

Interactive comment on “Geophysical and geochemical controls on the megafaunal community of a high Arctic cold seep” by A. Sen et al.

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General Comments

This is a well-written manuscript describing the geochemistry, physical habitat and fauna at a series of methane-seeps in the Arctic. The authors relate changes in faunal distribution, from photographic images, to the local chemical and physical environment and discuss the micro-habitats available at such seeps. The manuscript would be improved if it were possible to make comparisons with the background fauna away from seep influence. There are a number of problems with the analytical procedures, presentation and interpretation of results as described below.

Response: Thank you for appreciating the premise of the manuscript. Unfortunately, comparisons with background fauna away from seep influence is only possible qualitatively, and this is the approach we took in the manuscript. More details on this are below.

Specific Comments

Methane

Since the study is based on methane seepage it is essential to have some reliable measurements of methane concentrations available to the biota. Unfortunately the method described for methane analysis does not measure “dissolved pore water methane”, as stated, but a mixture of free methane plus methane sorbed to the sediment and released by the sodium hydroxide addition (Ertefai et al. 2010). Since authigenic carbonate is present, the concentration of the sorbed methane can be up to two orders of magnitude higher than the dissolved methane (Ijir et al. 2009). It is unclear why pore water obtained from the rhizons was not used for on-board methane analysis. There is no information available, to my knowledge, on the extent to which sorbed methane is available to the biota. Thus comparisons between sites based on methane availability are thus not valid.

Response: We have to disagree with the reviewer saying that what we measured is “...a mixture of free methane plus methane sorbed to the sediment...” therefore claiming absence of any dissolved gas in our samples. Methane is soluble in water and is always present in pore water samples of marine sediments typically demonstrating 50-80 % porosity (Abrams et al., 2017).

It is possible that some amount of free and sorbed gas is also present in the bottom sediments *in situ* and some occasional desorption occurred due to NaOH in our samples. Portions of methane purposefully extracted with alkaline technique from sediments at active thermogenic seepage locations are essentially unknown because (1) petroleum exploration prioritizes acidic extraction and vacuum desorption methods, and (2) existing works on alkaline extraction focus on “..environments that are not influenced by thermogenic processes.” (Ertefai et al., 2010) and not related to visible seabed seepage. Moreover, Ertefai et al., 2010 report inaccuracy of their sorbed gas analyses from non-seep sites of as much as 65%, implying difficulties during even targeted extraction efforts. During the analytical stage of our work we did not aim to extract any sorbed gases, therefore, we did not apply any long-duration techniques for mechanical disintegration of clayey assemblages (orbital shakers, mills, etc.).

The publication by Ijir et al., suggested by Dr. Dando states that content of adsorbed gas is twice higher in carbonate concretions compared to surrounding sediments. In our work we collected and analyzed the samples that are by all means equal to what Ijir et al., 2009 call surrounding sediments with absence of any macroscopically observed carbonates. Therefore, the ratio of adsorbed and extractable methane to bulk methane in even non-seep environment is poorly understood and lacks firm quantifications due to substantial analytical errors. In our study area where free gas escapes the seabed, the contribution of occasional desorption of gas strongly-bound to clay minerals is deemed to be negligible compared to abundance of its more labile forms (dissolved and free gas).

The issue of mixing free gas and dissolved gas in headspace samples is inevitable and, we believe, has become a condition well known in marine geoscience. Headspace gas analysis shows concentration of only dissolved gas if this concentration is lower than the solubility limit under P and T conditions of a laboratory where the samples are collected. Concentrations measured in our shallow sediments are below this critical value, thus representing dissolved gas only.

Analyses of pore water collected with rhizons are not optimal due to long exposure (at least an hour) of water drops to the air in the syringes that always have some dead volume. During such sampling dissolved gas gets equilibrated with atmospheric gas causing loss of methane in analyte that is hard to trace and account for.

Despite this, even if our method did result in some amount of free gas and sorbed gas getting included in our measurements, the same method was used for all the pingos and the point was to compare between them. Therefore, even if our method overestimates dissolved methane concentrations, it does so equally for pingo 5 and the other pingos. And our measurements, which were lower at pingo 5 compared to the other pingos, likely indicates that dissolved methane is also lower at pingo 5.

Sulphide

Dissolved reduced sulphur species are utilised by chemoautotrophic free-living and symbiotic bacteria as an energy source. The concentration of dissolved "sulphide" ($H_2S + HS^- + S=$) and thiosulphate is thus an important measurement. The authors state that sulphide was below the detection limit in the bottom water and in the upper "few" cm of the sediment of all the cores. However, sulphide must have been present to support the bacterial mats visible on the surface. The detection limit is not given but since the lowest standard used in the assay was 40 μM (Hong et al., 2017) the method described may not have been able to detect concentrations of a few μM . Many thiotrophic symbiotic associations exist in sediments with dissolved sulphide concentrations of $< 1 \mu M$. It is very difficult to prevent oxidation of low concentrations of sulphide in pore water and since the samples were not analysed immediately, it is probable that oxidation occurred during preservation and storage. H_2S would have been carried into the upper sediment and the water column in the methane bubbles as well as in the associated water plume (Reeburgh 2009, Dando et al. 1994a). In addition, the drawdown of seawater induced by the rising methane bubbles (O'Hara et al. 1995, Zimmermann et al. 1997) would have locally generated reduced sulphur species from iron sulphides within the sediment (Dando et al. 1994b) as well as producing a halo of less reducing areas surrounding the bubble outlets.

Response: Dr. Dando is correct, the detection limit was 40 μM and we agree that thiotrophic symbioses can exist with much lower concentrations of dissolved sulphide.

We also agree that the presence of bacterial mats indicate the presence of dissolved sulphide at the water-sediment interface (although the mats are very patchy and usually quite small). We can therefore remove the sentence on sulfide toxicity with respect to non-seep fauna.

However, the main points we were trying to make were with respect to comparing sulphide concentrations between the different pingos and they are: 1) sulphide concentrations likely do not differ between the pingos and 2) we might have been unable to detect sulphide in the bottom water, but that is the case for both pingo 5 and the other pingos. We believe that these points are still valid. However, we can emphasize our inability to detect low concentrations of sulphide, both within the sediment and in the bottom water, but doing this would not alter the conclusion we came to, that 'GHP5 is not deficient in this regard (sulphide) either, in comparison to the other pingos'. We also would like to emphasize that, even though we did not measure sulphide concentration immediately, the porewater samples were collected by rhizons which has been shown to prevent oxidation (Seeberg-Elverfeldt Jens et al., 2005) of the samples. We also preserved the samples by adding saturated $\text{Ac}(\text{OAc})_2$ < 30 minutes after the rhizons were disconnected from the syringes. Such preservation measure is widely used in the literature and can prevent the oxidation of sulphide.

With respect to the downward seawater irrigation due to rising bubbles in the sediments, Hong et al. (2017) have shown that it is unlikely to occur. Briefly, if seawater indeed intruded from the bottom seawater to the surficial sediments, one would expect seawater concentrations for all porewater species. Hong et al. (2017) have shown that this is not the case from the 100 fold higher ammonium detected in the first 5-10 cm of the sediments. We can therefore confidently exclude the impact on sulfide concentration by such so-called bubble irrigation mechanism.

Identification of biota

The statements "Visible fauna (at least a few centimeters across) were identified" (p5) and the statement in the following paragraph that "Numerous individuals of siboglinid worms were seen", appear contradictory. *Oligobrachia haakonmosbiensis* is large for a frenulate, with a tube diameter of 0.6-0.9 mm (Smirnov, 2014). It would be useful to have a high resolution image, perhaps as a supplementary file, to show how these individual siboglinids were visible in the photographs, since a lot of the Discussion is based on their presence or absence.

Response: We can add a supplementary figure, to show what the worms look like in the images. It should be kept in mind that individuals of *O. haakonmosbiensis* were not seen or marked. Clumps or mats of them were seen and these aggregations were outlined (Methods, pages 5-6). Since this point was not clear, we can change this portion of the Methods section to clarify this.

Most of the identifications relied on interpretations of images taken by a towed camera with a resolution of 16 million pixels and with stereo cameras mounted on a ROV with a resolution of 1.4 million pixels. We are not told the respective field of views photographed by these cameras so it is not possible to estimate the respective resolutions. It would help interpretation if the authors would calculate the sizes that the respective pixels represent. Rough calculations, from the dimensions given in Figure 2, suggest that the pixel size in images from

the stereo cameras may have been inadequate to resolve smaller organisms, such as *Oligobrachia* tubes, 0.6-0.9 mm in diameter (Smirnov, 2014) unless they occurred in clumps.

Response: As stated above, the worms did appear in clumps and these clumps were outlined as polygons in a GIS as opposed to every individual being marked. It is absolutely true that single individuals would not be visible in the images. Figure 2 does have scales in the mosaics/transects, but we can add scales in the individual images as well to give a better idea of the fields of view.

The core samples at GHP 5 were taken around the periphery of the pingo (Figure 1) so that it is not possible to deduce from these that *Oligobrachia* was absent from pingo 5.

Response: The deduction that *Oligobrachia* was absent from pingo 5 is not based only on the core samples. This deduction is based on the images and mosaics from the site. It is also based on our extensive surveys of the pingo before imaging for mosaicking purposes was conducted. Therefore, we did not conclude that *Oligobrachia* was absent from pingo 5 just because we did not recover them in the cores (and 1069 at least is not from the periphery). We concluded that they were absent because we spent a considerable amount of time surveying the pingo with the ROV's HD video camera, and did not see the clumps that are so abundant on the other pingos. We are quite confident that the worms are more or less absent at pingo 5. It is certainly possible that a few, scattered individuals are present and these solitary individuals would not be visible in any kind of imagery. However, this is one of the limitations that always exist with image-based studies and is the standard shortcoming that has to be taken into consideration when using an image based approach. We can add this caveat to the discussion. Nonetheless, mats of *Oligobrachia* are not present at pingo 5, and this is an important difference between pingo 5 and the other pingos, and the fundamental statement that we address in this manuscript.

Another problem in comparing tow cam and ROV pictures is that the ROV imaging was always from fairly discrete areas on pingo 5 while the tow cams were transects covering from the outside into the centre of the pingo (Figure 1). This might explain why Nothria, for example, was identified in all the tow cams but not in the ROV pictures. The tow cam epifaunal data, presented in Table 1, should therefore be divided into "on pingo" and "off pingo" sections. The reason that TC25 GHP3 clusters with the GHP5 ROV camera tracks is probably because tow cam 25 has the greatest proportion of offpingo track of any of the tow-cams.

Response: It is true that the tow cam images are transects whereas the ROV images cover more discrete areas (although ROV mosaic 3 is more a series of transects than a mosaic). That is why we chose to neither discuss the overall community structure, nor to make comparisons of overall community between pingo 5 and the other pingos, except very briefly, and to mention the diversity indices. We included a figure of the results of the community analyses in order for these results to be available for everyone, but we refrained from discussing these results, other than very briefly because we agree that the different ways in which the pingos were imaged could be a factor that muddles the overall community characteristics. This is why we instead chose to focus on the presence or absence of *Oligobrachia* since this is a trend that we feel confident of comparing between the different pingos, as stated above.

It is more likely that the absence of *Nothria* from the ROV images is due to the imaging capabilities, and not because the ROV images were discrete mosaics. This is because we could not see *Nothria* in ROV images from pingo 3, i.e., the ROV images over pingo 3 that could not be used because the navigation data was not useable. If the absence of *Nothria* from pingo 5 was simply due to the locations in which the images were taken, then they would be visible in the ROV images taken over pingo 3. Their absence from these images suggests that they are not visible in the ROV camera. Regardless, however, *Nothria* was not used in the community analysis, nor in comparisons between the pingos.

All of the images and faunal data from them is more or less ‘on-pingo.’ We did not use any ‘off-pingo’ images for our quantitative analyses, therefore the data presented in Table 1 cannot be divided into these two categories. There is no clear boundary that distinguishes the seafloor as being part of a pingo versus not. Therefore, we only used images that appear to be part of the different pingos. We know that our navigation data was not perfect for this, but we also looked at the images and the presence of obvious signs of seepage (carbonate crusts, bacterial mats, worm tufts, etc.) as evidence of the images being taken from the seeping pingos. It is entirely possible that some images included in our study are in fact, slightly off the pingo in question, but this error would exist for all the pingos from which the tow cam images were taken. It is true that this error would not occur for the ROV images over pingo 5 but, as stated above, that is why we did not go into any detailed discussions about comparing overall communities.

The only time that we did consider ‘off-pingo’ images was in a transect to the west of pingo 3 (at least 1 km away), which we discuss only qualitatively, where we introduce the idea that seepage areas might have higher species diversity than background, non seep affected seafloor.

In short, all the tow cam images are, to the best of our ability, with the given constraints of the study, taken over the pingos themselves. It would therefore be inappropriate to classify any of them as being ‘off-pingo.’ But we agree that off-pingo areas could have been included in the tow cam images, which makes comparisons with the discrete mosaics on pingo 5 difficult. However, we acknowledge this shortcoming and as a result, do not discuss community differences between pingo 5 and the other pingos. We stick only to the main difference, ie, the presence or absence of frenulates, which we do feel confident is a real difference between pingo 5 compared to the others.

The frenulates observed were identified from specimens in the core samples and density estimates for them were calculated from the densities observed in the cores. It would have helped the interpretations if information had been provided on the depth they reached in the sediment. This may be site specific, since the penetration depth of a species has been shown to vary between cores (Dando et al. 2008). *Oligobrachia haakonmosbiensis* were reported penetrating the sediment to a depth of 55 cm at the Håkon Mosby mud volcano (Lösekann et al. 2007).

Response: We did not include this because we did not conduct any good, exhaustive measurements on how far the tubes penetrated (and we had different types of cores,

which all affect the animals differently when they are retrieved). Roughly, we can say that the tubes reached 50-60 into the sediment, which is in the same range as what was seen at the Håkon Mosby mud volcano by Lösekann et al., (2008) and Gebruk et al. (2003). We can add this information to the text.

It would also be helpful to know whether other macrofauna were recovered from the cores, since most faunal species with chemoautotrophic bacteria at shallower seeps are infaunal and would not show on surface photographs. An example is at a methane seep at 170 m depth in the N. Sea where 3 such species were found living within the sediment and shells of a fourth, the bivalve *Lucinoma borealis*, were recovered (Dando et al. 1991, Dando 2001): no epifauna with chemoautotrophic symbionts were observed. Many frenulates have tubes completely buried within the sediment; thus chemosynthesis is probably more common at the pingo sites than this study of mainly epifauna suggests.

Response: We agree that there might be infaunal species that are chemosymbiotic, and we mention this in the text. We also tried to emphasize that we are only considering animals that are visible in images in this study. However, this might not have always been clear, especially when we talk about *Oligobrachia* being the only confirmed chemosymbiotic species. We can change the text to make sure that every time we talk about a trend like that, we specify that we are referring only to larger animals, visible in images and that smaller, infaunal animals are not taken into account in this study.

Discussion

On discussing the distribution of the frenulate *Oligobrachia*, the authors wrote: “the image transects containing siboglinid frenulates were much less even in terms of species abundances compared to the transect and mosaics which did not contain any frenulates”. This would be expected since it has been shown that, on the Rockall slope, frenulate distribution did not cluster with most other taxa and there was an inverse relationship between frenulate density and the density of other benthos (Dando et al. 2008). This was explained because sediment disturbance by other organisms would increase sulphide oxidation and displace, or bury, the thin tubes of the frenulates. It should be noted that in the latter study most of the frenulates had a low abundance and none of the tubes projected from the sediment, if they did at all, as far as those of *Oligobrachia haakonmosbiensis* and thus would not provide an epifaunal habitat. In the one obligate, methane seep frenulate species that occurred in high densities, *Siboglinum poseidoni* (Dando et al. 1994c), no epifauna were noted between or above the projecting tubes.

The authors consider that chemoautotrophic primary production at the pingos might exceed the photosynthetic primary production reaching the sea floor. The examples they cite are from deeper water, where less photosynthetic production reaches the sea floor. This is unlikely to be true at 400 m where much more photosynthetic production will reach the seabed. As shown in a comparative study (Bernadino et al. 2012), the isotopic difference between background and seep fauna was much lower at the Eel River seeps (250-500m) than at deeper seeps at 770 m depth and deeper. Isotopic evidence of food inputs is needed to support the authors’ hypothesis. Since methane solubility increases with pressure there will, potentially, also be more methane available to the biota at deeper sites.

Response: We do have isotopic evidence of chemosynthetic food input at the pingo site, but these results are part of a separate article and we are not really at liberty to discuss them yet. Our goal was not to show that chemoautotrophic production exceeds photosynthetic production per se, we just wanted to include the notion that local primary production, in the form of chemoautotrophy occurs at the site. Instead of saying that 'autochthonous chemoautotrophic primary production tends to exceed photosynthesis derived detrital food supply (page 12), we can change the text to say that both photosynthetically derived and chemosynthesis based organic matter is likely available at the pingos. The point here was to try to explain why certain animals were seen to appear to show a preference for seep based habitats such as the frenulates, carbonates or bacterial mats. With respect to nutrition, we mentioned that some animals might be grazing on bacterial mats, which could in turn affect higher order consumers and explain their distribution among bacterial mats. Therefore, changing the text so that it does not imply that chemoautotrophic input exceeds photosynthetic input will not change the overall message and so we can easily modify the text to avoid this confusion.

