Stable Isotopic Evidence in Support of Active Microbial Methane Cycling in Low-Temperature Diffuse Flow Vents at 9°50'N East Pacific Rise

ACCEPTED- w/ revised References

Giora Proskurowski[a,*], Marvin D. Lilley[a], E.J. Olson[a]

[a]School of Oceanography, University of Washington, Seattle, WA 98195

*Corresponding author.
E-mail Address: giora@whoi.edu (G. Proskurowski)
Current address: Woods Hole Oceanographic Institution, MS#4, Woods Hole, MA 02543

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ABSTRACT

A unique dataset from paired low- and high-temperature vents at 9°50'N East Pacific Rise provides insight into the microbiological activity in low-temperature diffuse fluids. The stable carbon isotopic composition of CH_4 and CO_2 in 9°50'N hydrothermal fluids indicates microbial methane production, perhaps coupled with microbial methane consumption. Diffuse fluids are depleted in ^{13}C by ~10‰ in values of $\delta^{13}C$ of CH_4, and by ~0.55‰ in values of $\delta^{13}C$ of CO_2, relative to the values of the high-temperature source fluid ($\delta^{13}C$ of CH_4 = -20.1 ± 1.2‰, $\delta^{13}C$ of CO_2 = -4.08 ± 0.15‰). Mixing of seawater or thermogenic sources cannot account for the depletions in ^{13}C of both CH_4 and CO_2 at diffuse vents relative to adjacent high-temperature vents. The substrate utilization and ^{13}C fractionation associated with the microbiological processes of methanogenesis and methane oxidation can explain observed steady-state CH_4 and CO_2 concentrations and carbon isotopic compositions. A mass-isotope numerical box-model of these paired vent systems is consistent with the hypothesis that microbial methane cycling is active at diffuse vents at 9°50'N. The detectable ^{13}C modification of fluid geochemistry by microbial metabolisms may provide a useful tool for detecting active methanogenesis.

1. INTRODUCTION

Although the genetic traces of suites of archaea and bacteria have been identified in hydrothermal fluids at more than 300°C (DEMING and BAROSS, 1993; HUBER et al., 2003; TAKAI and HORIKOSHI, 1999), it is unlikely that these organisms were active well above the ~120°C current upper temperature limit for life (KASHEFI and LOVLEY, 2003). While fluids at black smoker temperatures cannot be absolutely discounted as a potential microbial habitat (BAROSS et al., 2004; SCHRENK et al., 2003), it is much more plausible that these unique organisms were active in nearby lower temperature hydrothermal environments and entrained into the hot sampled fluid. Such lower temperature hydrothermal environments occur when high-temperature hydrothermal fluid is cooled, conductively or through mixing with seawater. The seafloor expressions of this low-temperature hydrothermal activity are diffusely venting fluids that provide a warm, mineral, metal, and volatile rich habitat that is amenable to microbial life.

The term "diffuse fluid", used interchangeably herein with "low-temperature fluid", explicitly refers to fluid venting directly out of cracks in the basaltic substrate (VON DAMM and LILLEY, 2004). This definition of diffuse fluid does not include fluids leaking out of vent structures or shimmering water that is a result of high-temperature fluids mixing above the seafloor. Diffuse fluids have been shown to be, primarily, a dilution of high-temperature fluids and crustal seawater (BUTTERFIELD et al., 1999; BUTTERFIELD and MASSOTH, 1994). Conductive cooling of high-temperature fluids in the shallow crust (COOPER et al., 2000), and three-component mixing between high-temperature fluids, seawater, and modified-seawater sources (RAVIZZA et al., 2001), are minor formation mechanisms of diffuse fluids.

The comparison of heat and chemical flux estimates from low-temperature and high-temperature fluids suggests a greater (BAKER et al., 1993; ELDERFIELD and SCHULTZ, 1996; SCHULTZ and ELDERFIELD, 1999) contribution to the global ocean basins from diffuse vents than from high-temperature vents. Despite the relative importance of diffuse fluids in hydrothermal systems, chemical data describing these low-temperature fluids have been sparsely reported, and the results have often been overshadowed by high-temperature revelations (BUTTERFIELD et al., 1999; BUTTERFIELD and MASSOTH, 1994). Although the groundwork for detailed biological and chemical work in diffuse fluids was laid in a comprehensive study in the late 1980's (JOHNSON et al., 1988), not until recently have diffuse fluids emerged as the focal point of hydrothermal investigations (BUTTERFIELD et al., 2004; COOPER et al., 2000; HOLDEN et al., 1998; HUBER et al., 2003; VON DAMM and LILLEY, 2004). Microbiological and geochemical interest in diffuse fluids has been invigorated in part because these fluids have been indicated as the closest environmental model for a subseafloor biosphere (SUMMIT and BAROSS, 2001). Despite the renewed focus, diffuse fluids remain challenging to study, both analytically and from a sampling standpoint.

As the name implies, diffuse fluids emanate from the seafloor in an unfocused wafting of warm water, unlike the focused flow of high-temperature fluids venting directly out of a chimney orifice. Thus, obtaining a sample of diffuse fluid without entraining large quantities of seawater is extremely rare, and diffuse samples are typically 90-95% seawater. The seawater dominated composition of diffuse fluids is problematic because large extrapolations (to a seawater-free endmember) are required to directly compare low-temperature and high-temperature fluid concentration data. A critical challenge in the evaluation of the microbiological impact on the chemistry of diffuse fluids is accurately assessing the modifications to the high-temperature

parent fluid, a difficult task when using considerably manipulated concentration data. Because the effects of mixing and dilution are inherently recorded in isotopic data, the error and complexity in normalizing concentration data is muted or absent when comparing diffuse and high-temperature stable isotope data.

Many biological and chemical processes preferentially utilize ^{12}C relative to ^{13}C, leaving substrates enriched in ^{13}C and metabolic products depleted in ^{13}C. Thus, evidence for microbial metabolic activity may be preserved in the isotope record. We attribute the differences in the carbon isotopic composition of CH_4 and CO_2 between the low- and high-temperature fluids to microbiological modifications by methanogens and, perhaps, methanotrophs. As many biological and chemical processes preferentially utilize ^{12}C relative to ^{13}C, microbial metabolisms typically yield substrates enriched in ^{13}C and metabolic products depleted in ^{13}C. Methanogens, obligate anaerobic archaea that produce CH_4 during the metabolism of an oxidized carbon source, are commonly detected and isolated from hydrothermal environments (JANNASCH, 1995). Methanotrophy, the microbial oxidation of methane, is mediated by methanotrophic bacteria in aerobic environments and by obligate anaerobic methanotrophic archaea in anaerobic environments. Although anaerobic methanotrophs have been identified in anoxic sediments (HINRICHS et al., 2000), indications that this group is active at hydrothermal vent environments is scarce, as anaerobic methane oxidizers have only been detected at the sedimented hydrothermal site Guaymas (TESKE et al., 2002).

To decipher the impact of microbial activity in hydrothermal fluids, links between specific microbial groups and specific geochemical modifications must be established. This requires the integration and reconciliation of molecular methods, experimental culture work, and geochemical fluid analysis. The intention of this manuscript is to present an isotopic dataset

suggestive of active microbial methanogenesis and methane oxidation in diffuse hydrothermal fluids as a baseline example of the microbiological influence on the volatile geochemistry of hydrothermal vent systems.

Here we present the results of a natural experiment examining the carbon isotopic composition of low-temperature fluids that are located proximal to, and are a chemical dilution of, a high-temperature "parent" fluid. We attribute the isotopic depletions in $\delta^{13}C$ of CH_4 from low-temperature fluids, relative to values from high-temperature fluids, to microbial methanogenesis. Despite limited microbiological evidence of methanotrophy in hydrothermal environments, we hypothesize that the observed isotopic depletions in $\delta^{13}C$ of CO_2 from low-temperature fluids, relative to values from high-temperature fluids, is suggestive of active methanotrophy.

2. MATERIALS AND METHODS

2.1. Geologic setting

The 9°50'N area of the EPR is perhaps the best example of a prototypical fast-spreading ridge (ALT, 1995). The symmetric ridge profile shallows to ~2500m water depth at the axis, and is characterized by a full spreading rate of 11 cm/yr (CARBOTTE and MACDONALD, 1992), a shallow magma chamber located ~1 km beneath the seafloor (DETRICK et al., 1987), extensive evidence of volcanism (FORNARI et al., 2004), and an ~60 m wide axial summit trough (AST) that supports active hydrothermal vents.

The area between 9°46'-51'N was impacted by seafloor eruptive events in 1991 and 1992 (HAYMON et al., 1993), triggering a series of research expeditions to chronicle the dynamic

chemical, biological, and geological responses to this volcanic perturbation. In the months preceding April 2006 a series of earthquakes signaled eruptive activity at this intensively studied area, and a series of response cruises sponsored by the Ridge2000 program verified extensive volcanism (COWEN et al., 2007; LILLEY et al., 2006; VON DAMM et al., 2006). At the time of writing, it is unclear the extent to which the eruptive events have modified the specific vent sites described here. It is important to note that all data and descriptions of vent sites presented here are based on measurements and observations prior to the most recent eruptive events, and may not accurately depict their present state.

Samples analyzed herein are from vents located within the AST along an ~8 km stretch of the EPR between 9°46'-51'N (Figure 1). Two diffuse vents, Bio9R and Y, are ideally suited to examining the processes that modify high-temperature fluids in diffuse flow environments. Bio9R and Y vents are located directly adjacent to the high-temperature vents Bio9 and TWP, respectively. The diffuse vent Biomarker9 *Riftia* (Bio9R) adjoins the Bio9 vent site; Y vent is diffusely venting at the base of a lava pillar upon which the TWP sulfide structure and vent are located. The close proximity of low- and high-temperature fluids, and the absence of other vent sites within 10m of Bio9/Bio9R and 20m of TWP/Y, allows for the safe assumption that the diffuse fluid (Bio9R, Y) is the diluted and modified analog of the proximal high-temperature fluid (Bio9, TWP) (VON DAMM and LILLEY, 2004).

2.2. Sample collection and analysis

Accurate analysis of hydrothermal volatiles requires that both diffuse fluid samples and high-temperature black smoker samples be obtained using "gas-tight" sampling vessels. As samples are collected under high hydrostatic pressures, gas-tight samplers (GT) are designed to prevent

degassing of the fluid during the subsequent release of pressure upon ascent. The gas-tight samplers (GT) employed in this study are fixed volume samplers that maintain the integrity of the fluid sample until it is quantitatively transferred to a shipboard vacuum extraction line where the volatile fraction is quantitatively removed. The GT samplers are constructed entirely of titanium and enclose a 150 mL sample volume that is spring sealed and hydraulically opened using a gold tipped piston that ensures degassing does not occur upon ascent. Gas-tight, samplers are most often deployed in high-temperature vent work as discrete samples where the ¼-inch titanium tube inlet is positioned in the vent orifice and triggered by the vehicle's manipulator arm. All samples presented here were collected using the DSV *Alvin*; the sample number reflects the Alvin dive number followed by the numerical identity of the gas-tight sampler employed. Due to the low flow rates and poorly defined orifices of diffuse vents, the acquisition of good-quality diffuse samples requires special consideration. All of the low-temperature fluids presented here were obtained using GT vessels mounted on the NOAA manifold system described in Massoth (1988) and Von Damm and Lilley (2004). The manifold system pumps diffuse fluids into a titanium manifold where temperature is monitored as a quality control, and multiple GT sample bottles may be triggered once a stable temperature has been reached.

Once shipboard, high-temperature and diffuse vent fluid samples were processed identically using the extraction procedure described here. Using internal standards this method was shown to be insensitive to the volatile concentrations, a concern as high-temperature samples can have orders of magnitude more gas, by volume, than diffuse samples. The fluid was quantitatively extracted from the GT sampler on a portable titanium and glass vacuum line. Extracted fluid was acidified to convert all carbonate species to $CO_{2(aq)}$, agitated ultrasonically to

move >97% of the CO_2 into the gas phase. This gas was subsequently passed through a -60°C cold trap to remove water, and, using a mechanical bellows pump, moved to a set of calibrated volumes. After manometric determination of the volume, several aliquots were preserved in 30mL pyrex breakseal vials. Remaining degassed fluid was stored in acid rinsed HDPE bottles for shore-based determination of normalization elements Mg and Si using methods described in Von Damm (2000).

