**General Comments to the referees:** 

We gratefully acknowledge the referees for their constructive advices to improve this

paper. In the following reply we tried to implement all referees comments to the text.

**Authors** reply to

**Anonymous Referee #1** 

Referee:

Page 12252, Line 24

What would be an abiotic mechanism to generate methane?

**Comment:** Abiotic generation of methane can occur by different pathways: e.g. thermogenic

generation of methane and serpentinization of olivine at hydrothermal systems ((Berndt et al.,

1996; Keir et al., 2008) and references therein).

We added references for the abiotic mechanisms to generate methane.

It now reads:

"It is generated in terrestrial, limnic and marine ecosystems by biotic (Segers, 1998;

Reeburgh, 2007) and abiotic mechanisms (Berndt et al., 1996; Keir et al., 2008)."

Referee:

Page 12253, Line 5

consumption??

Comment: changed

Referee:

Page 12259, Line 19-25

I see this way to show the variability of the data critical. I would suggest to calculate the

standard error (std dev in % of the average) for each water mass and give this number as

information. This info can than also be given in the figures for each data point (not only for

one). Because you assume that the variability calculated for one sample is also valid for the

others.

Comment: Due to the heavy amount of work during sample processing we restricted our

sample number to only one oxidation rate sample per depth excepting the depths of triplicates

to determine the oxidation rate error. Based on the suggestion of the referee we give the standard deviation in percent of the average for the triplicates. In addition, oxidation rate errors for water depths where no triplicates exists were derived from the calculated percentage error of the individual water body (see changed text).

#### It now reads:

"For selected water depths incubation experiments were performed in triplicates to determine the standard deviation (s in percentage of the average, n = 3) of the measured oxidation rates for the upper oxic-, deep anoxic-, and oxic/anoxic transition zone in intermediate water depth (80 - 145 m). For the Gotland Deep, the standard deviation for the oxic zone is 12.4 %, the oxic/anoxic transition zone 8.8 % and the anoxic zone 134.4 % derived from triplicates sampled in 70, 85 and 175 m water depth, respectively. For the Landsort Deep, the standard deviation for the oxic zone is 11.7 %, oxic/anoxic transition zone 11.2 % and the anoxic zone 173.2 % determined by triplicates sampled in 70, 80 and 175 m water depth, respectively."

# Referee:

# Page 12260, Line 9-10

I suggest to take the turnover time (1/k) given in days, as this is more catchy or more intuitive than only "k".

**Comment:** We agree with the referee and provided additionally the turnover time of methane (1/k) given in days. We modified the text.

It now reads: "The turnover rate constant (k) in Eq. (2) expresses the fraction of  $^{14}$ CH<sub>4</sub> that is oxidized per unit time, whereby the turnover time of methane is represented by its reciprocal (1/k)."

Therefore, we changed the text in the following:

# at page 12252, line 11-14

"In contrast, the turnover of methane within the redox zones showed strong differences between the two basins (GD: max. 0.12 nM d<sup>-1</sup> and LD: max. 0.61 nM d<sup>-1</sup>), with a nearly four times lower turnover time of methane in the LD (GD: 455 d, LD: 127 d)."

# at page 12262, line 17-18

"The highest rate was measured within the redox zone (0.12 nM d<sup>-1</sup> at 90 m water depth) with a methane turnover time of 455 d (k = 0.0022 d<sup>-1</sup>)."

# at page 12263, line 15-17

"The highest rate was measured within the redox zone (0.61 nM d<sup>-1</sup> at 90 m water depth) with a methane turnover time of 127 d (k = 0.0079 d<sup>-1</sup>)."

Parameter	Gotland Deep	Landsort Deep
depth interval of the redox zone	81 – 143 m	84 – 130 m
$\delta^{13}$ C CH <sub>4</sub> (redox zone)	-60 – -79 ‰	-20 – -72 ‰
max. CH <sub>4</sub> conc. (bottom water)	1233 nM, 223 m	2935 nM, 422 m
$\delta^{13}$ C CH <sub>4</sub> (bottom water)	-84 ‰, 223 m	-71 ‰, 422 m
max. methane oxidation rate $(r_{ox})$	0.12 nM d <sup>-1</sup> , 90 m	0.61 nM d <sup>-1</sup> , 90 m
max. turnover rate constant $(k)$	0.0022 d <sup>-1</sup>	0.0079 d <sup>-1</sup>
min. methane turnover time $(1/k)$	455 d	127 d
integrated methane oxidation rates in the redox zone ( $ir_{ox}$ )	1.77 μmol d <sup>-1</sup> m <sup>-2</sup>	4.85 μmol d <sup>-1</sup> m <sup>-2</sup>
vertical mixing rates ( $K_p$ , upper anoxic zone)	$2.5*10^{-6} \text{ m}^2 \text{ s}^{-1}$	$1.6*10^{-5} \text{ m}^2 \text{ s}^{-1}$
pmoA detection (DNA analysis)	not achieved	80 – 115 m
pmoA gene expression (mRNA analysis)	85 – 125 m	70 – 115 m

