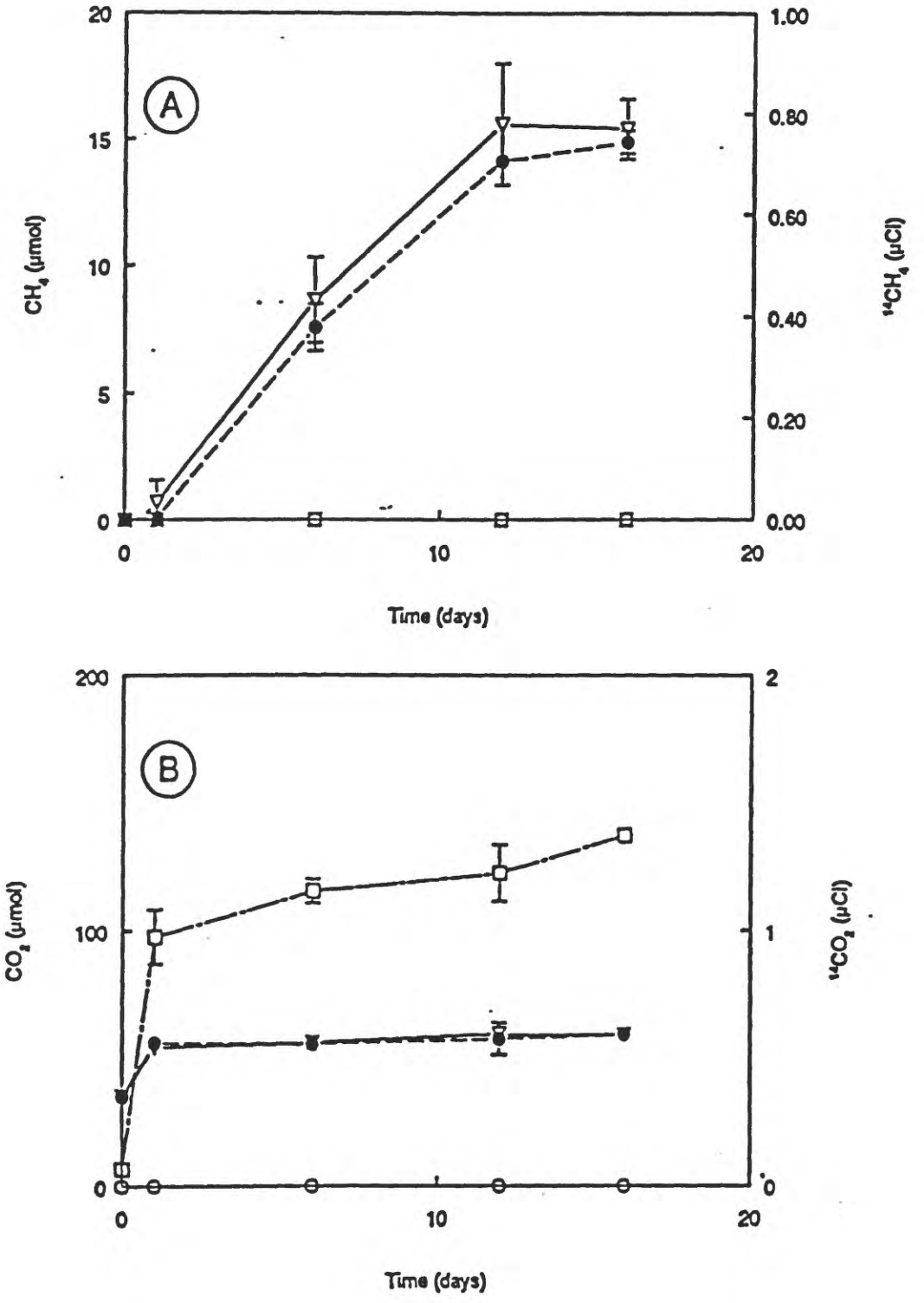


Figure 26. Searsville Lake sediment collected and slurried anaerobically and aerobically on 8/8/95. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 in unamended slurries; (●) 5 mM sulfate; (□) aerobic; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended anaerobic slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

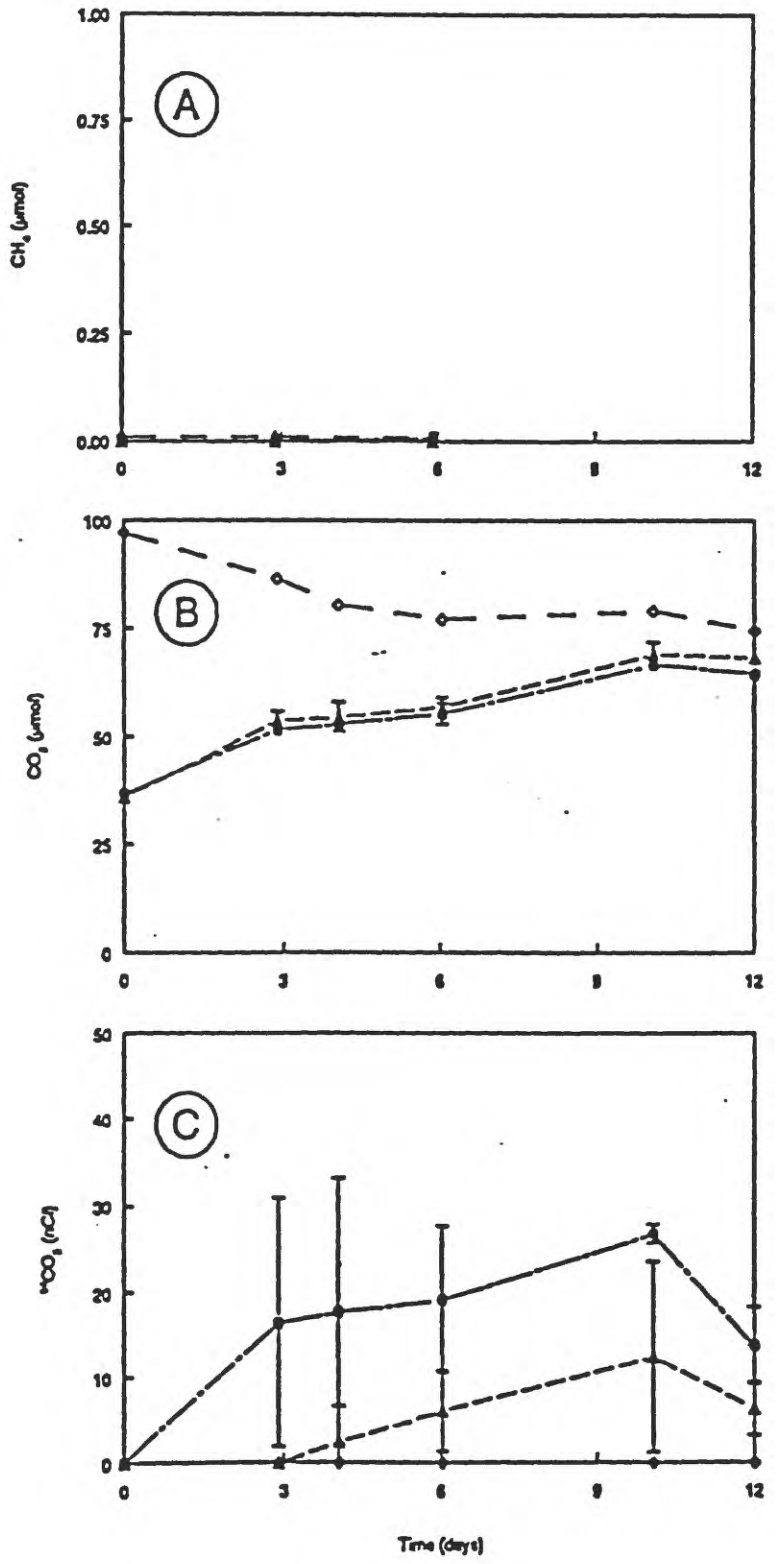


Figure 27. Sacramento Delta peat soil collected on 10/7/91 and slurried anaerobically on 4/1/94 with 20 mM nitrate. (A) CH_4; (B) CO_2; and (C) $^{14}CO_2$. Symbols: (▲) slurries with 4 µCi (3.8 µM) 2-^{14}C-TFA; (●) 10.2 µCi (9.4 µM) 2-^{14}C-TFA; (○) 4 µCi (3.8 µM) 2-^{14}C-TFA added after slurries were autoclaved. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