In a land-based laboratory glass vacuum line, breakseals were broken and the volatile samples were manipulated so that pure fractions of CO_2 and CH_4 could be measured by isotope-ratio mass spectrometery (IRMS). A bellows pump was used to adjust the volume of sample to be analyzed, ideally ~500uL CO_2 and ~200nL CH_4. After passing through a slush trap (to remove water), the condensable fraction (CO_2, H_2S) was frozen out in a liquid nitrogen trap, and methane was trapped in a valved u-trap on silica gel under liquid nitrogen temperatures. The non-condensable fraction (primarily H_2, and N_2) was slowly evacuated using a metered valve to vacuum. The methane fraction frozen in the u-trap was analyzed on a continuous flow Finnigan Delta+XL mass spectrometer, using a combustion and pre-concentrating inlet system (BRAND, 1996). The CO_2 and H_2S remaining in the vacuum line were separated by passing the gas over powdered Ag_3PO_4, effectively removing all H_2S by producing solid Ag_2S. The CO_2 was frozen into a valved finger and analyzed on a dual-inlet Finnigan 251 mass spectrometer. Using internal gas standards, mixed to approximate a hydrothermal sample and processed using the described method, analytical error was found to be ±0.3‰ for $\delta^{13}C$ of CH_4 and better than ±0.05‰ for $\delta^{13}C$ of CO_2. All samples were measured in triplicate, and the mean standard deviation of all triplicate measurements was in agreement with error analysis by internal

standards. All measured $^{13}C/^{12}C$ ratios are reported in standard δ notation and ‰ units, referenced to the vPDB scale.

2.3. Data reduction and normalization

During sampling, seawater is inevitably entrained into the sampler, and thus measured concentrations do not accurately reflect the composition of the pure vent fluid. For high-temperature samples, endmember concentrations presented here are calculated using established methods that assume pure vent fluid has a zero Mg concentration, and therefore seawater mixing during sampling is the source of all Mg present in the sample (BUTTERFIELD et al., 1994; LILLEY et al., 1993; VON DAMM, 2000). This method is effective for samples where minor amounts of seawater have been entrained during sampling because endmember values require only a small extrapolation. However, for diffuse fluids, where samples may only be a few percent by volume vent fluid, large extrapolations are required, and an alternate method for calculating endmember concentrations is preferred. The high precision of aqueous silica measurements (relative to magnesium measurements), and smaller extrapolations used in Si normalization (relative to Mg=0 extrapolations) encourages the use of Si to normalize fluid data in the comparison of high-temperature and diffuse fluids (VON DAMM and LILLEY, 2004). Here we assume that vent fluid Si is conservatively mixed with a 0.155 mmol/kg seawater endmember (as measured from sample 3547-12, a background seawater sample from 9°50'N). While Von Damm and Lilley (2004) normalized all fluid data to Si=1 mmol/kg, a value that approximates the Si concentrations measured in low-temperature fluids, here we normalize the low-temperature data to the endmember Si concentration of the adjacent high-temperature fluid. Although this method

of Si-normalization involves larger extrapolations for the low-temperature data (compared with normalization to Si=1 mmol/kg), this approach has the advantage that endmember high-temperature gas concentrations can be directly compared with previously reported endmember hydrothermal volatile data.

It should be noted that endmember CO_2 concentrations and $\delta^{13}C$ values of CO_2 have been corrected only for the seawater bicarbonate entrained during sampling and not for seawater bicarbonate that was present in the initial fluid. Although previous studies have made additional corrections to endmember CO_2 values to include the seawater bicarbonate contained in the original parcel of downwelled fluid (EVANS et al., 1988; LILLEY et al., 1993; WELHAN and LUPTON, 1987), recent evidence from the Endeavour hydrothermal system (PROSKUROWSKI et al., 2004), Baby Bare (SANSONE et al., 1998) and several ODP drill cores (ALT and TEAGLE, 1999), suggest that correcting for original seawater bicarbonate is not appropriate. The diffuse samples presented here have low measured CO_2 concentrations and high Mg concentrations, such that the molar amount of seawater bicarbonate entrained during sampling (as determined by Mg) added to the 2.3 mmol/kg of bicarbonate assumed to be present in the original fluid accounts for nearly all, and in some cases, more than, the CO_2 measured in the sample. This untenable result supports the hypothesis that seawater bicarbonate originally present in the downwelled fluid has been removed prior to venting at the seafloor. Assuming that the original bicarbonate has been removed, and applying just the Mg correction for entrained seawater to the ^{13}C measurement yields internally consistent values for the Y and Bio9R vents, whereas additional corrections for original bicarbonate result in implausible and inconsistent isotope values.

Although the low-temperature samples have near-seawater Mg values, the correction applied to the ^{13}C isotope value of CO_2 is done by the isotope-mass balance approach shown below, and does not involve large extrapolations:

$$R_{measured}[CO_2]_{measured} = R_{vent}[CO_2]_{vent} + R_{entrained}[CO_2]_{entrained} \quad (1)$$

$$[CO_2]_{measured} = [CO_2]_{vent} + [CO_2]_{entrained} \quad (2)$$

Where $R=^{13}C/^{12}C$, $R_{measured}$ and $[CO_2]_{measured}$ denote the raw measured vent fluid values, R_{vent} and $[CO_2]_{vent}$ denote the values for the pure vent fluid component, and $R_{entrained}$ and $[CO_2]_{entrained}$ denote the values for the entrained seawater bicarbonate component. The mass balance is solved for R_{vent}, as all other variables are known, given that $R_{entrained}$ was measured at 0.9996 (-0.40‰), and:

$$[CO_2]_{entrained} = \frac{[Mg]_{measured}}{[Mg]_{SW}}[CO_2]_{SW} \quad (3)$$

Where $Mg_{measured}$ is the measured Mg value reported in Table 1, Mg_{SW} is the measured background seawater Mg value (52.2 mmol/kg) and $[CO_2]_{SW}$ is the measured background seawater bicarbonate concentration (2.3 mmol/kg). The errors, involved in this approach are ±0.2‰ as determined by a Monte Carlo error analysis, and are tied mainly to the precision of the Mg measurement. Isotope values for CH_4 are not corrected, as seawater CH_4 concentrations are, at least, three orders of magnitude smaller than measured vent CH_4 concentrations.

Uncorrected (measured) data are presented in Table 1, and endmember data (concentrations of high-temperature fluids normalized to Mg=0; concentrations of low-temperature fluids normalized to the endmember Si value of the proximal high-temperature vent; and CO_2 isotope values corrected for the contribution of entrained seawater bicarbonate) are

presented in Table 2. Unless specifically noted, all subsequent discussion of data pertains to the endmember values.

3. RESULTS

3.1. Endmember gas concentrations

Endmember gas concentrations and isotope compositions from high-temperature black smoker vents (Bio9, TWP) and proximal low-temperature diffuse flow vents (Bio9R, Y) from the BIOGEOTRANSECT study area near 9°50'N along the EPR are presented in Table 2. For comparative purposes, original data from Q, A, and V vents (located within ~5 km the Bio9-Bio9R vents) and previously published data from sites along the East Pacific Rise, the Mid-Atlantic Ridge, and the Juan de Fuca Ridge are also presented. These samples represent a sub-set of a time series beginning in 1991, directly following a large eruptive event centered at 9°50'N (HAYMON et al., 1993), and ending in 2000.

The high-temperature vents in the study region have high endmember CO_2 concentrations, ranging from 45 mmol/kg up to 185 mmol/kg. The lowest 9°50'N CO_2 values approximate the observed gas concentrations at Endeavour during periods of sub-seafloor magmatism (LILLEY et al., 2003), and are significantly greater that 5-20 mmol/kg values typically reported from hydrothermal vents (VON DAMM, 1995). The highest CO_2 concentrations reported here aproximate the high values reported from Axial Seamount, a volcanically active vent site believed to have a near-continuous supply of magmatic CO_2 (BUTTERFIELD et al., 1990; BUTTERFIELD et al., 2004). The low-temperature diffuse vents at 9°50'N also exhibit high CO_2 concentrations when normalized to the Si content of the neighboring high-temperature vent. In general, the CO_2 concentration of the diffuse fluid approximates the concentration of the

proximal high-temperature endmember; however, the large extrapolations used in calculating diffuse endmember concentrations discourage a more detailed assessment.

Endmember CH_4 concentrations range from 0.05 to 0.12 mmol/kg for post-1991 high-temperature samples (Bio9 and TWP) and 0.06 to 1.87 mmol/kg for post-1991 low-temperature samples (Bio9R and Y). Methane concentrations in 1991, sampled directly after eruptive activity, are ubiquitously high, with the high-temperature vent samples averaging 0.13 mmol/kg and a 1991 mean diffuse vent CH_4 concentration of 5.86 mmol/kg. The 1991 Y and V vent values (10.20 and 1.52 mmol/kg, respectively) are similar to the mmolar CH_4 levels at sediment influenced vents such as those at Main Endeavour Field (LILLEY et al., 1993) and Guaymas Basin (VON DAMM et al., 1985; WELHAN and LUPTON, 1987). Methane concentrations of post-1991 Y vent samples are two to three times those of TWP, the adjacent high-temperature vent, while the low-temperature Bio9R vent has similar CH_4 concentrations to its high-temperature analog, Bio9 vent. The low CH_4 values, 0.05-0.16 mmol/g, associated with many of the vents near 9°50'N are similar to reported values along the EPR at 11°N, 13°N and 21°N (LILLEY et al., 1983; MERLIVAT et al., 1987; WELHAN and CRAIG, 1979; WELHAN and CRAIG, 1983), the Mid-Atlantic Ridge (MAR) at MARK, TAG and Broken Spur (CHARLOU et al., 1996; JAMES et al., 1995; JEAN-BAPTISTE et al., 1991), and the Juan de Fuca Ridge at South Cleft and Axial Volcano (BUTTERFIELD et al., 2004; EVANS et al., 1988).

Hydrogen concentration data reveal two trends: 1) low-temperature samples have extremely depleted H_2 levels compared with their high-temperature analogs, and 2) elevated H_2 concentrations observed in 1991 high-temperature samples systematically decrease in subsequent years. Hydrogen concentration plotted against measured vent temperature (Figure 2) clearly shows the difference in hydrogen concentration between high-temperature and diffuse vents.

Highly extrapolated endmember H_2 concentrations of 9°50'N low-temperature vents range from 0.01 mmol/kg to 0.16 mmol/kg, values that are low compared to basalt hosted sites not influenced by recent seafloor eruption events (BUTTERFIELD et al., 2004; CHARLOU et al., 2000; CHARLOU et al., 2002; EVANS et al., 1988; LILLEY et al., 1993; VON DAMM, 1995). Post eruptive high-temperature vents exhibit H_2 concentrations of 0.08 to 0.51 mmol/kg, excluding the anomalously high 3.42-8.36 mmol/kg concentrations observed at TWP. Hydrogen concentrations during periods of eruptive activity in 1991 and 1992 are similar to those observed at TWP, ranging from 1.40-2.80 mmol/kg at Bio9, to 3.01 mmol/kg at Q vent, and greater than 27 mmol/kg at A vent, the site of the highest reported hydrothermal vent H_2 concentrations (LILLEY et al., 2003).

3.2. Stable carbon isotopic composition

The carbon isotopic compositions of methane and carbon dioxide from 9°50'N samples are summarized in Figure 3 and Table 2. The reported $\delta^{13}C$ values of CO_2 have been corrected to a zero Mg endmember composition using measured background EPR seawater CO_2 concentration and $\delta^{13}C$ value of CO_2 (2.3 mmol/kg and -0.40‰, respectively). High-temperature vents sampled between 1992 and 2000 have a well-constrained carbon isotopic composition, a $\delta^{13}C$ value of CO_2 of -4.08 ± 0.16‰ and a $\delta^{13}C$ value of CH_4 of -20.1 ± 1.2‰. Compared to neighboring high-temperature vents, 9°50'N diffuse fluids are depleted in ^{13}C in both CO_2 and CH_4, with an average $\delta^{13}C$ value of CO_2 of -4.55 ± 0.53‰, and an average $\delta^{13}C$ value of CH_4 of -30.2 ± 2.7‰. Due to extremely large amounts of high-temperature fluids exiting the crust through cracks and fissures, rather than well-defined vent orifices (HAYMON et al., 1993), focused flow fluids were difficult to sample in 1991. Samples from the Bio9 cluster (368°C) and

A vent (396°C), taken shortly after the 1991 seafloor eruption, are isotopically distinct from samples taken on subsequent visits. Methane from Bio9 in 1991 (a $\delta^{13}C$ value of -34.6‰) is depleted in ^{13}C relative to high-temperature samples subsequent to 1991, resembling the methane isotopic composition of diffuse samples. Similarly, 1991 A vent CH_4 ($\delta^{13}C$ value of -26.5‰) is more depleted in ^{13}C relative to any other high-temperature vent CH_4 measured in this study.

