Methane fates in the benthos and water column at cold seep sites along the continental margin of Central and North America

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ARTICLE INFO

ABSTRACT

The potential influence of methane seeps on carbon cycling is a key question for global assessments, but the study of carbon cycling in surface sediments and the water column of cold seep environments is complicated by the high temporal and spatial variability of fluid and gas fluxes at these sites. In this study we directly examined carbon sources supporting benthic and planktonic food webs at venting methane seeps using isotopic and molecular approaches that integrate this variability. At four seep environments located along North and Central America, microorganisms from two size fractions were collected over several days from 2800 to 9050 l of seawater to provide a time-integrated measure of key microbial groups and the carbon sources supporting the overall planktonic microbial community. In addition to water column measurements, the extent of seafloor methane release was estimated at two of the sites by examining the stable carbon isotopic signature (δ^{13}C) of benthic metazoan infauna. This signature reveals carbon sources fueling the base of the food chain and thus provides a metric that represents a time-integrated view of the dominant microbial processes within the sediment. The stable carbon isotopic composition of microbial DNA (δ^{13}C-DNA), which had values between −17.0 and −19.5‰, indicated that bulk planktonic microbial production was not ultimately linked to methane or other ^{13}C-depleted seep-derived carbon sources. Instead these data support the importance of organic carbon derived from either photo- or chemoautotrophic CO_2 fixation to the planktonic food web. Results of qPCR of microbial DNA sequences coding for a subunit of the particulate methane monooxygenase gene (*pmoA*) showed that only a small percentage of the planktonic microbial community were potential methane oxidizers possessing *pmoA* (< 5% of 16S rRNA gene copies). There was an overall decrease of ^{13}C-depleted carbon fueling the benthic metazoan community from 3 to 5 cm below the seafloor to the sediment surface, reflecting limited use of isotopically depleted carbon at the sediment surface. Rare methane emission as indicated by limited aerobic methane oxidation acts to corroborate our findings for the planktonic microbial community.

1. Introduction

The water column and sediments of cold seeps host a variety of microbes with autotrophic and heterotrophic metabolisms (Valentine, 2011). The role of methane in fueling benthic microbial production is well known, and this methane oxidation in sediments appears to augment deep-sea food sources of benthic metazoan communities both at methane seeps and in surrounding habitats (e.g., Levin and Michener, 2002; MacAvoy et al., 2002, 2003; Levin and Mendoza, 2007; Thurber et al., 2010, 2012, 2013; Levin et al., 2013, 2016). The sediment filter for methane is often very efficient, yet methanotrophy in the water column of seep environments can also be widespread (Reeburgh, 2007; Valentine, 2011). Recent studies have shown that

reduced carbon sources other than methane, such as larger alkanes, organic acids, and other dissolved organic carbon (DOC) compounds may also be available to fuel benthic and planktonic food webs at these sites (Valentine et al., 2005; Pohlman et al., 2010).

In some locations the annual flux of seep-derived DOC into the overlying water column may exceed that of organic carbon inputs from the surface ocean (e.g., Sommer et al., 2002; Valentine et al., 2005; Pohlman et al., 2010). At Hydrate Ridge, OR, sediment pore water DOC concentrations exceeded typical deep ocean values by 100–400 times (Valentine et al., 2005). This pronounced elevation was most noticeable in sediments overlain by microbial mats, which are indicative of active fluid outflow through the sediment surface (e.g., Tryon and Brown, 2001; Levin et al., 2003). In these same samples pore water DOC
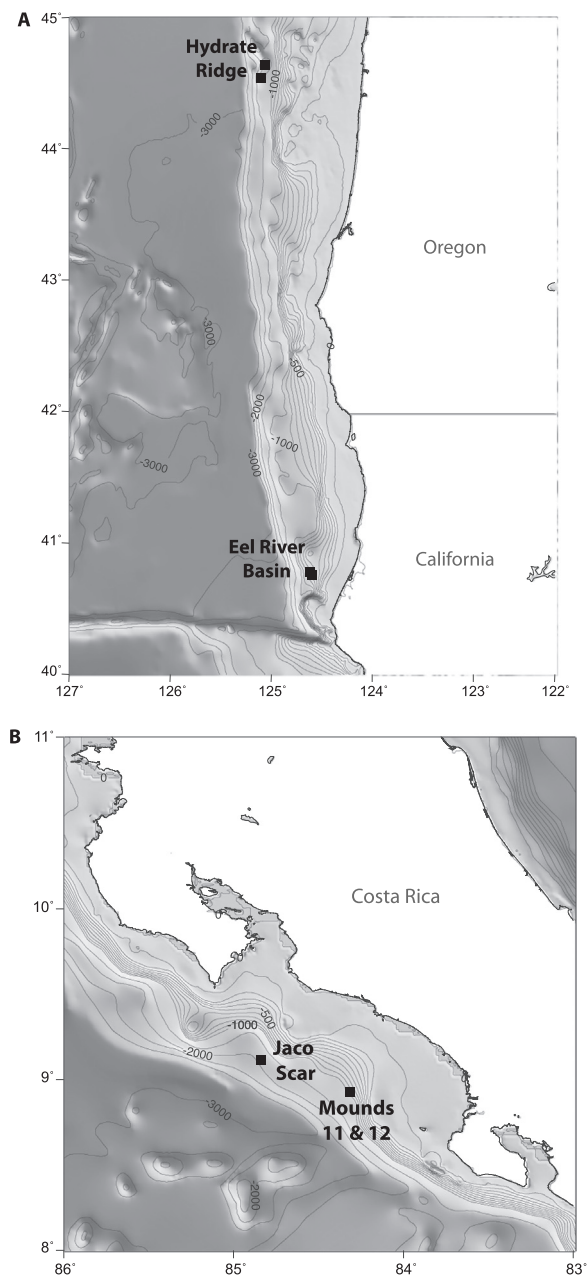
Fig. 1. Map of sampling locations: Eel River basin and Hydrate Ridge (a); Jaco Scar and Mounds 11 & 12 (b).