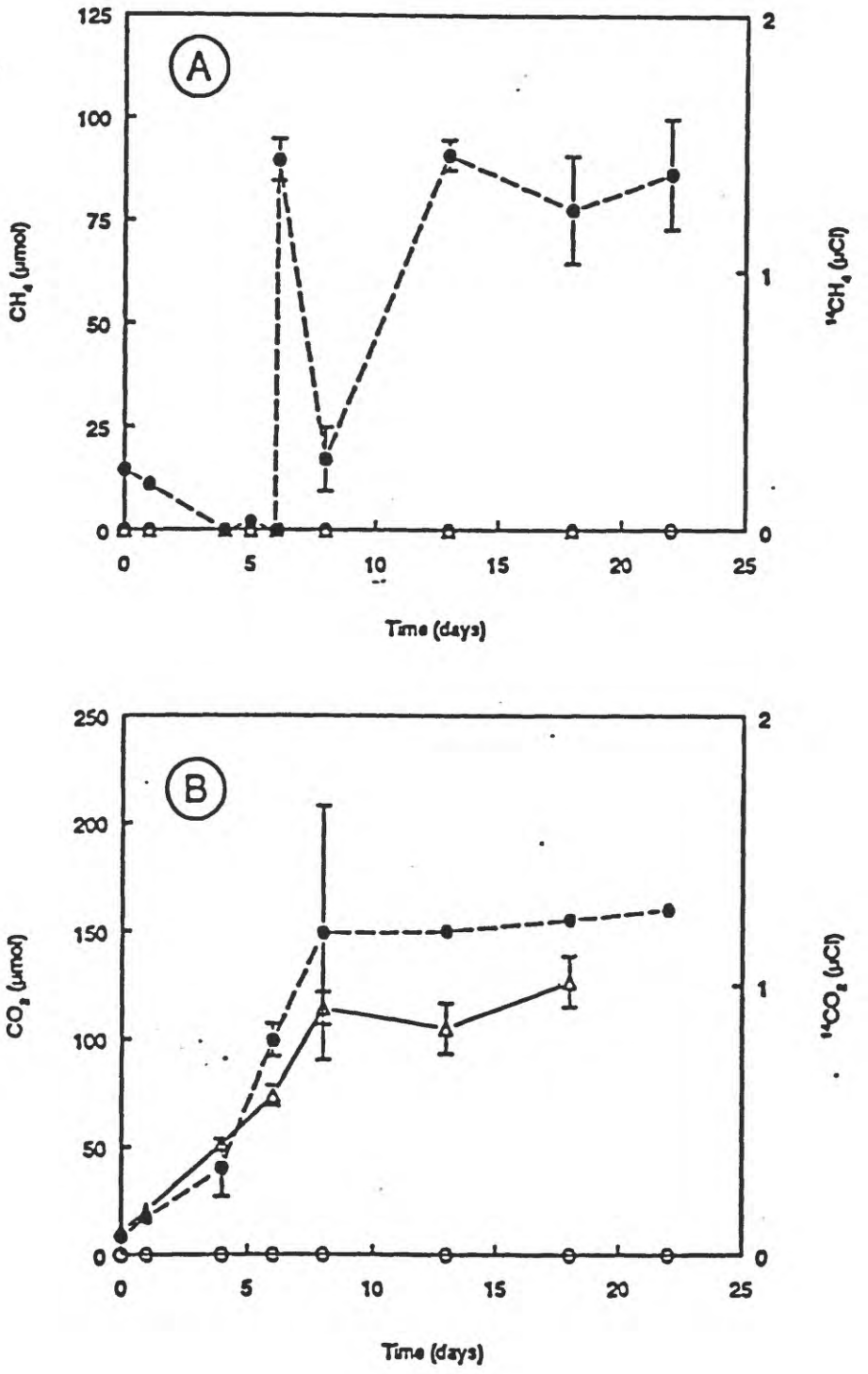


Figure 28. Sacramento Delta peat soil collected 8/9/95 and slurried aerobically on 8/24/95 with CH_4 and O_2. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. Methane and oxygen re-added on day 5, 8 and 18. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (Δ) slurries without methane or oxygen; (●) slurries with methane and oxygen; (○) $^{14}CH_4$ and $^{14}CO_2$ for slurries with methane and oxygen, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

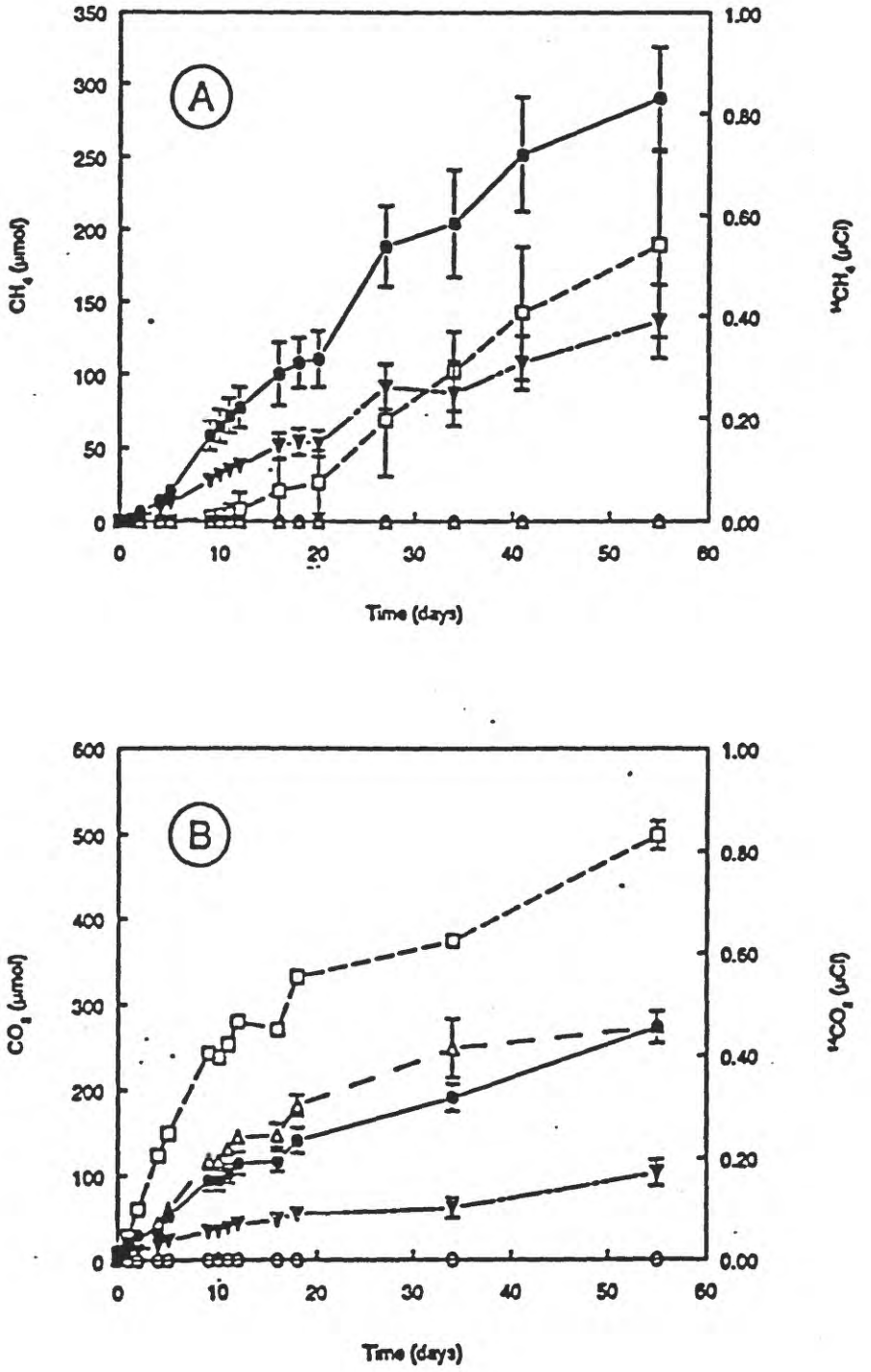


Figure 29. Forest soil collected 6/14/95 and slurried anaerobically or aerobically on 6/16/95. All received 1 μCi (0.93 μM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: CH_4 and CO_2 in (●) anaerobic surface soil slurries; (□) aerobic surface soil slurries; (▼) anaerobic subsurface soil slurries; (△) aerobic subsurface soil slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for anaerobic surface soil slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