The $\delta^{13}C$ values of CO_2 presented here are among the heaviest measured at hydrothermal vents (CHARLOU et al., 2002; CHARLOU et al., 1996; JEAN-BAPTISTE et al., 1991; LILLEY et al., 1993; SHANKS et al., 1995; TAYLOR, 1986; WELHAN and CRAIG, 1983; WELHAN and LUPTON, 1987), and are very similar to the -4‰ values measured in highly vessiculated basalt "popping rocks" thought to represent the average composition of undegassed CO_2 residing in the upper crust (JAVOY and PINEAU, 1991; PINEAU and JAVOY, 1994; SARDA and GRAHAM, 1990). The high concentrations of CO_2 enriched in ^{13}C relative to other hydrothermal sites suggest an undegassed, primordial, magmatic source of CO_2. This conclusion is supported by the geologic evidence of fast spreading rate (CARBOTTE and MACDONALD, 1992), active volcanism (FORNARI et al., 2004; FORNARI et al., 1998; HAYMON et al., 1993; VON DAMM, 2000; VON DAMM et al., 1995), and shallow heat source (LILLEY et al., 2003; VON DAMM, 2004) at 9°50'N. The constant CO_2 isotope and concentration values at high-temperature vents over the 7+ year sampling period imply that the magmatic source of CO_2 is often replenished so that the melt reservoir is not appreciably degassed, a process that would deplete both the concentration and the isotopic composition of CO_2 (BUTTERFIELD et al., 1990; JAVOY et al., 1978; KELLEY and FRÜH-GREEN, 1999; KELLEY and FRÜH-GREEN, 2000; KELLEY and FRÜH-GREEN, 2001).

4. DISCUSSION

The aim of this manuscript is to report the isotopic compositions of CH_4 and CO_2 at diffuse vents and proximal high-temperature sites, and interpret the observed isotopic depletions of the low-temperature volatiles in the context of relevant chemical, and biological processes. The sample set presented here was carefully chosen to create a seafloor experiment with the high-temperature fluid as the control. The close proximity (<1m) of TWP to Y vent and of Bio9 to Bio9R, as well as the absence of other vents within 20m of TWP/Y and 10m of Bio9/Bio9R, suggests that the low-temperature fluid is derived locally from the high-temperature fluid. Furthermore, the ratios of conservative tracers such as temperature, Si, and Mn (VON DAMM, 1995) are invariant (for T/Si and Mn/Si) between the high-temperature fluid and the diffuse fluid, supporting the hypothesis that the diffuse fluids at Bio9R and Y vents are a dilution of the high-temperature endmember (VON DAMM and LILLEY, 2004). In contrast, concentrations of bio-active and chemically reactive elements such as Fe, H_2S, CO_2, CH_4, and H_2 show dramatic differences between the low-temperature and high-temperature vent environments. Von Damm and Lilley (2004) conclude that iron in diffuse fluids is lost to mineral precipitation, and that biological activity at the low-temperature sites leads to consumption of H_2S, H_2, and CO_2 and production of CH_4. Here we present isotopic data that support Von Damm and Lilley's (2004) hypothesis of microbial methanogenesis at the diffuse sites and further suggest that microbial methanotrophy may be an active process in the low-temperature fluids.

4.1. Potential Mechanisms of Isotopic Modification of Vent Fluids

The depletions in ^{13}C of CH_4 and CO_2 observed at diffuse sites relative to high-temperature sites at 9°50'N vent sites may be attributed to three mechanisms: temperature dependent isotopic

equilibration, the mixing of isotopically distinct sources, and isotopic fractionation during chemical or metabolic reactions.

Temperature dependent isotopic fractionation can be confidently ruled out as a mechanism to deplete the carbon isotopic composition of low-temperature fluids based on data presented in Figure 3. The expected isotopic fractionations at given temperatures are plotted based on the calculations of Horita (2001), demonstrating that the isotopic composition of neither the high-temperature (300-400°C) nor the low-temperature fluids (20-55°C) is accurately approximated by the isotopic equilibrium temperature. The fractionation between CO_2 and CH_4 from high-temperature samples from 9°50'N (with the notable exception of the 1991 sample) is compatible with equilibrium fractionation at temperatures ranging from 420-585°C. While these predicted temperatures fall within the range between the highest measured hydrothermal vent temperatures and temperatures observed in deeply penetrating hydrothermal fluids (GILLIS and ROBERTS, 1999; MANNING et al., 1996), they are, on average, 140°C greater than the corresponding measured temperature. At low-temperature sites the equilibrium isotopic fractionation predicted temperatures ranges 240-375°C, with the predicted temperature being, on average, 270°C higher than the measured value. The result of apparent CO_2-CH_4 isotopic disequilibrium is not unexpected, as the kinetics of the uncatalyzed CO_2-CH_4 isotope exchange reaction at temperature less than 400-500°C, and the metal catalyzed reaction at temperatures less than 200°C, are exceedingly slow in laboratory experiments (HORITA, 2001; SACKETT, 1993). The increasingly sluggish kinetics of CO_2-CH_4 isotope exchange as temperature decreases precludes temperature dependent isotope fractionation at diffusely venting low-temperature sites, and suggests that CO_2-CH_4 carbon isotopic equilibrium is not well established even at high-temperature vent sites.

The mixing of isotopically distinct sources is a common mechanism by which the isotopic signature of a natural sample can be modified. For the purposes of this discussion "mixing" refers to the addition of externally sourced CO_2 and CH_4 to the high-temperature vent fluid (rather than CO_2 and CH_4 produced by thermal or biological processes within the high-temperature fluid and subsequently incorporated). Because the low-temperature fluids are depleted in ^{13}C in both CH_4 and CO_2 relative to the high-temperature fluid, an external source must be depleted in ^{13}C in both CH_4 and CO_2 relative to the low-temperature fluid if a mixing model is assumed. Seawater cannot be this external source, as there is no appreciable CH_4 contribution from seawater, and seawater bicarbonate is enriched in ^{13}C relative to the high-temperature CO_2. Moreover, because the mixing trajectories described by the $\delta^{13}C$ values of CH_4 and CO_2 of the Bio9/Bio9R and Y/TWP vent pairs are divergent, a mixing model with a single external source is not a viable model. Thus, either a) multiple sources, variably depleted in ^{13}C in both CO_2 and CH_4, relative to the high-temperature samples, are available for mixing at each site, or b) reaction based fractionating mechanisms must be considered to explain the observed depletions in ^{13}C at the diffuse sites relative to the high-temperature sites.

We suggest that there are only two possible external sources of CO_2 depleted in ^{13}C relative to the -4.5 to -3.7‰ $\delta^{13}C$ values of CO_2 from 9°50'N high-temperature vents: highly evolved magmatic CO_2 and thermogenic CO_2. Magmatic CO_2 becomes increasingly depleted in ^{13}C as it degasses (BOTTINGA and JAVOY, 1989; PINEAU and JAVOY, 1983; PINEAU et al., 1976); however, a scenario where highly evolved magmatic CO_2 is input solely to diffuse fluids is extremely unlikely. Therefore, the thermal maturation of organic matter, producing both CO_2 and CH_4, is required if a mixing model is to be invoked to describe the isotopic data.

4.2. Assessing a Thermogenic Source of CH_4 and CO_2 to Bio9R and Y vents

We suggest that thermogenic production of carbon at 9°50'N diffuse vents is unlikely to be more than a minor source for the following reasons: a) the lack of a stable and sufficeintly large source of organic carbon, b) the lack of increased nutrient concentrations in diffuse vents, suggesting that fresh biomass is not being pyrolyzed, c) the thermal alteration of organic matter is constrained to temperatures above 50°C and, d) the observed depletions in ^{13}C of CO_2 in diffuse fluids relative to high-tempearture fluids are not adequately described by a thermogenic source.

The lack of sediments along the fast-spreading ridge precludes the necessary accumulation of sedimentary organic matter (HAYMON et al., 1993). Despite the lack of sediments along the ridge axis the potential for organic matter to interact with hydrothermal fluids does exist. In 1991, a 1-10 cm thick lava flow at the aptly named Tubeworm BBQ site (<1km north of Bio9) was observed to envelop areas colonized by vent megafauna (HAYMON et al., 1993), creating a potential reservoir of organic matter. However, the organic matter reservoir created by infrequent eruptive events is likely too small and too episodic to account for the steady-state isotopic depletion seen in diffuse fluids six years after the 1991 eruption.

Another potential source of organic carbon to this system is dissolved organic carbon (DOC), which has typical deep Pacific seawater concentrations of 33-36 μmol/kg (HANSELL and CARLSON, 1998; LANG et al., 2006). Measurements from the Main Endeavour Field show that high-temperature fluid DOC concentrations (~14 μmol/kg) are less than seawater concentrations, while diffuse fluid DOC concentrations (~46 μmol/kg) are greater than seawater concentrations (LANG et al., 2006). Thermal degradation of DOC at high-temperatures is hypothesized as a potential sink of DOC at high-temperature vent sites; however, elevated DOC concentrations at

diffuse sites suggest that these thermogenic processes are not active at low-temperatures and that biological production is the likely source of the additional DOC (LANG et al., 2006). Even if seawater DOC was being thermogenically converted to CH_4 in diffuse fluids, a ~30 μmol/kg DOC source of organic carbon is still only a fraction of the 100-260 μmol/kg differences in CH_4 concentrations observed at TWP/Y vents.

Low-level "nutrient" concentrations argue against a thermogenic source of CH_4 via the pyrolysis of microbial biomass at 9°50'N diffuse vents. The pyrolytic products of living biomass should include nitrogen and phosphorous approximating the Redfield ratio. However, measurement of NH_4^+ and PO_4^{-3} concentrations show no enrichment in 9°50'N diffuse fluids when compared to the high temperature fluid and are often depleted relative to the background seawater value (VON DAMM and LILLEY, 2004).

The potential for thermogenic CH_4 and CO_2 production by the pyrolysis of microbial biomass or DOC exists where microbial communities intersect transient fluxes of heat, such as the shallow ocean crust and, in particular, diffuse flow sites. However, the thermogenic production of volatile carbon during sediment diagenesis at less than 50°C is extremely limited (HUNT, 1995; SCHIMMELMANN et al., 2006; SEEWALD, 2003). Analysis of organic matter exposed to temperatures of 50-80°C at Middle Valley, a sedimented hydrothermal system along the northern segment of the Juan de Fuca Ridge, showed the absence of typical petroleum markers of thermogenic activity such as polycyclic aromatic hydrocarbons (SIMONEIT et al., 1992). These results suggest that a possible source of thermogenic volatiles to the diffuse vents described here must be constrained to a stable zone of mixing 80-400°C with a steady supply of organic matter. Despite counter-indications from direct seafloor observations, it is concievable that thermogenically favorable conditions do exisit at 9°50'N, such as in sub-surface

pockets rich in microbial biomass. However, we suggest that the observed $\delta^{13}C$ values of CO_2 are not compatible a thermogenic source. Thermal organic matter degradation experiments at temperatures >100°C, show that in addition to CH_4, CO_2 is produced in a near 1:1 ratio, with $\delta^{13}C$ values of CO_2 -15 to -5‰ (ANDRESEN et al., 1993; CHUNG and SACKETT, 1979; HUNT, 1995). Considering the maximum increase in CH_4 between a high-temperature fluid and its diffuse fluid analog is 0.2 mmol/kg, mixing a similar amount of byproduct CO_2 with the large (100+ mmol/kg) reservoir of CO_2 present in the fluid would deplete the $\delta^{13}C$ values of CO_2 by a maximum of 0.03‰, an order of magnitude less than the 0.5‰ mean isotopic shift observed.

If a thermogenic source is only a minor contributor to the isotopic depletions at Y and Bio9R vents, then a pure mixing model between high-temperature endmembers and external CH_4 and CO_2 sources cannot fully describe the observed isotopic data.