Regarding sulphide in the upper sediment, p.14 line 11: only 1 measurement at 5 cm depth is shown in Fig. 4 (for core 1045, off the edge of pingo 3). The exact value for this sample is not shown. However, a single measurement does not justify the statement that "sulfide was not detectable - - - even in the upper 5 cm of the sediment at the pingo site". Should this read "sites"?

Response: The value is 0. The 0 values for the other cores should also be shown in this figure and we apologize that they are not there. Even at 10 cm, we often could not detect sulphide in the cores, which is the reason for the statement on page 14, line 11, that sulphide was not detectable in the bottom water or even in the first 5 cms. That is, this statement was not based on a single measurement from one core at all, but we agree that the 0 values for the other cores should be shown on this figure and we will rectify that.

As mentioned above, it is probable that sulphide was present at significant concentrations for the biota, including the bacterial mats at the surface, but was not detected using the stated analytical procedure. Serov et al. (2017 Fig. S2C) shows a picture from one of the pingos with a white bacteria mat, presumably of sulphur-oxidising bacteria, on top of "tubeworms" that project approximately 4 cm above the sediment, if the scale on the photograph is correct. If these are sulphur-oxidising bacteria then sulphide or thiosulphate must be present in the water column. The "tubeworms" are approximately 10 mm across, measured against the scale on the photograph, and thus cannot be *Oligobrachia*.

Response: As acknowledged above, we will change the text so that we clarify that we did not detect sulphide, but there could be concentrations lower than what we detected, including using bacterial mats as evidence for this.

With respect to the image in Serov et al., based on previous descriptions of *Oligobrachia*, it would appear that the worms with filamentous bacteria on them are too large to be *Oligobrachia*. However, we have sampled these worms and they are, in fact, *Oligobrachia* (manuscript in preparation). The bacteria can form large, dense colonies on the tubes of the worms, so that they appear much larger than the tubes themselves. Below is an image that shows this. This was also seen in nearby sites such

as in Storfjordrenna (Åström et al., 2016) and a site of pingo-crater complexes in Bjronøyrenna (publication in preparation).



P14, line 21 and subsequent text: "this particular image transect did not contain frenulates." *Oligobrachia haakonmosbiensis* has large tubes for a frenulate, many species have tubes 100 μM or less across and would not be visible if they did project above the surface, although many species are completely buried within the sediment. More than one species frequently occur in the same core sample (Dando et al. 2008) so that it is not possible to state that frenulates were absent. To be correct you should write that "this transect did not show any 'visible' frenulates".

Response: We can change this as suggested. As we mentioned before, we were trying to emphasize that only animals visible in images are considered in this study, but obviously, we have not emphasized this enough and we will make sure that this point is very clear.

GHP5 gas release: in many submarine seeps gas is only released at low tide when slight differences in bottom pressure cause the sub-surface gas volume to increase. At other frequently visited methane seep sites, such as the Scanner pockmark, continuous gas release may, or may not, be present during any given cruise. In the absence of data on the tidal conditions when observations were made over GHP5 it is not possible to state that gas was not released from this pingo.

Response: It is indeed true that gas seepage can be induced by tidal effects. We however do not think this can explain the contrast in gas flare activity between pingo 5 and the other pingos as tidal changes should pose the influence on all pingos that have almost the same water depth and are within an area of only about 2km^2 . Gas flare data was acquired in 2016 together with the 3D seismic survey, and all in all, the survey took two days. During this time-span, we detected no gas flares from GHP5, and thus we can rule out a potential tide-controlled leakage. Furthermore, as is mentioned in the manuscript, several cruises were conducted, over three years and during different seasons, all of which consistently documented the absence of any flare activity over pingo 5.

The enhanced reflectors below GHP5 indicate subsurface gas and, on enlargement of Figure 6, it is possible to see a small gas “chimney” towards the edge of the pingo (see Fig 6 section), although this is considerably smaller than the chimneys below the other pingos. Core 920 on the edge of GHP 5 contains methane of thermogenic origin (Serov 2017, Table S1), implying a deep source for the gas.

Response: The enhanced reflectors below GHP5 may indicate pockets of subsurface gas, buried carbonates or gas hydrate (but not necessarily active gas migration feeding the GHP). The seismic data show lower amplitude dipping reflectors underneath the unconformity below GHP5. Active seismic chimneys are normally represented by distinct acoustically masked areas (such as under pingos 1-3) or a vertical pipe structure often accompanied by velocity-related anomalies, which we don't observe below GHP5. A narrow zone of weak acoustic blanking under the margin of pingo 5 may indicate either very low to negligible fluid migration, or a fault zone. On the neighboring inlines and crosslines in our seismic volume, this feature appears even less prominent; therefore, we conclude that there is no significant fluid/gas migration underneath GHP5. On the other hand, underneath the other pingos, prominent seismic chimneys occur. We can change the text so that instead of writing “lack of chimney..” in the manuscript we can write “no prominent seismic chimney” underneath GHP5.

Active release of methane from the sediment will channel the methane into the higher porosity release channels. The sediment at the sides of these channels will have a low methane concentration, due to the down-flow of seawater from the sediment surface (Dando et al. 1994a, O’Hara et al. 1995). Thus it is not correct to argue that methane release will stimulate overall sulphate reduction and methanotrophy in a seeping pingo when compared to a non-seeping pingo with a high sediment methane concentration (p17 first paragraph). Microbes may also be removed from the system by the rising fluids.

Response: As our reply for the previous comments and the results from Hong et al. (2017), from the concentration of ammonium in the porewater, we can confidently exclude the possibility of downward seawater flow into the sediments. We therefore do not think such argument is relevant in our study sites.

Furthermore, sulfate reduction rates were measured independently of methane. Therefore, our argument that sulfate reduction rates are different at pingo 5 is still valid. Overall, the point is that pingo 5 is different from the other pingos. There is lower methane concentration in the sediment (whether this is strictly dissolved methane or not), there were no hydrates recovered from pingo 5, there are no prominent seismic chimneys below pingo 5, sulfate flux rates were lower at pingo 5, and no gas flares rising into the water column were seen at pingo 5. Together, these seem to indicate that the geochemical conditions at pingo 5 are different, which could account for the absence of frenulates. We even have preliminary results indicating that the microbial community at pingo 5 is different, including that ANMEs make up less of the total bacterial/archaeal community at pingo 5 (Klasek et al., in prep, which is referred to in the manuscript), which further supports our argument.

In short, we believe that our overall conclusion, of different geochemical conditions at pingo 5, compared to the other pingos, is nonetheless valid, and could account for the absence of frenulates from pingo 5.

Sulphate reduction (p16): “In most cores, the ratio of inorganic carbon to sulfate consumption was found to be close to 1:1 regardless of depth (both GHP5 and GHP3). The one exception was core 1048 from GHP5, for which, almost all values were closer to the 2:1 ratio.” Core 1048 is shown in Figure 1 to be the furthest away from any pingo, i.e. it is in background sediment. Thus it should be no surprise that in this core sulphate reduction is not dependent upon the presence of methane.

Response: This is correct.

“The dual need for inorganic and organic carbon sources (plus thiotrophic chemoautotrophy) likely results in frenulates **in general**, and, *O. haakonmosbiensis* specifically, relying heavily on a highly active sediment methanotrophic microbial community” (p16 line 31). This is not true as a general statement. Dando et al. (2011), in a study of the relationship between 10 species of frenulates and the chemistry of their habitat, found that, with the exception of 1 obligate methane seep species, all occupied sediments where the dissolved methane concentration was $< 1 \mu\text{M}$.

Response: We did not phrase this correctly. The idea was to introduce a hypothesis, that *O. haakonmosbiensis* relies on an active microbial community because they might be obtaining their nutrition from both their symbionts and the surrounding sediment. We can rephrase this.

“On the other hand, at GHP5, seepage of methane is low due to the lack of a sub-surface gas chimney. Methane is still present in the sediment, but in lower concentrations and as a result, methanotrophic microbes are likely less abundant and methanotrophic activity is considerably lower, as evidenced by lower AOM linked sulfate flux rates” p17. As mentioned earlier the authors do not know the concentration of available methane in the sediment and hence cannot make such comparisons regarding different methane concentrations at different sites. The values in Figure 4 may just equate to the amount of authigenic carbonate in the samples. A small gas chimney appears to be visible below GHP5 in Figure 6.

Response: As stated above, we disagree that we did not measure dissolved methane. Additionally, we must emphasize that the presence of gas hydrates was observed in most of the sediment cores recovered from pingos 1-3, while no gas hydrate was recovered from any of the sediment cores in pingo 5. Dissolved methane concentration must be high enough (i.e., at saturation level) to allow for the presence of gas hydrates, which is the case for most the sediment cores, but not pingo 5. This is quite solid evidence to support our inference and therefore contrasting methane concentrations between pingo 5 and the other pingos.

We do not understand the rationale behind “methane concentration may just equate the amount of authigenic carbonates in the sediments”. Precipitation of authigenic carbonates depends on the saturation state of carbonate minerals, which is a function of the availability of DIC, calcium, and magnesium in the porewater. The supply of calcium and magnesium in the porewater is independent of the supply of methane in the sediments. Of course a faster turnover of methane through AOM can accelerate the precipitation of authigenic carbonate precipitation but there is no sign showing the absolute amount of methane and authigenic carbonate should be in any way be related.

As discussed above, despite there being some blanking under pingo 5, the seismic data indicates that there are pockets of gas, but not necessarily active gas migration. Furthermore, no hydrates were recovered from pingo 5, which also suggests less seepage there. Combined, we believe that this suggests that dissolved methane concentrations are lower in the sediment at pingo 5, irrespective of whether one is convinced we measured dissolved methane in our samples or not. In any case, sulfate flux rates were measured independently of methane, and they suggest lower methanotrophic activity at pingo 5, which is the main crux of our argument.

The discussion regarding hydrothermal vents is not very appropriate for this paper with respect to *O. haakonmosbiensis*. This is a cold-water species that penetrates approximately 0.5 m into the sediment. At vent sites the temperature within the sediment would, almost certainly, be lethal for the species.

Response: We were referring to the ‘lower temperature’ zone of seeps where *Sclerolinum* is found, but we agree, we can remove the discussion related to hydrothermal vents.

Although *O. haakonmosbiensis* was the only metazoan with chemoautotrophic symbionts found, it does not mean that it was the only one present, since the infauna, where, for example, other frenulates and thyasirid and lucinid bivalves might be expected, was not studied. It is therefore also not correct to state that “the community at the pingos does not contain specialized seep endemics” (p22 line 13) since the infauna were not studied and *O. haakonmosbiensis*, if distinct from *O. webbi*, is probably a seep obligate species. “Endemic” is incorrect in this context since it refers to geographic regions, not habitats.

Response: We can change to say seep specific or seep obligate. And yes, we agree that there might be infaunal community members that are seep obligate, and we do mention this in the text (thyasirids). Once again, we will have to make sure that we clearly state that we are talking about larger, visible fauna. We acknowledge that the frenulates at the site might be seep obligate (e.g., page 19, page 12). But we can also change any discussions about seep obligates in the overall community and make sure that we do not say that they are absent or completely lacking, but rather, that only one species has so far been seen (and again, make sure that the scale we are referring to is large animals visible in images).

Figure 1

It would aid interpretation if the positions of the observed gas flares were pinpointed in figures b-e.

Response: We can add these.

Figure 2, 8 & 9

These would benefit from scales in the camera pictures, since the laser spots, when present, are difficult to see.

Response: We can add them.

Figure 3

Figure 3b has TC21 and TC18GHP3 plotted on top of each other, including the labels, so it is not clear what this point represents.

Response: This is because they are so similar, that they end up being right on top of each other. We can include an explanation in the figure caption.

Figure 4

The lines after the final points appear to be extrapolated randomly. If this is because the graphs are part of larger ones and have been cut off at 60 cm then it would be sensible to give the depth and values of the next points in parenthesis at the end of the lines. The coloured open circles are not well differentiated at the scale of the Figure and should be replaced by coloured filled circles to differentiate the cores.

Response: We can make these changes.

Figure 6

I think the vertical scale is m depth below the sea surface and not sediment depth. Fig, 6b is too small to be useful without enlargement.

Response: We can make 6b larger. Yes, the vertical scale should be meters below sea level and we can change this.

Discussion

The term “megafaunal taxa” is used in the Discussion. Megafauna are large animals such as cetaceans and large fish. The correct term for the taxa observed is “macrofauna”

Response: The distinction between megafauna and macrofauna is somewhat subjective and different people have different opinions on how to use the two terms. We use megafauna for this manuscript since we refer to animals large enough to be seen easily with the naked eye. We consider macrofauna to be smaller animals that are retained on a 0.3 mm or 0.5 mm sieve (this cut off seems to vary between studies) and are not easy to see through imagery. This definition is certainly subjective as well, but it is in accordance with many other similar seep and vent studies and we chose to use this terminology to be consistent with other studies with similar methodologies (Amon et al., 2017; Baco et al., 2010; Bowden et al., 2013; Hessler et al., 1988; Lessard-Pilon et al., 2010; Marcon et al., 2014; Podowski et al., 2009, 2010; Rybakova (Goroslavskaya) et al., 2013; Sellanes et al., 2008).

P14 lines 20 & 21, Fig. 2 should read Fig. 3

Response: Thanks for pointing this out, we can change this.

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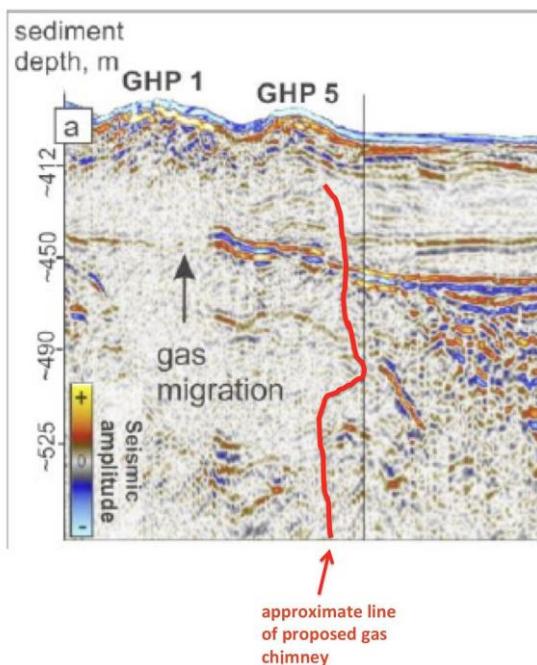
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Figure 6 section



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Interactive comment on “Geophysical and geochemical controls on the megafaunal community of a high Arctic cold seep” by Arunima Sen et al.

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Relating the geochemistry to the species distribution of the macrofauna at seeps is always very difficult. Concentrations of possible energy sources, both in the surface layers, and within the sediment depth to which most macrofauna occur, can change by an order of magnitude or more over a distance of 10-50 cm from the seep outlet. In this study the positions of the cores with respect to the camera tracks are not that well constrained. Core 920 is shown here as NE of GHP5 while in Hong et al. (2017) it is shown as SE of GHP5.

Response: We agree completely, but we urge readers to remember that constraining the biology based on the geochemistry at a fine scale was not the aim of this study. We were instead looking at more general patterns over a larger scale. Namely, that pingo 5 does not have extensive mats of siboglinids like the other three pingos. We initially thought that lower sediment concentrations would explain this trend, but as it turns out, pingo 5 does not have lower sediment sulfide concentrations compared to the other pingos. However, there are multiple lines of evidence that suggest that overall geochemical conditions at pingo 5 are different, and we believe that these could explain the absence of siboglinids from pingo 5. These differences are: 1) lower methane concentrations at pingo 5 (even if it is not strictly only dissolved methane), 2) no hydrates were recovered from pingo 5 but were from the other pingos, 3) there are no prominent seismic chimneys below pingo 5, 4) sulfate flux rates are lower at pingo 5 compared to the others, 5) there are no rising gas flares into the water column from pingo 5, but there are from all the other pingos, and 6) ANMEs make up less of the total microbial community at pingo 5 compared to the others. Together, we believe that these differences suggest that overall at pingo 5, there is likely to be lower methane flux and a less active methanotrophic microbial community (i.e. lower AOM rates).

We checked our coordinates and it appears that there is a mistake in Hong et al., 2017.

We should mention that cores were not taken in sync with the imaging efforts. We are well aware that the cores do not line up with the mosaics or transects. And therefore, fine scale comparisons of geochemistry with biology is not possible. But we do believe that at the scale of one pingo as a whole compared to another, our sampling efforts were sufficient. In fact, the similar sulfide profiles from cores taken at pingo 5 and the other pingos indicate that our sampling efforts were adequate to obtain a general overview of geochemical conditions at the scale of individual pingos. Had we only got ‘peripheral’ data from pingo 5, then the sulfide concentrations from pingo 5 would be consistently lower, but this was not the case (instead, it was very similar to the other pingos).