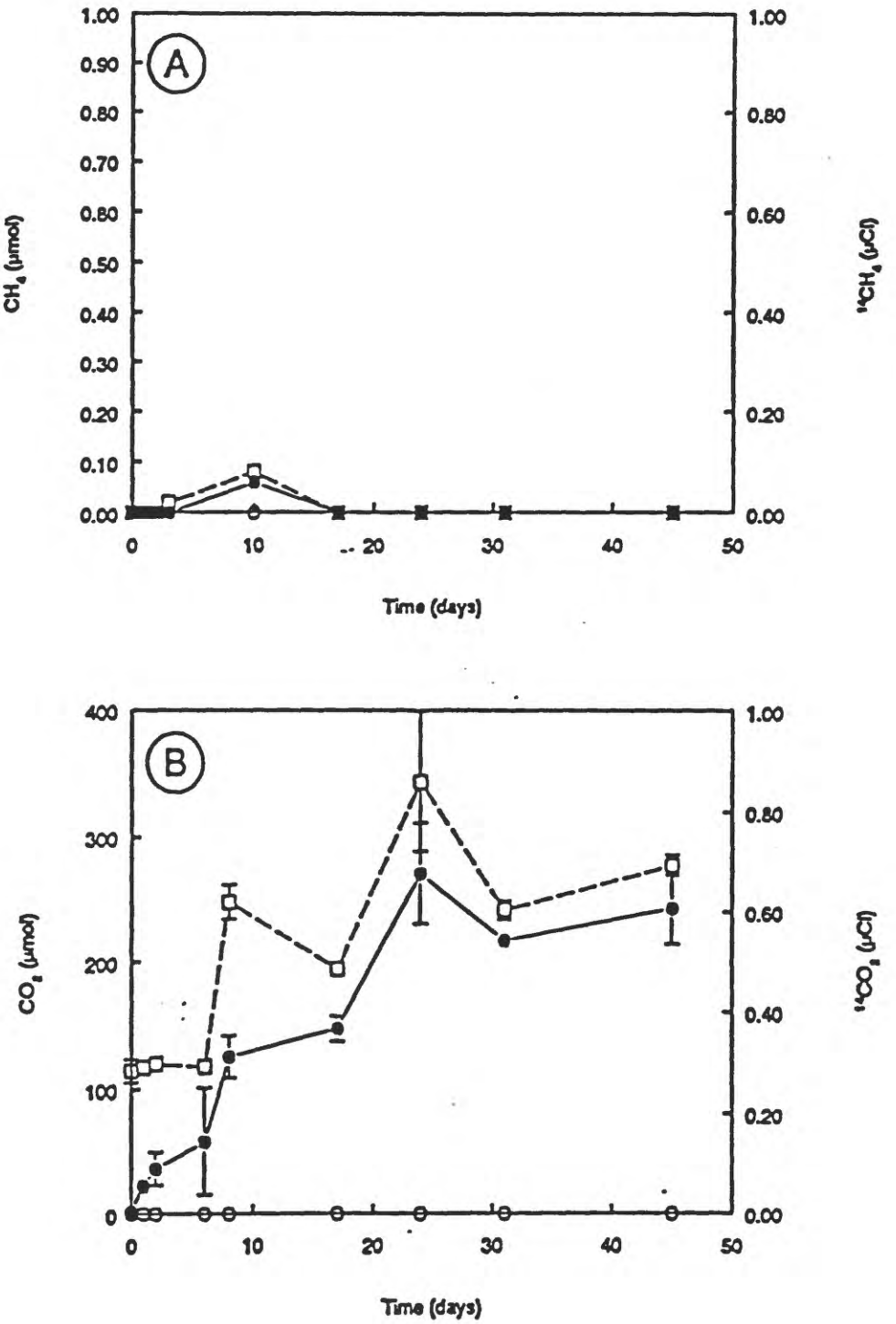


Figure 30. Forest soil collected 6/14/95 and slurried aerobically on 6/26/95. All received 1 μCi (0.93 μM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Oxygen addition made at beginning of experiment and periodically throughout. Symbols: (●) CH_4 and CO_2 in live aerobic surface soil slurries; (□) autoclaved aerobic surface soil slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for live slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

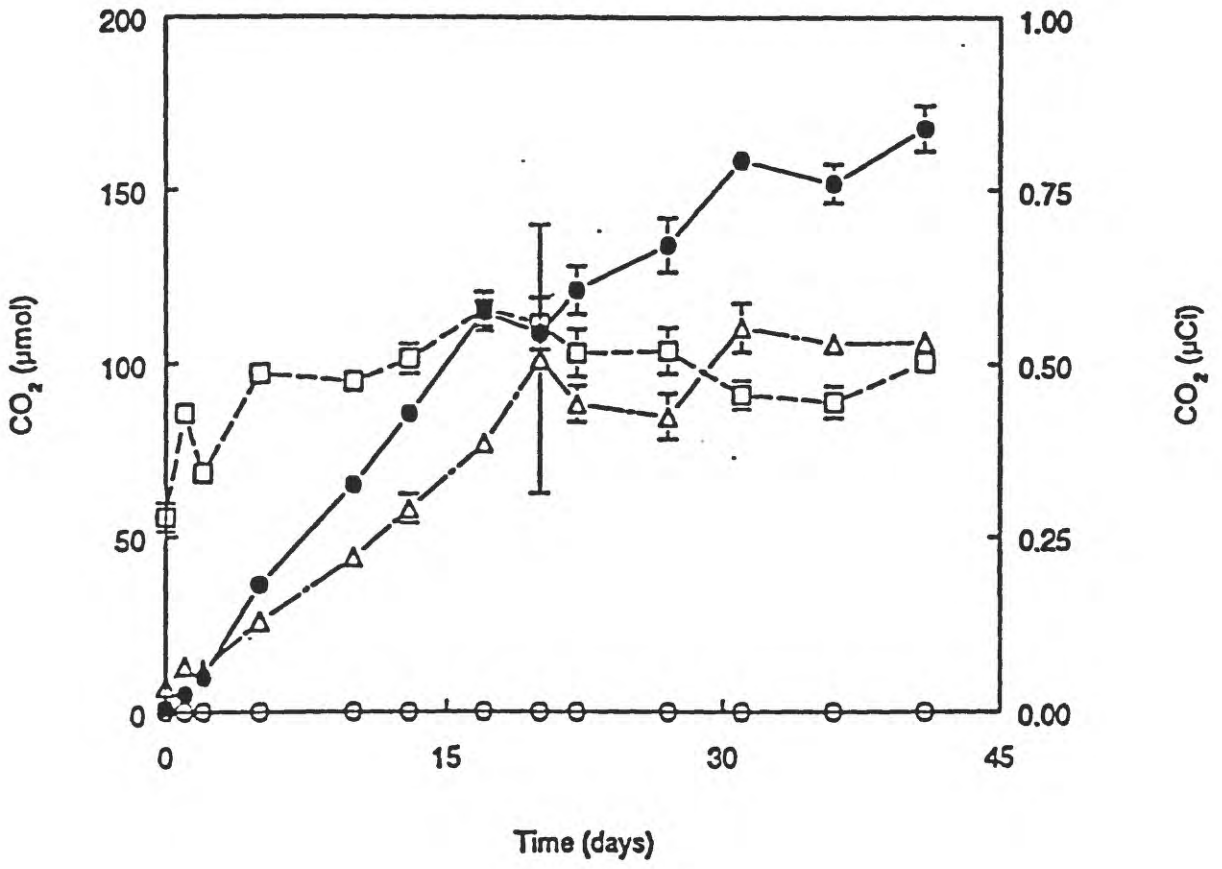


Figure 31. Forest soil from site 1, collected 7/7/95. Slurried aerobically and anaerobically on 7/11/95. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. Symbols: CO_2 for (●) anaerobic soil slurries; (Δ) aerobic slurries; (□) autoclaved aerobic soil slurries; (○) and $^{14}CO_2$ for anaerobic slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

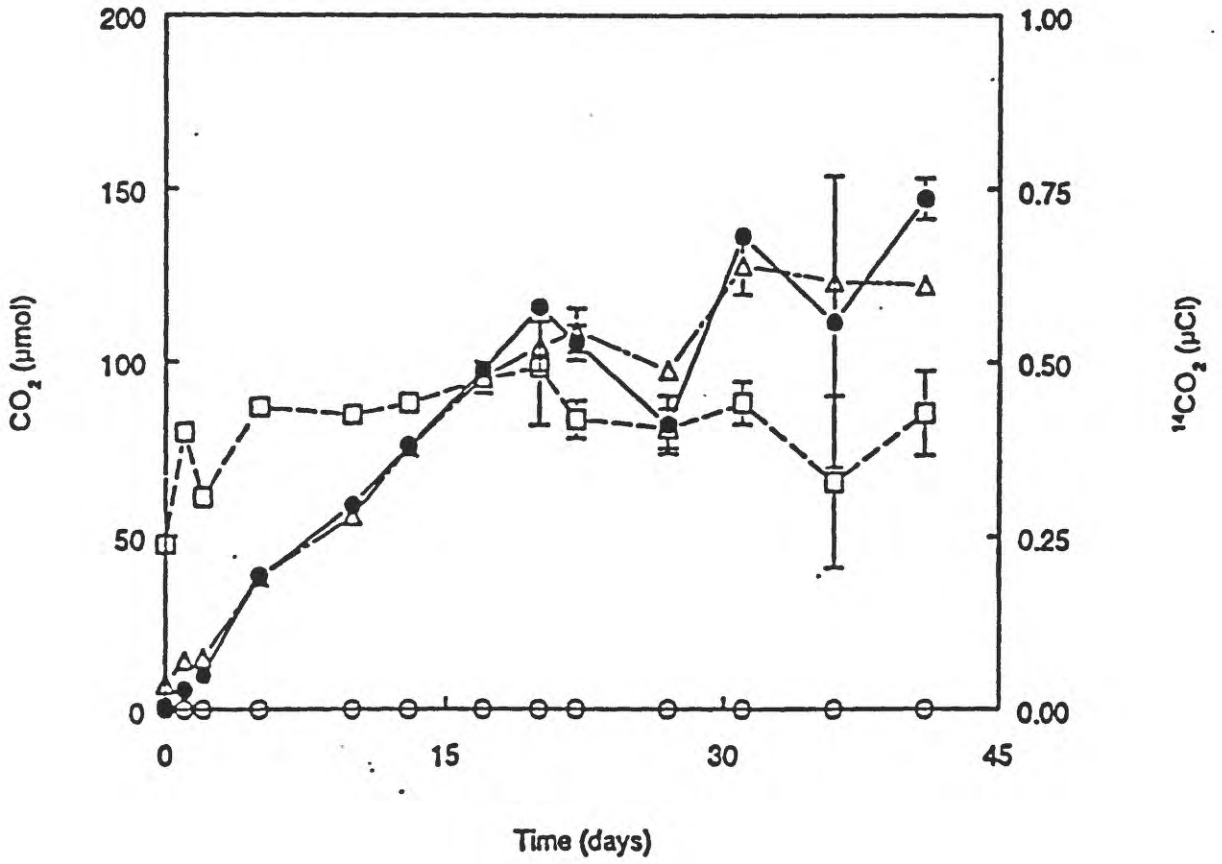


Figure 32. Forest soil from site 2, collected 7/7/95. Slurried aerobically and anaerobically on 7/11/95. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. Symbols: CO_2 for (●) anaerobic soil slurries; (△) aerobic slurries; (□) autoclaved aerobic soil slurries; (○) $^{14}CH_4$ and $^{14}CO_2$ for anaerobic slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