4.3. Origin of CH_4 at 9°50'N

High-temperature samples are characterized by low (<0.1 mmol/kg) CH_4 concentrations and $\delta^{13}C$ values of CH_4 averaging -20‰, strongly suggesting that the CH_4 is of magmatic origin. Methane venting at high-temperature 9°50'N sites is similar in stable carbon isotopic composition to fluids measured at unsedimented mid-ocean ridge hydrothermal systems, such as the Southern Juan de Fuca Ridge (-20.8 to -17.8‰) (EVANS et al., 1988), the EPR at 21°N (-17.6 to -15.0‰) (WELHAN and CRAIG, 1983), the EPR at 13°N (-19.5 to -16.6‰) (MERLIVAT et al., 1987), the MAR at Broken Spur (-19 to -18‰) (JAMES et al., 1995), and the MAR at Menez-Gwen (-19.6 to -18/8‰) (CHARLOU et al., 2000) (see Table 2).

In order to explain the general trend of diffuse volatiles being depleted in ^{13}C relative to high-temperature samples, we invoke the microbial processes of methanogenesis and methane

oxidation. Methanogenesis must be the dominant microbial process as both low-temperature sites presented in this study are characterized by elevated CH_4 concentrations and decreased H_2 concentrations relative to their high-temperature analog. Methanogenesis, the microbial reduction of low-molecular weight oxidized carbon species (primarily CO_2 and acetate) to methane, has been cited as a classic example of high-temperature chemolithoautotrophy since the first hyperthermophile was cultured from 21°N EPR hydrothermal vent fluid (JONES et al., 1983). Methanogens are obligate anaerobes that include mesophiles, thermophiles, and hyperthoermophiles (WHITMAN et al., 1992). In addition to the successful isolation and enrichment of methanogens from ridge-crest low-temperature environments (HOLDEN et al., 1998; JEANTHON et al., 1999; JEANTHON et al., 1998; JONES et al., 1989; SUMMIT and BAROSS, 1998), methanogens have been detected using molecular methods in the high-temperature samples from vent fluids and sulfide chimneys (HUBER et al., 2002; SCHRENK et al., 2003; TAKAI and HORIKOSHI, 1999).

Microbiological methanogenesis, in its simplest form, proceeds according to the straightforward reduction of carbon dioxide:

$$CO_2 + 4H_2 \Leftrightarrow CH_4 + 2H_2O \qquad (3)$$

While an alternate, aceticlastic, pathway is important in many methanogenic environments, the reduction of CO_2 is favored over acetate in moderately thermal environments (FEY et al., 2004), and extreme thermophiles capable of aceticlastic methane production have yet to be isolated (VALENTINE et al., 2004). As illustrated in the above reaction, biogenic methane production consumes large amounts of hydrogen relative to the carbon dioxide reduced or the methane produced. At the Bio9/Bio9R and TWP/Y sites milli-molar decreases in hydrogen concentration at the diffuse site relative to the high-temperature site do not consistently correspond with the

appropriate differences in methane and CO_2 concentrations. The lack of a precise stoichiometric CH_4 gain and CO_2 loss relative to hydrogen consumption during methanogenesis is likely due to the error involved in calculating comparable high-temperature and low-temperature endmember concentrations, and may be further obscured by the potential for hydrogen consumption by other microbial processes (e.g. thermophilic sulfate reduction).

Unlike the concentration data, the ^{13}C composition of CH_4 is unaffected by seawater mixing and thus can be a sensitive and robust indicator of microbial activity. Figures 4 and 5 contrast the isotopic composition of adjacent high-temperature and diffuse fluids, illustrating the concentration and isotopic differences between Y and TWP and Bio9R and Bio9 fluids. The low-temperature fluid at Y vent is depleted in ^{13}C by an average of 11.7‰ in $\delta^{13}C$ of CH_4, and 0.37‰ in $\delta^{13}C$ of CO_2, relative to TWP over a sampling period of three years. Similar results are observed at Bio9R, where the low-temperature fluid is depleted in ^{13}C by 4.3‰ in $\delta^{13}C$ of CH_4, and by 0.90‰ in $\delta^{13}C$ of CO_2, relative to samples taken at Bio9 between 1994 and 1997.

Natural methane samples with $\delta^{13}C$ values of CH_4 less than -50‰ are typically ascribed to methanogenic production (CICERONE and OREMLAND, 1988; SCHOELL, 1980; SCHOELL, 1988; WHITICAR, 1990). However, the majority of natural samples are from low-temperature environments such as rice paddy soils and marine sediments utilizing CO_2 substrates with typical $\delta^{13}C$ values near -20‰ (SCHOELL, 1980). Methanogens in hydrothermal environments primarily utilize magmatic CO_2 with $\delta^{13}C$ values of -9 to -4‰, and thus would be expected to produce CH_4 enriched in ^{13}C relative to CH_4 from low-temperature methanogenesis in sediments. The -32 to -25‰ $\delta^{13}C$ values of CH_4 from 9°50'N diffuse fluids are enriched in ^{13}C relative to values typically ascribed to methane of biogenic origin, but are compatible with methanogenesis of a substrate -4‰

in $\delta^{13}C$ of CO_2 and a fractionation factor at the low end of the range $1.023 \leq \alpha \leq 1.064$ determined by Valentine et al. (2004).

4.4. Is methanogenesis coupled with methane oxidation?

The depletions in ^{13}C observed in CO_2 from diffuse fluids, relative to CO_2 from high-temperature fluids, cannot be explained if just methanogenesis is invoked. Methanogenesis alone would either a) have no observable effect on the ^{13}C composition of CO_2, as the amount of CO_2 consumed is insignificant compared with the large CO_2 pool, or b) slightly enrich the CO_2 reservoir in ^{13}C as light CO_2 molecules are is preferentially metabolized. However, the observed $\delta^{13}C$ values of CO_2 in diffuse fluids are depleted up to 0.9‰, a result that requires an additional process.

We hypothesize that methanogenesis is coupled with methane oxidation. Microbial methane oxidation can occur both aerobically and anaerobically. Aerobic microbial methane oxidation has been investigated extensively in numerous environments, including hydrothermal plumes (COWEN et al., 2002; DE ANGELIS et al., 1993), and basalt-hosted hydrothermal vent fluids (ELSAIED et al., 2005; NERCESSIAN et al., 2005). Anaerobic methane oxidation is less well understood, as no anaerobic methane oxidizers have been cultured (ALPERIN et al., 1988; GIRGUIS, 2003; VALENTINE et al., 2000). Although oxygenated seawater is mixed into diffuse fluids, oxygen is rapidly consumed heterotrophically or by reaction with reduced chemicals, and is not detectible in diffuse fluids above 8-12°C (CORLISS et al., 1979; JOHNSON et al., 1988). Although diffuse fluids hosting methanogens are requisitely anaerobic, based on the frequent identification of microaerobic or denitrifying microbes in diffuse fluids >20ºC (CAMPBELL et al.,

2006), it is likely that microaerobic zones exist in the subseafloor where methane could be oxidized aerobically.

Both aerobic and anaerobic methane oxidation metabolisms isotopically fractionate the CH_4 substrate, producing CO_2 relatively depleted in ^{13}C. Carbon isotope fractionation factors vary from 1.005 to 1.031 for aerobic methane oxidation (determined experimentally) (BARKER and FRITZ, 1981; COLEMAN et al., 1981) and from 1.0088 to 1.014 for anaerobic methane oxidation (determined from natural sediment analysis) (ALPERIN et al., 1988; MARTENS et al., 1999; WHITICAR and FABER, 1986). Simply, the CH_4 substrate will be enriched in ^{13}C as methane oxidation proceeds, and the CO_2 produced will be depleted in ^{13}C relative to the CH_4 pool. This pattern of isotopic fractionation is consistent with the $\delta^{13}C$ values of diffuse fluid CO_2 that are depleted in ^{13}C relative to the high-temperature fluid, as well as $\delta^{13}C$ values of diffuse fluid CH_4 that are enriched in ^{13}C relative to values predicted from a mid-range methanogenic fractionation factor (α=1.044) (VALENTINE et al., 2000).

Unlike the abundant evidence for methanogenesis in hydrothermal environments, evidence of methanotrophy, and specifically anaerobic methane oxidation, has been elusive. Although anaerobic methane oxidizers have not yet been identified in mid-ocean ridge hydrothermal vent fluids, recent investigations have shown ANME groups to be present in deep-sea environments such as the cool (<10°C) carbonate chimneys at the Lost City Hydrothermal Field (BRAZELTON et al., 2006), the sediments of a mud volcano (NIEMANN et al., 2006), and the gas seeps of the Black Sea (MICHAELIS et al., 2002). Anaerobic methanotrophs have yet to be successfully isolated in culture, likely because a consortium of archaea and sulfate-reducing bacteria mediate the oxidation of CH_4 (BOETIUS et al., 2000; MICHAELIS et al., 2002; NIEMANN et al., 2006; ORPHAN et al., 2001). The coupling of anaerobic methane oxidation to the presence of

sulfate is important in the context of diffuse fluids which, due to seawater mixing, have high sulfate concentrations relative to sulfate depleted hydrothermal fluids (HUBER et al., 2006). Incubation experiments of Guyamas Basin sediments indicate that anaerobic microbial methane oxidation is most active at 30-60°C (KALLMEYER and BOETIUS, 2004).

While the temperature range and isotopic composition of 9°50'N diffuse fluids are compatible with methane oxidation, other metabolic process that produce CO_2, e.g. sulfate reduction coupled to oxidation of organic carbon other than methane (KNIEMEYER et al., 2007), cannot be discounted. The evidence presented here suggests that microbial methane oxidation may cycle methanogenic methane, and that further microbiological investigations of methanotrophy in diffuse fluids are warranted.

An evaluation of the potential for microbial modification of diffuse fluid composition can be made if fluid residence time of the diffuse fluid, quantity of biomass, and in-situ metabolic rate are known. At present the residence times of diffuse fluids are unknown, as are estimates of biomass and metabolic rates. As these data become available they will provide an important verification of the conclusions presented here.

4.5. Numerical box model of microbial methane cycling

The impact of microbial CH_4 production and subsequent oxidation on the isotopic composition and concentration of CH_4 and CO_2 was modeled using a numerical-box model depicted in Figures 6 and 7. This model uses mass- and isotope-balance calculations to track carbon through the reservoirs of the box-model, and ignores kinetics, diffusion, and speciation. This model serves as a numerical method of providing a qualitative answer to the question: Are observed

isotopic and concentration trends at Bio9/Bio9R and TWP/Y vents consistent with the microbial processes of methanogenesis coupled with methanotrophy?

The model is a spreadsheet-based series of iterative calculations of four different pools of carbon that are related through the processes of dilution, advection, methanogenesis and methanotrophy. Each carbon pool (geometric shapes in Figure 6) is represented in the model as a mass-isotope balance equation of the inputs and outputs to the pool, where each input or output (arrows in Figure 6) is expressed as a rate and an isotopic fractionation factor. As the goal of the model was to relate the observed $\delta^{13}C$ values of CH_4 and CO_2 to the competing isotopic fractionations of microbial methanogenesis and methane oxidation, the model was tuned to reasonable initial conditions then run for ~2500 combinations of fractionations (α _methanogenesis and α_AMO). The high-temperature pools of CH_4 and CO_2 are considered infinite reservoirs with concentrations and isotope values set to the average measured values, while the low-temperature pools are initially empty. Less well defined are the relative rates of methanogenesis (k_methanogenesis) and methane oxidation (k_AMO), dilution of the high-temperature fluid, and venting of the diffuse fluid to the surrounding ocean. The rate of methanogenesis was constrained by assuming that methanogenesis in the diffuse fluid is limited by the concentration of H_2 available from the high-temperature fluid, after dilution, and considering a $4H_2$:$1CH_4$ stoichiometry for methanogenesis (ZINDER, 1993). Rates of anaerobic methane oxidation relative to methanogenesis have been observed to range from 1% in coastal sites to over 1000% in gas hydrate environments (ORCUTT et al., 2005). Thus, the rate of methane oxidation was adjusted to approximate the rate of methanogenesis in terms of absolute amount of carbon processed per time step, and tuned to yield reasonable concentrations of low-temperature CH_4. The transfer of high-temperature CH_4 and CO_2 to the low-temperature fluid

was constrained based on temperature estimates of dilution, adjusted to account for conductive cooling. For example, a 30°C diffuse fluid represents a 300°C fluid diluted ten-fold, a dilution factor of 10. The rate at which low-temperature CO_2 and CH_4 are vented to the ocean (advection) was constrained by the model result for low-temperature CO_2 concentration, and thus is primarily linked to the input of CO_2. The modeled isotopic results are fairly insensitive to advection (as advection is increased from 5% to 50%, $\delta^{13}C$ values of CH_4 change by -1.5‰ and $\delta^{13}C$ of CO_2 changes by +0.05‰, a 5% and 1% change, respectively). However, the concentrations of CH_4 and CO_2 are highly sensitive to advection, decreasing advection from 50% to 5% leads to a 500% increase in the amount of CH_4 and an 800% gain in CO_2. An advection rate of 10% resulted in modeled diffuse CH_4 and CO_2 concentrations most similar to those measured at Bio9R and Y vents. Although this advection rate is suggestive of short residence times for the diffuse fluid, this value should be considered an artifact of the mass-balance approach rather than an observation-based constraint.