My earlier comment regarding false dissolved methane readings by adding equal volumes of 1 M sodium hydroxide to samples for the analysis of free methane was influenced by my own early studies in which samples were not analysed at sea and had a longer storage time in contact with NaOH. I accept that a short exposure may not bias the results to the extent I believed, although it would be good to have results from an untreated pore water sample for comparison.

Indeed Ertefai et al. also used 1 M NaOH treatment before measuring free methane and then continued the treatment for a longer period to measure adsorbed methane. Timing is everything!

Response: We agree with Dr. Dando that timing is crucial. In the future research we will conduct test measurements with and without NaOH solution in samples at different times after sampling.

It is known that results of FID GC measurements of headspace samples require interpretation. We interpret measured concentrations from the samples collected in extremely active seepage site with massive gas hydrate layers within 1-3 m of sediment column, bacterial mats on the seabed and authigenic formations on the seabed as concentrations of the labile (dissolved) methane. GHP 5 clearly shows indications of some gas seepage (mats, fauna, etc.) making us confident that dissolved gas is present in the subsurface sediments.

The GHPs are located within an area of 10 km² uniformly influenced by one sediment source and ocean currents implying no evidence of any appreciable heterogeneity in clayey mineral content and composition. Macroscopic observations of sediments from different pingos are in agreement with this. It means adsorption potential of bulk sediments is uniform within the area. Therefore, if some adsorbed gas contaminated our measurements, this contamination is likely uniform throughout the whole set of the samples. Thus, the trend of lower methane concentration in GHP5 compared to other GHPs should remain.

Head space methane concentrations is one line of evidence for different methane seepage activity and geochemical conditions in pingos along with reflective seismic data, echosounder data, pore water chemistry results and video surveys. Complex interpretation of these data supports our conclusion of modest methane supply in superficial sediments of GHP5 as opposed to larger methane discharge in other GHPs.

There is at least one paper on free methane concentrations in sediments off Spitzbergen, that did not use sodium hydroxide pre-treatment for dissolved methane measurements, and should be cited Kneis et al. (2004). These authors analysed 26 sediment samples between 15 and 30 cm depth and found a thermogenic methane signature, d13C of -50.8 (mean) in the adsorbed methane but a d13C of -65.2 (mean) in the free methane. The free methane concentrations, 0.5 – 5.5 micromol/litre were lower than measurements from a similar sediment depths in the pingo areas, 6-330 micromol/litre (Serov et al. 2017). It was suggested, Kneis et al. (2004), that the adsorbed methane was not available to the microbes and that the free methane was probably a mixture from both thermogenic and biogenic sources. However, the methane isotope data reported from the pingo area (Serov et al. 2017) was taken from a greater sediment depth so that a direct comparison of the sources of the free methane cannot be made.

Response: We appreciate the suggestion to refer to a paper of our colleague from CAGE Dr. Kneis. Despite, unravelling the source of gas is indeed an important topic, it is not a focus of our study. We submit that expanding the discussion chapter of our paper to cover isotopic compositions of adsorbed and dissolved gas in bottom sediments around Svalbard archipelago would dilute the focus of our work. However, one important conclusion may be drawn from comparing results of Kneis et al., and Serov et al.,: concentrations of methane in pingos at the same subsurface depth are up to 660 times higher. As opposed to regional study of Kneis et al., not targeting seeps, our study site demonstrates drastically different style of methane release with greater abundance of

labile methane detected not only geochemically, but with direct and indirect geophysical observations.

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Comment on bg-2017-540 Geophysical and geochemical controls on the megafaunal community of a high Arctic cold seep

Sen A et al.

This manuscript presents data on the megafaunal community structure associated with cold seep sites in the western Barents Sea inferred from high resolution seabed imagery, and relates these data to available geochemical information. The manuscript is well-written, and as information on the ecology and biogeochemistry of cold seeps in the Arctic is still quite rare, it makes an important contribution to the field.

Response: Thank you for appreciating the manuscript.

At the same time, there are some methodological constraints that limit the interpretability of the data. In particular, the conclusions drawn with regard to microhabitats are in my view not fully supported by the available data and the respective part of the discussion could be shortened.

Response: We hope that we have addressed your specific concerns adequately below. Please note that we were not referring to or studying microhabitats in this manuscript. We were mainly looking at larger scale patterns and processes, as opposed to small scale variability in habitats and conditions.

Seepage rates/ volumes at cold seep sites often vary strongly over small spatial scales, and the seafloor pictures shown in this ms indicate that the same seems to be true for the GHPs under study, with patches of bacterial mats, for example, probably indicating higher than average methane/ sulphide availability. In fact colonisation by bacterial mats and certain megafauna species (Calyptogena, Acharax; siboglinids etc), are often linked to relative flux rates (e.g. at HMMV or Hydrate Ridge) and can even serve as (crude) indicators for seepage intensity. The limited number of sediment cores taken in this study (and I fully accept the limits that can be placed on sampling in Arctic and deep waters) will likely not have been sufficient to resolve this spatial pattern which is likely to impact on microhabitat preferences.

Response: We completely agree that seepage and concentrations/fluxes of different compounds demonstrate heterogeneity over very small spatial scales at chemosynthesis based habitats such as seeps. We also agree that animal and microbial distributions can therefore serve as crude indicators of seepage patterns. However, as mentioned in our response to Dr. Dando, we did not attempt to use the images or the cores to examine patterns over small spatial scales. Our aim was to compare the different gas hydrate pingos, specifically to compare pingo 5 to the other three pingos, which are on the scale of several hundred meters in diameter. Similar numbers of cores were taken from pingo 5 as well as from the others, and we believe that these cores are representative of conditions at those pingos as a whole, which is what we examine and discuss. Furthermore, we used additional lines of evidence, in addition to geochemical measurements, to support our conclusions of inter-pingo differences, such as presence/absence of gas flares and seismic data that demonstrates different types of sub-surface gas/gas hydrate reservoirs.

An analogy can perhaps explain this issue. Mountain tops and valleys have very different kinds of vegetation because they experience different environmental conditions overall, and this difference corresponds to what we were demonstrating, i.e. that pingo 5 has a different animal community from the other pingos that corresponds with overall different geochemical conditions. For example, high altitude trees, say, pines, might be absent in the valleys, which is similar to the siboglinids being absent from pingo 5. Within a mountain top or a valley, there are additionally small scale differences in species distributions related to various factors, such as soil type and quality, the presence of streams or rivers, steepness of slope, etc. Similarly, within a pingo that contains siboglinid worms, small scale differences can determine if the worms are present in a location or absent a few centimeters away. We would like to emphasize that we are not tackling this second question and we agree that our methodology is insufficient for doing so.

In addition, temporal variability of seepage - even at sites considerably deeper than those under investigation here - is often strongly linked to tidal rhythms and longer term observations are likely to be necessary to conclude with certainty whether active seepage occurs at a specific site or not.

Response: It is true that we cannot assess temporal variability in seepage. However, as addressed in Dr. Dando's review, in terms of gas flares, we are confident that pingo 5 does not show any flare activity because this data was collected over multiple years, across different seasons, and each time, over long periods of time that well encompassed tidal rhythms. Furthermore, we do not say that pingo 5 exhibits no active seepage in the sediment, quite the contrary, in fact, we argue that pingo 5 does show enhanced flux of methane and sulfide, which could even account for the bacterial mats seen there (page 11, lines 18-19). Instead, what we argue is that methane flux, sulfate flux and sulfide flux and therefore AOM rates, are lower at pingo 5. In addition, we did not recover any gas hydrates from the +5 gravity cores at pingo 5 whereas we recovered gas hydrates from the gravity cores from the other pingos. Such difference in gas hydrate abundance also clearly demonstrates the contrast in methane supply from pingo 5 compared to the other pingo features.

In this context I was also surprised to see that all pictures taken along each of the specific transects seem to have been 'lumped together' for analysis and no attempt was made to distinguish between fauna at more or less active seepage sites.

Response: In fact, this is what we did and was the aim of the manuscript: to compare the 'less active' pingo 5 to the 'more active' pingos 1-3.

Judging from fig. 1, the photographic transects seem to have included (or at least could have been extended to include) reference areas without seepage and I was disappointed to see no comparison between fauna at seep and reference sites. Is there a reason for this? This would add a very valuable and relevant dimension to the manuscript.

Response: We did compare with images that were far enough away from the pingos (at least 1 km away) to be considered more representative of 'reference' sites. However, bacterial mats were nonetheless seen in this area and since we are unaware of the extent to which lateral diffusion takes place, we cannot confidently state that any of our image transects included true

reference, non-seep impacted areas. That is why even though we did make some comparisons to 'non-seep' areas, we were very cautious with these comparisons.

While it is likely that AOM consortia provide the sulphide utilised by chemoautotrophic bacteria, it seems unlikely that sulphide can be both abundant enough to support the bacterial mats visible in the photographs while being removed so efficiently from the substrate that concentrations in the upper sediment and bottom water were below detection limit. Could this be an analytical error ?

Response: As we clarified for Dr. Dando, the detection limit was 40 μM . Bacteria can survive on lower concentrations, therefore, they might be present even when we are unable to quantify sulfide through our measurements. As we agreed to do, based on Dr. Dando's discussion of this topic, we will remove the section that says that sulfide flux to the bottom water is so low as to allow non-seep specific fauna from colonizing the area without getting poisoned by sulfide, since it is true that sulfide might be reaching the bottom water despite our inability to detect it.

Explanation of changes made to the manuscript:

We thank Dr. Paul Dando and an anonymous reviewer for their comments and suggestions regarding the manuscript. Following the interactive discussions with Dr. Dando and our reply to comments made by reviewer two, we present a substantially revised manuscript wherein we have made a number of changes in order to incorporate all the suggestions and to address every issue brought up by the reviewers. These are outlined briefly below:

1. One of the issues brought up was that it was not always clear that the study focused on animals visible in images. We have made this much clearer now, and have stressed repeatedly, throughout the manuscript, that the scale being addressed in the study is large animals visible to the naked eye in images. Additionally, whenever we discussed any community scale dynamics, we clearly stated that for the most part, we are talking about larger animals, but also took into account and referred to other animals that were not covered in the images.
2. Both reviewers brought up the issue of our flare data not capturing temporal trends. We explained in our comments that our flare data was collected over multiple visits in different seasons and conducted over long periods of time that would have covered tidal rhythms and patterns. In the manuscript now, this has now been stressed, therefore it should now be clear to readers that we are not missing a temporal component to our conclusion of no flares from GHP5.
3. Dr. Dando brought up, correctly, that at the resolution of the images, individual tubes of *Oligobrachia* worms would not be visible. We have now provided explanations of how *Oligobrachia* was seen in the images, namely, that aggregations were seen and marked, as opposed to individual worms. In order to illustrate this, we have added a supplementary figure, as suggested, to show what these aggregations look like in the images.
4. Dr. Dando was concerned that our headspace measurements overestimated concentrations of dissolved methane. We have provided explanations as to why we believe that for the most part, our measurements are reflective of dissolved methane concentrations. In short, our syringes might have contained some free gas, however, in seafloor conditions, this gas would have been dissolved. Therefore, we likely analyzed mostly dissolved methane if we talk about in-situ conditions. Nonetheless, we added the caveat that some amount of free gas might have been included in our measurements as well.
5. Dr. Dando pointed out that the seismic data indicates the presence of some amount of gas underneath GHP5. We have changed the text throughout the manuscript, so that instead of saying that the seismic data reveals nothing of consequence below GHP5, we now say that there is weak blanking and therefore limited methane advection below GHP5.
6. Both reviewers were concerned about our datasets being inadequate for examining heterogeneity of animals and geochemical conditions over small spatial scales, and the distribution of animals with respect to microhabitats. We responded in our comments that this was not our intention- instead, we were comparing individual pingo features and therefore, were examining larger scale differences in both animal distributions and geochemical conditions. In the manuscript, we have altered the text to stress that the focus of the study was broader scale patterns and we hope that this confusion will now be avoided.
7. Both reviewers brought up some issues with respect to sulfide measurements, particularly concerning detection limits and the presence of sulfide in the bottom water. We have clarified in the text now, that our detection limit was 40 μM and we have changed our approach to bottom water measurements. We acknowledge that sulfide could be present in

the bottom water in concentrations below our detection limit and have removed the section where we had mentioned that non-seep animals might not be exposed to toxic levels of sulfide at the study site.

8. All discussion with hydrothermal vents have been removed.
9. Specific comments were addressed and all suggestions towards this end were incorporated (e.g., adding scales to figures where lasers were not very visible, changing figure details and appearances, adding details to figures such as flare locations, etc.).

We hope that the reviewers and the editor will be satisfied with the made to the manuscript and we thank them for expressing their views that have helped to improve the manuscript.

Sincerely,

Arunima Sen and co-authors

Geophysical and geochemical controls on the megafaunal community of a high Arctic cold seep

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Abstract

Cold seep megafaunal communities around gas hydrate mounds (pingos) in the Western Barents Sea (76°N, 16°E, ~400 m depth) were investigated with high resolution, geographically referenced images acquired with an ROV and towed camera.

Four pingos associated with seabed methane release hosted diverse biological communities of mainly non-seep (background)

15 species including commercially important fish and crustaceans, as well as a species new to this area (the snow crab *Chionoecetes opilio*). We attribute the presence of most benthic community members to habitat heterogeneity and the occurrence of hard substrates (methane derived authigenic carbonates), particularly the most abundant phyla (Cnidaria and Porifera), though food availability and exposure to a diverse microbial community is also important for certain taxa. Only one chemosynthesis based species was confirmed, the siboglinid frenulate polychaete, *Oligobrachia* cf. *haakonmosbiensis*.

20 Overall, the pingo communities formed two distinct clusters, distinguished by the presence or absence of frenulates. Methane gas advection through sediments was absent low below the single pingo that lacked frenulates, while seismic profiles indicated abundant gas saturated sediment below the other frenulate colonized pingos. The absence of frenulates could not be explained by sediment sulfide concentrations, despite these worms likely containing sulfide oxidizing symbionts. We propose that high levels of seafloor methane seepage linked to sub-surface gas reservoirs support an abundant and active sediment 25 methanotrophic community that maintains high sulfide fluxes and serves as a carbon source for frenulate worms. The pingo currently lacking a large sub-surface gas source and lower methane concentrations likely has lower sulfide flux rates and limited amounts of carbon, insufficient to support frenulates. Two previously undocumented behaviors were visible through the images: grazing activity of snow crabs on bacterial mats, and seafloor crawling of *Nothria conchylega* onuphid polychaetes.

1 Introduction

Cold seeps, where hydrocarbons and reduced gases emerge from the seafloor, are ubiquitous in the world's oceans and despite being discovered only a few decades ago (Paull et al., 1984), they have been studied intensively in a variety of settings around the world (Levin, 2005; Levin et al., 2016; Sibuet and Olu, 1998; Sibuet and Olu-Le Roy, 2002). However, cold seeps in the

5 Arctic Ocean have received less attention and the literature on Arctic seep communities is limited to a few studies in the Barents and Beaufort Seas (Åström et al., 2016, 2017b, 2017a; Gebruk et al., 2003; Lösekann et al., 2008; Paull et al., 2015; Pimenov et al., 2000; Rybakova (Goroslavskaya) et al., 2013). The most well studied seep site in the Arctic is the Håkon Mosby mud volcano (HMMV), which has practically become synonymous with Arctic seep biology. Paradoxically, high thermal gradients in the sediment have led researchers to conclude that HMMV does not really constitute a typical cold seep
10 (Gebruk et al., 2003).

Another limitation to our current understanding of cold seeps is the focus on mainly deep-sea sites. It should be noted that the terms 'shallow' and 'deep' are relative, and a strict, universally accepted cutoff value separating the two does not exist. Nonetheless, relatively shallow seeps, such as those on continental shelves and upper continental slopes, have not been studied nearly as well as their deep-sea counterparts. In their reviews of cold seeps, Sibuet and Olu (1998; 2002) only considered sites
15 at a minimum of 400 m water depth and even the more recent review of Levin et al. (2016) refers to cold seeps within the context of the deep sea. Yet studies of seeps in comparatively shallow water (< 400 meters) are crucial to resolve depth-related trends in biodiversity, chemosymbiotic species and seep-obligate fauna (Carney et al., 1983; Dando, 2010; Sahling et al., 2003).

