**Answers to reviewers of the Biogeoscience Manuscript (bg-2012-547) “**Limitations of microbial hydrocarbon degradation at the Amon Mud Volcano (Nile Deep Sea Fan)” by J. Felden et al.

## Reviewer #1:

Reviewer 1: The manuscript described the shifts of sedimentary microbial activities, geochemistry and temperature associated with activity of a mud volcano within 3 years, and dis- cussed about factors that regulate microbial hydrocarbon degradation especially for anaerobic methane oxidation. Observation of activity changes of deep-sea cold seep environments including mud volcano is interesting, and this study provide novel in- sights into geochemical and microbiological processes of surface and subsurface hydrocarbon degradation in mud volcano environments. On the other hand, observation for transition of geochemical fluxes in subseafloor environments associated with cold seep environments including mud volcano is a challenging project because geochemical fluxes in cold seep sedimentary environments sometimes vary within a meter even at the same expedition as the authors described in 3.2 (Methane and other pore water constituents) of the manuscript. I think the information of each sampling and sample characterization presented in this manuscript is not sufficient to conclude that the observed differences between 2006 and 2009 were the results of activity changes of the mud volcano but not the local variation of each site.

Reply: We agree with the referee that the assessment of temporal changes in activities of mud volcanoes is highly challenging and limited by spatial heterogeneities. However, we have based our suggestion on the careful evaluation of several independent observations, including changes in the surface smoothness and erosion of mud blocks as observed by ROV video observations, as well as decrease in subsurface temperatures commonly interpreted as related to declining geological activity of mud volcanoes (e.g. Feseker et al. 2008) and local trends in cell counts and process rates. The way we have formulated the conclusion, it remains a suggestion/hypothesis and we would like to stick to this interpretation of our data, as it makes most sense in light of all measurements combined. We have revised the abstract accordingly, and added a sentence as to the limitations of quantitative observations to acknowledge the referee’s input.

Reviewer 1: Specific comments History of the mud volcano should be described and summarized in the introduction, results or discussion section.

Reply: We have referred to relevant publications in the introduction discussing the geological origin of this mud volcano (Dupre et al 2007, 2008, 2010; Foucher et al., 2009; De Lange and Krijgsman, 2010), including findings of extensive carbonate crusts surrounding the MV. We are not aware of further published information on the history of the MV. As to the biological observations: the presence of relatively large tubeworm bushes (Duperron et al. 2009) suggests that the mud volcano had been active for a few decades, as these organisms depend on sulphide flux.

Reviewer 1: **2.1** Sampling site: Were the sediment cores taken from the "same" sampling sites in 2006 and 2009? Did the authors determine specific sampling site by deploying markers or apply another technique to determine the exact sampling site? Especially for the central dome site, only the map presented in Fig1 is not likely sufficient, and more precise information about the sampling sites and sampling strategies should be given in this section.

**Reply:** In both years we used a ROV for geo-referenced sampling and in situ instrument deployment. The vehicle and the ship were equipped with a positioning system, which enabled us to return to the same sampling sites in 2009, and to carry out precise sample retrieval (enabled by Posidonia transponders on winch operated tools and the ROV). We also deployed physical markers at the seafloor to enhance further the relocation of transects and specific sampling sites.
We added the following statement:
L 99: "Precise positioning and operation of the in situ tools as well as targeted sampling of the different mud volcano habitats were achieved by using the Posidonia navigation system on the ship, the winch operated tools and the remotely operated vehicle (ROV) "Quest 4000" (Marum, Bremen, Germany). Also, physical markers were deployed at the seafloor for orientation during both expeditions."

Reviewer 1: **3** Results: The presence of mud blocks in the central dome area is described in this section. Such blocks may differ from surrounding sediments in physical properties and sedimentological features (grain size etc.) that influence pore water chemistry and/or heat flow (temperature gradient). Moreover such mud blocks could also be taken by both ROV and gravity core operations. Thus, sediment description in gravity coring including temperature measurements, and seafloor observation in PC sampling are important for data interpretation. I am also interested in the origin of the mud blocks. The mud volcano activity has likely continued in tens of years as described below while mud blocks we observed might recently occur.

**Reply:** In the framework of our study, the only physical sediment parameter we have determined was porosity, which showed averaged values of 0.7 upper most 20 cm of the sediment (Now added to the MS, L178ff). In our study, we had to limit the assessment of mud blocks to comparative visual observations (2006 – 2009). Within this time frame, the observed mud blocks showed signs of erosion and thus we assume the mud blocks must have been deposited shortly before our first sampling campaign, by a fresh eruption (now clarified in L 222ff).