showed a depleted stable carbon isotope (δ^{13}C) signature indicative of being derived from biogenic methane (Valentine et al., 2005). In the Barkley Canyon region of the Cascadia Margin, DOC in bottom waters also exhibited elevated concentrations and depleted δ^{13}C signatures relative to off-seep sites (Pohlman et al., 2010). Based on a carbon

isotope mass balance, ^{13}C-depleted DOC (δ^{13}C =−35‰) was found to represent up to 60% of the deep water DOC inventory at this site, with methane-derived DOC (δ^{13}C =−43.6‰) representing up to 50% of the flux of DOC from sediments into bottom waters here (Pohlman et al., 2010). Thus, the available evidence suggests that cold seep sites can be a major source of ^{13}C-depleted DOC into the overlying water column, including bioavailable organic acids (particularly acetate and formate; e.g., Valentine et al., 2005; Lang et al., 2006, 2010), which can be incorporated by bottom water microbial communities.

The goal of this study was to combine space- and time-integrated approaches to develop a coupled benthic-planktonic understanding of carbon cycling in these environments, which have highly variable vertical and horizontal solute fluxes (Tryon et al., 2002; Levin et al., 2003; Heeschen et al., 2005). Four active methane seeps [Eel River (CA), Hydrate Ridge (OR), and two sites on the Costa Rica margin; Fig. 1, Table 1] were chosen to represent a range of biogeochemical environments defined by differing bottom-water methane flux, over-lying water column productivity, and water column depth (Valentine et al., 2001; Heeschen et al., 2005; Schmidt et al., 2005; Mau et al., 2006, 2012; Tables 1, 2). Our approach capitalized on the ^{13}C-depleted isotopic signature of seep-derived carbon sources relative to carbon derived from photosynthetic CO_2 fixation (e.g., Alperin and Hoehler, 2009; Table 2) to trace the possible incorporation of seep-derived carbon into planktonic (via isotopic composition of microbial DNA) and benthic food webs (via isotopic composition of metazoan infauna). We also compared these data with molecular biological measurements to examine genetic proxies for methane availability. Ultimately, our study presents a unique approach to constraining the role of seep-derived, isotopically depleted carbon sources in the dark ocean's carbon cycle.

2. Methods

2.1. Sample collection

During cruises on the RV *Atlantis* benthic fauna and planktonic microbes were collected at locations within the Eel River basin (ER), California and Hydrate Ridge (HR), Oregon seep systems (July 2006/ AT 15-7) and within the Costa Rica Dome system [Mounds 11 and 12, and Jaco Scar; June 2005/ AT 11−28 (planktonic sampling only); February-March 2009/ AT 15−44 (benthic sampling only)] (Fig. 1, Table 1). The DSV *Alvin* was used to collect a total of 38 push cores that resulted in a vertical profile of fauna at the Eel River basin and Hydrate Ridge in both clam bed (8 cores at ER and 15 cores at HR) and microbial mat (6 mat and near mat at ER and 9 at HR) habitats. These cores were sectioned vertically allowing resolution of the isotopic composition of the fauna to be resolved as a function of depth in the sediment. Cores included did not all have all vertical horizons represented and there was differential replication with depth as in many instances the greatest diversity and abundance of fauna, sometimes the only fauna present, were in the top layer of sediment. For analysis, we treat these data as both a mean composite to represent the community as well as outliers from these means. At no point were

Table 1.
Locations, depths, temperature, salinity, oxygen concentrations, and volumes of collected water column samples. mab, meters above bottom.

Methane seep system	Location	Avg. bottom depth (m)	Avg. sampling depth (mab)	Avg. temp. (°C)	Avg. salinity	Avg. [O$_2$] (μmol kg^{-1})	Volume filtered (L)
Eel River basin, CA	40°48.7′N, 124°36.7′W	523	29	6.3	34.15	38.6	4600
	40°47.1′N, 124°35.8′W	531	13	6.0	34.17	30.8	1000
Hydrate Ridge, OR	44°34.2′N, 125°8.8′W	780	77	4.7	34.28	12.2	5760
	44°40.0′N, 125°6.0′W	607	52	5.3	34.15	25.4	3290
Mounds 11 & 12, Costa Rica	8°55.7′N, 84°18.8′W	1003	103	5.3	34.58	24.0	3100
Jaco Scar, Costa Rica	9°7.1′N, 84°50.5′W	1814	149	2.9	34.63	66.6	2800