# Page 12260, Line 12

I am not convinced that this method to calculate the integrated MOX is appropriate. The curve of MOX in the redox zone of figure 2 and 3 is rather bumpy. If you take the maximal MOX and interpolate this over the whole depth, this will in result in a overestimation. Thus I suggest to calculate the area below the curve in several steps or to integrate over the curve.

**Comment:** The integrated oxidation rates were calculated by subdividing the redox zone in several depth intervals to gain an accurate integral under the curve. We modified the text. It now reads:

"Integrated methane oxidation rates (ir<sub>ox</sub>) were calculated according to the trapezoid-rule displayed in Eq. (3). Therefore we subdivided the redox zone in depth intervals. Each depth interval was defined by two consecutive sampling depths, whereas dz [m] is the vertical distance between the two samples. f(z) and f(z + dz) are the corresponding oxidation rates ( $r_{ox}$ ) [µmol d<sup>-1</sup> m<sup>-3</sup>] for each sampling depth. The total oxidation rate within the redox zone (ir<sub>ox</sub>) was obtained by summing up the individual oxidation rates of each depth interval."

# Page 12261, Line 6-7

(Why??)

**Comment:** Besides our transcript analysis (detection of active methanotrophs) we also wanted to determine the total methanotrophic assemblages by the detection of the *pmoA* gene. To point out this fact we changed the text:

It now reads:

"In addition 50 ng DNA of each water sample was processed via PCR (30-35 cycles) and DGGE under the same conditions to determine the total methanotrophic assemblages within the entire water column."

#### Referee:

# Page 12262, Line 23

Can you give any information on the sensitivity of the PCR? What is detection limit in cell number or amount of DNA??

A detection limit between 10<sup>1</sup> and 10<sup>2</sup> copies of the *pmoA* gene was determined by Kolb et al. (2003). However, molecular studies also showed that PCR-based methods are strongly affected by the type of the target gene sequence, type and composition of the sample matrix, the type of target organism as well as the number and diversity of bacteria in the sample (Löffler et al., 2000; Rodrigues et al., 2002). Furthermore, a molecular study which investigated 16S rRNA genes in the Gotland Deep showed that the theoretical and practical detection via PCR analysis differ significantly (Labrenz et al., 2004).

We modified the text accordingly.

In the results part it now reads: "pmoA genes could not be detected in this study."

This has been discussed in more detail in the discussion part now (see section 5.1.2):

"In contrast to the GD where no *pmoA* genes could be amplified in PCR reaction and thus probably were below the detection limit, DNA analysis for the identification of *pmoA* genes on the samples obtained in the LD yielded in PCR products. Thus, the *pmoA* gene copy number at GD was below the detection limit of our approach. Within this study we were not able to determine this limit directly. But for soil methanotrophs it ranged between  $10^1$  and  $10^2$  copies of the *pmoA* gene per reaction (Kolb et al., 2003), which could be a realistic number also for our study."

Page 12266, Line 6-7

But most values are quite near the oxidation trend, only one data point is completely aside.

How exactly do the points have to be on the oxidation trend???

**Comment:** There is no regimentation how exactly the points have to fit with the oxidation

trend. The calculated theoretical oxidation trend is just an indicator to classify each point into

the groups: below the mixing line (data points affected by mixing processes), between the

mixing line and oxidation trend (impact of mixing and partly influenced by oxidation) and

above the oxidation trend (data points are clearly related to oxidation processes). We changed

the text at page 12266, line 5-9 to clarify our assumption on the data points.