Several sites of methane seepage have been discovered on the continental shelf offshore Svalbard and in the northwest Barents
20 Sea (Andreassen et al., 2017; Åström et al., 2016; Portnov et al., 2016; Sahling et al., 2014; Serov et al., 2017). An abundance of cold seeps in the Arctic is important, because the Arctic is connected to both the Pacific and the Atlantic Oceans. This setting provides an excellent opportunity to study the establishment of biogeographic provinces, migration and connectivity between seep populations that are otherwise disconnected from each other at lower latitudes. The presence of numerous cold seeps on the Barents Sea shelf could also be pertinent to the overall ecology and economy of the Arctic. The Barents Sea is
25 considered an ecological hotspot for the circumpolar Arctic and an economically important region supporting one of the richest fisheries in the world (Carroll et al., 2018; Haug et al., 2017; Wassmann et al., 2011). The interaction between cold Arctic and warm Atlantic water masses, seasonal sea ice cover and the interplay of pelagic-benthic coupling creates a highly productive region (Degen et al., 2016; Ingvaldsen and Loeng, 2009; Sakshaug et al., 2009; Tamelander et al., 2006). Moreover, the Arctic and particularly the Barents Sea, are predicted to experience amplified impacts of climate warming such as shrinking sea ice
30 cover, changing oceanographic patterns and increasing ocean acidification (Haug et al., 2017; Onarheim and Årthun, 2017; Węsławski et al., 2011). Such climatic and environmental changes in the region and the associated impact of newly established invasive and northward migratory species may cause major ecological shifts in the Barents Sea (Cochrane et al., 2009; Degen

et al., 2016; Johannessen et al., 2012). With our limited knowledge of the biology and ecology of Arctic seeps, predictions about how these methane based ecosystems will respond to a warming Arctic are difficult to make.

This study examines the megafaunal community associated with a cold seep site on the Arctic shelf in the western Barents Sea 5 (Fig. 1). Our results serve as a first step towards addressing some of the existing gaps of knowledge regarding cold seep and Arctic ecology, i.e., with respect to seeps on the continental shelf in relatively shallow water (<400 m) in the high Arctic (76°N) (Åström et al., 2016, 2017b; Dando, 2010; Paull et al., 2015).

High resolution, georeferenced seabed imagery was used for analyzing the communities of visible megafaunal animals 10 associated with four gas hydrate bearing mounds (pingos) exhibiting active methane seepage. All animals visible in the images (i.e., at least a few cm in size) were examined, thereby resulting in the inclusion of different categories of animals such as epifauna, infauna and even some pelagic species. Multiple long-term Hydroacoustic surveys were conducted at the pingos 15 over three years and across different seasons. These surveys revealed flares of gas rising into the water column from around the summits of three of the four investigated pingos, suggesting different seepage regimes and sediment geochemical conditions between the free gas emitting pingos and the single pingo from which no hydroacoustic flares were seen. We hypothesized that megafaunal communities at the free gas emitting and non-emitting pingos would differ. Further, we expected 20 differences in the concentrationsmposition of sulfide and methane in sediment pore water between the gas emitting pingos and non-emitting pingo, to account for differences in associated megafaunal communities. The setting for this study is particularly useful for teasing apart the factors affecting the large-scale distribution of chemosynthesis based species, since these animals are directly reliant on seeping chemicals (Levin, 2005; Sibuet and Olu, 1998). Chemosynthesis based animals are often considered ecosystem engineers within cold seep systems and their presence or absence may subsequently affect community structure as a whole (Cordes et al., 2010; Levin, 2005; Levin et al., 2016). Our approach consisted of linking overall seepage patterns to sediment geochemistry and the distribution patterns of chemosynthesis based animals in the context of the overall 25 community structure.

25 2 Methods and Materials

2.1 Study site

The area of focus for this study is a site on the Arctic shelf (hereafter referred to as the ‘pingo site’), about 50 km south of Sørkapp (South Cape), Spitsbergen, characterized by sub-circular, domed seabed structures (Fig. 1) from which gas hydrates have been recovered in sediment cores (Hong et al., 2017). The morphological similarity of these mounds to terrestrial and 30 offshore pingos have resulted in them being referred to as ‘gas hydrate pingos’ (GHPs) (Serov et al., 2017). Originally, the term pingo refers to mounds of earth-covered ice in permafrost regions, formed by the hydrostatic pressure of water in the

permafrost (Pissart, 1985). Similar features in marine systems, where sediment gas hydrates are analogous to ice in terrestrial systems have been referred to as gas hydrate pingos or submarine pingos (Chapman et al., 2004; Hovland and Svensen, 2006; Paull et al., 2007; Serié et al., 2012). In this study, the term gas hydrate pingos (GHPs), or simply, pingos, will be used for the four⁴ features of interest

5 The pingo site is located at a depth of about 380 m, on the flank of the glacially eroded Storfjordrenna cross shelf trough. A stable grounded ice sheet over Storfjordrenna, followed by alternating warm and cold periods resulted in both the accumulation of gas hydrates as well as their episodic dissociation over the past 22,000 years (Serov et al., 2017). The GHPs themselves are proposed to have been formed ~15,500 years ago, when deglaciation followed by a warm Heinrich H1 event had a particularly debilitating effect on the gas hydrate stability zone (GHSZ) and resulted in the large scale release of methane from gas hydrates
10 that had accumulated during the prior thousands of years. Since about 8,000 years ago, however, the region experienced a steady transition to current conditions of stable gas hydrates (Serov et al., 2017).

This study focuses on a cluster of four GHPs, within an area of 2 km². These GHPs rise gradually above the surrounding seafloor (8-12 m) with diameters ranging between 280 m and 450 m. Hydroacoustic, seismic and geochemical surveys show persistent and continuous release of predominantly thermogenic methane gas around the summits of three of the four GHPs
15 (GHPs 1,2 and 3) (Serov et al., 2017). No such free gas emissions were seen over GHP5 during repeat on-site observations over 3 years (2013-2016) across⁴ during different seasons.

2.2 Imagery

Two sets of seafloor imagery were collected in 2015 and 2016 (Fig. 1). The first set was taken in 2015, with the MISO-WHOI (Multidisciplinary Instrumentation in Support of Oceanography, Woods Hole Oceanographic Institution) towed camera (tow
20 cam for short) aboard the R/V *Helmer Hanssen* (cruise number CAGE-15_2). The tow cam consisted of a 16 mMegapixel digital still camera with optical image stabilization (photo resolution: 4928 x 3264 pixels). It was mounted on a frame that also contained 6 cores (~ 1 m long) and 6 Niskin bottles. Due to space limitations and the logistical difficulties with mounting the cores and the camera together on the main body of the frame, the downward facing camera was tilted by 25 degrees. Images
25 were taken every 10-15 seconds. Despite slow ship speeds, overlap between successive images could not be achieved, therefore the tow cam image surveys were essentially transects over the different GHPs. The dataset consisted of one transect each over GHP1 and GHP2, and two transects over GHP3. Transects were named with an acronym for tow cam, followed by the dive number and the pingo number (e.g., TC25 GHP1). Navigation files from additional transects over GHP3 were inadequate for georeferencing purposes, therefore the images associated with these transects were only used qualitatively to ascertain species' presence or absence.

The second set of images was taken in 2016, also aboard *Helmer Hanssen* (cruise number CAGE-16_5). During this cruise, images were acquired via a pair of stereo cameras mounted on a remotely operated vehicle (ROV), 30K, operated by the Norwegian University for Science and Technology (NTNU). The stereo cameras (GC1380 digital still cameras, image resolutions of 1360 x 1024 pixels) were spaced 40 cm apart linearly, ensuring more than 50% overlap between left and right cameras, and faced downward at an angle of 35 degrees. Due to higher maneuverability and control over an ROV in comparison to the tow cam, the imagery surveys in 2016 were conducted with the purpose of constructing mosaics (i.e., overlapping images taken in a lawn mower fashion). Three mosaicking surveys were conducted over GHP5. Mosaics were named ROV, followed by the mosaic number and GHP5 (e.g., ROV1 GHP5). Navigation at GHP3 was unreliable, therefore the corresponding images were unusable for quantitative analyses. However, these images were used to conduct a comparison of animals visually identifiable in the tow cam and ROV images.

2.3 Mosaicking and georeferencing

Neither the tow cam system nor the ship had closed loop positioning systems during the 2015 cruise. Using the length of the tow cam system's cable to correct image location proved unsuccessful, therefore the ship's coordinates were used for positioning the tow cam images in space. At the scale of the site, this level of georeferencing is more than adequate, for it could be used to differentiate between different pingos and overall locations over them (summits, flanks, etc.). The ROV images were georeferenced based on coordinates obtained through an ultra-short baseline (USBL) closed positioning system. Images were mosaicked with the IFREMER software, Matisse v3 (courtesy Aurélien Arnaubec). This software takes angles of tilt into account for estimating the footprints of images on the seafloor and uses navigation data for placing the mosaics in space. In the case of the tow cam images, since no overlap existed between images, the GeoTIFF output from Matisse v3 consisted of single images in space based on the coordinates of the image (Fig. 2). With the ROV images, the software produced a georeferenced mosaic as the GeoTIFF output. Due to the low quality blending process of Matisse, higher quality seamless mosaics using a customized mosaicking script within Matlab (Pizarro and Singh, 2003; Singh et al., 2004) were constructed, which were subsequently georeferenced by matching and lining up easily identifiable features to the same features in the Matisse mosaics (Fig. 2). All georeferenced images and mosaics were displayed within ArcGIS (ArcMap 10.3 and 10.5)

2.4 Faunal identification and community analyses

Visible fauna (at least a few centimeters across) were identified to the lowest possible taxonomic division and marked manually (Table 1). Different categories of fauna were included While the majority of fauna would be considered epifauna, animals partially buried in the sediment were also included, and a few species were present on the seafloor that could more generally be considered pelagic (e.g., ctenophores). and Therefore the biological communities examined in this study are referred to as megafauna and by that, we mean animals large enough to be seen with the naked eye (Danovaro, 2009), which is consistent with a number of other image based studies (Amon et al., 2017; Baco et al., 2010; Bowden et al., 2013; Hessler et al., 1988;

Lessard-Pilon et al., 2010; Marcon et al., 2014; Podowski et al., 2009, 2010; Rybakova (Goroslavskaya) et al., 2013; Sellanes et al., 2008; Sen et al., 2013, 2014, 2016, 2017). ~~While the majority of fauna would be considered epifauna, animals partially buried in the sediment were also included and a few species were present on the seafloor that could more generally be considered pelagic (e.g., ctenophores).~~ Each individual was marked and the raw numbers were standardized to the different 5 areas of the mosaics and transects by converting to densities based on the size of the mosaic or transect area.

Numerous mats or aggregations of individuals of siboglinid worms were seen (Supplementary Fig. 1) and since specimens collected in core samples were found to be frenulates lacking pinnules on the tentacles, the species identity was narrowed down to two possibilities: *Oligobrachia haakonmosbiensis* or *Oligobrachia webbi* (Brattegard, 1966; Smirnov, 2000). These two species are similar in terms of morphology and while officially they are considered different species (Smirnov, 2000, 10 2014), a consensus does not exist on this separation (e.g., Meunier et al., 2010). Since no DNA sequences are available for *O. webbi*, similar worms from other Arctic seeps have been referred to as *O. haakonmosbiensis* due to close affinities in mitochondrial COI sequences with this species (Lösekann et al., 2008; Paull et al., 2015). For the sake of consistency with other Arctic seep studies, we will refer to the species in this study as *O. cf. haakonmosbiensis*. A comprehensive picture of the penetration depth of the worms was not obtained for this study, however, rough estimates indicate that individuals reached up to 50-60 cm in length. The tube diameter of these worms is about 500 µm, therefore single individuals were not visible in the images, making it impossible to count or mark individual ~~It was not feasible to count individual~~ frenulates in the mosaics or image transects. Therefore, aggregations were outlined and the average density of 7000 individuals/m² obtained from core samples taken during ~~in~~ 2016 was applied to the aggregation outlines in order to estimate total numbers of individuals within 15 aggregations and densities in the transects or mosaics as a whole.

20 Similarly, Though single individuals were visible every individual of the small solitary *Caryophilia* coral, ~~it was not feasible to mark every individual of this taxon in the images either~~ ~~could not be marked in the images due to aggregations containing large numbers of individuals~~. Aggregations of the two morphotypes (pink and white) of *Caryophilia* coral~~this taxon~~ were outlined, similar to the siboglinids, however, densities from samples could not be applied to the outlined aggregations for estimating numbers of individuals since *Caryophilia* tends to populate hard surfaces which were avoided during core sampling.

25 Instead, six *Caryophilia* aggregations were selected at random for each morphotype from the mosaics and image transects, and the number of individuals present in each aggregation were counted. Among the randomly chosen aggregations, on average, 27 individuals of the pink morphotype were seen (within an average aggregation size of 116 cm²) and 28 individuals of the white morphotype were seen (within an average aggregation size of 34 cm²). These averages were used to estimate total numbers and overall densities for all remaining aggregations outlined in the mosaics and image transects.

30 An exception to the standard methodology of marking every visible taxon, was a type of encrusting animal, possibly a bryozoan. Reddish, brownish and greenish morphotypes of this organism~~animal~~ were seen, but given the difficulty in identifying them or even visualizing them sufficiently, these animals were not marked in the image transects and mosaics nor discussed in this study.

The tow cam images captured more detail than the ROV stereo cameras. Therefore, before inclusion in the community analysis, the different taxa were evaluated both in terms of size and shape as well as their overall ability to be seen in images collected by the ROV stereo cameras. Twenty taxa identified in the tow cam images were not seen in the ROV images. Of these taxa, twelve were rare, i.e. only seen once, or at one site. These rare taxa were included in the community analyses because their absence from the ROV mosaics is likely not due to their inability to have been seen on the images, but rather due to their rare appearance. In order to determine whether the remaining 8 should be included in the community analyses, the ROV images from pingo 3 (with unreliable navigation data) were examined. Comparing the ROV GHP3 with the other and ROV GHP5 images (from GHP5) made it possible to explore whether animals not seen in the ROV GHP5 images was due to an inherent inability to see them in ROV images because of their lower resolution. Of these, juvenile fish, *Molpadias borealis* sea cucumbers, white possible scaphopods, the two unidentifiable polychaete species and the onuphid worm, *Nothria conchylega* were not seen in the ROV GHP3 images. *M. borealis* sea cucumbers and juvenile fish tend to stick out more, and are larger than some of the smallest animals seen in the ROV images such as stick sponges and *P. borealis* shrimp. Therefore they were considered detectable through the ROV stereo cameras and their absence from an ROV image was attributed to their actual absence and not due to their inability to be seen in those images. They were therefore included in the community analyses. The other animals that were not seen in the ROV GHP3 images were excluded from the community analyses because they likely would have been missed despite being present either because of their small size (e.g. white possible scaphopod) or because they blended into the background sediment (e.g. *N. conchylega*). In a few cases, this comparison between ROV images from GHP5 and GHP3 determined whether higher level taxa should be used. For example, pycnogonids were grouped together into a single category for community analyses despite at least three different morphotypes being visible in the tow cam images. Gastropods were all grouped together despite 10 morphotypes being visible in the tow cam images. Furthermore, hermit crabs (*Pagurus* sp.) were included in the gastropod category since it was not always clear whether gastropod shells were occupied by the original inhabitants or by hermit crabs. Similarly, all ophiuroids were grouped together, as were three morphotypes of some of the zoarcid fish.

Overall densities of visible fauna were used in creating a Bray Curtis similarity matrix, which was the basis for multi-dimensional scaling (MDS) and cluster analyses. A fourth root transformation was applied on the abundance data due to the vast range of densities among faunal groups, in order to balance the impact of both highly abundant and rare taxa within the same dataset. MDS/cluster analysis was conducted both with and without the inclusion of frenulates. In both iterations of community analyses, ANOSIM tests were run in order to test the significance of the cluster results.

2.5 Geochemical measurements

Gravity cores were recovered from the different GHPs in order to determine the general geochemical characteristics of sediment pore fluids (Fig. 1 and Table 2). In 2015, six gravity cores were taken: cores 911, 912 and 940 at GHP3, core 913 at

GHP1, core 914 at GHP2 and core 920 at GHP5. Sulfide, sulfate, dissolved inorganic carbon (DIC) and methane were measured in cores 911 and 920. Methane was not measured in core 940, and only methane was measured in cores 912, 913 and 914. In 2016, 5 gravity cores were taken at GHP3 and GHP5. Core 1045 at GHP3 and cores 1048, 1068, 1069 and 1070 at GHP5. All 2016 cores were subjected to the full array of geochemical analyses with the exception of core 1048, for which 5 methane was not measured.

2.5.1 Porewater measurements

Details of the porewater sampling and analyses are given in Hong et al. (2017). Briefly, porewater samples were collected by inserting acid-washed rhizons into pre-defined depths of the sediment cores in the refrigerated room onboard. Quantities of 5-15 ml of water were collected in acid-washed syringes. The total alkalinity was measured by Gram titration method within 10 two hours after the syringes were disconnected from the rhizons. For the determination of total hydrogen sulfide concentration (ΣHS), Aan aliquot of water sample was preserved with saturated $Zn(OAc)_2$ solution to prevent re-dissolution of sulfde within half an hour. Samples were stored frozen until analyses were conducted in the lab. Concentrations of hydrogen sulfide were analyzed spectrophotometrically using the 'Cline method' (Cline, 1969). The detection limit for this method is 40 μM . Sulfate concentrations were measured from the same samples for sulphide analyses by ion chromatography. Concentrations of 15 dissolved calcium and magnesium were measured by ICP-AES. Both analyses were performed in the laboratory of the Geological Survey of Norway (NGU).