The model, initialized with values given in Table 3 and iterated until steady state was achieved, was run for thousands of combinations of methanogenic and anaerobic methane oxidation fractionation factors (α_methanogenesis and α_AMO) to determine the best fit of measured isotope data from Bio9/Bio9R and TWP/Y vents (Table 4). The model accurately replicates the isotopic composition and steady-state concentrations of CH_4 and CO_2 at Y vent when fractionations for methanogenesis and methane oxidation are 1.035 and 1.008, respectively. The fractionations predicted by the box model for TWP/Y vent are within the $1.023 \leq \alpha \leq 1.064$ range of values for methanogenesis, as reported by Valentine et al. (2004), and within the $1.007 \leq \alpha \leq 1.012$ range reported for anaerobic methane oxidation in sediments (ALPERIN et al., 1988; MARTENS et al., 1999; WHITICAR and FABER, 1986).

The low H_2 and CH_4 concentrations at Bio9/Bio9R relative to TWP/Y suggest that less CH_4 is cycled through Bio9R than Y vent. The diminished throughput of CH_4 at Bio9R requires increased isotopic fractionations in order to fit the observed CH_4 and CO_2 isotopic data. Best-fit results suggest that a methanogenic fractionation of 1.071 and a CH_4 oxidation fractionation of 1.053 to accommodate the low throughput of CH_4. The increased methanogenic fractionation predicted at Bio9R is at the high end of experimental values (VALENTINE et al., 2004). Valentine et al. (2004) report increased values for methanogenic fractionation under H_2-limiting conditions, a result that is consistent with the low H_2 concentrations at Bio9 relative to TWP. Although model predicted CH_4 oxidation isotopic fractionation at Bio9R is greater than fractionations observed for anaerobic CH_4 oxidation in sediments (ALPERIN et al., 1988; MARTENS et al., 1999), the predicted values are within the range for aerobic CH_4 oxidation (BARKER and FRITZ, 1981; COLEMAN et al., 1981). However, justifying an aerobic environment is difficult considering active methanogenesis necessitates the absence of oxygen. One possibility, albeit speculative, is that aerobic microenvironments could be more likely to occur in the less reducing fluids of Bio9/Bio9R (lower H_2 concentrations than TWP/Y), yielding the increased isotopic fractionations associated with aerobic methanotrophy.

In summary, a box model that accounts for a flux of CO_2 and CH_4 from the high-temperature vent coupled with microbial CO_2 reduction and CH_4 oxidation in the low-temperature vent, is consistent with the observed isotope and concentration data.

5. CONCLUSIONS

The vent sites located near 9°50'N along the EPR were the subject of close investigation by the AdVenture cruise series during the 1990's and a comprehensive set of volatile samples was

collected. We report stable carbon isotope values for CH_4 and CO_2 from two vent pairs, both consisting of a high-temperature vent situated directly next to a low-temperature vent. The vent pairs are assumed to have the same source, and thus insight can be gained from the compositional and isotopic differences.

Examination of the isotopic differences between low-temperature fluids and their modified high-temperature suggests that microbial methanogenesis is active in 9°50'N diffuse fluids, perhaps coupled with methane oxidation. The diffuse fluids from Y and Bio9R are, on average, 10‰ lower in $\delta^{13}C$ of CH_4 and 0.55‰ lower in $\delta^{13}C$ of CO_2 than the source fluid measured at TWP and Bio9 vents. Microbial methanogenesis is consistent with diffuse fluid CH_4 depeted in ^{13}C relative to the high-temperature source. Methanotrophy, a process yet to be identified in hydrothermal fluids, is consistent with diffuse fluid CO_2 depeted in ^{13}C relative to the high-temperature source; although so are other CO_2 producing metabolic reactions, such as microbial sulfate reduction. A numerical box model confirms that the competing processes of microbial methanogenesis and methane oxidation can result in the isotopic compositions and concentrations observed at 9°50'N.

The -31 to -26‰ $\delta^{13}C$ values of microbially cylced CH_4 reported here are enriched in ^{13}C relative to the <-50‰ values typically ascribed to "biogenic methane" (CICERONE and OREMLAND, 1988; SCHOELL, 1980; SCHOELL, 1988; WHITICAR, 1990). Recent evidence showing methanogenic fractionation factors as low as 1.022 (VALENTINE et al., 2004), and evidence reported here suggesting the potential for microbial methane oxidation to enrich the methane pool in ^{13}C, should serve as strong caveats when attempting to define the source of a natural methane sample according to its carbon isotope composition. The continued integration of

molecular and genetic work, experimental culture studies and geochemical analysis will yield less equivocal interpretations of complex natural systems such as diffuse fluids.

This dataset demonstrates that microbial activity in diffuse fluids modifies the fluid geochemistry systematically, and detectably. As such, similar isotopic investigations may prove useful in the detection and assessment of methanogenic activity during future exploration.

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Table 1. Comparison of measured hydrothermal fluids, see Table 2 for calculated endmember values. As no correction is applied to the measured $\delta^{13}C$ values of CH_4, that data is presented in Table 2 as an endmember value.

Vent	Year	T°C	[Mg] mmol/kg measured	[Si] mmol/kg measured	[Si] mmol/kg end-member	$[H_2]$ mmol/kg measured	$[CH_4]$ mmol/kg measured	$[CO_2]$ mmol/kg measured	$\delta^{13}CO_2$ measured
Bio9R	1994	30	51.9	0.8	13.2[a]	0.001	0.006	9.5	-4.18
Bio9R	1995	33	48.5	1.2	14.8[a]	0.002	0.004	9.2	-3.42
Bio9R	1997	27	51.6	0.7	13.0[a]	0.001	0.003	4.3	-2.62
Bio9	1991	368	25.2	5.1	9.9	1.448	0.082	24.2	-3.70
Bio9	1992	388	5.7	6.5	7.0	1.258	0.043	119.3	-4.01
Bio9	1994	359	2.1	11.9	13.2[a]	0.313	0.088	180.3	-4.02
Bio9	1995	364	2.2	14.2	14.8[a]	0.328	0.081	128.1	-4.05
Bio9	1997	369	2.3	12.6	13.0[a]	0.201	0.087	109.5	-3.95
Y	1991	55	46.7	0.7	12.7[b]	0.002	0.468	10.1	-3.02
Y	1992	22	51.4	0.7	12.7[b]	0.001	0.087	4.6	-2.33
Y	1994	20	50.6	0.7	12.8	0.007	0.016	5.0	-2.64
Y	1995	25	51.4	0.9	13.8[c]	0.000	0.014	5.9	-3.14
Y	1997	18	51.9	0.5	18.1	0.001	0.004	3.8	-2.16
TWP	1994	358	26.9	6.3	12.8	4.049	0.060	53.4	-4.14
TWP	1995	341	36.0	4.6	13.8[c]	1.592	0.033	33.2	-4.17
TWP	1997	307	2.7	17.2	18.1	3.245	0.093	73.1	-4.17
V	1991	70	43.6	2.8	7.6[d]	0.030	0.540	6.8	-2.42
Q	1991	371	21.5	n/a	n/a	1.772	0.050	130.0	-4.14
A	1991	396	45.2	0.6	n/a	3.630	0.020	8.8	-1.86

(a) endmember Si values reflect averages from multiple samples as reported in Von Damm (2004)
(b) endmember Si values reflect average of all 1994 Y vent data, as no data exist for TWP (the neighboring high temperature vent) in 1991 and 1992
(c) endmember Si values reflect averages of Si and Mg data from multiple 1995 TWP samples
(d) as V vent has no proximal high-temperature vent the endmember Si value reflects an average of all 1991 endmember Si values reported by Von Damm (2004)

Table 2. Comparison of endmember hydrothermal fluids. Endmember values correct the measured concentration data of high temperature vents for seawater entrained during sampling by normalizing data to a zero Mg value. Further corrections are applied to low temperature concentration data so that the proximal high-temperature vent data can be directly compared. Low temperature concentration data are normalized to the endmember Si value of the neighboring high temperature vent (presented in this table as Si endmember). CO_2 isotope data is corrected for all samples for entrained seawater, using a mass-isotope balance approach and assuming the endmember vent fluid has a zero Mg concentration.

Sample	Vent	Year	T°C	Measured [Mg] mmol/kg	Measured [Si] mmol/kg	Endmember $[H_2]$ [a,b] mmol/kg	Endmember $[CH_4]$ [a,b] mmol/kg	Endmember $[CO_2]$ [a,b] mmol/kg	$\delta^{13}C$ CH_4 [c] ‰	$\delta^{13}C$ CO_2 [d,e] ‰
2752-2	Bio9R	1994	30	51.91	0.8	0.02	0.12	188.35	-26.8	-5.36
3025-3	Bio9R	1995	33	48.48	1.2	0.03	0.06	134.36	-25.2	-4.31
3154-10	Bio9R	1997	27	51.60	0.7	0.02	0.06	104.66	-26.2	-5.03
2351-3	Bio9	1991	368	25.24	5.1	2.80	0.16	44.76	-34.6	-3.86
2498-3	Bio9	1992	388	5.73	6.5	1.41	0.05	133.76	-19.6	-4.02
2735-4	Bio9	1994	359	2.07	11.9	0.33	0.09	187.66	-19.4	-4.02
3030-2	Bio9	1995	364	2.21	14.2	0.34	0.09	133.70	-19.0	-4.06
3157-9	Bio9	1997	369	2.31	12.6	0.21	0.09	114.44	-18.9	-3.95
2372-3	Y	1991	55	46.71	0.7	0.04	10.20	220.02	-31.5	-3.68
2499-2	Y	1992	22	51.41	0.7	0.03	1.87	98.11	-27.5	-4.17
2852-7	Y	1994	20	50.55	0.7	0.16	0.38	121.16	-33.8	-4.42
3020-3	Y	1995	24.7	51.35	0.9	0.01	0.28	115.56	-32.6	-4.81
3158-10	Y	1997	18.2	51.89	0.5	0.03	0.21	194.40	-33.6	-4.64
2850-5	TWP	1994	358	26.92	6.3	8.36	0.12	107.83	-21.0	-4.22
3035-6	TWP	1995	341	36.00	4.6	4.89	0.10	102.00	-22.5	-4.36
3169-11	TWP	1997	307	2.65	17.2	3.42	0.10	76.91	-21.4	-4.18
2366-2	V	1991	70	43.56	2.8	0.09	1.52	19.28	-34.8	-3.80
2368-3	Q	1991	371	21.47	n.m.	3.01	0.08	219.25	-19.9	-4.16
2755-7	Q	1994	297	2.43	n.m.	0.51	0.12	168.78	-16.83	-3.83
3176-11	Q	1997	319	2.56	n.m.	0.27	0.13	153.75	-18.96	-3.70
2366-4	A	1991	396	45.20	0.6	27.07	0.14	51.07	-26.5	n.m.
3547-12	9°N seawater [f]	2001	2	52.0	0.155	b.d.l.	b.d.l.	2.3	n.m.	-0.4
	SJdFR [g]		285			0.53	0.12	4.5	-19.3	-8.3
	21°N EPR [g]		350			1.7	0.09	5.7	-16.0	-7.0
	13°N EPR [g]		300			0.14	0.051	18.4	-18.1	-4.8
	MARK [g]		350			0.48	0.06	6.7	n.m.	n.m.
	Menez Gwen [g]		284			0.05	2.63	20	-19.2	-9.1
	Broken Spur [g]		360			1.03	0.13	7.1	-18.5	-9.0

(a) High-temperature endmember concentrations determined by extrapolation to a zero-Mg value, see text for details, total cumulative error ± 3%

(b) Low-temperature endmember concentrations determined by extrapolation to a zero-Mg adjusted Si value, see text for details, total cumulative error ± 5%

