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Discussions

Interactive comment on "Carbon isotopic evidence for microbial control of carbon supply to Orca Basin at the brine-seawater interface" *by* S. R. Shah et al.

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We thank both reviewers for their thoughtful comments and careful reviews and particularly appreciate the suggestions from Anonymous Referee #1 to better explain the significance of this work. The introduction has been re-ordered and partially re-written according to the following:

"Active and diverse microbial communities have been reported in deep-sea brines of the Gulf of Mexico, Red Sea and Mediterranean Sea (reviewed in Antunes et al., 2011; Joye et al., 2010). These anoxic brines test the limits of microbial adaptation across a range of temperatures, salinities and pH conditions. Orca Basin, a deep basin lo-

cated on the Texas-Louisiana slope, is the largest brine pool yet identified in the Gulf of Mexico (Pilcher and Blumstein, 2007; Shokes et al., 1977; Trabant and Presley, 1978). Although moderate conditions are found in Orca Basin brine compared to other deep-sea brines (Antunes et al., 2011; Joye et al., 2005), studies of microbial abundance and activity in Orca Basin have concluded that very little physiological activity can be measured below the seawater-brine interface (Dickins and Van Vleet, 1992; LaRock et al., 1979; Tuovila et al., 1987; Wiesenburg et al., 1985). This lack of microbial activity stands in contrast to other Gulf of Mexico brines in the Gulf of Mexico (Joye et al., 2009, 2010). The continental slope of the northern Gulf of Mexico is characterized by numerous basins and pockmarks extending from the shelf edge to the basin floor (Bouma and Bryant, 1994 and references therein). This complex bathymetry is produced by the interaction of sediments and underlying salt, which drives salt deformation (Humphris, 1978), a process also associated with brine formation and brine seepage along seafloor fault networks (Reilly et al., 1996; Roberts and Carney, 1997). Although brine seepage is considered widespread in the northwestern Gulf of Mexico, only a few brine pools have been detected and studied (Joye et al., 2005; Macdonald et al., 1990; Shokes et al., 1977). Orca Basin, an intraslope depression at a water depth of approximately 2,000 meters on the northwestern continental slope of the Gulf of Mexico, was the first among these to be identified (Shokes et al., 1977; Trabant and Presley, 1978). Below 2,240 meters depth, the basin is occupied by an anoxic, hypersaline brine. Both the geochemistry of the brine and composition of underlying sediments indicate the $\sim$200-m thick brine originates from dissolution of a nearby salt exposure and lateral advection of brine to the basin (Addy and Behrens, 1980; Pilcher and Blumstein, 2007; Sheu et al., 1988; Shokes et al., 1977; Trabant and Presley, 1978). This formation mechanism is unlike many other Gulf of Mexico brines that have in situ sources of dissolved ions and reduced gases (Joye et al., 2005, 2009, 2010). Within the Orca Basin brine, oxygen and nitrate are not detectable while phosphate concentrations are elevated compared to overlying seawater resulting from organic-matter decomposition (Leventer et al., 1983; Shokes et al., 1977; Van Cappellen et al.,