2.5.2 Estimation of dissolved inorganic carbon (DIC) concentrations measurements

The concentration of DIC was approximated based on carbonate alkalinity which itself was estimated by subtracting total alkalinity from the concentration of ΣHS hydrogen sulfide. This is a reasonable assumption for the slightly basic porewater as 20 bicarbonate ions should be the dominantated by carbonate species in solution. To differentiate the different pathways of sulfate reduction, either through organic matter degradation or through coupling with anaerobic oxidation of methane (AOM), we estimated the values of ΔDIC and ΔSO_4 based on the following equations (Claypool et al., 2006; Suess and Whiticar, 1989):

$$\Delta DIC = ([DIC]_{spl} - [DIC]_{BW}) + \Delta Ca^{2+} + \Delta Mg^{2+}$$

$$25 \Delta SO_4 = [SO_4]_{spl} - [SO_4]_{BW}$$

$$\Delta Ca^{2+} = [Ca^{2+}]_{spl} - [Ca^{2+}]_{BW}$$

$$\Delta Mg^{2+} = [Mg^{2+}]_{spl} - [Mg^{2+}]_{BW}$$

where $[]_{spl}$ and $[]_{BW}$ are the concentrations of various chemical species in porewater samples (spl) and bottom water (bw), 30 respectively. We applied these calculations only to samples above the depth of the sulfate methane transition zone (SMTZ). Such an operation assumes that DIC is produced by organoclastic sulfate reduction and/or AOM-sustained sulfate reduction

while carbonate precipitation, which consumes both calcium and magnesium, decreases DIC concentration. By plotting ΔDIC against ΔSO_4 , it is possible to differentiate the primary DIC production reactions based on different stoichiometric relationships. For every mole of organic matter degraded, one mole of sulfate is consumed and two moles of DIC will be produced. On the other hand, While for AOM-sustained sulfate reduction, one mole of DIC is produced for every mole of sulfate consumed.

2.5.3 Methane measurements

Concentrations of dissolved pore water methane were obtained through conventional headspace sample preparation (Kolb and Ettre, 2006) and flame-ionized detector gas chromatography. The bulk sediment volume of 5ml was placed in 20 ml headspace vials, 5 ml of 1 molar NaOH solution was added, the vials were capped with rubber septa, sealed with aluminum crimp caps and shaken for 2 minutes. The measurements were carried out using TG-BOND Alumina (Na_2SO_4) 30m x 0.53mm x 10 μ column on ThermoScientific Trace 1310 gas chromatograph. Free gas can mix with dissolved gas in this method, however, this may take place only if overall headspace concentrations measured in laboratory conditions exceed the solubility limit of methane under in-situ pressure and temperature conditions. The concentrations in this study were below this critical value, therefore, our measurements are reflective of dissolved methane concentrations, although it should be kept in mind that free gas might also have been included to a small extent.

2.6 Seismic data

A seismic profile was generated from a broadband (10-350 Hz) high-resolution (~6 m lateral- and 2-3 m vertical resolution of the shallow subsurface) P-Cable 3D seismic cube (R/V *Helmer Hanssen* cruise number CAGE 16-6, 2016). This P-Cable 3D seismic system consisted of fourteen, 25 m long, streamers each containing 8 hydrophones. The streamers were spaced 12.5 m apart along a cross-cable towed perpendicular to the ships steaming direction. Seismic energy was generated using one mini-GI air gun of 45 in³ volume, operated with an air gun pressure of 160 bar -(Petersen et al. (2010) and Waage et al. in prep.).

3 Results

25 3.1 Community characteristics

Bacterial mats were seen in every image transect or mosaic, confirming the presence of reduced chemicals in the sediment and seepage activity at every pingo including GHP5. Fewer bacterial mats were seen on GHP5 in comparison to the other GHPs (Table 1). Hard substrates were also seen in every image transect or mosaic. Most of these hard substrates were clearly

authigenic carbonates, distinguishable by their pitted texture. The texture of all rock like features was not always visible in the images, either due to resolution issues or because of animals colonizing the surfaces. Therefore, some of the hard features could possibly be dropstones or other substrates not formed as a result of seepage activity. Nonetheless every GHP hosted carbonate structures, indicative of long-term gas seepage.

5

A total of 60 taxa were identified and marked in the image [transects and mosaics](#) (Table 1). Of these, 56 were used in the community analyses (see Section 2.4). [In addition to the 60 taxa seen and marked in the mosaics/transects, two individuals of *Chionoecetes opilio* \(snow crab\) and a few individuals of the wolffish \(*Anarhichas minor*\) were seen in the images over GHP3, \(Table 1\).](#)

10 On average, 29 taxa were seen in single mosaics or image transects over the different pingos (this average is based on raw richness counts and does not account for the differences in areas covered by the mosaics and image transects). Many morphologically distinct taxa were grouped together as a single taxon on a number of occasions. For example, at least 10 morphologically distinct types of gastropods and three types of pycnogonids were seen. Therefore, the total taxonomic richness of the pingo site is likely considerably higher than the 620 taxa listed in Table 1. Richness counts were similar between the various transects or mosaics, and furthermore, richness counts of the mosaics from the non-gas emitting pingo 15 (GHP5) were very similar to those at the gas emitting pingos (Table 3). On the other hand, the diversity indices revealed that the gas emitting pingos (except the TC25 transect over GHP3) were much less even than GHP5. This trend was only observed with siboglinids included in the analysis, with their removal, diversity indices did not display much variation between the individual pingos, mosaics or image transects. [In addition to the 60 taxa seen and marked in the mosaics/transects, two individuals of *Chionoecetes opilio* \(snow crab\) and a few individuals of the wolffish \(*Anarhichas minor*\) were seen in the images over GHP3, \(Table 1\).](#)

20 In total, 11 animal phyla were seen (Porifera, Cnidaria, Ctenophora, Nemertea, Priapulida, Sipuncula, Annelida, Arthropoda, Mollusca, Echinodermata, and Chordata). Of these, Cnidarians were represented by the largest number of taxa (18), followed by Echinoderms (11 taxa) and Chordates (10). The most abundant taxon by far was the frenulates, despite their absence from

25 GHP5. The frenulate worms are the only known chemosynthetic species observed in [the images used in](#) this study and also the only potential seep specific [or obligate or obligate](#) species. Following frenulates, the next most numerous taxa were *Gersemia* corals and *Thenea* sponges (likely *Thenea valdiviae*, Cárdenas and Rapp, 2012; Steenstrup and Tendal, 1982). The onuphid worm, *Nothria conchylega* was also abundant in the tow cam images and trails in the sediment were also seen behind these worms. Most of the cnidarians and the [non-*Thenea*](#) sponges were seen on hard substrates. A number of other animals were 30 also seen on or near hard substrates, such as the Northern shrimp (*Pandalus borealis*), ophiuroids and various gastropods. Pycnogonids were often seen among frenulate worm tufts. Conversely, pycnogonids were rare or absent in image transects or mosaics where frenulates were not present. Among the various taxa, several hold economic value, such as Atlantic cod (*Gadus morhua*), Northern shrimp, haddock (*Melanogrammus aeglefinus*) and Greenland halibut (*Reinhardtius hippoglossoides*) (Norwegian Directorate of Fisheries, 2017).

The community analysis including frenulates resulted in communities on mosaics/transects separating into two distinct clusters ($R = 0.926$, $p = 0.03$, Fig. 3A). This clustering corresponded to communities containing the frenulates and communities without frenulates. In other words, the GHP5 mosaics, and the TC25 transect over GHP3 formed one cluster, while the other GHP3 5 transect and the transects over GHP1 and GHP2 formed a separate cluster. When frenulates were excluded from the analysis, a similar result was obtained, with two clusters corresponding to communities/mosaics from GHP5 and communities/transects from the other GHPs. This clustering was also significant ($R = 0.704$, $p = 0.03$, Fig. 3B).

3.2 Sediment geochemistry and sub-surface features

Sulfide was below the detection limit ($40 \mu\text{M}$) in the bottom water. ~~In most cores, the first sediment layers where measurements were taken tended to contain undetectable or very low concentrations as well, as well as in the upper few centimeters of the sediment from all analyzed cores. It was first detected between depths of 5 and 10 cm, depending on the rate of Fe (II) production through iron reduction in the top layer of sediments.~~ Subsequently, sulfide concentrations rapidly increased downcore, with high millimolar level concentrations measured across all cores (Fig. 4A). The sulfide profiles of cores taken from GHP5 did not appear to differ substantially from sulfide profiles of cores from GHP3 (Fig. 4A). Methane was detectable 10 at the sediment surface and often remained more or less constant, until large increases were measured below 40 cm (50 cm for GHP5 cores). At depth, methane concentrations tended to be lower in cores taken from GHP5 in comparison to the cores from the other pingos (Fig. 4B).

At both GHP3 and GHP5, by and large, a 1:1 correspondence was observed between increase in inorganic carbon and increase 20 in sulfate (ΔDIC and ΔSO_4) in both shallow and deeper sediment, suggesting that most sulfate reduction in the sediment, is linked to anaerobic oxidation of methane (AOM, Fig. 5). In some cores, a switch from a 2:1 to a 1:1 ratio was observed (e.g., core 940, Fig. 5). Core 1045 from GHP5 was the only core in which even values from deeper in the sediment corresponded more closely to the 2:1 ratio, indicative of organic matter degradation via sulfate reducing bacteria being the major consumer 25 of sulfate. Therefore, at both GHP3 and GHP5, sulfate reduction is coupled to methane oxidation, though in the shallow sediment, sulfate can also be consumed by though the use of organic matter (Fig. 5). Further, the rate of sulfate consumption appears to differ between GHP3 and GHP5. Sulfate flux rates ranged from $-0.31 \text{ mol/m}^2/\text{day}$ to $-2.08 \text{ mol/m}^2/\text{day}$ in cores from GHP3. The lowest rate of sulfate flux was measured in a core from GHP5 ($-0.12 \text{ mol/m}^2/\text{day}$) and the maximum rate of sulfate flux measured in cores from GHP5 was only $-0.9 \text{ mol/m}^2/\text{day}$. On average, the rate of sulfate flux measured in cores from GHP5 ($-0.57 \text{ mol/m}^2/\text{day}$) was lower in comparison to cores from GHP3 ($-1.22 \text{ mol/m}^2/\text{day}$, Table 4).

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Beneath the three pingos emitting gas into the overlying water column, the seismic data revealed vertical zones of acoustic blanking in the shallow subsurface (up to $\sim 150 \text{ m}$ depth) and adjacent local high amplitude anomalies. In contrast, lower

~~amplitude dipping reflectors, no anomalies and only a very narrow zone of weak or zones of acoustic blanking were observed underneath GHP5 (Fig. 6).~~

4 Discussion

Four gas hydrate pingo features within a 2 km² area on the Arctic shelf were examined for this study. Split beam echosounder

5 data, collected over multiple years and seasons revealed gas bubbles in the water column above three of the four GHPs, often reaching impressive heights of a hundred meters above the seafloor (Serov et al., 2017). The emitted gases are primarily methane of thermogenic origin, confirming that these GHPs (GHP1, GHP2, GHP3) represent highly active methane seep sites. Although gas emissions into the water column were not detected at GHP5, the presence of bacterial mats indicates that this pingo also exhibits seepage, and analyses indicate that it is also of thermogenic origin (Serov et al., 2017). Carbonate 10 formations, including large slabs, were seen on all four pingos, suggestive of long term seepage (Berndt et al., 2014; Crémière et al., 2016; Seabrook et al., 2017). In this study, imagery was used to unveil key attributes of these shallow water (<400 m) Arctic cold seep megafaunal communities were revealed and the large-scale distribution of chemosynthesis based community members was linked to overall seepage patterns and sub-surface features. Comparisons of features of shallow water Arctic 15 cold seeps identified through the present study with other seep sites indicate major differences between cold seep communities at high and low latitudes. We conclude by considering cold seeps within the larger perspective of the Arctic, both from an ecological and economic point of view.

4.1 Community characteristics

Sixty-two megafaunal taxa were identified in total at the pingo site and on average, 29 taxa were seen within a single mosaic

20 or image transect. No major differences in taxonomic richness between individual pingos was seen, though the image transects containing siboglinid frenulates were much less even in terms of species abundances compared to the transect and mosaics which did not contain any frenulates (Table 3). This is clearly due to the extremely high abundance of frenulates (thousands of individuals), and with this group excluded, diversity indices of the various pingos are comparable. It should be noted that species richness, and even morphospecies richness counts are considerably higher because in certain cases, morphospecies 25 were grouped together under a single category. In one instance, this larger level grouping even lumped two different phyla together (gastropod molluscs with hermit crab arthropods). Among the taxa list generated for the pingo site, cnidarians accounted for the largest number of taxa (18), followed by echinoderms (11 taxa) and then chordates (10 taxa). After the frenulates, *Gersemia* corals and spherical *Thenea* sponges were the next most numerous groups of animals. A few different types of commercial species were seen, including Atlantic cod (*Gadus morhua*), Northern shrimp (*Pandalus borealis*), haddock 30 (*Melanogrammus aeglefinus*) and Greenland halibut (*Hippoglossoides platessoides*). Only one chemosynthesis based species

was seen, the siboglinid frenulate worm, *O. cf. haakonmosbiensis*. This species is also the only animal seen in the images at of the site that could possibly be a seep obligate species. However, the generalist lifestyle of frenulates (Hilário et al., 2011; Southward, 2000; Southward et al., 2005) and the debate around the consideration of *O. haakonmosbiensis* as a separate species from the fjord frenulate *Oligobrachia webbi* (Meunier et al., 2010), means that it is possible that, despite the cold seep setting, 5 the entire visible megafaunal pingopinge community—consists solely of background benthic species, regardless of whether community members are chemosynthesis based or conventionally heterotrophic. Nonetheless, the animals at the study site appear to take advantage of, and utilize the seep environment and its inherent characteristics.

In some cases, such as for hard substrate dwelling animals like sponges or corals, the benefits of a seep system on the benthic 10 landscape is evident: it provides hard settlement surfaces, in the form of authigenic carbonates, in a predominantly soft sediment seafloor (Becker et al., 2009; Cordes et al., 2008; Levin et al., 2015). The advantage of abundant hard settlement substrates likely corresponds with cnidarians and sponges being the most abundant and speciose of the phyla seen at the site. Mobile species, such as *P. borealis* shrimp and ophiuroids were also often seen among carbonates and for these taxa, the 15 advantage of these structures likely lies in the increase in habitat heterogeneity created by them, which can provide shelter or protection (Åström et al. 2017b).

Other taxa might make use of the other major advantage of the seep environment, i.e., autochthonous chemoautotrophic primary production, which, combined with detrital, photosynthetically derived material (Sibuet and Olu-Le Roy, 2002), tends to exceed the photosynthesis derived, detrital could lead to a high food supply at the pingo site, of the surrounding seafloor (Sibuet and Olu-Le Roy, 2002). Indeed, certain taxa appeared to show affinities for chemosynthesis based seep habitats, i.e., the frenulate worms and bacterial mats, both of which constitute the base of the local food chain. One of the snow crabs, for example, was seen grazing among the bacterial mats (Fig. 7). Importantly, this is likely the first record of such behavior in snow crabs, since they are not normally associated with cold seeps. Other crabs in the Majidae family have been seen at cold seeps (Martin and Haney, 2005) and are considered to either be grazers of filamentous bacteria or predators of vesicomyid 25 clams (Barry et al., 1996). While the chemosynthetic members themselves could serve as a food source for certain animals, the combination of high primary production and settlement surfaces together could lead to higher food availability for other, and particularly, higher order consumers. Siboglinid worms, including frenulates, are known to enhance local infaunal diversity and density (Bernardino et al., 2012), and samples from the pingo study site contained numerous instances of foraminifera, polychaetes and caprellids adhering to the tubes of the worms. Pycnogonids were largely associated with frenulate worms, and 30 mosaics or image transects without frenulates contained the lowest numbers of pycnogonids. *P. borealis* individuals were often present among bacterial mats and frenulates, in addition to often being in and around carbonate concretions. Since these animals are known to be predators and scavengers (Arnaud and Bamber, 1988; Bergström, 2000), the advantage of the frenulate habitat is likely enhanced prey availability. Similarly, certain gastropods were seen perched atop carbonate structures and it is unlikely that the hardness of the concretions, or their sheltering properties are of particular significance for this shelled group

of animals. Rather, it is probably the dense colonization of these structures by various animals that accounts for this association with carbonates, since the observed gastropods likely also have predatory or scavenging feeding styles (Aguzzi et al., 2012).

One of the numerically most abundant taxa at the site was the spherical sponge, *Thenea* sp. (Fig. 8B) Individuals did not appear to associate with any seep specific features or habitats. Instead, they were seen on soft sediment and this genus is known to use fascicles of spicules to anchor itself into sediment and mud (Vacelet and Donadey, 1977). Similarly, the onuphid polychaete, *Nothria conchylega* was seen in large numbers at the study site (Table 1), but did not display an affinity for any seep habitat such as carbonates, bacterial mats or frenulate worms. Both *Thenea* sponges and *N. conchylega* are common benthic taxa and their quantities at the study site could simply be due to the site falling within their natural distributional range.