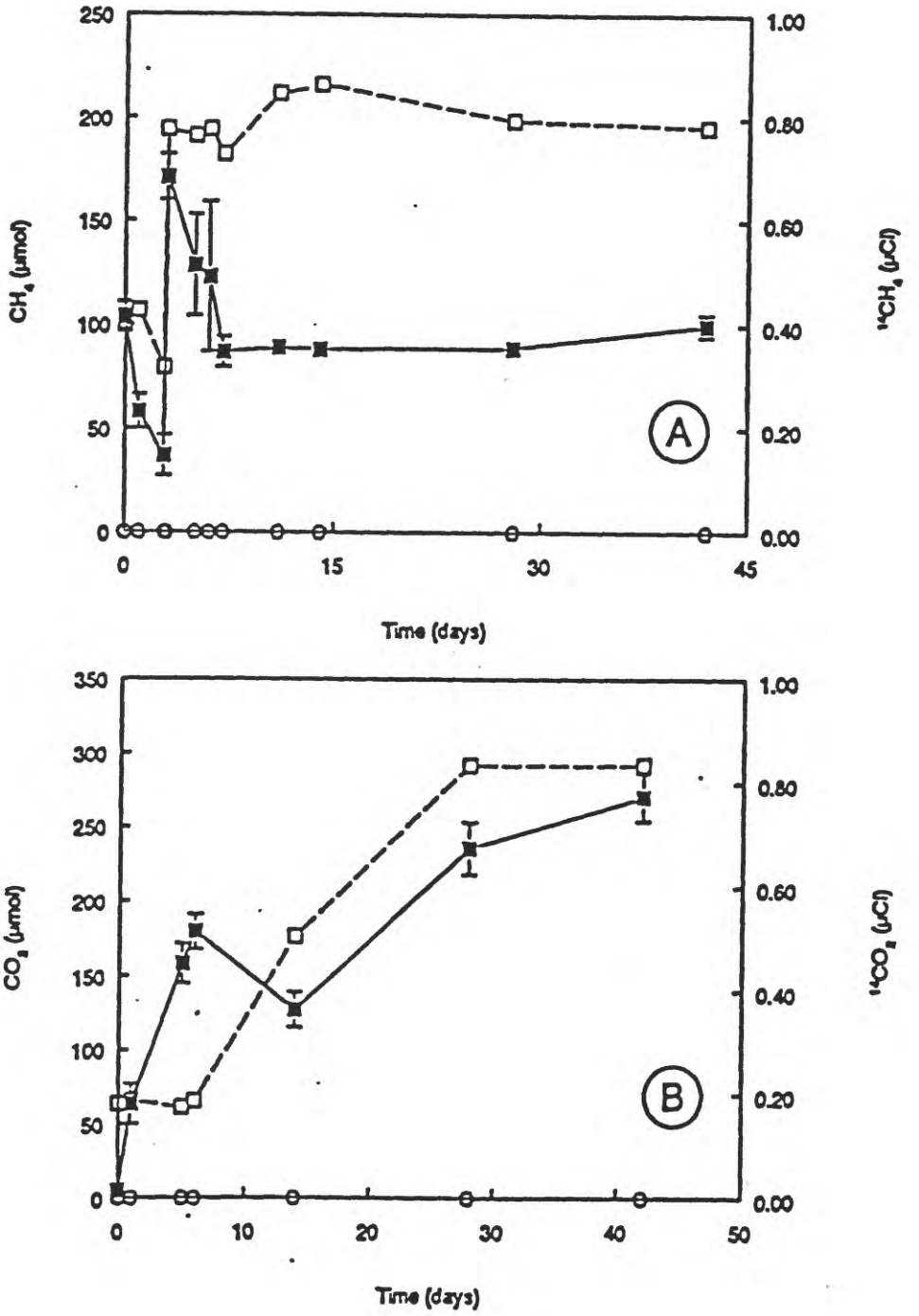


Figure 33. Forest soil collected 6/14/95 and slurried aerobically on 6/30/95 with CH_4 and O_2. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. Methane and oxygen re-added on day 3. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (■) slurries methane or oxygen; (□) autoclaved slurries with methane and oxygen; (○) $^{14}CH_4$ and $^{14}CO_2$ for live slurries with methane and oxygen, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation, except for autoclaved slurries (□) where multiple samples were not done.

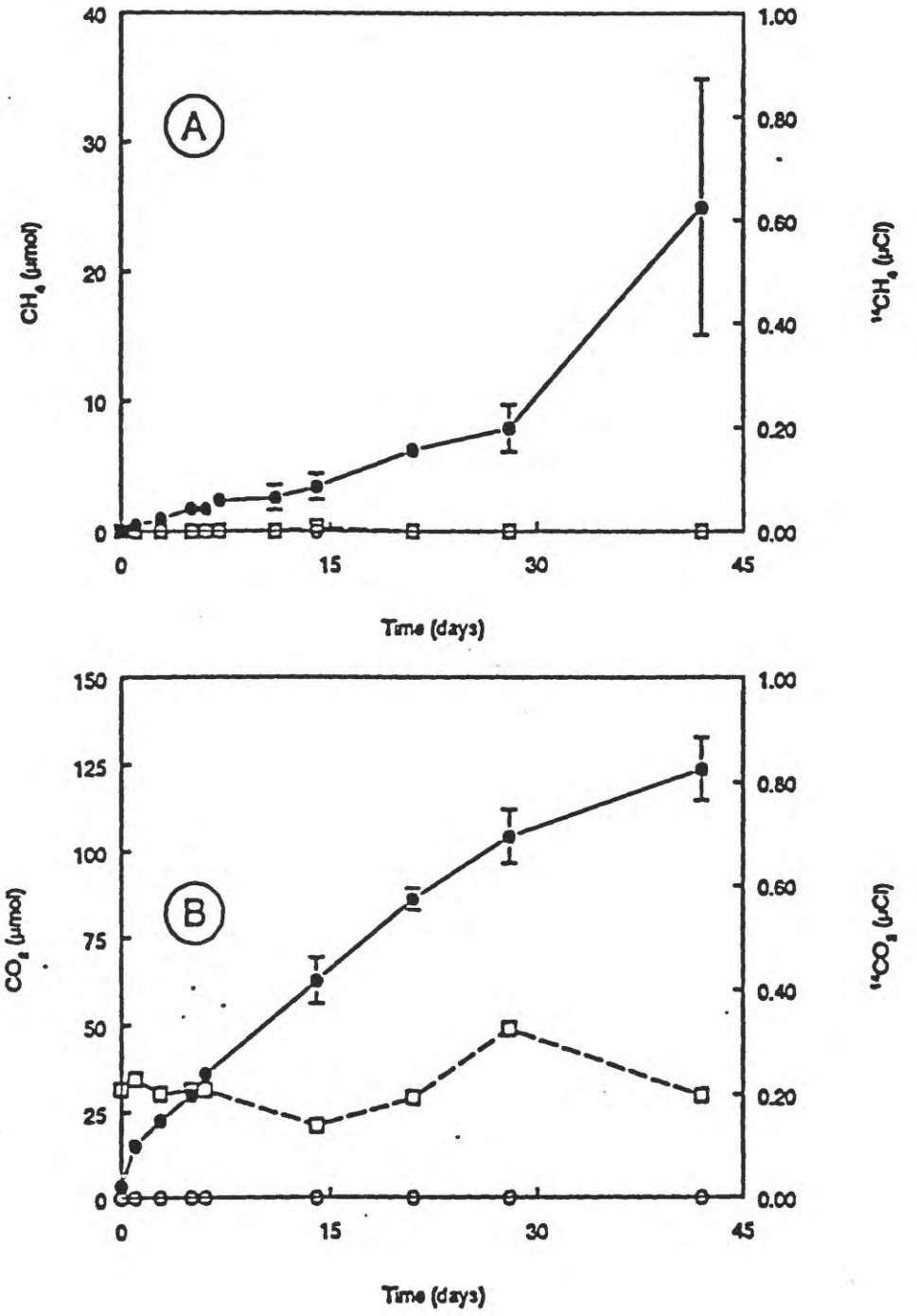


Figure 34. Forest soil collected 6/14/95 and slurried anaerobically on 6/30/95 with 5 mM sulfate. All received 1 µCi (0.94 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (●) slurries with sulfate; (□) autoclaved slurries with sulfate; (○) $^{14}CH_4$ and $^{14}CO_2$ for live slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation, except for autoclaved slurries (□) where multiple samples were not done.