Some work on the mud blocks at the Amon MV was done before by Dupré et al., (2007; 2008), which we cite in our study. For your information (but not further expanded in the MS): she explains that the high backscatter signal is mostly produced by metre-scale mud breccia blocks, that make up parts of the central rough area. The homogeneous and structureless mud breccia which correspond to a soft and unconsolidated material contain millimetric rock clasts and authigenic carbonate concretions.

Reviewer 1: **3.2** Methane efflux and temperature gradients P345, L27: Authors concluded that microbial activity in the bacterial mat zone in 2009 was higher than in 2006. Considering the heterogeneity of subseafloor environments in such area, number of samples used for the comparison; just one and two cores (Fig 3) in 2009 and 2006, is not likely sufficient.

**Reply**: L268; We have revised the MS accordingly. Nevertheless, it seems relevant to consider that sulphate in the pore water shows depletion in 2009, in line with the cooling of temperatures in the fluids. We assume that this is caused by a combination of reduced fluid flow delivering sulphate from the deep subsurface and high consumption rates, most likely limited by sulphate depletion in 2009. This hypothesis is discussed in more details in the discussion section 4.2, last paragraph.

Reviewer 1: Additional information such as seafloor observation in the microbial mat site, sampling location, methane efflux in water column etc. that suggest the increasing microbial activity in 2009 should be given for this discussion if possible.

**Reply:** We have provided an overview of the sampling scheme in Figure 1D; and all precise sampling locations are deposited in PANGAEA (http://doi.pangaea.de/10.1594/PANGAEA.804779). The heat flux and water column data cannot be further resolved than on the habitat level (center vs surrounding biogenic mound area), the sediment coring was precise enough to also distinguish the bacterial mat zone of the MV center, so we have limited the discussion to comparative observations in these habitats.

Reviewer 1: **4.1.2 P351, L12**-: The site description should be given as an independent paragraph. I believe the finding such as tubeworm and carbonate crust did not occur after 2006 but the structure of the paragraph may mislead the interpretation of the discussion.

**Reply:** The site description of the sulfur-band including the observed carbonate crusts, associated faunal communities (e.g. tubeworms) have been described in detail before (Duperron et al., 2009; Ritt et al., 2011; Girnth et al., 2011), and are hence restricted to a short description and citation of these works. We have clarified now that we found tubeworms and carbonate crust in 2006 and 2009 (L399).

"Surrounding the mud flow, active chemosynthetic communities were observed in 2006 and 2009, consisting mainly of Lamellibrachia tubeworms (Duperron et al., 2009), and bivalves associated with carbonate crusts (Ritt et al., 2011)."

Reviewer 1: **4.2** Kinetic limitation by disturbance and heat in the central area. Sedimentary characterization of sediment cores is very important for the discussion in this section. If the sediments taken from consolidated mud blocks or lacked enough pore space for microbial life, such structure could also influence subseafloor anaerobic microbial community (eg. Rebata-Landa V & Santamarina JC 2006).

**Reply:** The samples we have obtained from the center of the MV showed a porosity of on average 0.7 even down to a sediment depth of 3 m bsf, and thus provided sufficient pore space to support a microbial community (This observation is now added in L178). Therefore, we concluded that pore space is not a limiting factor for microbial activity at the Amon MV, and did not further discuss this point.

Reviewer 1: **P353, L5-:** Authors concluded that the temperature changes influence microbial biomass. However, they did not provide exact temperature of each sample, and temperature at seafloor at 2006 might be not too high for the growth of methanotrophs. In addition, SR in 2009 was generally lower than that in 2006, data from activity measurement were not provided at the center dome site for the 2009 samples, and cell abundance was measured for each only one PC in both years. Therefore, the data presented here is not enough to lead the conclusion. Constrains of habitability for subsurface life such as pore space and availability of organic compounds also could explain the difference between the cores in 2006 and 2009.

**Reply:** In our sampling strategy we aimed for replicate in situ temperature measurements in the different habitats. These were taken with an in situ lance, which does not retrieve samples. It is not possible to determine correct fluid temperatures after sampling in retrieved cores, due to the cooling during recovery from great depths. However, due to the precise spatial sampling, we believe we can combine the results, and suggest that the cooling measured is reflected in e.g. lower fluid flow rates and increasing cell numbers and activities in some habitats. Pore space did not differ between years or cores. In any case, we are very careful with conclusions and use this finding mostly as a suggestion.

Concerning habitability, we suggested that subsurface fluids of more than 70°C could have prevented the growth of anaerobic methanotrophic communities, despite the presence of sulphate and methane in the seep fluid. Currently, no anaerobic methanotrophs are known to thrive at higher temperatures (see L515).

Reviewer 1: **Table 2:** Some of the AOM and SR rates present minus values (eg. 0.6 ± 6.6). Do they mean methane and sulfate production?

**Reply:** We have revised the Table 1 with AOM (anaerobic oxidation rates) and SR (sulphate reduction rates) to show ranges, rather than averages and standard deviations.