It reads now:

"The calculated theoretical oxidation trend can be used to classify each CH<sub>4</sub> data point into

three main groups: below the mixing line (CH<sub>4</sub> affected by mixing processes), between the

mixing line and oxidation trend (CH<sub>4</sub> influenced by mixing and partly influenced by

oxidation) and above the oxidation trend (CH<sub>4</sub> clearly related to oxidation processes). Our

results show that the CH<sub>4</sub> data points in both basins fit reasonably well in the <sup>13</sup>C CH<sub>4</sub>

depleted part of the oxidation trend. However, within the redox zone of the eastern Gotland

Basin a deviation is visible in the <sup>13</sup>C CH<sub>4</sub> enriched part (Figure 5B). Based on oceanographic

studies, which indicated a stronger perturbation of the redox zone in the eastern compared to

the western Gotland Basin (Dellwig et al., 2012; Kamyshny et al., 2013), we assume that the

observed deviation from the oxidation trend results from enhanced mixing within the redox-

zone of the eastern Gotland Basin."

Referee:

Page 12268, Line 4-5

For better comparison between the 2 sites, use the same scales for methane and MOX in the

figures.

Comment: We have modified the scales (uniform scaling) for methane concentrations and

oxidation rates in the figures 2B), 2C), 3B), and 3C). See figures 2 and 3 below.

Referee:

Page 12278, Table 1.

add: in the redox zone

**Comment:** We added "in the redox zone" in table 1. See table 1 above.

# Authors reply to

Referee #2 (T. Pape)

# **General comments**

The introduction contains some information potentially irrelevant for the present study, so that there is potential for shortening (see specific comments).

**Comment:** We tried to implement all specific comments. See reply on specific comments below.

In the manuscript I missed a brief discussion on known occurrences of aerobic methanotrophs within aquatic O2-depleted zones. For instance minimum O2 concentrations tolerable for aerobic methanotrophs, knowledge on methanotrophic populations in redox zones in other regions etc.

Comment: For permanently stratified water columns in limnic habitats it is known that aerobic  $CH_4$  oxidation is most active in the vicinity of oxic–anoxic interfaces, where both  $CH_4$  and  $O_2$  are available (Biderre-Petit et al., 2011). Previous studies in marine environments showed an elevated abundance of aerobic methanotrophs together with increased oxidation rates at the oxic/anoxic transition zone (Blumenberg et al., 2007; Wakeham et al., 2007; Biderre-Petit et al., 2011). Rudd and Hamilton (1975) has been shown that aerobic methanotrophs achieve their maximal activity under low oxygen conditions (0.1 - 1 mg/l). However, to our knowledge there is no minimum or specific  $O_2$  concentration known under which activity aerobic methanotrophs is highest.

In this the Black Sea, the Cariaco Trench and Lake Pavin (freshwater habitat) are given as examples for regions with active methane oxidation together with the relevant references. Please see comment below on the comparison of methane oxidation rates.

• In addition, the origin of methane ascending in the water column, underlying geochemical processes and explanations for the significant differences in d13C-CH4 in anoxic waters in the two deeps investigated would be of interest for the readership of Biogeosciences.

**Comment:** Unfortunately, we have no verified methane data from the sediments available to clarify the underlying geochemical processes of the origin of methane. The obtained  $\delta^{13}$ C CH<sub>4</sub>

data (GD: -84 ‰, 223 m; LD: -71 ‰, 347 m) in the anoxic water columns indicate a biogenic origin of methane in both deeps. The stable carbon isotope ratios measured in the deep waters are in the range ( $\delta^{13}$ C CH<sub>4</sub> from -110 ‰ to -50 ‰) of those which are representative for microbially formed methane (Whiticar, 1999).Compare to the GD the determined  $\delta^{13}$ C CH<sub>4</sub> values in the anoxic deep water of the LD were strongly affected by vertical mixing processes. A comparison of both deeps to explain significant differences in  $\delta^{13}$ C CH<sub>4</sub> values cannot be given exactly because of the unavailable water sample (not taken for this study) close to the LD sediments (see Fig. 3) which would not be influenced by vertical mixing. The investigation of the stable carbon isotopic composition in the sediments of the GD and LD will be subject of a further study.

Furthermore, we added to the text (at page 12264, line 3) the information of the origin of methane.

#### It now reads:

"The measured  $\delta^{13}$ C CH<sub>4</sub> values (GD: -84 ‰, 223 m; LD: -71 ‰, 347 m) in the anoxic waters of both deeps are in the range of those which are representative for a biogenic origin of methane (Whiticar, 1999). This stable isotope signature is modified by microbial methane turnover in the overlain water column as this consumption impacts the concentration distribution of methane and its stable carbon isotope pattern (Whiticar, 1999; Reeburgh, 2007; Schmale et al., 2010)."