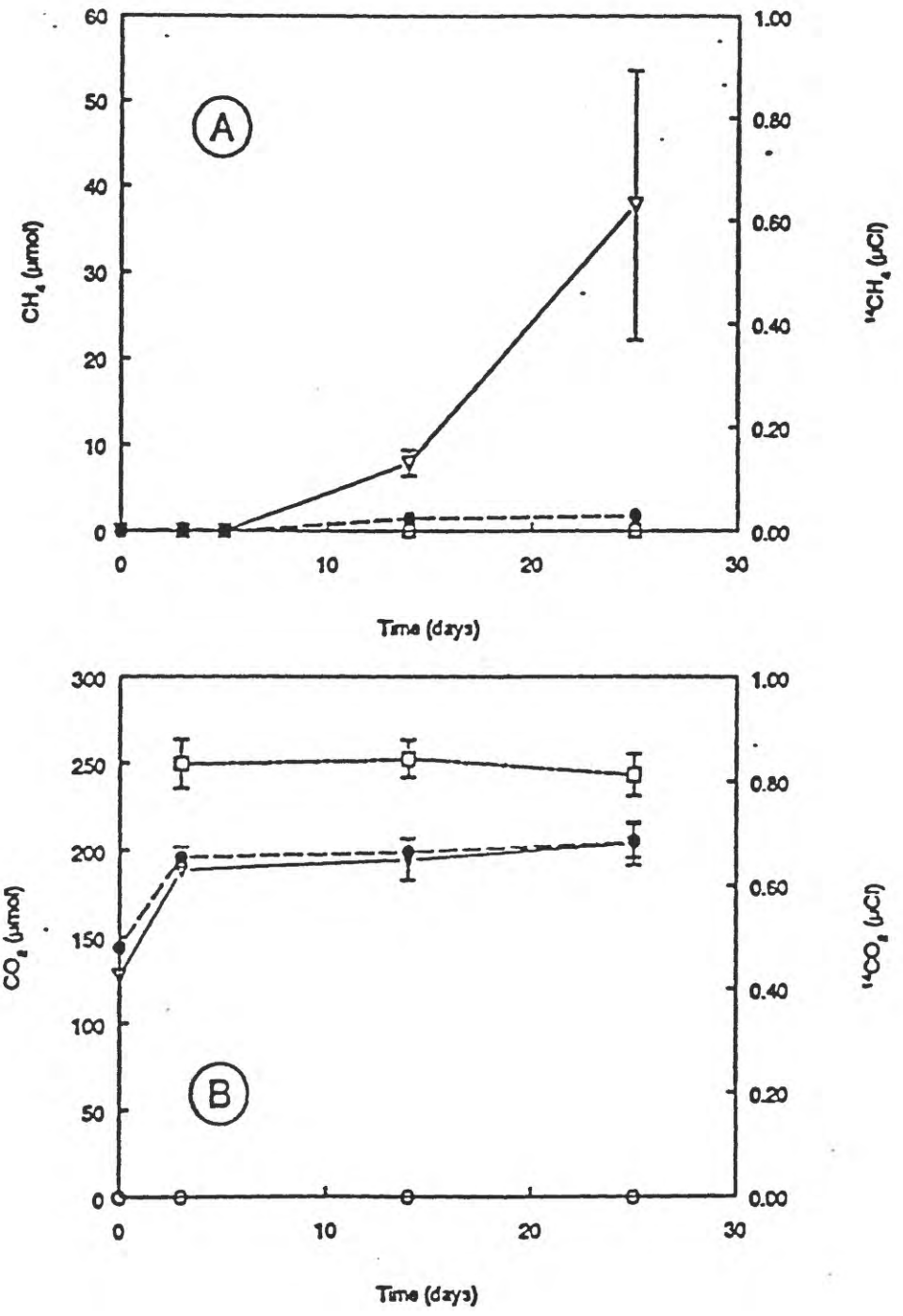


Figure 35. Forest soil collected 7/7/95 and slurried anaerobically on 8/25/95 with 5 mM sulfate in phosphate buffered medium, pH 6.8. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) slurries without sulfate; (●) slurries with sulfate; (□) autoclaved slurries with sulfate; (○) $^{14}CH_4$ and $^{14}CO_2$ for slurries without sulfate, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

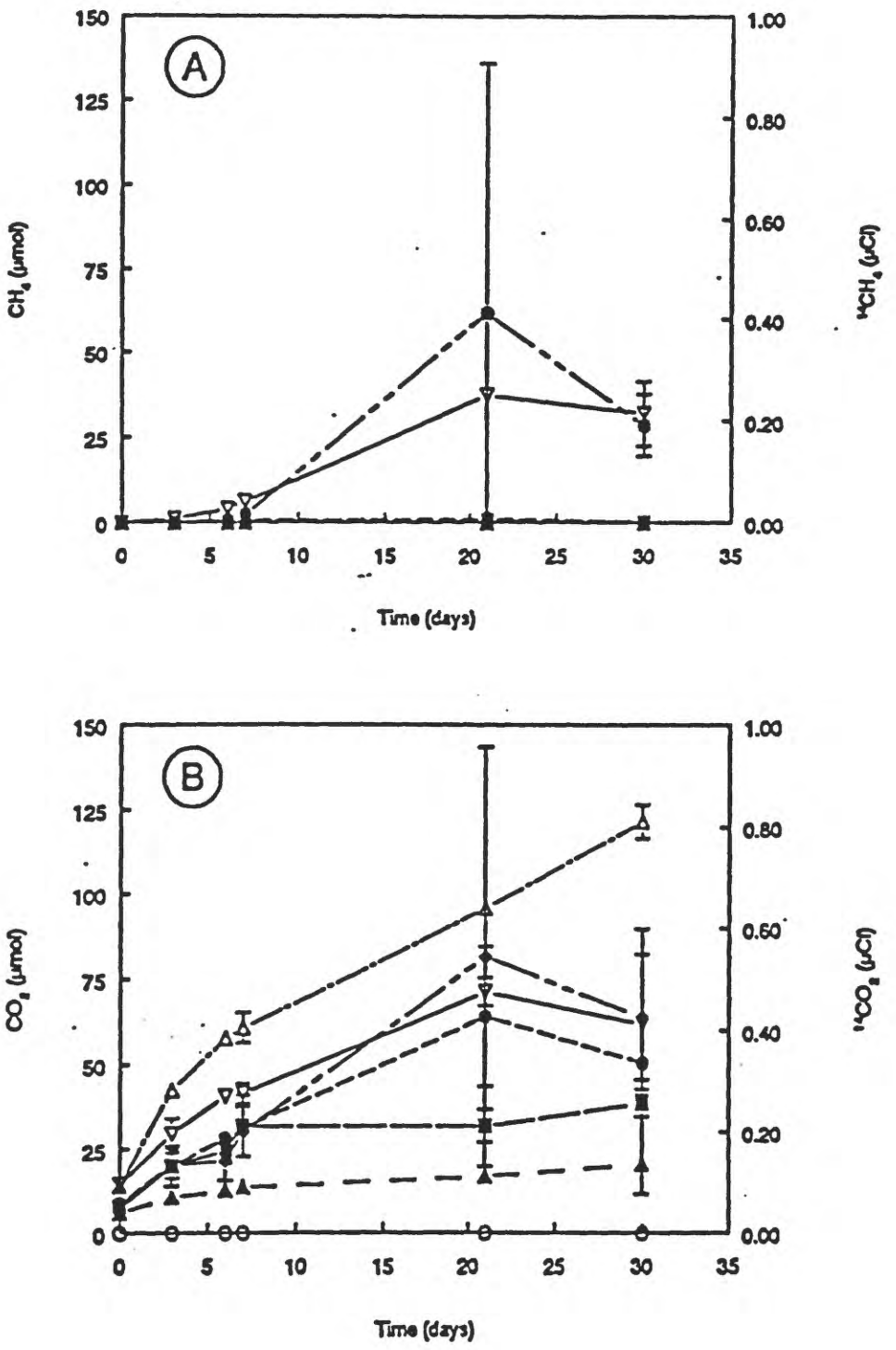


Figure 36. Forest soil collected 7/7/95 and slurried anaerobically on 8/17/95 with 5 mM sulfate, 5 mM nitrate, 0.5 mM NH_4Cl, 5 mM acetate, or 0.1 mmol/L FeOOH, where indicated. All received 0.91 µCi (0.86 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) slurries without addition; (●) slurries with sulfate; (△) nitrate; (▲) NH_4; (■) acetate; (◆) FeOOH; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended slurries, which are representative results for all conditions. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