On the other hand, the local productivity of the seep system could be beneficial for them and account for their high numbers at the study site (since the increased availability of hard substrate is of no particular consequence to these soft sediment dwelling animals). Indeed spherical sponges (*Stelletta* and *Pseudosuberites* genera) occur in high abundances in New Zealand seeps on the Hikurangi margin, where they are associated with sulfidic sediments and areas of active seepage (Baco et al., 2010; Bowden et al., 2013; Thurber et al., 2010). Similarly, the onuphid polychaete, *Hyalinoecia artifex* has been observed at US Atlantic seeps, where they maintain a carnivorous, epibenthic lifestyle, crawling and dragging their tubes along the seafloor (Meyer et al., 2016). Trails in the sediment were seen behind *N. conchylega* individuals in this study, which is evidence for crawling behavior on the seafloor of this species as well (see Fig. 8A9). Clearly visible trails associated with *N. conchylega* is of significance since this species has been postulated to exhibit crawling behavior (Budaeva and Paxton, 2013; Hayward and Ryland, 1995, 1995), but to our knowledge, this is the first time such behavior has actually been documented.

Other than food and substrate availability, another possible advantage of the seep environment that could be capitalized upon by the resident animals is a diverse and abundant microbial community, including members that are less abundant in background sediment. For example, seep sediment is dominated by sulfate reducing and sulfur oxidizing bacteria as well as methanotrophs, whereas seafloor sediment from non-seep areas is dominated by more cosmopolitan bacteria (Seabrook et al., 2017). Spherical *Pseudosuberites* sponges from New Zealand seeps are even hypothesized to be chemoautotrophic (Thurber et al., 2010). In general, sponges and corals tend to have a highly diverse bacterial microbiome (Blackall et al., 2015; Bourne et al., 2016; Vacelet and Donadey, 1977). The dominant members of coral microbiomes are Proteobacteria (particularly Gamma and Alpha) (Bourne et al., 2016; Littman et al., 2009; Rohwer et al., 2002), and Gammaproteobacteria are known to be common members of seep sediment communities (Valentine, 2011), including at HMMV (Lösekann et al., 2007; Niemann et al., 2006). Archaea, including anaerobic methanotrophs and nitrate reducers are also known to associate with corals (Siboni et al., 2008; Wegley et al., 2004), and archaeal anaerobic methanotrophs (known as ANME) are key players in the anaerobic oxidation of methane (AOM) that is so fundamental to seep geochemistry (Boetius and Wenzhöfer, 2013; Knittel et al., 2005; Knittel and Boetius, 2009). Therefore, the pingo seeps could be beneficial for certain species that associate with bacteria

because they provide access and exposure to a more diverse array of bacterial strains than is present in the non-seep benthic seafloor.

5 ~~Importantly, sulfide was not detectable in bottom water or even in the upper 5 cm of the sediment at the pingo site. Therefore, background species are capable of colonizing the site and utilizing its associated resources without the complication of having to avoid sulfide toxicity. It should be noted that though certain associations between animal groups and niches or habitats are visible to a certain extent through this study, confirmation of such associations requires further study.~~

4.2 Factors controlling the distribution of chemosynthesis based community members (frenulates)

10 We hypothesized that the lack of free gas ebullition at GHP5 was representative of this pingo being substantially different from the other three, in terms of both abiotic and biotic features. As hypothesized, community analysis based on data from the georeferenced images and mosaics indicate that the communities on the three free gas emitting GHPs differ, and cluster separately from those on GHP5 (Fig. 32). The TC25 transect over GHP3 appeared to be an exception, because it clustered with the GHP5 communities (Fig. 32). However, ~~no frenulates were visible this particular image transect did not contain frenulates in this particular transect~~, a feature shared with the GHP5 mosaics. Since frenulate abundances were in the order of thousands of individuals, community analyses were also conducted with them excluded which resulted in GHP5 communities forming a distinct group from the other pingo communities. Nonetheless, the most obvious difference between GHP5 communities and the communities on the other pingos was the absence of frenulates. ~~This difference was further confirmed through cores and extensive ROV based surveys. GHP5 was surveyed with the ROV before mosaic based imaging was conducted with the explicit purpose of locating siboglinid worms, since they were considered to be representative of locations with active seepage. Despite these efforts, no aggregations of these animals were seen. Furthermore, siboglinids were not recovered in any of the gravity cores taken from GHP5. Combined, these results suggest that aggregations of siboglinids are truly absent from GHP5, as opposed to them simply not being present in the mosaics of GHP5 which covered more discrete areas than the transects over the other pingos.~~

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25 The lack of frenulates from GHP5 has important ecological implications since they are the only confirmed chemosynthethesis based ~~bie~~ animal at the study site. All frenulates have obligate, nutritional symbiotic associations with bacterial endosymbionts (Fisher, 1990; Hilário et al., 2011; Southward, 1982; Southward et al., 2005) and molecular data and electron micrographs suggest that thiotrophy is the dominant metabolic mode for symbionts of *O. haakonmosbiensis* (Lösekann et al., 2008; Pimenov et al., 2000). Thus we expected sediment sulfide concentrations at GHP5 to be lower than those at the other pingos, and too low to sustain the frenulate worms and their symbionts.

Contrary to our expectations, sediment sulfide concentrations at GHP5 were not lower in comparison to GHP3 (Fig. 3A). Sulfide (and sulfate) measurements were only possible in cores from GHP3 and GHP5, but due to the other similarities between GHP3 and the other free gas emitting pingos, we consider sulfide/sulfate profiles of GHP3 to be representative of conditions at GHP1 and GHP2 as well. The sulfide concentrations measured on GHP5 were at the millimolar level, which is likely not limiting with respect to supporting chemosynthesis based fauna of this size. Though the exact sulfide needs of frenulates and *O. cf. haakonmosbiensis* have not been quantified, significantly larger chemoautotrophic symbioses are known to be found in environments with lower in situ concentrations of dissolved sulfide (Decker et al., 2017; Podowski et al., 2010; Sarrazin et al., 1999; Sen et al., 2013; Urcuyo et al., 2003). Therefore, the sediment at GHP5 contains more than enough sulfide to theoretically support *O. cf. haakonmosbiensis*, and yet, they are absent from this pingo.

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Therefore, ~~other~~another factors likely accounts for the absence of frenulates on GHP5, overriding the advantage of an abundant energy source to this chemo-obligate worm. Colonization being inhibited by an inadequate larval supply can be eliminated because GHP5 is in the vicinity of the other three pingos. In experiments conducted on *Siboglinum fiordicum* frenulates, only larvae reared in containers with 10 cm of sediment grew well (Bakke, 1974), and in general, soft sediment is considered the preferred substrate of frenulates (Southward, 1999, 2000; Southward et al., 2005). Soft sediment is the primary sediment type at GHP5, therefore a lack of suitable substrate does not explain the absence of frenulates at GHP5 either. The settlement cues for frenulates are not known, but methane and sulfide have been hypothesized to serve as such cues for seep animals in general (Cordes et al., 2010). Only sulfide has been tested experimentally, and was shown to positively correlate with settlement of seep associated polychaetes (Levin et al., 2006). ~~We were unable to detect However, sulfide was not detected~~ in the bottom water at any of the study pingos, ~~nor was it present in the first few centimeters of the sediment in any of the cores~~ (Fig. 3). ~~However, our detection limit for sulfide was 40 μ M and frenulate larvae could potentially detect, or be attracted to concentrations lower than this. Nonetheless, it is unlikely that GHP5 is deficient with respect to sulfide in the bottom water, in comparison with the other pingos, since GHP sediment sulfide profiles were similar to those from the other pingos. Additionally, bacterial mats that rely on sulfide reaching the sediment-water interface, were seen at GHP5, as they were seen at the other pingos. If sulfide serves as a settlement cue for *O. haakonmosbiensis*, then GHP5 is not deficient in this regard either, in comparison to the other pingos.~~

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Nevertheless, GHP5 does differ from the pingos in other respects. The geophysical setting of GHP5 was different, with ~~clear~~ zones of acoustic blanking below GHPs 1-3 absent beneath GHP5 (Fig. 5). Such regions of acoustic wipe-outs are interpreted as gas saturated sediment. Therefore, a sub-surface gas reservoir is likely connected to GHPs 1-3, which allows for advection of gas through the sediment and up into the water column. The absence of acoustic blanking, ~~except in the form of a narrow zone of weak blanking~~ underneath GHP5 suggests reduced sub-surface gas transport, or alternatively, a deeper barrier for upward gas migration, and subsequently, lower upward methane flux. In accordance with this, sediment methane concentrations were lower at GHP5 in comparison to the other pingos (Fig. 3B), and, gas hydrates were not recovered from

GHP5, though they were recovered in cores from the other pingos. Correspondingly, AOM rates would be expected to be lower at GHP5. Comparative AOM rates are not available for the different study pingos, but AOM occurs in concert with sulfate reduction, therefore sulfate fluxes can be used to make inferences about AOM rates. Sulfate reduction can and does take place in the absence of AOM as well (Dale et al., 2008; Hong et al., 2014; Wallmann et al., 2006), therefore it is important 5 to differentiate between AOM and the breakdown of organic material as being the processes that consume sulfate in a specific system. This can be done by utilizing the stoichiometric relationship of the two sulfate reduction pathways (Claypool et al., 2006; Suess and Whiticar, 1989), specifically, by measuring the ratio of increase in DIC~~carbon dioxide~~ to the decrease in sulfate. A 2:1 ratio is typical when sulfate is consumed through the anaerobic breakdown of organic matter. This ratio changes to 1:1 when sulfate reduction is linked to AOM. (Masuzawa et al., 1992). Though some of the cores displayed values close to 10 the 2:1 mark in the shallower depths of the sediment, deeper into the sediment, this ratio changes, and approaches the 1:1 correspondence. In most cores, the ratio of DIC~~inorganic carbon~~ to sulfate consumption was found to be close to 1:1 regardless of depth (both GHP5 and GHP3). The one exception was core 1048 from GHP5, for which, almost all values were closer to the 2:1 ratio. These results indicate that at both pingos 3 and 5, sulfate consumption is linked primarily to AOM, particularly in deeper sediment. With the exception of core 940 (which was on the periphery of GHP3), all the cores from GHP5 exhibited 15 lower sulfate flux rates than those at GHP3 (Table 4). Therefore, our data suggest that AOM rates are lower within GHP5, compared to the other pingos.

Lower AOM rates would result in lower rates of generation~~production~~ of two important product~~compounds~~: hydrogen sulfide, and bicarbonate~~carbon dioxide~~ (Boetius and Wenzhöfer, 2013; Knittel et al., 2005). Constant replenishment of sulfide, or, a 20 fresh supply, i.e., high sulfide fluxes, might be more important for the frenulate worms than simply high concentrations in the sediment. Additionally, the generation of bicarbonate~~ions~~ carbon dioxide might be important for these worms. Because of the carbon fixation activities of their symbionts, frenulates need inorganic carbon, and indeed, RubisCO coding genes have been found in *O. haakonmosbiensis* (Lösekann et al., 2008). Lösekann et al. (2008) hypothesized that *O. haakonmosbiensis* uses DIC~~carbon dioxide~~ produced either by AOM or the aerobic oxidation of methane. This hypothesis was put forward to explain 25 the extremely negative carbon isotope signatures in *O. haakonmosbiensis* biomarker lipids measured by the authors and by Gebruk et al. (2003), values that had never before been measured in thiotrophic symbioses and which cannot be explained by chemoautotrophic carbon fixation alone, but can be explained by the worms incorporating isotopically light carbon dioxide (DIC) produced by sediment microbes.

30 The authors further suggested that assimilation of microbial dissolved organic carbon (DOC) by the worms could also account for the depleted carbon isotope values. In fact, frenulates differ from other chemosymbiotic siboglinids, such as vestimentiferans, in that they appear to supplement their symbiont provided, chemosynthetic diet with dissolved organic material (Southward et al., 1979, 1981; Southward and Southward, 1970). The larvae of *Siboglinum fiordicum*, was seen, in laboratory experiments, to grow from the larval stage to the juvenile stage over the course of 13 months in which it only used

food from yolk reserves and from the surrounding sediment (Bakke, 1977). Furthermore, Dando et al., (2008) noted that in situ, the tubes of frenulates are often turned towards where there are locally high concentrations of reduced organic matter in the sediment.

5 The dual need for inorganic and organic carbon sources (plus thiotrophic chemoautotrophy) ~~could mean that likely results in frenulates in general, and, *O. cf. haakonmosbiensis* specifically, relying~~ heavily on a highly active sediment methanotrophic microbial community. We propose that this, ~~in addition to, or in combination with high sulfide flux~~, is the overriding factor that limits or excludes *O. cf. haakonmosbiensis* from GHP5 despite high sulfide availability. At the free gas emitting GHPs, the ~~large~~ sub-surface gas chimneys lead to constant seepage of methane, which in turn, supports abundant methanotrophic

10 microbes and microbial activity, such as AOM linked to sulfate reduction and sulfide production, thereby providing desirable conditions for *O. cf. haakonmosbiensis*. On the other hand, at GHP5, seepage of methane is low due to the lack of a sub-surface gas chimney. ~~The seismic data reveals enhanced reflectors below GHP5, which may indicate pockets of sub-surface gas or gas hydrates and indeed, Mmethane~~ is still present in the sediment, but in lower concentrations. ~~and a~~ As a result, methanotrophic microbes are likely less abundant and methanotrophic activity is considerably lower, as evidenced by lower AOM linked

15 sulfate flux rates. A smaller and less active microbial community could mean that GHP5 is deficient in the carbon compounds (either organic or inorganic, or both), required by the worms, and sulfide flux rates might be too low to sustain them as well. ~~A summary of this hypothesis is presented in Fig. 7. Further studies, with a focus on the microbial community and their activity in the sediment will be required to test this hypothesis, though early results indicate that the microbial community of GHP5 is significantly different from those at the other pingos and that ANMEs make up less of the total microbial community at GHP5~~

20 compared to the others (Klasek et al., in prep.). Furthermore, at HMMV, high rates of sulfate reduction and AOM were measured and high numbers of anaerobic methanotrophs were found around the bases of *O. haakonmosbiensis* tubes (Lösekann et al., 2008; Niemann et al., 2006). Similarly, high sulfate fluxes were measured at the Beaufort Sea *O. haakonmosbiensis* site (sulfate concentration decreased from seawater values to <0.1 mmol within 0.5 m of the sediment) (Paull et al., 2015). ~~A dependency on sediment methanotrophic microbes and their activity could also account for the absence of *O. haakonmosbiensis* at Arctic hydrothermal vents (Kongsrud and Rapp, 2011; Pedersen et al., 2010), despite its wide distribution across the Arctic because at vents, methane rises quickly into the water column due to the heat of hydrothermal emissions, thereby reducing the amount available for sediment microbes.~~

25 ~~Based on the geochemical data, we propose an additional factor that could contribute to the absence of *O. haakonmosbiensis* at GHP5. Hong et al. (submitted) found that the porewater from cores taken at GHP5 had a greater contribution from fluids that have undergone high temperature water rock interactions in comparison to the porewater from the other pingos. The product of such reactions is a higher abundance of dissolved heavy metals, such as lithium, which was measured in higher concentrations in GHP5 porewater compared to the porewater from the other three pingos (Hong et al., submitted). Other metals also tend to accumulate as a result of high temperature~~

rock water interactions such as barium, cesium and rubidium (James et al., 2003). Hydrothermal vent members of the siboglinid clade, such as vestimentiferans, have to deal with heavy metal rich hydrothermal fluids and at least *Riftia pachyptila* is known to contain metal binding proteins that can be used for detoxification purposes (Childress and Fisher, 1992; McMullin et al., 2007). In general, little is known about how vent animals cope with the toxic effect of high levels of heavy metals and frenulates are one of the least studied groups of siboglinids (Hilário et al., 2011). However, frenulates are considered basal within the siboglinid radiation, and a popular hypothesis regarding siboglinid evolution is that it has been driven by a trend of increased habitat specialization (Halanych et al., 2001; Hilário et al., 2011). Based on this, frenulates would be more generalistic than vestimentiferans in their habitats and could subsequently lack the highly specialized adaptations found in vestimentiferans. Therefore it is possible that *O. haakonmosbiensis* is not equipped to deal with heavy metal rich fluids. This hypothesis too could explain why the worm is absent both from GHP5 and hydrothermal vent fields in the Arctic.