(c) Average $^{13}CH_4$ measurement error ± 0.3‰

(d) Average $^{13}CO_2$ measurement error ± 0.05‰

(e) Measured $^{13}CO_2$ corrected to account for entrained seawater bicarbonate, average corrected $^{13}CO_2$ error ± 0.2‰, see text for details
(f) 9°50'N bottom seawater, sampling and handling identical to other samples
(g) SJdF(Evans et al., 1988), 21°N EPR (Welhan and Craig, 1983), 13°N EPR (Merlivat et al., 1987), MARK (Jean-Baptiste et al., 1991), Menez Gwen (Charlou et al., 2000), Broken Spur (James et al., 1995), concentrations are maximum reported, isotope values are median of range reported
n.m.: not measured
b.d.l.: beyond detection limits

Table 3. Best fit results and boundary conditions of box model presented in Figure 6

parameter	model Bio9/Bio9R	average Bio9/Bio9R	model TWP/Y	average TWP/Y
k_methanogenesis [a,b]	**0.0015**		**0.0045**	
k_AMO [c]	**0.8**		**0.6**	
k_recycled [d]	**0.015**		**0.045**	
dilution of HiT fluid [e]	**10**		**10**	
k_vent to ocean [f]	**0.10**		**0.10**	
$\delta^{13}CO_2$_HiT (‰)		**-3.96**		**-4.3**
$\delta^{13}CH_4$_HiT (‰)		**-19.3**		**-21.6**
[CO_2]_HiT (mmol/kg)		**120**		**95**
[CH_4]_HiT (mmol/kg)		**0.10**		**0.11**
[H_2]_HiT (mmol/kg)		(1.19)		(5.63)
$\delta^{13}CO_2$_LoT (‰)	*-4.87*	**-4.90***	*-4.73*	**-4.55***
$\delta^{13}CH_4$_LoT (‰)	*-26.05*	**-26.07***	*-31.90*	**-31.90***
α_methanogenesis [g]	*1.071*		*1.0373*	
α_AMO [h]	*1.053*		*1.0101*	
[CO_2]_LoT (mmol/kg)	*81*	(142)	*126*	(132)
[CH_4]_LoT (mmol/kg)	*0.24*	(0.080)	*1.07*	(0.68)

Boundary conditions are in **bold**, results in *italics*, data used for tuning of initial conditions in (parenthesis)

* Average measured isotope values of diffuse fluids were benchmark that determined the best fit of model results. Best fit determined by minimizing deviation of model isotope compositions from observed low-temperature isotope compositions (see Table 4).

(a) k_methanogenesis = [CH_4]_produced/[CO_2]_HiT

(b) rate constrainted by maximum CH_4 produced by drawdown of [H_2] according to a $4H_2$:$1CH_4$ stoichiometry; e.g. if 1mmol/kg H_2 consumed, 0.25 mmol/kg CH_4 is produced, and original CO_2 is 142 mmol/kg, then k_methanogenesis = .25/142 = .17

(c) k_AMO constrained to yield lower oxidation rates (in terms of total amount carbon) than methanogenesis and tuned to model results for [CH_4]_LoT

(d) rate of recycling between pool of low-temperature CO_2 and CH_4, the methanogenic rate is 10x that of methane oxidation rate, and is set to k_methanogenesis (with no dilution)

(e) dilution factor approximated using temperature constraints and estimates of conductive cooling

(f) rate of loss to ocean constrained by model results for [CO_2]_LoT

(g) α_methanogenesis = R_CO_2/R_CH_4, as determined by best fit to boundary conditions, where R = $^{13}C/^{12}C$

(h) α_AMO = R_CH_4/R_CO_2, as determined by best fit to boundary conditions

Table 4. Best-fit model results for fractionation factors and isotopic composition selected from 2500 simulations using unique combinations of fractionation factors for methanogenesis and methane oxidation.

Bio9/Bio9R				
α_methanogenesis (R_{CO2}/R_{CH4})	α_AMO (R_{CH4}/R_{CO2})	$\delta^{13}CO_2$_LoT (‰)	$\delta^{13}CH_4$_LoT (‰)	deviation[a]
1.071	1.053	-4.87	-26.05	0.03
1.079	1.062	-4.97	-26.10	0.08
1.072	1.054	-4.89	-26.18	0.11
TWP/Y				
α_methanogenesis (R_{CO2}/R_{CH4})	α_AMO (R_{CH4}/R_{CO2})	$\delta^{13}CO_2$_LoT (‰)	$\delta^{13}CH_4$_LoT (‰)	deviation[a]
1.035	1.008	-4.73	-31.87	0.18
1.040	1.013	-4.82	-31.96	0.27
1.036	1.009	-4.75	-32.09	0.28

(a) deviation calculated as square root of the total sum of squares between model result and measured isotope value (for CH_4 and CO_2)

Figure 1. Bathymetry and vent locations of 9°50'N East Pacific Rise study. Courtesy of D. Fornari.

Figure 2. Hydrogen concentration plotted on a log scale. The dramatic depletion in hydrogen at low-temperature vents is hypothesized to be the result of microbial consumption during methanogenesis (Von Damm and Lilley, 2004).

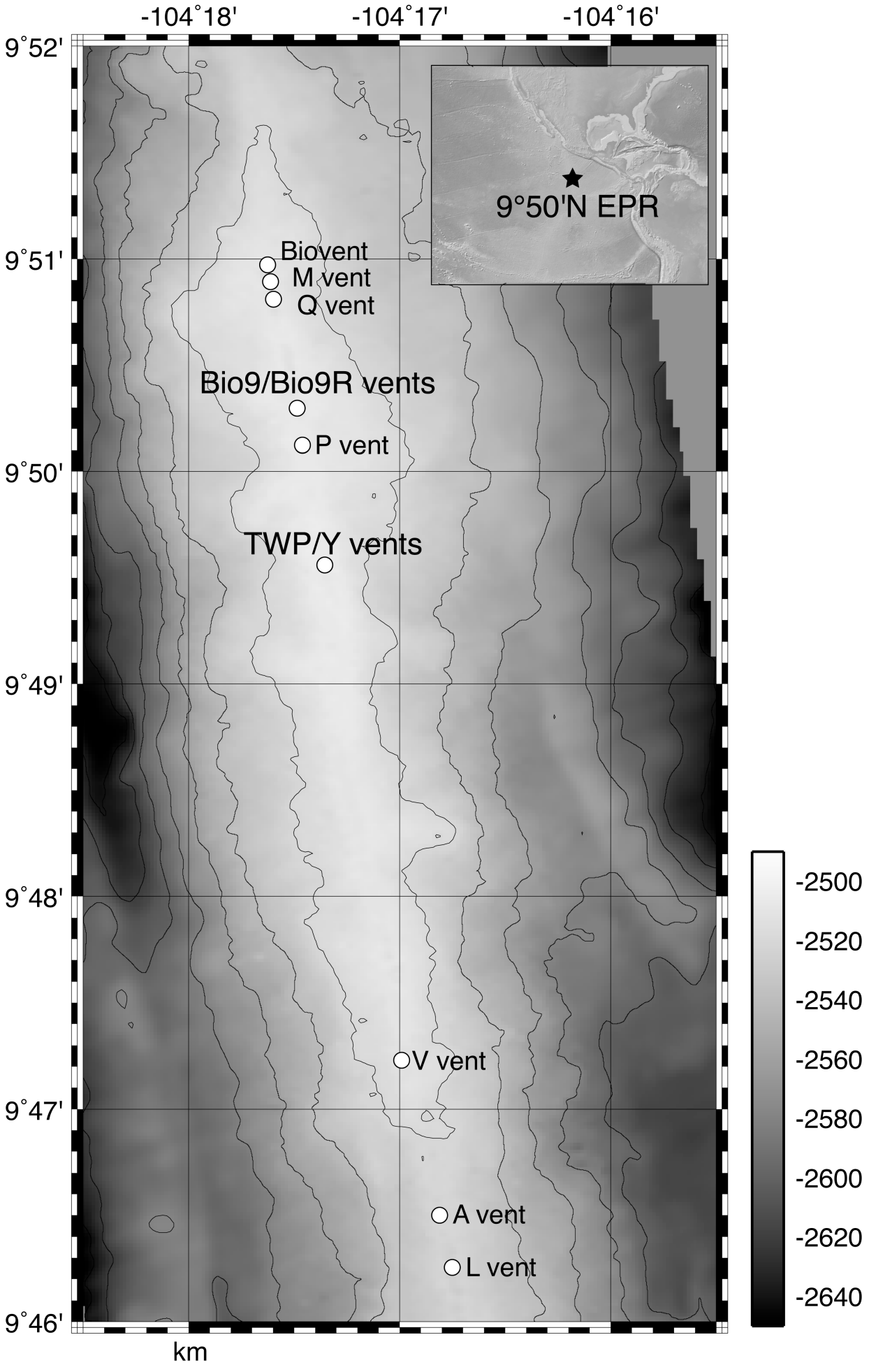
Figure 3. Expected CH_4-CO_2 equilibrium isotopic fractionations from Horita (2001). Both high-temperature and low-temperature vents appear to be out of isotopic equilibrium (high-temperature vents measured at 300-370°C have isotopic signatures indicating equilibrium temperatures 420-585°C, while the isotopic signature of diffuse fluids at 20-50°C, indicate equilibrium temperatures of 240-375°C). Note anomalous $\delta^{13}CH_4$ value of 1991 Bio9 386°C sample, where isotopic depletion is due to unique environmental conditions immediately following the 1991 seafloor eruption.

Figure 4. Comparison of TWP and Y vents. Plots of a) H_2 concentration, b) CH_4 concentration, c) $\delta^{13}C$ of CO_2 and d) $\delta^{13}C$ of CH_4 from 1994, 1995, and 1997. Concentration data and $\delta^{13}C$ values of CH_4 are compatible with active methanogenesis at Y vent. The depletions in $\delta^{13}C$ of CO_2 at Y vent indicate microbial methane oxidation.

Figure 5. Comparison of Bio9 and Bio9R vents. Plots of a) H_2 concentration, b) CH_4 concentration, c) $\delta^{13}C$ of CO_2 and d) $\delta^{13}C$ of CH_4 from 1994, 1995, and 1997. Consumption of hydrogen, and the depletion in $\delta^{13}C$ of CH_4 at the low-temperature Bio9R vent is compatible with methanogenesis. The depletions in $\delta^{13}C$ of CO_2 at Bio9R vent indicate microbial methane oxidation. The combined effect of microbial methanogenesis and methane oxidation may account for the apparent increase in 1994 and decrease in 1995 and 1997 in CH_4 concentrations.

Figure 6. Numerical box model describing active microbial methane cycling in low-temperature diffuse fluids. All arrows are associated with a rate and the indicated fluxes are associated with an isotopic fractionation factor corresponding to the microbiological process. Rates were estimated according to relative concentrations, and included dilution effects (seawater mixing with high-temperature fluids). Rates, fractionation factors, boundary conditions and results are shown in Table 3.

Figure 7. Plot of $\delta^{13}C$ of CH_4 vs $\delta^{13}C$ of CO_2 simplifying the result of a numerical box model run (final composition) as the sum of its principle components- methanogenesis, methanotrophy, and mixing. Arrows indicate microbial processes, dashed lines indicate mixing of microbial product with reservoir of carbon. Because of high concentrations of CO_2 input from high-temperature fluids, the final $\delta^{13}C$ value of CO_2 is only slightly depleted in ^{13}C (~0.55‰) relative to the initial value, despite the production of CO_2 highly depleted in ^{13}C. Note that methanogenesis causes the initial pool of CO_2 to be slightly enriched in ^{13}C as CH_4 depleted in ^{13}C is produced.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-104˚17'
-104˚16'
9˚52'
9˚51'
9˚50'
9˚49'
9˚48'
9˚47'
9˚46'
9°50'N EPR
Biovent
M vent
Q vent
Bio9/Bio9R vents
P vent
TWP/Y vents
V vent
A vent
L vent
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-2520
-2540
-2560
-2580
-2600
-2620
-2640
km
0
1
2

Figure 1.