1998). Sulfate concentrations, however, are not depleted within the brine suggesting a limited role for microbial sulfate reduction in the anoxic brine (Hurtgen et al., 1999; Leventer et al., 1983; Trefry et al., 1984; Wiesenburg et al., 1985) despite high concentrations of organic carbon and methane (Sackett et al., 1979). The most intense microbial activity is focused in the seawater-brine interface region (LaRock et al., 1979; Sheu et al., 1988; Van Cappellen et al., 1998; Wiesenburg et al., 1985), a particle trap where successive depletion of oxygen, nitrate and oxidized manganese has been observed (Trefry et al., 1984; Van Cappellen et al., 1998) along with narrowly-defined peaks of methane, ethane, propane and hydrogen sulfide concentrations (Wiesenburg et al., 1985). These authors propose that accumulation of reduced gases and anoxia within the brine result from the long residence time of brine in Orca Basin combined with slow in situ decomposition of organic carbon (Wiesenburg et al., 1985). The onset of brine accumulation in Orca Basin is thought to be signaled by a transition from shallow, black anoxic sediments to more deeply-buried gray sediments. This transition, reported in both slope sediments and in deeper basin sediments (Addy and Behrens, 1980; Leventer et al., 1983; Meckler et al., 2008; Northam et al., 1981) has been dated by three methods: radiocarbon analysis of total carbonate (Addy and Behrens, 1980); radiocarbon analysis of foraminifera (Leventer et al., 1983; Meckler et al., 2008); and biostratigraphy (Leventer et al., 1983), yielding similar ages of approximately 7900 to 8500 calendar years for Orca Basin brine. Here we use radiocarbon measurements of dissolved inorganic (DIC) and organic carbon (DOC) and box modeling to document slow, cumulative microbial processes driving organic carbon cycling within the basin."

We also modified the conclusion, changing the final sentences to: "Aside from anaerobic methane oxidation, carbon isotopic evidence cannot indicate specific microbial pathways important in DOC or DIC cycling. It also cannot determine the limiting factor that prevents organic carbon re-mineralization by microbial sulfate reduction in Orca Basin brine. Further microbial and compound-specific isotopic investigations of the brine and seawater-brine interface are needed to form a better understanding of specific microbial processes contributing to dissolved carbon storage in the Orca Basin

brine."

In Specific Comments, Referee #1 argues for not stating the conclusion early in the manuscript. We have changed this sentence to describe the work in the following sections rather than the conclusion as follows: "Here we use radiocarbon measurements of dissolved inorganic (DIC) and organic carbon (DOC) and box modeling to document slow, cumulative microbial processes driving organic carbon cycling within the basin."

Referee #1 also points out a confusing sentence where we have included refractory deep-ocean DOC in our discussion of in situ sources of brine DOC. We have removed the mention of refractory DOC from that sentence and moved it to the end of the paragraph with more explanation. The re-written section reads as follows: "These sources could include incorporation of DOC produced from DIC by chemoautotrophy at the brine interface (Joye et al., 2009), microbial conversion of POC to DOC and disaggregation of POC to DOC. The d13C value of DOC does not allow for a significant 13C-depleted source from methane oxidation as it is not more 13C-depleted than DOC in the water column above. A fraction of brine DOC is also likely to come from deep-ocean refractory DOC incorporated with bottom water upon brine formation, but this may only represent one seventh of total brine DOC."

We also agree with all technical comments from Referee #1 and incorporate all suggested changes.

In response to Referee #2's request for additional discussion about the differences in d13C values that we obtained using different measurement methods, we have included the following additional statement in Results Section 3.2: "Our parallel d13C measurements may differ due to the difference in chemical composition between brine samples and seawater calibration standards." We feel that determining the possible causes of the bias are beyond the scope of this paper. Each laboratory used different methods for d13C analysis, and, in the case of NOSAMS and SkIO, the extraction systems were tested using standard seawater and enriched sodium bicarbonate. The brine samples

analyzed here have a significantly different chemical composition and it is possible this is affecting the accuracy of one or both of these systems.

Referee #2 asks for the inclusion of two additional references, Tribovillard et al., (Mar Geol, 2008) and Hurtgen et al. (Am J. Sci, 1999). We have included a reference to Hurtgen et al. (1999) when discussing sulfate reduction rates (see rewritten introduction above). We also wish to thank Referee #2 for pointing out additional literature that describes the transformation and fate of organic carbon at the seawater-brine interface. We include a phrase about "extensive organic matter degradation" and cite Tribovillard et al. (2008) and Wong et al. (Mar Chem, 1984) when discussing DIC production at the seawater-brine interface (Page 17921 Line 17 and Page 17925 Lines 22-23).

As Referee #2 requests, we have also edited the entire text with an eye towards clarity and readability.

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