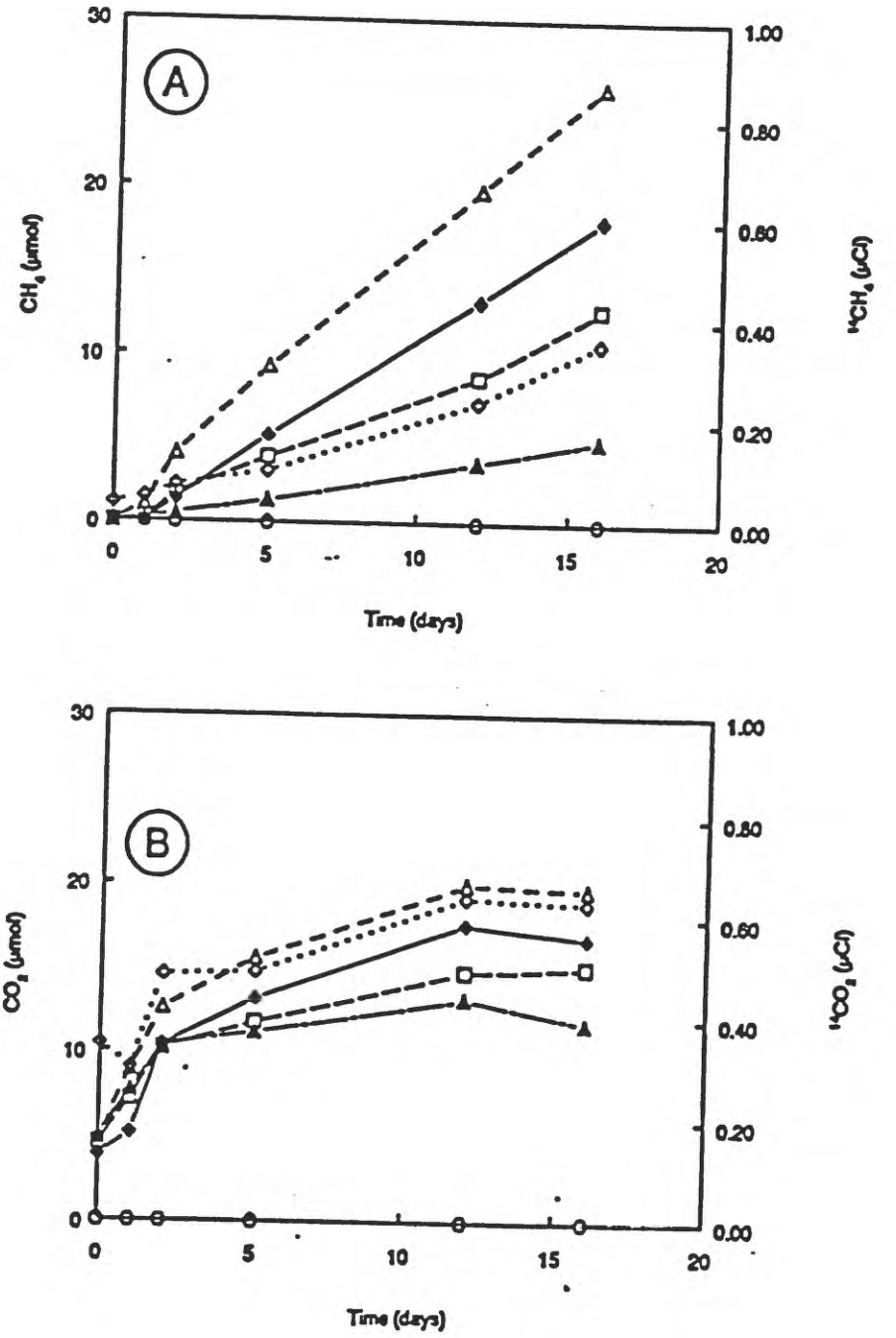


Figure 37. South San Francisco Bay saltmarsh small core experiment. Core collected on 3/31/95, incubated on the bench in sunlight until it was subcored on 6/6/95. All received 0.2 µCi (1.2 nmol/mL) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (◆) CH_4 and CO_2 for subcores from depth 0-2 cm; (△) 2-4 cm; (□) 4-6 cm; (◇) 6-9 cm; (▲) 9-12 cm; (○) $^{14}CH_4$ and $^{14}CO_2$ for 0-2 cm subcores, which are representative results for all depths.

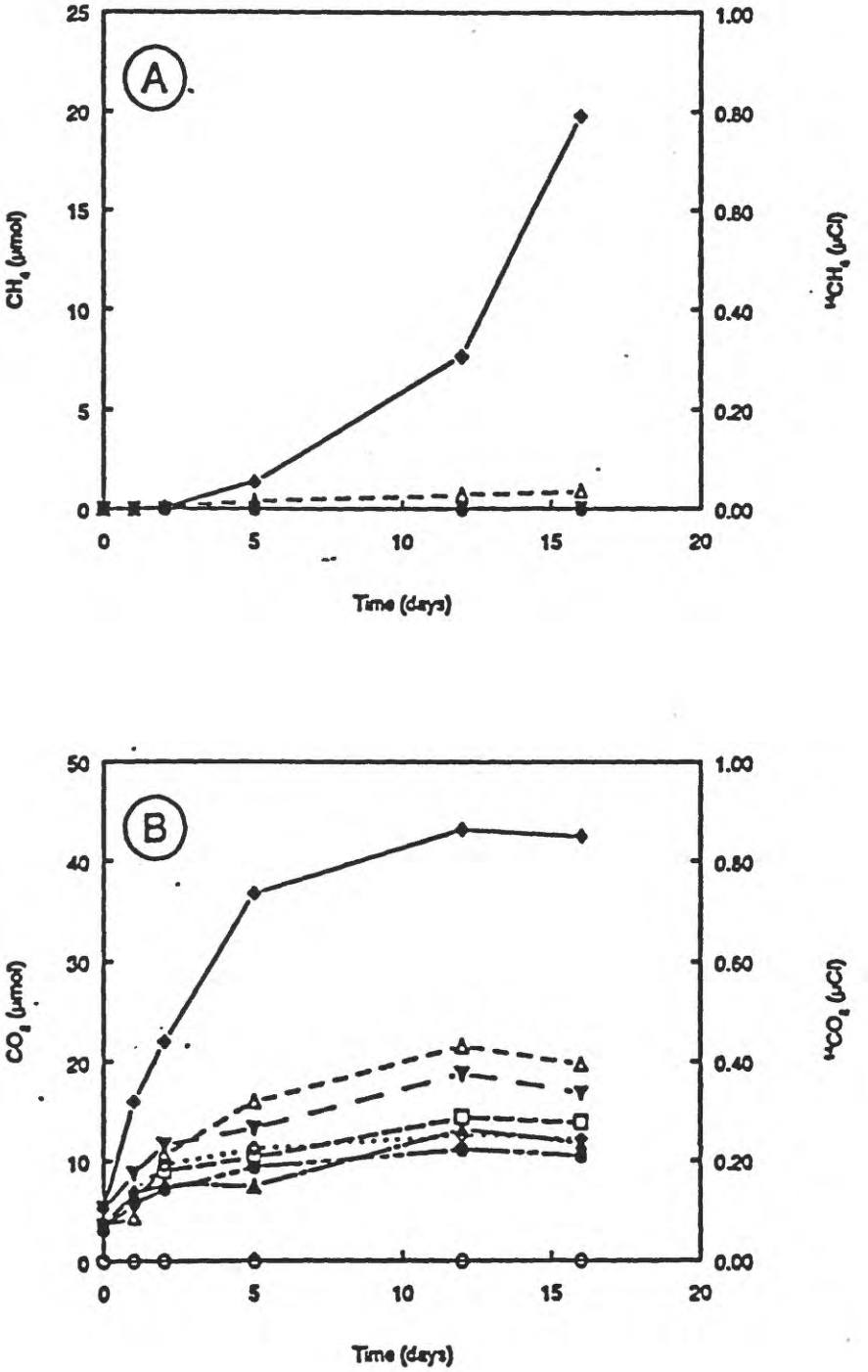


Figure 38. South San Francisco Bay saltmarsh small core experiment. Core collected on 5/3/95, incubated on the bench in sunlight until it was subcored on 6/6/95. All received 0.2 µCi (1.2 nmol/mL) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$, only depths 0-2 cm and 2-4 cm produced CH_4; (B) CO_2 and $^{14}CO_2$. Symbols: (◆) CH_4 and CO_2 for subcores from depth 0-2 cm; (△) 2-4 cm; (□) 4-6 cm; (◇) 6-9 cm; (▲) 9-12 cm; (●) 12-15 cm; (○) $^{14}CH_4$ and $^{14}CO_2$ for 0-2 cm subcores, which are representative results for all depths.