I also missed a comparison of methane oxidation rates in the redox zone investigated in this study and those established for oxic and suboxic waters in other regions found in the literature. The difference in oxidative strength between oxic and suboxic regimes would be of interest.

Comment: In section 5.1.2 at page 12264 line 21-23 we give the range of determined oxidations rates (from 1x10<sup>-3</sup> to 1.6 nM d<sup>-1</sup>) for the Black Sea which were presented by Reeburgh et al. (1991) and Durisch-Kaiser et al. (2005). For the Cariaco Trench, a permanently anoxic basin located on the continental shelf of Venezuela, oxidation rates model calculation suggests methane oxidation rates near the oxic-anoxic interface amounts to 0.4 – 0.8 nM d<sup>-1</sup>. In addition, the measured oxidation rates in this study are also in line with the calculated oxidation rate (GD redox zone: 0.28 nM d<sup>-1</sup>) by Schmale et al. (2012).

We want to give further information (in section 5.1.2) about detected oxidation rates in the Cariaco Trench.

We changed the sentence at page 12264, line 21-23

It now reads:

"Oxidation rates measured in the suboxic zone of anoxic basins like the central Black Sea:

1×10<sup>-3</sup> – 1.6 nM d<sup>-1</sup> (Reeburgh et al., 1991; Durisch-Kaiser et al., 2005) or the Cariaco

Trench: 0.4 - 0.8 nM d<sup>-1</sup> (Scranton, 1988) are in the same order of magnitude as the data

obtained in this study."

We added the sentence at page 12266, line 25

It now reads:

"A phylogenetically affiliated phylotype of the Uncultured GotDeep pmoA1 was also

detected in a meromictic crater lake (Lake Pavin) which is characterized by a permanently

stratified water column (Biderre-Petit et al., 2011). Apart from the identified phylotype in the

present study, two main phylotypes of type I methanotrophs have been found in the

meromictic lake."

I was slightly confused by inconsistent usage of varying terms for apparently identical matters

(e.g. redox zone = oxic/anoxic transition zone?; oxic/anoxic transition = oxic/anoxic interface?;

lower edge of redox zone = chemocline?). I strongly recommend unifying such terminologies for

quick understanding.

**Comment:** We unified the terms accordantly.

**Specific comments:** 

**Referee:** 

*Page 12251, title* 

**Title** is not fully representative for the manuscript, because focus is laid on microbial methane

oxidation in the redox zone (instead of anoxic basin), although data are additionally

presented for the anoxic and the oxic water body as well

Comment: We changed the title.

It now reads: "Comparative studies of pelagic microbial methane oxidation within the redox

zones of the Gotland Deep and Landsort Deep (central Baltic Sea)"

Referee:

Page 12252

- lines 2-3

specify 'differing environmental conditions'

**Comment:** hydrographic conditions (e.g. gas chemical parameters, vertical mixing) We changed the text.

It reads now: "Pelagic methane oxidation was investigated in dependence on differing hydrographic conditions within the redox zone of the Gotland Deep (GD) and Landsort Deep (LD), central Baltic Sea."

### - lines 4-10

due to length sentence hard to understand - potentially separate into two sentences. Indicate water depth for deep water masses, d13C-CH4 in surface water, O2 conc. in redox zone

**Comment:** We separated the sentence and indicated the water depths for deep water masses,  $d^{13}C$  CH<sub>4</sub> in the surface water. In this study the redox zone cannot defined by O<sub>2</sub> concentrations. We define the redox zone using the turbidity anomalies. Therefore, the characteristics of turbidity anomalies are very complex to give a brief explanation for the redox zone.

It reads now: "The redox zone of both deeps, which indicates the transition between oxic and anoxic conditions, was characterized by a pronounced methane concentration gradient between the deep water (GD: 1233 nM, 223 m; LD: 2935 nM, 422 m) and the surface water (GD and LD < 10 nM). This gradient together with a  $^{13}$ C CH<sub>4</sub> enrichment ( $\delta^{13}$ C CH<sub>4</sub> deep water: GD -84 ‰, LD -71 ‰; redox zone: GD -60 ‰, LD -20 ‰; surface water: GD -47 ‰, LD -50 ‰;  $\delta^{13}$ C CH<sub>4</sub> vs. Vienna Pee Dee Belemnite standard), clearly indicating microbial methane consumption within the redox zone."