4.3 Comparisons to other seep sites

Few studies have been conducted at other seep sites around the world using imagery and photomosaics to characterize megafaunal communities. Among the few that do, most report substantially lower species/taxa counts compared to the 62 taxa seen (conservatively) in this study: Lessard Pilon et al. (2010) recorded 15 taxa at a Gulf of Mexico seep site (approx.. 2000 m water depth) and 13 taxa were seen in the density study by Olu et al. (2009) at the West African Regab pockmark. In their review, Sibuet and Olu (1998) summarized macrofaunal and megafaunal species counts from all the known seep sites at the time (400 to 6000 m water depth) and found the highest such count to be 42, and on average to be 12. Bowden et al. (2013) on average saw 20 species per site among seeps off New Zealand on the Hikurangi margin (depth range: 744 m -1120 m), although species richness counts were related to the different numbers of images analyzed per site. On the other hand, Rybakova et al. (2013) counted 31 taxa at HMMV (water depth about 1200 m) and Amon et al. (2017) found 36 morphospecies on average across four Caribbean seeps in water depth ranging from 998 to 1600 m.

However, the high diversity at the pingo site is not completely unexpected, given the shallow (less than 400 m) water depth at which it is located. In general, diversity and species richness at cold seeps tend to decrease with increasing water depths (Sibuet and Olu, 1998). This usually applies to symbiont containing species as well, so that shallow seeps have more symbiont containing species than deeper seeps (Sibuet and Olu, 1998). The pingo site could represent a major deviation from this general trend, since so far, only one it contains only one chemosynthesis based, symbiont containing species has been confirmed at this site. Details on the macrofaunal community composition is currently being compiled, but across the 200+ taxa seen, only four taxa (thyasirid bivalves) were encountered that could possibly contain symbiotic bacteria. It is possible that one or more of the thyasirid bivalves or Additionally, the spherical *Thenea* sponges at the site could contain symbionts, but even if that were the

case, it is unlikely that the numbers of symbiont containing species at the study site would rival the numbers (10-15 species) recorded at shallow sites. It should be noted, though, that only seep sites at water depths of 400 m or more were included in this review, therefore, shallow in this context nonetheless refers to sites often considered the deep sea. In fact, Dando (2010) noted the opposite trend in shallower seeps, i.e., a decrease in numbers of symbiotic species with decreasing water depths. This 5 particular review also does not cover sites at water depths of the study site: it focuses specifically on seeps in water depths of 200 m or less. Sahling et al. (2003) examined depth related trends in seeps along the Sakhalin shelf in the Sea of Okhotsk along a depth gradient of 160 m to 1600 m and observed similar patterns to Dando (2010). These studies and our results illustrate that the relationship between depth and numbers of symbiont containing species at cold seeps is yet to be resolved. It is possible 10 that at least two switching points exist: the shallowest sites have very few symbiont containing species but at some point, possibly 400-500 m, the numbers of symbiont containing species rapidly increases and reaches a maximum, after which, deeper sites again see a drop in the numbers of symbiont containing species. More studies on seeps at intermediate depths, such as those on continental shelves like the present study site will be required to fully explore these trends.

Shallow seeps tend to be populated by a subset of the local, background benthic community (Dando, 2010), and this appears 15 to be true for the pingo study site as well. At the megafaunal scale, *O. cf. haakonmosbiensis* appears to be ~~is~~ the only possible exception and potential seep obligate species, although Smirnov (2014) reports *O. haakonmosbiensis* ~~this species~~ from a muddy bottom site in the Laptev Sea, without any mention of it being a seep site (and furthermore, *O. haakonmosbiensis* might not be separate from *O. webbi* which has been found in fjords). Background fauna and frenulates, is also what has been observed 20 at other Arctic seep sites, such as pockmarks on the Vestnesa Ridge (Åström et al., 2017b) in the Barents Sea and mud volcanoes in the Beaufort Sea (Paull et al., 2015). At HMMV as well, the community conforms to this basic structure, with the addition of the chemosynthesis ~~based~~^{is} moniliferan worm, *Sclerolinum contortum* (Gebruk et al., 2003; Lösekann et al., 2008). These seeps all vary considerably in terms of water depth: 380 m at the pingo site, 282-740 m for the Beaufort Sea pockmarks, and ~1200 m at both Vestnesa and HMMV. This indicates that in the Arctic, regardless of depth, soft-sediment 25 seeps tend to have similar overall community structure, of chemosynthesis based siboglinids and background taxa. There is no transition to communities dominated by large, chemosymbiotic seep fauna such as vestimentiferan tubeworms, vesicomyid clams and bathymodioline mussels, seen at about 400 m in seeps at lower latitudes (Sahling et al., 2003). Intriguingly, this separation between Arctic seeps and seeps in other parts of the world with respect to large, chemosymbiotic species, is likely only a modern trend. The shells of large bodied chemosymbiotic bivalves (thyasirids and vesicomyids) have been recovered 30 in cores from the pingo study site (Åström et al., 2017a), Vestnesa Ridge (Ambrose et al., 2015; Hansen et al., 2017; Sztybor and Rasmussen, 2017) and methane seep deposits on the Gakkel Ridge (Kim et al., 2006) and in the Laptev Sea (Sirenko et al., 1995). Shells from the pingo site have been estimated to be up to 7-14 thousand years old (M. Carroll, unpublished data), and based on the Vestnesa and Gakkel Ridge samples, the extinction event for these animals has been estimated to have taken place around 15,000 years ago (Ambrose et al., 2015; Hansen et al., 2017; Kim et al., 2006; Sztybor and Rasmussen, 2017). This coincides with deglaciation following the Heinrich H1 cold event and the accompanying environmental changes,

including extensive releases of methane such as is hypothesized to have created the pingos at the study site (Serov et al., 2017). This could have led to the local extinction of large chemosymbiotic bivalves in the Arctic. Since recolonization has not taken place despite Atlantic water inflow, (and at least Vestnesa and the pingo site fall within the path of the North Atlantic current), the changes that triggered the presumed demise of the Arctic chemosynthetic bivalves likely persist today. Sztybor and Rasmussen (2017) proposed the drop of bottom water temperatures to sub-zero levels at Vestnesa to be the explanatory factor. However, bottom water temperatures are about 2°C on average at the pingo site and mean annual bottom water temperature at the Beaufort Sea pockmarks is 0.2°C (although temperatures as low as -1.1°C were also recorded, Paull et al., 2015). Vesicomyids of the genus *Isorropodon* have also been sampled at the Nyegga seep site in the Norwegian Sea (Krylova et al., 2011), where bottom water temperatures are -0.7 °C (Portnova et al., 2014). These data make it difficult to use modern bottom water thermal regimes as a sufficient reason to explain the death and subsequent lack of recolonization of large chemosynthetic bivalves in the Arctic, although it could play a role. The precise causes for the disappearance and continued absence of large, chemosynthetic bivalves in the Arctic are still unclear. Nonetheless, based on the existing data, Arctic seeps appear to form a distinct biogeographical entity, exhibiting the same, general seep community structure, but one that is different from seep communities in other parts of the world.

Another way in which the pingo site appears to deviate from generalized seep trends relative to lower latitude seeps is with respect to the factors that promote successional progression of the communities. The presence of carbonate slabs on GHP5 indicates that this site could have experienced higher levels of seepage activity in the past and in fact, based on detailed geochemical and geophysical analyses, Hong et al. (2018)(submitted, 2017) concluded that this pingo represents a later stage in the geophysical history of these features. Therefore, it is likely that the community on this pingo also represents a later stage in the succession of the pingo seep communities. At present, the chemoautotrophic frenulate worms no longer exist, but the products of seepage, such as carbonate rocks, provide settlement substrates, making this community diverse and densely colonized. This is similar to what has been observed or predicted at lower latitude seeps, where carbonates and the tubes of vestimentiferans provide a substrate for hard bottomed animals such as sponges or cnidarians (Bergquist et al., 2003; Bowden et al., 2013; Cordes et al., 2005). The major difference is that in these studies and models of succession in lower latitude seeps, a major driving force from an active to a senescent or background fauna dominated community, is a cessation or displacement of fluid flow, often accompanied by a decrease in sediment sulfide concentrations. However, the sediment at GHP5 does not have lower sediment sulfide concentrations than the sediment hosting the earlier successional communities on the free gas emitting pingos (although methane seepage has likely decreased or even ceased due to the exhaustion of the sub-surface gas chimney). As discussed above, lower latitude seeps at comparable or greater depths are characterized by the presence of more than one type of large bodied chemoautotrophic faunal group, each with different geochemical needs and niches. This is not true for the study site, where so far, *O. cf. haakonmosbiensis* alone makes up the entirety of the megafaunal metazoan chemoautotrophic community. Infaunal thyasirid bivalves could possibly expand the chemosymbiotic repertoire of the study site, however, only small bodied species were found that usually only reached a maximum size of about 5 mm in length.

Therefore these species are unlikely to be considered megafauna (1 cm in maximum dimension is the lowest level at which animals have been considered megafauna (Amon et al., 2017). This difference, of one, compared to multiple chemoautotrophic megafaunal species being present, could account for the pingo site deviating from the trend of successional progression at seeps paralleling changes to sulfide concentration and availability. A highly limited chemosynthetic based megafaunal community appears to be the norm at Arctic seep sites, therefore, the pattern of successional progression in the absence of depleted sulfide reserves observed at the pingo site, though currently quite unique, could be representative of the Arctic in general, although further studies are needed in order to confirm this.

4.4 Arctic perspectives

Among the diverse assemblages of background benthic species present at the pingo site are a number of commercially important species. Atlantic cod (*Gadus morhua*) and the northern shrimp (*Pandalus borealis*) were particularly numerous, but haddock (*Melanogrammus aeglefinus*) and various flat fishes such as Greenland halibut (*Hippoglossoides platessoides*) were also seen. In addition, two individuals of snow crab (*Chionoecetes opilio*) were seen in the images over GHP3 (Table 1, Fig. 7). Commercial species have been observed at seep sites around the world (Baco et al., 2010; Bowden et al., 2013; Grupe et al., 2015; Higgs et al., 2016; Niemann et al., 2013; Sellanes et al., 2008) and the importance of methane seeps to local fisheries is recently gaining attention (Levin et al., 2016). In at least two cases, diets with a significant chemosynthetic component have been established for commercial species (Higgs et al., 2016; Niemann et al., 2013). Additionally, seep or site specific characteristics (three dimensional carbonate structures, proximity to oxygen minimum zones, chemical environments excluding predators or parasites) have been hypothesized to account for the enhanced densities of commercial fish species at seeps in comparison to non-seep environments (Levin et al., 2016; Sellanes et al., 2008). At the pingo site, no data currently exists on whether chemosynthesis derived material constitutes any part of the diets of the observed commercial species, or which features of the seep environment draws them to the location. Nonetheless, species targeted for commercial fishing are abundant at the pingo site. Methane seeps have not been studied intensively in the Arctic and their potential contributions to the Norwegian fishing industry have never been explored. Our results, for the first time, indicate that methane seeps could function as a habitat for multiple economically important species.

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Nearby background areas not affected by seepage were not imaged during this study, therefore quantitative comparisons between the pingo seeps and the surrounding seafloor with respect to megafauna were not possible. However, the tow cam image transects covered some area outside and to the west of GHP3 (although bacterial mats were seen in this area, so it likely does not constitute a truly non-seep environment). Every individual of every visible taxon was not marked in this area, although the total number of taxa seen in this area was recorded. In total, 28 taxa were seen in this non-pingo area of 2330 m², which amounts to 1.2 taxa per 100 m². This is considerably lower than the richness counts recorded in the pingo mosaics and transects (average 4.1 species per 100 m², Table 3). Density and abundance data could not be compared with the pingos because

this data was not compiled for this area, but the pingos appeared to be more densely colonized than the non-seep area. Qualitative comparisons of faunal abundances and a single comparison of richness counts are not sufficient for drawing robust conclusions about the differences between pingo-seep communities and the surrounding seabed. Nonetheless, our results suggest the possibility of the pingos creating a biomass and diversity hotspot on the seafloor with respect to megafauna. In 5 fact, this has been suggested (despite a similar absence of quantitative data) for the Concepción seep on the continental slope off Chile (Sellanes et al., 2008), and for HMMV, where Gebruk et al. (2003) noted that the background community appeared to be much 'poorer' than the HMMV community. Åström et al. (2017b) also found higher species richness, biomass and diversity at Vestnesa Ridge seep sites in comparison to non-seep sites.

10 Therefore, the seep sites such as the studied pingos could hold ecological significance. The Norwegian government has prioritized protection and mapping of the shelf and areas where coral, sponge, sea pen or other communities of high importance to the Barents Sea-Lofoten ecosystem (Norwegian Ministry of the Environment, 2010). Our results indicate that methane seeps could constitute one of these communities important to the ecology of the Barents Sea and the Arctic. Sites with gas hydrate reservoirs and seafloor methane emissions appear to be quite extensive along the Arctic shelf in the Barents Sea (Bünz et al., 15 2012; Sahling et al., 2014; Vadakkepuliyambatta, 2014; Westbrook et al., 2009), therefore, the impact of methane seeps on the larger benthic community could be widespread. However, Arctic shelf seep communities have not been systematically mapped, nor has their effect on seabed ecosystem dynamics been assessed, therefore we suggest their inclusion into current monitoring, mapping and conservation efforts.

20 In the Arctic, recognition of and maintenance of diversity hotspots is particularly relevant, because Arctic communities are experiencing substantial disruptions such as species replacements or trophic shifts due to the northward range shifts of many subarctic or temperate species (Degen et al., 2016; Johannessen et al., 2012; Wassmann et al., 2011). It is debatable whether true Arctic biodiversity hotspots exist at all since the meaning of the term *sensu stricto* refers to areas with high concentrations of endemic species (Myers et al., 2000) and relatively few species are considered as being endemic to the Arctic (Barry et al., 25 2013). Nonetheless, certain locations in the Arctic do tend to contain elevated numbers of 'true' Arctic species (Barry et al., 2013), and the diversity of the pingo site suggests that shelf cold seeps could fall under this category. Under most circumstances (i.e., in other parts of the world, at lower latitudes), one would not expect a seep site to be affected by the arrival of new species to the surrounding region, because the new arrivals would not be considered capable of successfully establishing themselves within the specialized seep environment. However, the community at the pingos does not contain specialized seep 30 inhabitants endemics and rather, consists almost entirely of background, benthic species. Furthermore, snow crabs were seen at the pingo site, even grazing on a bacterial mat, and this species has only been seen in the Barents Sea since 1996 (Kuzmin et al., 1998, 1999) and has spread to the north west, reaching west Spitsbergen fjords in 2017 (P. Renaud personal communication). The presence of snow crabs at the site indicates that species new to the area are capable of establishing themselves at the site, which suggests that these communities could experience the same types of upheavals documented at

benthic sites along the path of northward migration of southern latitude species (Cochrane et al., 2009; Johannessen et al., 2012; Węsławski et al., 2011).

In short, our results, indicate that the pingo study site and by extension, other shelf seeps could constitute important habitats for multiple commercial species, possibly serve as biomass and diversity hotspots on the seafloor, and could be threatened by climate change induced ecological disturbances. Therefore, it is crucial that benthic mapping efforts and long-term monitoring projects proposed to understand the response of a changing Barents Sea (Barry et al., 2013; Jørgensen et al., 2015) take shelf seep communities into account as well. Since seeps are long lived systems whose effect on the benthos can extend beyond the lifespan of seepage activity itself (Bowden et al., 2013; Cordes et al., 2008; Levin et al., 2016) , careful management policies will need to be drafted, in order to successfully maintain the juxtaposition between maintenance of the seep habitat and its economic exploitation.

4.5 Conclusions

Studies focused on the biology and ecology of Arctic cold seeps are rare. Therefore, the natural laboratory conditions of multiple pingos within a limited spatial extent at the study site provided an unprecedented opportunity to study the response of Arctic seep communities, and particularly, chemosynthetic members, to variable physical factors. Our results show that despite the likelihood of sulfide being the dominant energy source, concentrations of sulfide in the sediment do not necessarily correlate with the presence or absence of the only confirmed chemosynthetic animal at the site, *O. cf. haakonmosbiensis* frenulates. We hypothesize that high sulfide flux, and/or dissolved inorganic or organic carbon produced by microbial methanotrophic activity in the sediment constitute the major carbon source for these worms and small microbial communities and low methanotrophic activity in the sediment limit the presence of these worms even when sulfide is abundant. ~~Alternatively, site specific conditions, such as deeply sources, heavy metal rich porewater fluids could affect the settlement and development of *O. haakonmosbiensis* at certain locations within the study site. *Oligobrachia* worms~~ This worm is ~~are~~ ubiquitous across Arctic seeps and this is the first time that its distribution could be correlated with variable physical conditions. Overall, the pingo communities are characterized by a diversity of background species and a lack of seep obligate species, both of which are likely a function of the location of the study site on the shallow (less than 400 m) shelf. This study is the first to document seafloor crawling behavior of *Nothria conchylega* onuphid worms, behavior which has only been hypothesized before. Commercially important fish and crustacean species were seen in large numbers and surprising for a seep site, a species that is relatively new to this area (the snow crab) was seen grazing among bacterial mats. Further investigation of the pingo site and others like it is important to understand shallow water and shelf cold seeps, their effect on the benthos and their responses or possible susceptibility to a changing and warming Arctic.