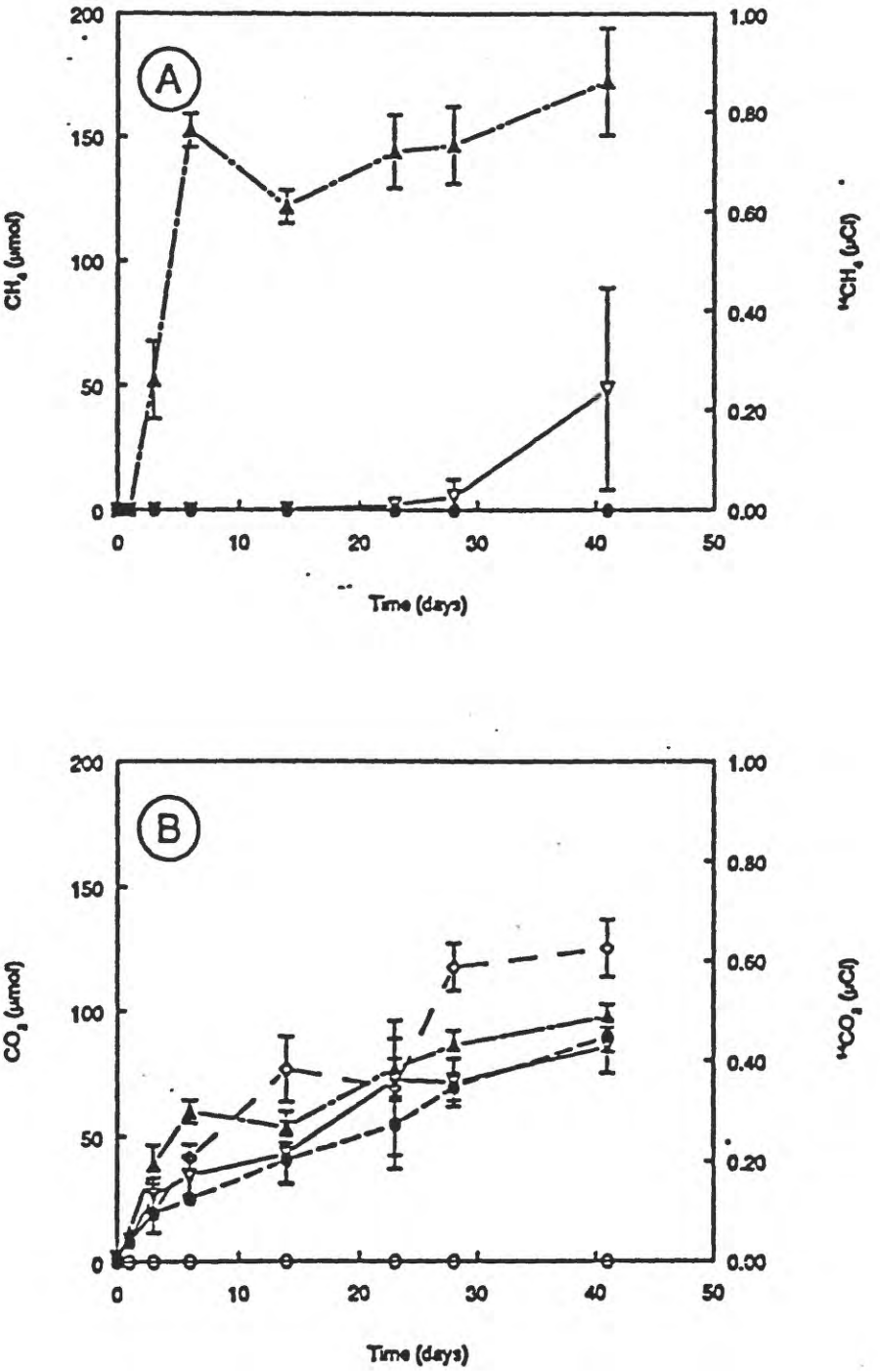


Figure 39. San Francisco Marina small core experiment. Core collected on 5/12/95, stored at 4 °C, and subcored aerobically and anaerobically on 5/16/95. Where indicated 20 mM sulfate, or 10 mM TFA were added to anaerobic cores. All received 0.24 µCi (1.5 nmol/mL) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (▽) CH_4 and CO_2 for anaerobic subcores without sulfate or TMA; (●) anaerobic, sulfate-amended; (▲) anaerobic, TMA-amended; (◇) aerobic; (○) $^{14}CH_4$ and $^{14}CO_2$ for unamended anaerobic subcores, which are representative results for all depths. Symbols represent the mean of three individual slurries and bars indicate ±1 standard deviation.

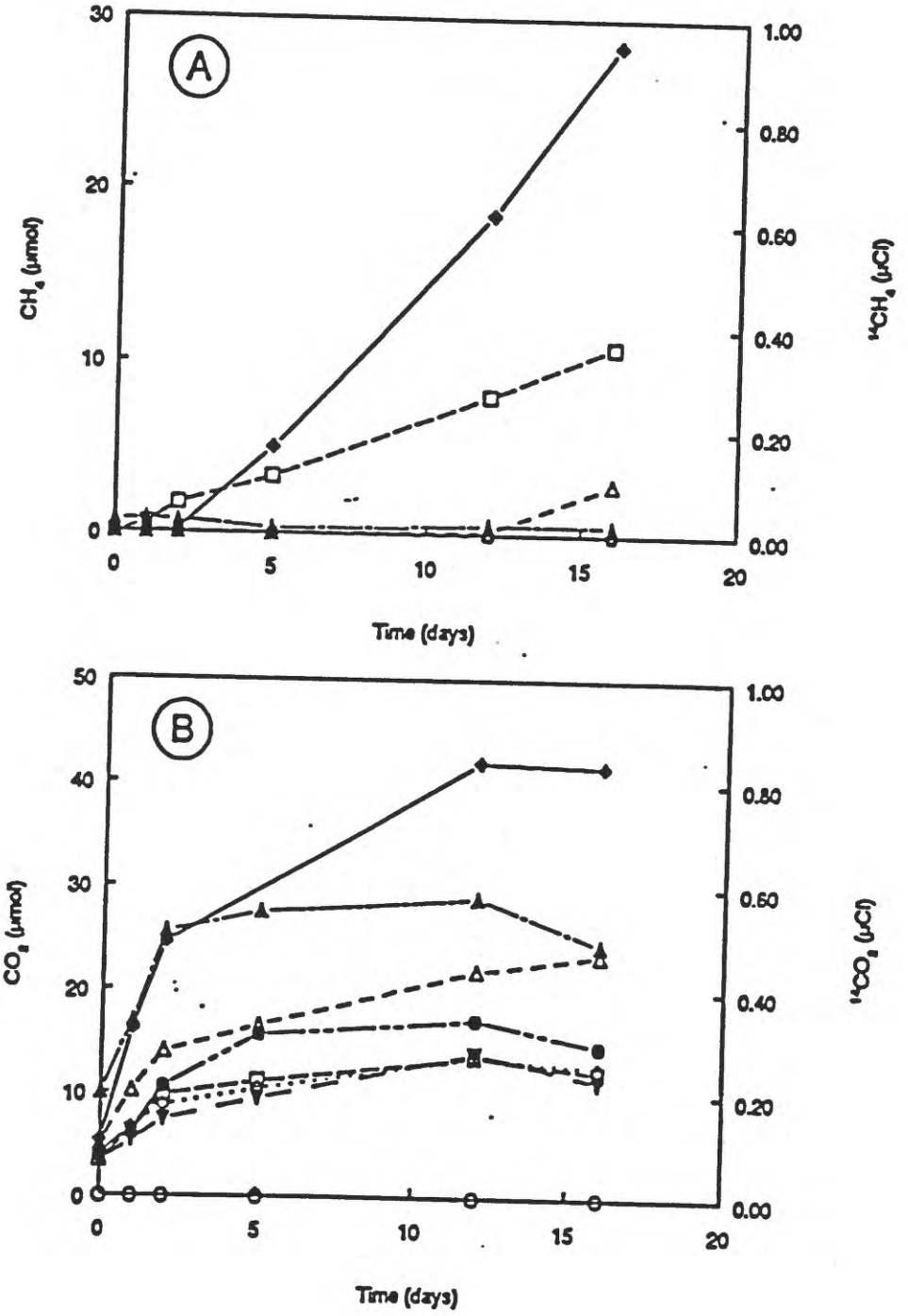
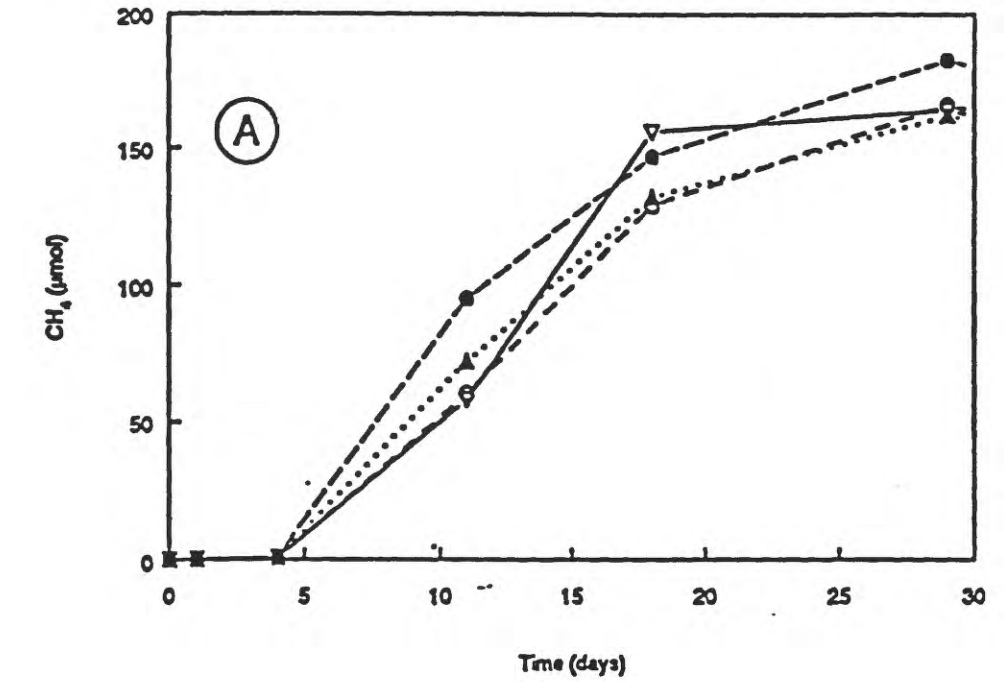