### - lines 18-19

specify 'differing hydrographic conditions'
potentially replace 'oxic/anoxic transition zone' by 'redox zone'

**Comment:** hydrographic conditions such as basins structures, lateral intrusions and vertical mixing; oxic/anoxic transition zone replaced by redox zone

It reads now: "Our study identified vertical transport of methane from the deep water body towards the redox zone as well as differing hydrographic conditions (lateral intrusions and vertical mixing) within the redox zone of these deeps as major factors that determine the pelagic methane oxidation."

#### Referee:

Page 12253

- lines 11-12

because thermophilic MOB type X methanotrophs have not been identified in this study this

sentence might be removed

**Comment:** MOB type X could have been also a potential candidate besides type I and II in

this study. Furthermore, our *pmoA* gene analysis were designed to detect type X too.

- lines 15-16

sentence relevant? (Only the particulate form of this enzyme (pMMO) is present in all three

groups.)

**Comment:** Yes, the particulate enzyme (pMMO) which is encoded by the *pmoA* gene is the

basis for our molecular analysis. Only the particulate form of this enzyme was found

universally in methanotrophs.

- lines 22-24

because methane-related archaea were not investigated in this study, sentence might be removed

**Comment:** The explanation of the methyl coenzyme M reductase (MCR) is needed for the

discussion in section 5.1.3.

- lines 25-28

because this is general information sentence might be moved to earlier section of the

Introduction (From marine water column studies it is known that methane is oxidized in the

aerobic environment by MOB of type I, II and X, whereas in the anoxic waters this process is

mediated by ANME I and II (Durisch-Kaiser et al., 2005; Schubert et al., 2006a; Blumenberg

et al., 2007).

**Comment:** We did not move the sentence to earlier section of the introduction because of the

description of 'MOB', which is firstly mentioned in the central part of the section.

Referee:

Page 12254

- lines 3-5

move sentence to top of paragraph

Comment: done

- line 19

'alternative' in this context unclear. With regard to which other organic matter degradation processes?

**Comment:** We removed "alternative" and give another process for the degradation of organic matter.

It reads now:

"Especially the downward diffusion of oxygen is affected by this density boundary leading to a vertical biogeochemical zonation with oxygen-limiting conditions in the intermediate and deep water body and the microbial turnover of organic matter by sulfate reduction or denitrification for example (Lass and Matthäus, 2008)."

### - line 21

remove 'described as'

# Comment:done

It reads now: "This zone is a smooth transition between oxic and anoxic conditions with an overlap of oxygen and hydrogen sulfide containing waters (Nausch et al., 2008; Labrenz et al., 2010, Dellwig et al., 2012)."

### - lines 21-23

potentially state typical vertical thicknesses of redox zones and oxygen concentrations

**Comment:** We cannot generalize the thickness or oxygen concentrations of the redox zone because the redox zone is a very variable transition zone which is depending on seasonal and spatial hydrographic conditions. See also page 12270, line 1-3.

#### - line 23

'suboxic zone' = 'redox zone'? Unify usage of terms throughout manuscript (see remark on Abstract, line 19)

**Comment:** We unified the usage of the terms.

### - line 27

In order to avoid confusion state that 'Gotland Basin' comprises 'Gotland Deep' and 'Landsort Deep' at the beginning of sentence

**Comment:** We changed the text.

It reads now:

"In the Gotland Basins, comprehensive water column investigations revealed a widespread release of methane from the sediments with strong methane enrichments in the stagnant anoxic water bodies (eastern Gotland Basin (Gotland Deep) and western Gotland Basin (Landsort Deep); max. 504 nM and 1086 nM, respectively; Schmale et al., 2010)."

### Referee:

# Page 12255

### - lines 1-3

state concentration ranges

Comment: A concentration range of 3 - 5 nM CH<sub>4</sub> in the surface water can be stated. We changed sentence.

It reads now: "Compared to the atmospheric equilibrium only slightly elevated methane concentrations  $(3 - 5 \text{ nM CH}_4)$  prevail in the surface waters (Bange et al., 1994; Schmale et al., 2010; Gülzow et al., 2012)."

# - lines 3-7

shorten sentence; remove: 'pelagic', 'activity related to the', 'under suboxic conditions'

Comment: 'pelagic', 'activity related to the' removed; 'under suboxic conditions' retained

It reads now: "High resolution gas chemistry studies in the water column of the Gotland Basins showed a pronounced methane gradient and an enrichment of <sup>13</sup>C CH<sub>4</sub> within the redox zone that indicates microbial methane oxidation in that water depth (Schmale et al., 2012)."