Data availability

The data are stored at the CAGE Centre for Arctic Gas Hydrate, Environment and Climate's data repository and requests for access should be directed to cage_data@list.uit.no.

Author contributions

5 AS constructed the mosaics, marked animals and conducted the community analyses. EÅ identified most species/taxa. WLH analysed the geochemical data and MW and AP analysed the geophysical data. PS quantified methane concentrations. All authors actively engaged in scientific discussions regarding the study and were all involved in writing the manuscript.

Competing interests

The authors declare they have no conflict of interest.

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Table 1: Total numbers of individuals/aggregations and densities of fauna seen, listed by mosaic/transect. For each taxon, the first number represents the number of individuals, or the number of aggregations in the case of fauna where counting and marking each individual was not possible. Aggregated taxa are marked by a + sign. For these taxa, the number in parentheses refers to the density, calculated based on the area of each mosaic/transect (in the case of frenulates, densities were calculated based on collections and in the case of *Caryophelia* corals, densities were calculated based on selecting a few aggregations per site and counting individuals within aggregations). In the case of bacterial mats, the number in parentheses refers to the density of mats per unit area, since density of individuals of bacteria cannot be counted. Taxa with a * were not used in community analyses. Taxa marked with ^ were seen in images over GHP3 that could not be used due to navigational difficulties associated with that particular lowering of the towed camera.

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Table 2: Properties of all the gravity cores taken and analyzed for the study. The measurements taken for each core are listed (sulfide concentration, sulfate concentration, excess inorganic carbon and methane concentration).

Table 3: Diversity indices and taxonomic richness counts (total and normalized for area) for the different mosaics and transects over the study pingos.

Table 4: Sulfate flux rates measured in cores from GHP3 and GHP5.

Figure 1: Location of the gas hydrate pingo (GHP) study site in the Barents Sea and overview of the site (a). Panels b through e are close-up views of the individual pingos. Free gas plumes were observed at all GHPs except GHP5 and their locations are marked with large black circles in panels b-e. Image transects are visible as lines over the pingos where each constituent image is shown as a single dark rectangle. Mosaics on GHP5 are shown as larger, irregular sized polygons. The small colored Dots represent the locations of the gravity core samples: white represents cores in which all geochemical measurements were made (sulfide, sulfate, DIC, magnesium, calcium and methane), yellow dots are cores in which all geochemical measurements except methane were made, and purple dots represent cores from which only methane was measured.

Figure 2: Examples of image transects and mosaics used in the study. (a): A portion of the TC25 image transect over GHP5. Individual images positioned in space are shown and close up views of two of the images are shown to the right. (b): ROV mosaic 3 over GHP5, with a close up view of one of the images used to construct the mosaic.

Figure 3: Dendrograms and MDS plots of the communities seen in the mosaics and transects over the different gas hydrate pingos. The top panel (a) are the results with frenulates included in the community analysis ($R = 0.926, p = 0.03$) while the bottom panel (b) are the results without frenulates ($R = 0.704, p = 0.03$) In both cases, a fourth root transformation was applied

to the densities, and Bray-Curtis similarity was used. In the bottom MDS plot, TC21 GHP2 and TC18 GHP3 are so similar that their representative triangles almost completely overlap.

Figure 4: Concentrations of sulfide (a) and methane (b) in gravity core samples. Cores from GHP5 are represented with red, 5 dashed lines connecting individual measurements (filled triangles) within the cores. Solid, black lines and filled circles represent cores from the other GHPs (GHP1, GHP2 and GHP3). Methane data from cores 911, 912, 913, 914, and 920 are reproduced from Serov et al., 2017 (*The post-glacial response of Arctic Ocean gas hydrates to climatic amelioration*, Proceedings of the National Academy of Sciences). Sulfide data from cores 911, 920, and 940 are reproduced from Hong et al., 2017 (*Seepage from an arctic shallow marine gas hydrate reservoir is insensitive to momentary ocean warming*, Nature 10 Communications).

Figure 5: Excess inorganic carbon ($\Delta\text{CO}_2 + \text{Mg} + \Delta\text{Ca}$) plotted against sulfate flux (consumption) in gravity cores from GHP3 and GHP5. The sediment depth range from where measurements were taken in the cores is listed individually for each core in Table 4. The solid lines represent the theoretical relationships for a 2:1 and 1:1 ratio of inorganic carbon:sulfate, where a 2:1 15 relationship represents use of sulfate by sulfate reducing bacteria in the breakdown of organic matter, and a 1:1 relationship represents sulfate reduction linked to methane oxidation.

Figure 6: Seismic profile of the four study pingos. The transect for the seismic profile is shown in the map of the study site, starting near GHP1 (point a) and ending near GHP3 (point b). Zones of acoustic blanking beneath the three free gas emitting 20 pingos are highlighted with arrows. hollow circles represent cores. This kind of blanking was not seen below GHP5.

Figure 7: Snow crabs (*Chionoecetes opilio*) seen at GHP3. Note the presence of the crab within a microbial mat in the lower image.

Figure 8: (a): Examples of the onuphid worm, *Nothria conchylega* (a few individuals are highlighted with black arrows). Note 25 trails in the sediment behind the onuphids, indicating they crawl on the seafloor surface. (b): Examples of *Thenea* sponges (possibly *Thenea valdiviae*). A few individuals are circled in yellow.

Supplementary Figure 1: Example of aggregations of *Oligobrachia* cf. *haakonmosbiensis* frenulate worms seen in the images used in this study. The worms are visible as dark patches in the sediment. Individual worms could not be differentiated; 30 however, aggregations are clearly visible. The top panel is an unaltered image and the bottom panel is a georeferenced version of the same image, with the frenulate aggregations outlined.

Phylum and taxon	Number of individuals/aggregations (density)						
	TC21 GHP2	TC18 GHP3	TC25 GHP1	TC25 GHP3	ROV1 GHP5	ROV2 GHP5	ROV3 GHP5
Non-animals							
Bacterial mats*+	1078 (0.63)	1313 (0.96)	216 (0.27)	40 (0.09)	26 (0.03)	6 (0.01)	2 (0.01)
Carbonates (and other rock features)*	1558 (0.91)	93 (0.07)	4161 (5.28)	2 (0.004)	918 (1.16)	296 (0.46)	985 (3.08)
Porifera							
<i>Phakellia</i> sp. (Elephant ear sponge)	5 (0.003)	0 (0)	6 (0.01)	0 (0)	7 (0.01)	2 (0.003)	5 (0.02)
<i>Thenea</i> sp. (possibly <i>valdiviae</i>)	1381 (0.81)	772 (0.57)	74 (0.09)	265 (0.57)	102 (0.13)	103 (0.16)	47 (0.15)
<i>Stylocordyla borealis</i> (stalked sponge)	1 (0.001)	0 (0)	2 (0.003)	1 (0.002)	1 (0.001)	0 (0)	1 (0.003)
Unknown species 1 (white)	0 (0)	0 (0)	8 (0.01)	0 (0)	5 (0.01)	0 (0)	0 (0)
Unknown species 2 (encrusting, yellow)	0 (0)	0 (0)	5 (0.01)	0 (0)	2 (0.003)	0 (0)	41 (0.13)
Unknown species 3 (stick sponge)	0 (0)	0 (0)	6 (0.01)	0 (0)	0 (0)	0 (0)	5 (0.02)
Unknown species 4 (encrusting, white)	78 (0.05)	15 (0.01)	187 (0.24)	1 (0.002)	200 (0.25)	264 (0.41)	374 (1.17)
Unknown species 5 (white)	0 (0)	0 (0)	5 (0.01)	0 (0)	0 (0)	0 (0)	1 (0.003)
Cnidaria							
<i>Bolocera</i> sp.	185 (0.11)	96 (0.07)	197 (0.25)	4 (0.01)	137 (0.17)	77 (0.12)	149 (0.47)
<i>Caryophyllia</i> sp. (pink)*	2 (0.01)	13 (0.39)	18 (1.38)	0 (0)	5 (0.17)	6 (0.01)	4 (2.24)
<i>Caryophyllia</i> sp. (white)*	88 (1.94)	4 (0.001)	646 (11.56)	1 (0.02)	2 (0.08)	0 (0)	3 (0.69)
<i>Cerianthus</i> sp. (soft bottom anemone)	117 (0.07)	76 (0.06)	42 (0.05)	2 (0.004)	19 (0.02)	9 (0.01)	7 (0.02)
<i>Corymorphida</i>	29 (0.02)	6 (0)	54 (0.07)	0 (0)	16 (0.02)	10 (0.02)	31 (0.1)
<i>Difa glomerata</i> (Cauliflower coral)	0 (0)	0 (0)	6 (0.01)	0 (0)	0 (0)	0 (0)	1 (0)
<i>Edwardsiidae</i>	47 (0.03)	22 (0.02)	187 (0.24)	1 (0)	7 (0.01)	0 (0)	0 (0)
<i>Gersemia</i> sp. (orange)	85 (0.05)	104 (0.08)	206 (0.26)	31 (0.07)	1 (0.001)	0 (0)	2 (0.01)
<i>Gersemia</i> sp. (white)	1001 (0.58)	410 (0.3)	621 (0.79)	240 (0.51)	380 (0.48)	313 (0.49)	328 (1.03)
<i>Hormathia</i> sp.	120 (0.07)	34 (0.02)	82 (0.1)	0 (0)	65 (0.08)	58 (0.09)	0 (0)
Juvenile anemones	189 (0.11)	351 (0.26)	404 (0.51)	199 (0.43)	118 (0.15)	5 (0.01)	120 (0.38)
<i>Liponema multicornis</i> (Pom pom anemone)	38 (0.02)	35 (0.03)	15 (0.02)	14 (0.03)	17 (0.02)	5 (0.01)	4 (0.01)

<i>Lucernaria quadricornis</i> (stalked jellyfish)	4 (0.002)	1 (0.001)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown actinarian 1 (small solitary corals)	15 (0.01)	20 (0.01)	50 (0.06)	8 (0.02)	4 (0.01)	0 (0)	3 (0.01)
Unknown actinarian 2 (bright orange)	43 (0.03)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.003)	0 (0)
Unknown medusa	0 (0)	0 (0)	13 (0.02)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown octocoral 1 (orange)	103 (0.06)	5 (0.004)	25 (0.03)	6 (0.01)	0 (0)	0 (0)	1 (0.003)
Unknown octocoral 2 (yellow)	0 (0)	10 (0.01)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ctenophora							
<i>Beroe cucumis</i>	0 (0)	0 (0)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)
Nemertea							
Nemertean, species unknown	36 (0.02)	36 (0.03)	7 (0.01)	10 (0.02)	2 (0.003)	0 (0)	0 (0)
Priapulida							
Priapulid, species unknown	0 (0)	0 (0)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)
Sipuncula							
Sipunculid, species unknown	81 (0.05)	53 (0.04)	53 (0.07)	0 (0)	0 (0)	0 (0)	0 (0)
Annelida							
<i>Aphrodita</i> sp. (sea mouse)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Nothria conchylega</i> (onuphids)*	270 (0.16)	170 (0.12)	311 (0.39)	559 (1.2)	0 (0)	0 (0)	0 (0)
<i>Oligobrachia haakonmosbiensis</i> (siboglinids)+	619 (1059.92)	947 (2144.19)	339 (671.45)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown species 1*+	0 (0)	15 (N/A)	11 (N/A)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown species 2*	4 (0.002)	0 (0)	10 (0.01)	24 (0.05)	0 (0)	0 (0)	0 (0)
Arthropoda							
<i>Chionoecetes opilio</i> (snow crab)^	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>Euphausiacea</i> (krill)	54 (0.03)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Pandalus borealis</i> (Northern shrimp)	359 (0.2)	155 (0.11)	227 (0.29)	38 (0.08)	277 (0.35)	59 (0.09)	34 (0.11)

Pycnogonids	483 (0.28)	249 (0.18)	76 (0.1)	12 (0.03)	0 (0)	0 (0)	1 (0.003)
Mollusca (/Arthropoda)							
Gastropods/Hermit crabs	64 (0.04)	53 (0.04)	54 (0.07)	9 (0.02)	6 (0.01)	3 (0.005)	1 (0.003)
Echinodermata							
<i>Chiridota</i> sp.	0 (0)	0 (0)	3 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Cucumaria</i> sp.	2 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Elpidia</i> sp. (sea pig)	0 (0)	1 (0.001)	0 (0)	0 (0)	1 (0.001)	1 (0.002)	0 (0)
<i>Henricia</i> sp. (pink)	4 (0.002)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Henricia</i> sp. (white)	2 (0.001)	0 (0)	0 (0)	0 (0)	1 (0.001)	0 (0)	1 (0.003)
<i>Henricia</i> sp. (orange)	3 (0.002)	1 (0.001)	4 (0.01)	0 (0)	0 (0)	1 (0.002)	0 (0)
<i>Henricia</i> sp. (yellow)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.003)	0 (0)	0 (0)
<i>Holothuridae</i> (species unknown)	2 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Molpadia borealis</i>	1 (0.001)	2 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ophiuroids	123 (0.07)	106 (0.08)	208 (0.26)	0 (0)	0 (0)	0 (0)	9 (0.03)
<i>Poraniomorpha</i> sp.	0 (0)	0 (0)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)
Chordata							
<i>Anarhichas minor</i> (spotted wolffish)^	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>Gadus morhua</i> (Atlantic cod)	335 (0.2)	16 (0.01)	0 (0)	2 (0.004)	2 (0.003)	99 (0.16)	77 (0.24)
Gray tunicates+	0 (0)	0 (0)	8 (0.01)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Hippoglossoides platessoides</i> (American plaice)	5 (0.003)	5 (0.003)	1 (0.001)	0 (0)	2 (0.003)	0 (0)	0 (0)
<i>Leptagonus</i> sp. (snake blenny)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Lycodes reticulatus</i>	1 (0.001)	1 (0.001)	0 (0)	0 (0)	1 (0.001)	0 (0)	0 (0)
<i>Melanogrammus aeglefinus</i> (Haddock)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.003)
<i>Reinhardtius hippoglossoides</i> (Greenland halibut)	3 (0.002)	0 (0)	0 (0)	1 (0.002)	0 (0)	0 (0)	0 (0)
Skates	4 (0.002)	1 (0.001)	0 (0)	1 (0.002)	1 (0.001)	1 (0.002)	1 (0.003)
Zoarcids (small)	1 (0.001)	4 (0.003)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)

Others/Unknown

White, possible scaphopod*	46 (0.03)	89 (0.07)	56 (0.07)	44 (0.09)	0 (0)	0 (0)
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Core number	GHP number	Year	Measurements taken
911	3	2015	sulfide, sulfate, DIC, methane, calcium, magnesium
912	3	2015	methane
913	1	2015	methane
914	2	2015	methane
920	5	2015	sulfide, sulfate, DIC, methane, calcium, magnesium
940	3	2015	sulfide, sulfate, DIC, calcium, magnesium
1045	3	2016	sulfide, sulfate, DIC, methane, calcium, magnesium
1048	5	2016	sulfide, sulfate, DIC, calcium, magnesium
1068	5	2016	sulfide, sulfate, DIC, methane, calcium, magnesium
1069	5	2016	sulfide, sulfate, DIC, methane, calcium, magnesium
1070	5	2016	sulfide, sulfate, DIC, methane, calcium, magnesium

Mosaic/Transect	Mosaic/transect area (m ²)	Total Richness	Richness/100 m ²	Margalef's index (d)	Pielou's evenness (J)	Shannon diversity (H')	Simpson's index (1-λ)
with siboglinids included							
TC21 GHP 2	1714.23	41	2.4	5.74	0.007	0.026	0.006
TC18 GHP 3	1363.22	33	2.4	4.17	0.003	0.009	0.002
TC25 GHP 1	787.63	39	5.0	5.83	0.016	0.057	0.013
TC25 GHP 3	467.56	20	4.3	32.04	0.578	1.732	1.703
ROV1 GHP 5	787.99	28	3.6	47.99	0.636	2.119	1.948
ROV2 GHP 5	637.52	18	2.8	36.32	0.666	1.926	2.156
ROV3 GHP 5	319.50	27	8.5	19.04	0.613	2.022	1.089
without siboglinids							
TC21 GHP 2	1714.23	40	2.3	35.79	0.673	2.483	1.301
TC18 GHP 3	1363.22	32	2.3	46.43	0.684	2.37	1.756
TC25 GHP 1	787.63	38	4.8	24.78	0.726	2.641	1.159
TC25 GHP 3	467.56	20	4.3	32.04	0.578	1.732	1.703
ROV1 GHP 5	787.99	28	3.6	47.99	0.636	2.119	1.948
ROV2 GHP 5	637.52	18	2.8	36.32	0.666	1.926	2.156
ROV3 GHP 5	319.50	27	8.5	19.04	0.613	2.022	1.089

Core number	GHP number	Sulfate flux (mole/m ² /day)	Depth range for measurements (cm)
911	3	2.08	15-74
1045	3	1.27	10-110
940	3	0.31	5-313
920	5	0.37	10-240
1048	5	0.12	10-324
1068	5	0.90	12-308
1069	5	0.58	8-206
1070	5	0.90	8-266