Figure 40. Bolinas Bay small core experiment. Cores collected on 5/23/95 and subcored anaerobically on 6/7/95. All received 0.2 µCi (1.2 nmol/mL) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) CO_2 and $^{14}CO_2$. Symbols: (◆) CH_4 and CO_2 for subcores from depth 0-2 cm; (△) 2-4 cm; (□) 4-6 cm; (◇) 6-9 cm; (▲) 9-12 cm; (●) 12-15 cm; (▼) 15-19 cm; (○) $^{14}CH_4$ and $^{14}CO_2$ for 0-2 cm subcores, which are representative results for all depths.



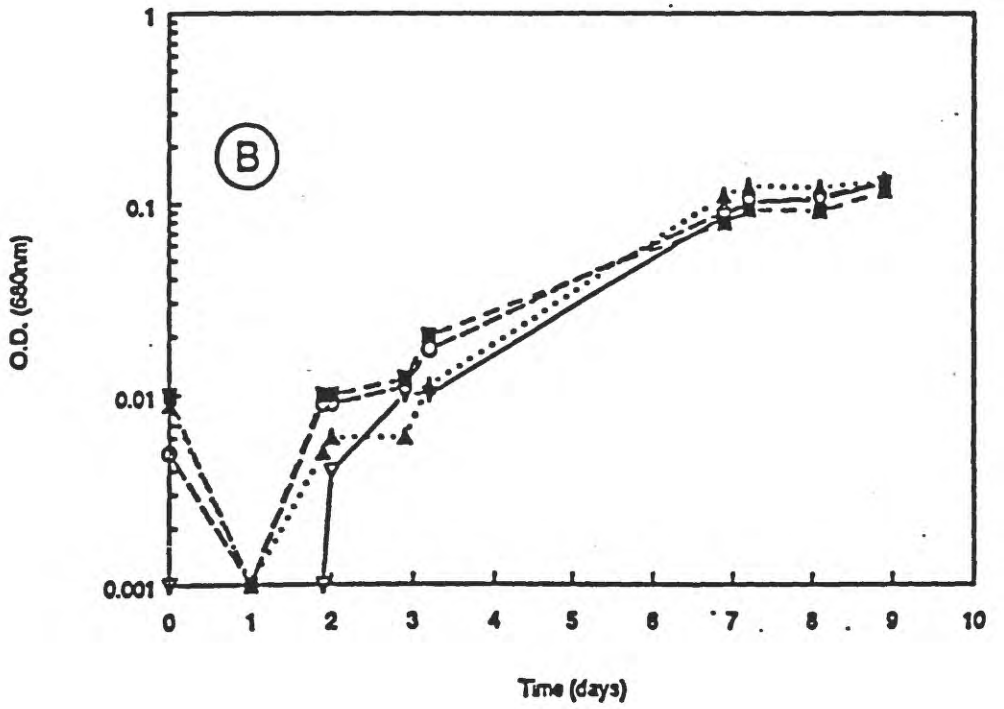


Figure 41. Effects of 0 to 1 mM TFA concentrations on (A) methanogenesis of *Methanosarcina mazei* S-6 growing on 20 mM acetate; and (B) optical density of *Desulfobacter curvatus* growing on 20 mM acetate. Both incubated statically at 28 °C. Symbols: (▽) CH_4 for cultures without TFA addition; (■) 0.1 μM TFA; (○) 10 μM TFA; (●) 100 μM TFA; (▲) 1 mM TFA.

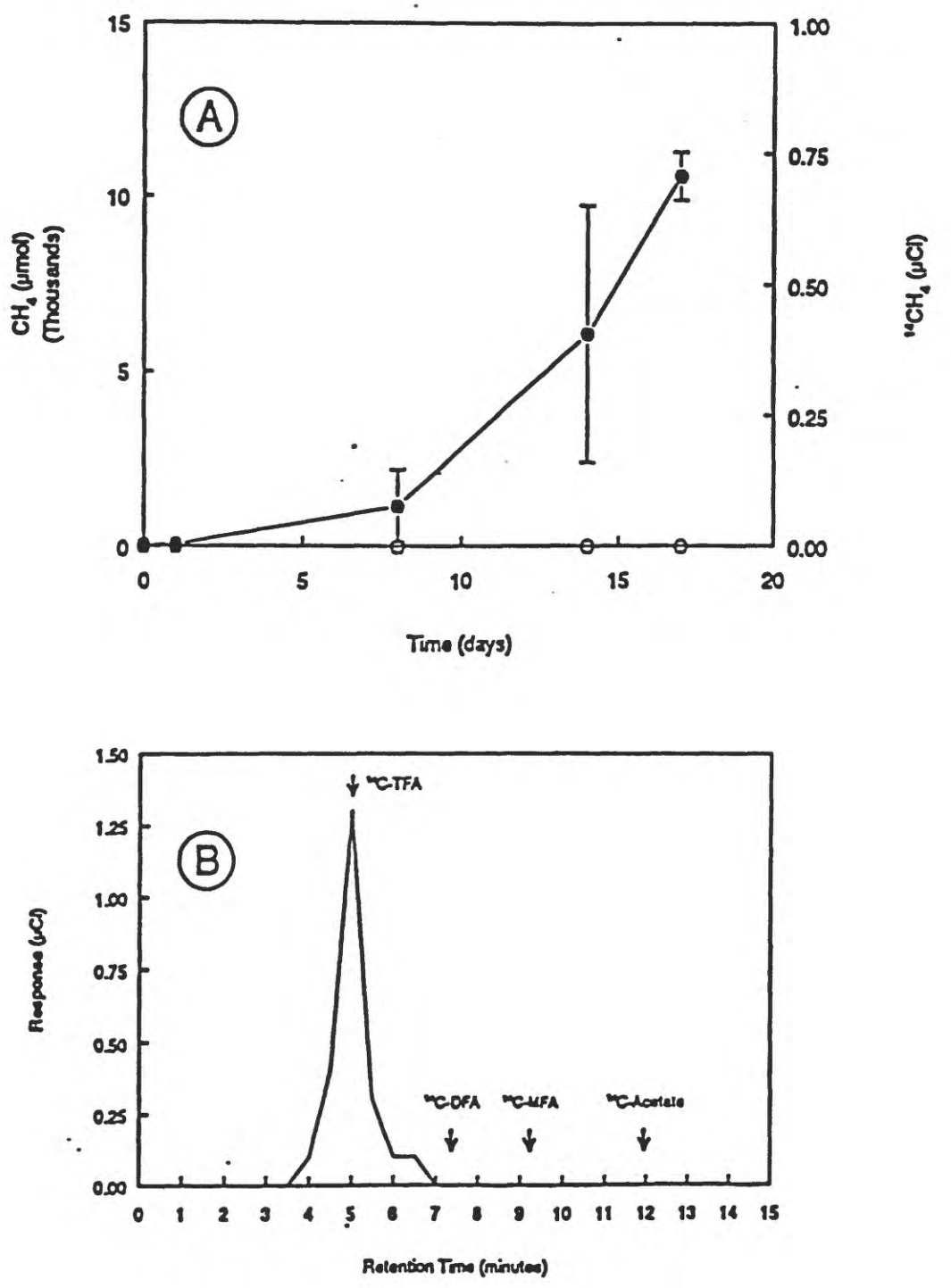


Figure 42. *Methanosarcina mazei* S-6 growing on 20 mM acetate in the presence of 0.4 µCi (0.75 µM) 2-^{14}C-TFA. (A) CH_4 and $^{14}CH_4$; (B) HPLC analysis of a liquid-phase sample taken at end of experiment, showing presence of ^{14}C-TFA and absence of ^{14}C-DFA, ^{14}C-MFA, and ^{14}C-acetate. Symbols in (A) represent the mean of three individual cultures and bars indicate ±1 standard deviation. Duplicate analyses not shown in (B).