### - lines 11-16

statement of specific compound classes is apparently insignificant for the present study; relevance of second part of sentence for this study unclear

**Comment:** We removed the second part of sentence. The first part of the sentence represents an independent evidence for the occurrence of type I methanotrophic bacteria in the Gotland Deep besides the molecular analysis by Schmale et al. (2012).

It reads now: "Furthermore, biomarker analysis could identify specific bacteriohopanepolyols (BHP) and phospholipid fatty acids (PLFA) that are characteristic for the presence of active type I methanotrophic bacteria (Berndmeyer et al., 2013)."

### - lines 23-24

remove 'our work'; specify 'different environmental ... conditions'

**Comment:** done

It reads now: "In this study we focus on the influence of different hydrographic conditions (lateral intrusions and vertical mixing) on the methane turnover within the pelagic redox zone."

#### - line 27

state nature of sample investigated by molecular analysis

**Comment:** A detailed description is given in section 3.3. Furthermore, we replaced 'molecular analysis' by 'molecular biological analysis'.

It reads now.

"The combined data on methane chemistry, methane oxidation rates and molecular biological analysis will gain first insights into the temporal stability and regional transferability of microbial processes related to pelagic microbial methane consumption in the central Baltic Sea."

## Referee:

# Page 12256

#### - line 5

specify 'different basin structures'

**Comment:** "Different basin structures" is specified in line 6-9 ("The Landsort Deep represents the deepest areal in the western Gotland Basin (max. depth 460 m) with a relatively small spatial dimension. In contrast, the eastern Gotland Basin represents the largest basin of the Baltic Sea with a maximum water depth of about 250 m at the Gotland Deep.")

#### - lines 10-13

it would helpful for readers understanding if flow directions of saline water from the North Sea would be illustrated in Fig. 1B

**Comment:** Please, see comments to Figure 1 below.

### - line 15

'decreasing' salt content unclear. With respect to distance from source?

**Comment:** Yes, with respect to distance from the source. We changed the text.

It reads now: "The travel of inflowing saline water from the North Sea along different basins and sills promotes the mixing between saline bottom water and less saline overlying water

masses, resulting in a decreasing salt content of the intruding water along its way into the central Baltic Sea."

#### - line 20

title of paragraph might be amended by 'and physico-chemical measurements' or equivalent **Comment:** We will leave the title as it is. The paragraph 3.1 is just focused on the strategy. Details on the different measurements are given in following chapters 3.2-3.4.

# - line 21

remove brackets

Comment: removed

## - line 22

remove 'procedures'

**Comment:** removed

### Referee:

Page 12257

- line 19

replace 'distribution' by 'concentration'

**Comment:** replaced

# - line 21

replace 'according to' by 'using' or equivalent

**Comment:** replaced

### - line 22

replace 'first detection of hydrogen sulfide' by 'for samples virtually devoid of hydrogen sulfide' or equivalent

**Comment:** replaced

It reads now: "Oxygen concentrations were only determined for samples virtually devoid of hydrogen sulfide."

Page 12258

- *line* 7

remove 'phase'

**Comment:** removed

3.3 Methane oxidation rates

This section requires some re-organization to separate information on water sampling from those dealing with the tracer preparation and labeling procedure which in the current version of the manuscript are intermixed. To achieve a better readability the chapter might be separated into

sub-chapters

Comment: We re-organized and separated into sub-chapters (see text below). Into subchapter 3.3.1 "Sampling and <sup>14</sup>CH<sub>4</sub> tracer preparation" and 3.3.2 "Sample processing" to

achieve a better readability. See re-organized chapter 3.3 in the manuscript.

- line 16-17

remove '(Glasgerätebau....sealing material)

**Comment:** removed

It reads now: "According to the sampling procedure by Reeburgh et al. (1991) water samples were directly transferred from the rosette sampler into transfusion bottles and sealed air-free

with aluminum screw caps and natural rubber septa."

- line 16-17

move sentence to top of paragraph

**Comment:** done

- line 21

remove 'gas'

**Comment:** done

- line 27-1 (page 12259)

explain 'residual tracer liquid'

**Comment:** phrase removed

Page 12259

- lines 10-11

exemplify 'dissolved inorganic carbon compounds'

Comment: done

It reads now: "The microbial activity was terminated by injection of sodium hydroxide (500 μl, 50 % (w/w)) which led to the precipitation of dissolved inorganic carbon compounds (CO<sub>2(aq)</sub>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2</sup>-)."