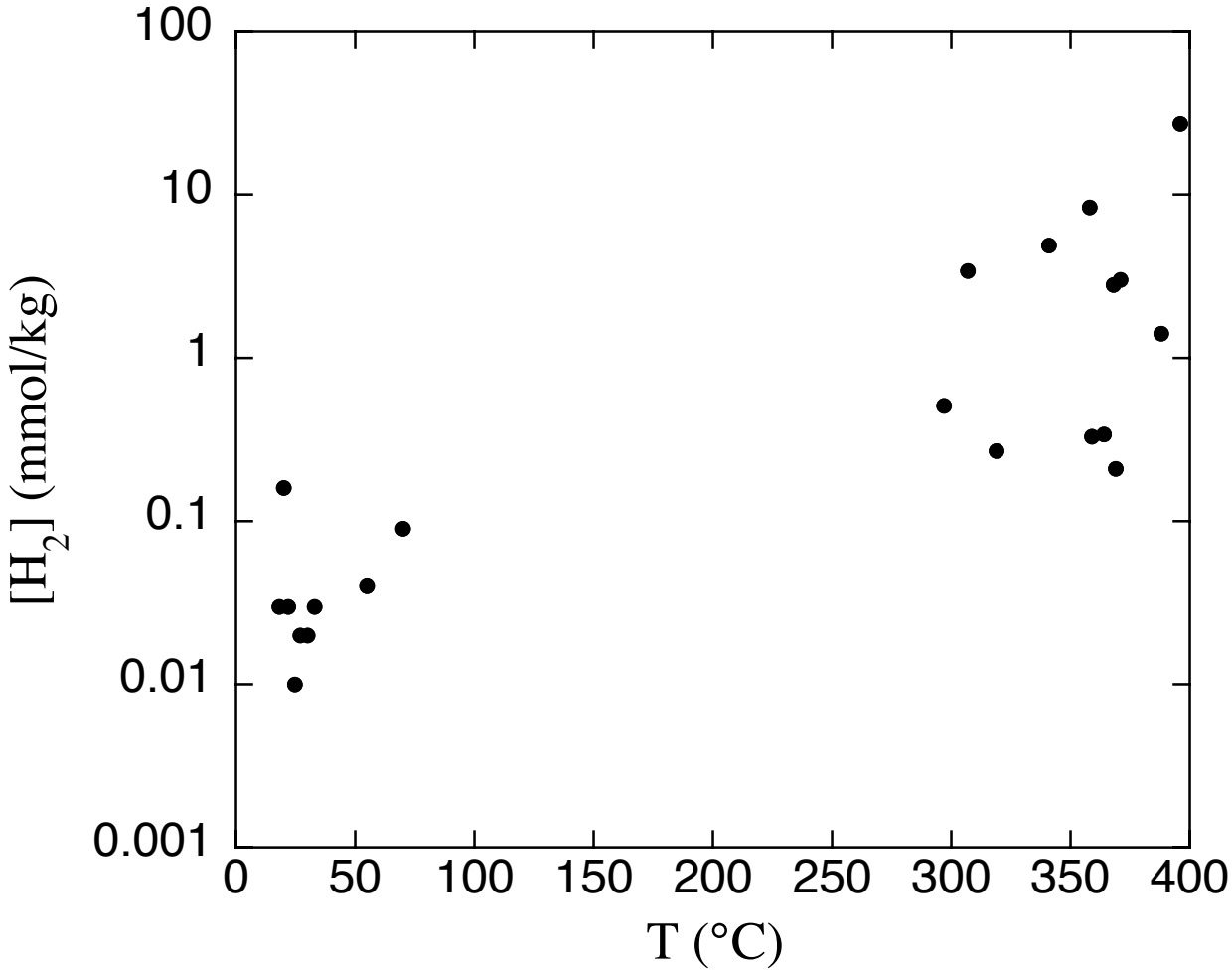

Figure 2.

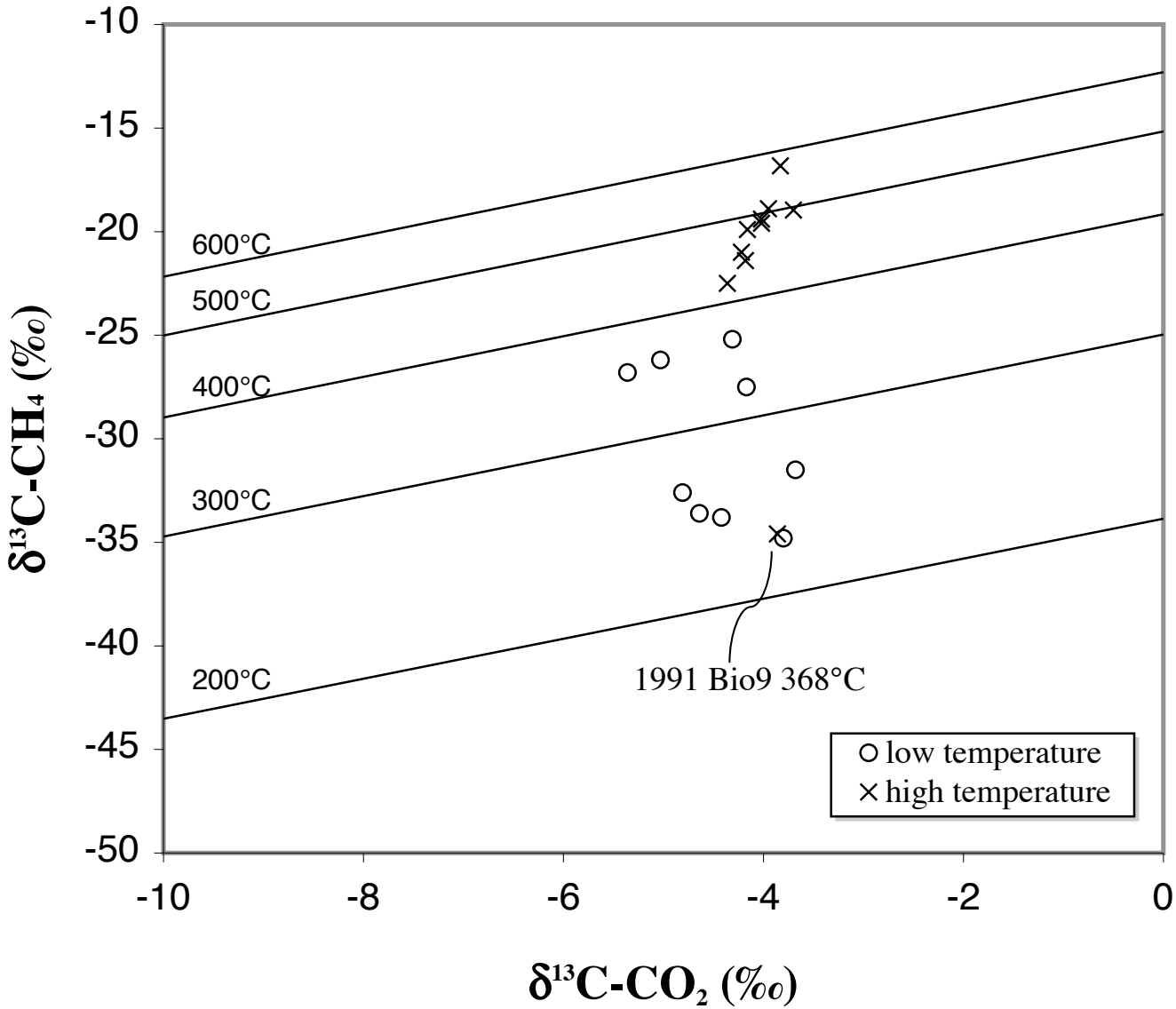
-10
-15
-20
-25
-30
-35
-40
-45
-50
-10
-8
-6
-4
-2
0
600°C
500°C
400°C
300°C
200°C
1991 Bio9 368°C
low temperature
high temperature
δ13C-CH4 (‰)
δ13C-CO2 (‰)

Figure 3.

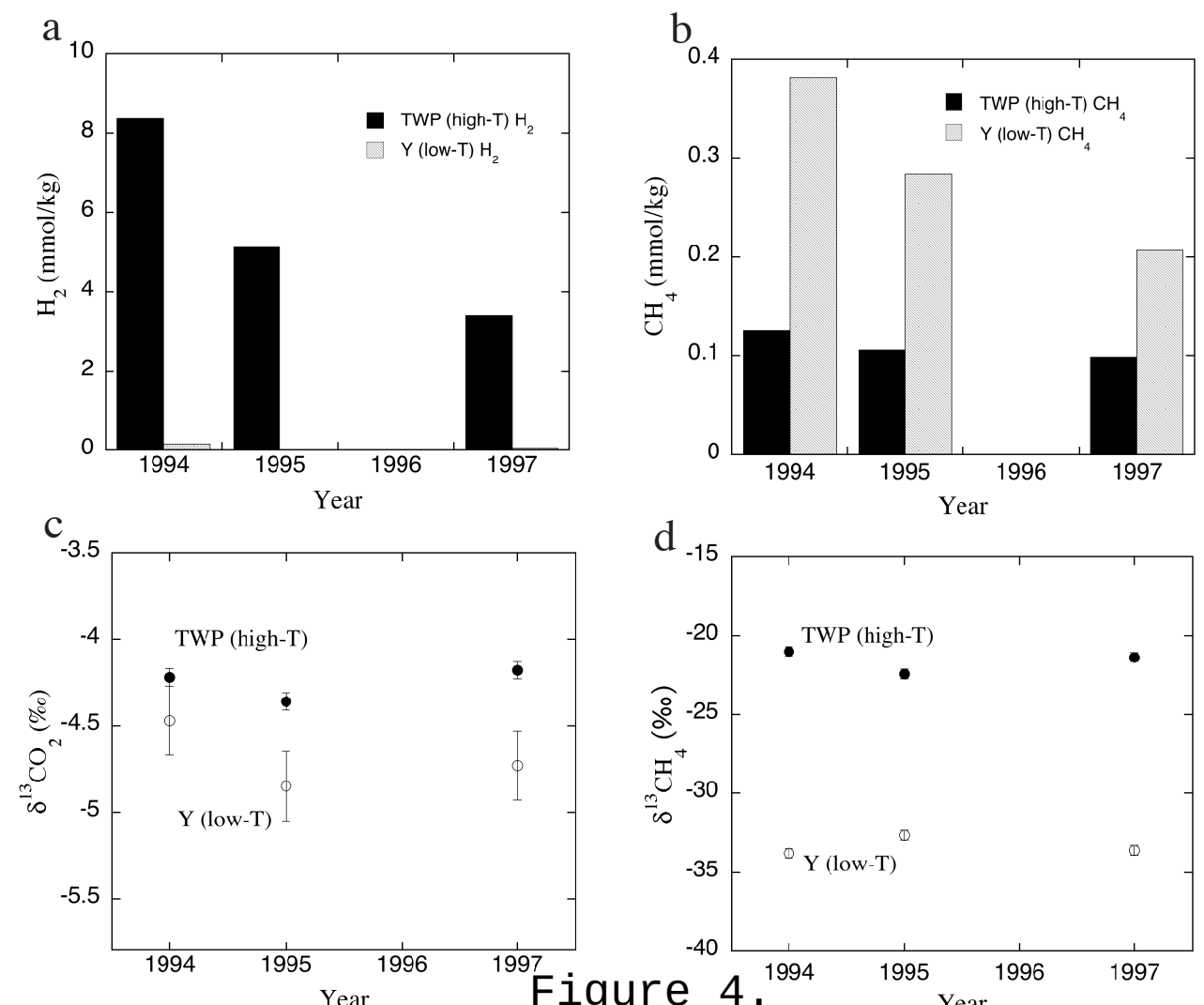
a
10
8
6
4
2
0
H_2 (mmol/kg)
TWP (high-T) H_2
Y (low-T) H_2
1994
1995
1996
1997
Year
b
0.4
0.3
0.2
0.1
0
CH_4 (mmol/kg)
TWP (high-T) CH_4
Y (low-T) CH_4
1994
1995
1996
1997
Year
c
-3.5
-4
-4.5
-5
-5.5
$\delta^{13}CO_2$ (‰)
TWP (high-T)
Y (low-T)
1994
1995
1996
1997
Year
d
-15
-20
-25
-30
-35
-40
$\delta^{13}CH_4$ (‰)
TWP (high-T)
Y (low-T)
1994
1995
1996
1997
Year

Figure 4.