- lines 11-12

remove 'previously'

Comment: done

- line 20

define 'oxygenated' and 'oxygen-deficient' with regard to O2 concentrations

**Comment:** We changed the text.

It reads now:

"For selected water depths incubation experiments were performed in triplicates to determine the error (standard deviation s in percent of the average, n = 3) of the measured oxidation rates for the upper oxic-, deep anoxic-, and oxic/anoxic transition zone in intermediate water depth (80 - 145 m)."

- lines 24-25

'The standard deviations are given as error bars in' might be removed removed

**Comment:** removed

- line 25

replace 'performed' by 'obtained' or similar

**Comment:** replaced by obtained

# Page 12260

#### - line 3

define 'dpm'

**Comment:** dpm = disintegrations per minute. We added the definition to the text.

It reads now: "...where  $^{14}CO_2$  is the radioactivity [dpm = disintegrations per minute] of the microbial formed carbon dioxide,..."

#### Referee:

### Page 12261

### - line 10

'field studies': a single cruise only is mentioned in the Method section

**Comment:** We changed to singular.

### - line 11-12

data of water densities are not presented in the manuscript

**Comment:** We didn't want to overload Figure 2a and 3a with an additional parameter. Density of seawater is defined as a function of salinity and temperature and the resulting stratification can be derived by the shown temperature and salinity profiles. We would like to leave the figure without that parameter.

### - line 14

replace 'different distinct peaks with two maxima' by 'two peaks' or

**Comment:** We changed the text.

It reads now: "The vertical turbidity profile showed two maxima, one in the surface water and the other in around 127 m water depth."

# - line 18

replace 'suboxic zone' by 'redox zone'; indication for lower boundary of redox zone (143 m) based on O2 concentrations unclear, since lowermost sample analyzed for O2 concentrations is located at approx. 100 m

**Comment:** suboxic zone by redox zone replaced, Indication for lower boundary of the redox zone based not on  $O_2$  concentrations. In chapter 4.1 we defined the depth interval of the redox zone using turbidity anomalies.

- line 22

clarify 'suboxic zone (redox zone)'; see comments on usage of various terms above

**Comment:** We unified the various terms to 'redox zone'

It reads now: "The turbidity anomalies covered a depth interval of approx. 62 m (81 - 143 m),

reflecting the redox zone and the subsequent transition to anoxic conditions (chemocline);

Kamyshny et al., 2013.

- line 25

remove 'based on the fact'

**Comment:** removed

It reads now: "However, since O<sub>2</sub> concentrations were only measured until first detection of

H<sub>2</sub>S, the co-occurrence of O<sub>2</sub> and H<sub>2</sub>S is not documented in our dataset."

Referee:

Page 12262

- lines 16-17

Because methane oxidation rates were determined in relatively high resolution at comparably low standard deviation, it might be worth to mention that rates peaked at two depth intervals in the

redox zone (as was also found for the Landsort Deep)

**Comment:** We could not clarify why the rates peaked at two depths and thus excluded that

point in our discussion. Therefore we think that it should be adequate to show these peaks in

the figures without pointing to that feature in the text. However, these additional maxima

were certainly recognized in our calculation of the integrated oxidation rates along the redox

zone at both sampling sites (see section 5.1.2).

- line 18

refer to Fig. 4 with respect to turnover rate constant; explain why methane turnover at Gotland

Deep is restricted to the redox zone, while at Landsort Deep it takes also place in shallow waters

belonging to the oxic zone

Comment: In the depth interval above the redox zone the turnover rates constants at the

Landsort Deep are approx. 1-2 order of magnitude higher compared to the rates obtained in

the Gotland Deep. In contrast to the Gotland Deep the occurrence of active methanotrophic

bacteria in the Landsort Deep is not only restricted to the redox zone. The elevated turnover rate constants in Fig. 4 are in good agreement with the depth intervals of active methanotrophs at both sampling sites (see black bars in Fig. 2c and 3c below). Furthermore, this relationship is not reflected by the calculated methane oxidation rates due to the CH<sub>4</sub> concentrations which differ slightly above the redox zone (Gotland Deep: 60-80 m, 10-17 nM CH<sub>4</sub>; Landsort Deep: 60-80 m, 8-10 nM CH<sub>4</sub>).

We added "Figure 4" in line 18. Furthermore, we added the explanation (at page 12265, line 18) why the methane turnover at Gotland Deep is restricted to the redox zone, while at Landsort Deep takes place also in shallow waters.