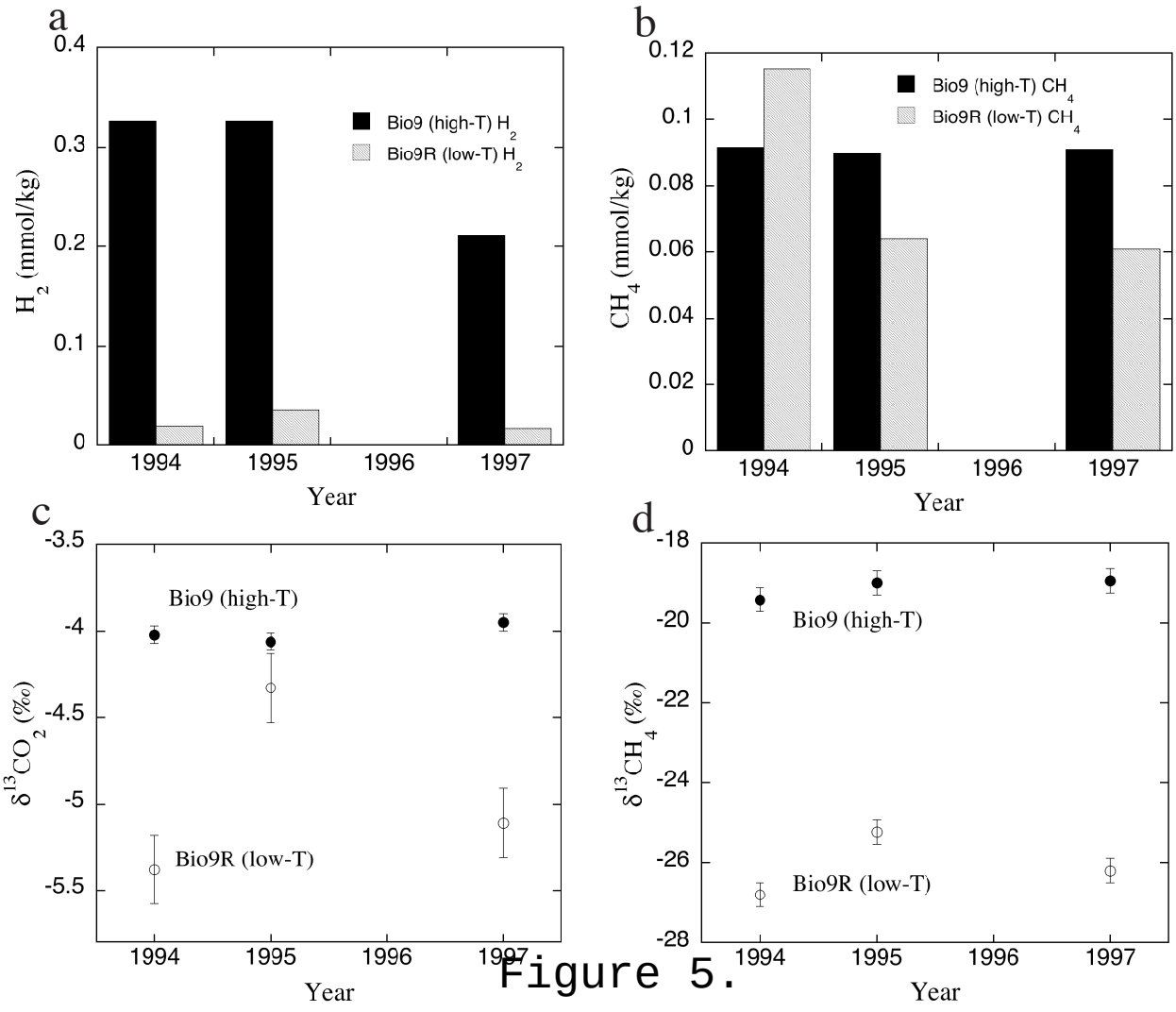
a
0.4
0.3
0.2
0.1
0
H2 (mmol/kg)
Bio9 (high-T) H2
Bio9R (low-T) H2
1994
1995
1996
1997
Year
b
0.12
0.1
0.08
0.06
0.04
0.02
0
CH4 (mmol/kg)
Bio9 (high-T) CH4
Bio9R (low-T) CH4
1994
1995
1996
1997
Year
c
-3.5
-4
-4.5
-5
-5.5
δ13CO2 (‰)
Bio9 (high-T)
Bio9R (low-T)
1994
1995
1996
1997
Year
d
-18
-20
-22
-24
-26
-28
δ13CH4 (‰)
Bio9 (high-T)
Bio9R (low-T)
1994
1995
1996
1997
Year

Figure 5.

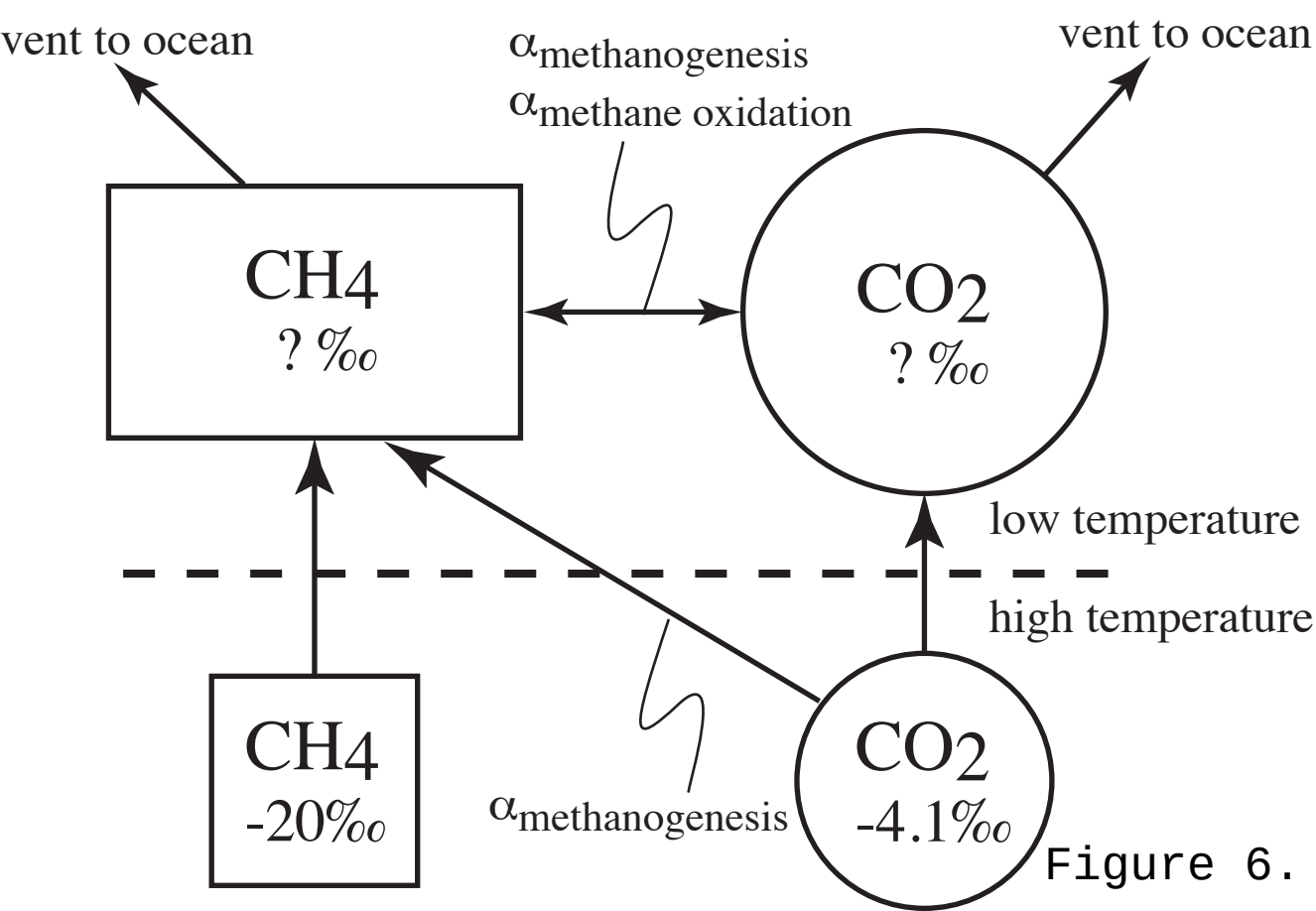
vent to ocean
αmethanogenesis
αmethane oxidation
vent to ocean
CH4
? ‰
CO2
? ‰
low temperature
high temperature
CH4
-20‰
αmethanogenesis
CO2
-4.1‰

Figure 6.

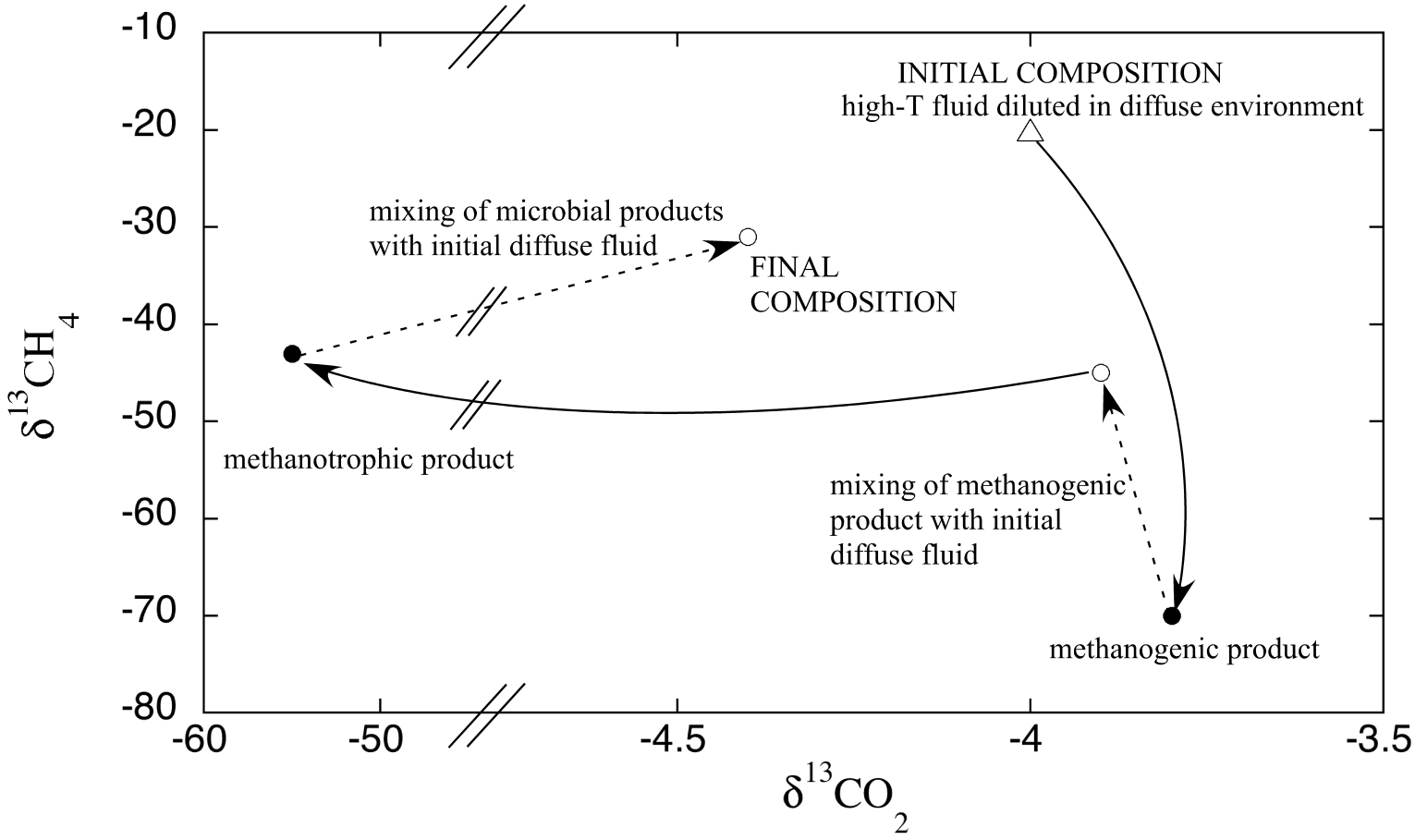
-10
-20
-30
-40
-50
-60
-70
-80
-60
-50
-4.5
-4
-3.5
δ13CH4
δ13CO2
INITIAL COMPOSITION
high-T fluid diluted in diffuse environment
mixing of microbial products
with initial diffuse fluid
FINAL
COMPOSITION
methanotrophic product
mixing of methanogenic
product with initial
diffuse fluid
methanogenic product

Figure 7.