The added explanation reads:

"Furthermore, elevated turnover rate constants were also detected in the oxic zone of the LD and not only within the redox zone as it was observed in the GD. This is in accordance with the transcript analysis which could confirm the expression of the *pmoA* gene above the redox zone of the LD, indicating active methanotrophs also in the oxic zone."

#### - line 23

potentially give more information on the meaning of the fact that 'pmoA genes could not be detected' for readers inexperienced in this field

**Comment:** We changed the sentence at page 12261, line 6-7 to point out the meaning of *pmoA* detection already in the method chapter.

This sentence reads now:

"In addition 50 ng DNA of each water sample was processed via PCR (30-35 cycles) and DGGE under the same conditions to determine the total methanotrophic assemblages within the entire water column.

This has been discussed in more detail in the discussion part now (see section 5.1.2):

"In contrast to the GD where no *pmoA* genes could be amplified in PCR reaction and thus probably were below the detection limit, DNA analysis for the identification of *pmoA* genes on the samples obtained in the LD yielded in PCR products. Thus, the *pmoA* gene copy number at GD was below the detection limit of our approach. Within this study we were not able to determine this limit directly. But for soil methanotrophs it ranged between 10<sup>1</sup> and 10<sup>2</sup> copies of the *pmoA* gene per reaction (Kolb et al., 2003), which could be a realistic number also for our study."

# Page 12263

#### - line 12

insert ,stable carbon' before ,isotopic shift'

Comment: done

#### - line 17

refer to Fig. 4 with respect to turnover rate constant

**Comment:** done

It now reads: "The highest rate was measured within the redox zone (0.61 nM d<sup>-1</sup> at 90 m water depth) with a methane turnover time of 127 d (k = 0.0079 d<sup>-1</sup>, Figure 4)."

### - lines 19-20

remove 'This time'

**Comment:** removed

#### Referee:

### Page 12264

### - line 3

replace 'Pelagic' by 'Microbial'; sentences 1 (lines 3-5) and 3 (lines 6-8) may be combined by specifying trends in concentration distribution and stable carbon isotope pattern that are indicative for methane consumption in first sentence

**Comment:** We replaced 'pelagic' by 'microbial' and changed the first part of the paragraph 5.1.1.

## It now reads:

"The measured  $\delta^{13}$ C CH<sub>4</sub> values (GD: -84 ‰, 223 m; LD: -71 ‰, 347 m) in the anoxic waters of both deeps are in the range of those which are representative for a biogenic origin of methane (Whiticar, 1999). This stable isotope signature is modified by microbial methane turnover in the overlain water column as this consumption impacts the concentration distribution of methane and its stable carbon isotope pattern (Whiticar, 1999; Reeburgh, 2007; Schmale et al., 2010)."

## - lines 8-11

What might be explanation for decreasing CH4 concentrations with depth between ca. 100 and 125 m at Landsort Deep, while d13C-CH4 values remain relatively constant in this depth

interval?

Comment: The stable carbon isotopic pattern shows a slightly enrichment of <sup>13</sup>C CH<sub>4</sub> from 125 to 100 m water depth. In addition, the determined oxidation rates as well as the Rayleigh fractionation approach (plot of δ<sup>13</sup>C CH<sub>4</sub> versus 1/CH<sub>4</sub> in Figure 5A) clearly indicate methane oxidation processes in this depth interval.

### - line 11

indicate depth interval considered in this sentence

**Comment:** We changed the text.

It now reads: "In the LD, the steeper methane gradient within the redox zone together with the stronger <sup>13</sup>C CH<sub>4</sub> enrichment in the lower oxic zone indicates more pronounced and efficient methane consumption compared to the GD assuming similar Eddy diffusion."

#### - line 16

'outstanding position' with respect to what?

**Comment:** As an important depth interval for microbial methane oxidation.

## - lines 19-21

state potential reason for the much lower CH4 in GD or refer to respective subsequent chapter

Comment: We assume that the reviewer means "much lower CH<sub>4</sub> oxidation rates" The potential reasons (stability of the intermediate water body as well as the differing methane concentration) are explained in detail at the end of the chapter and at the beginning of the next chapter (page 12265, lines 2-10).

#### Referee:

# Page 12265

### - line 1

word choice: turnover rate constant was not detected in but calculated for the LD

**Comment:** We changed the text.

It now reads: "At this zone the maximum turnover rate constant (k) calculated for the LD (0.0079 d<sup>-1</sup>) is nearly four times as high as the constant in the GD (0.0022 d<sup>-1</sup>, Figure 4)."

- line 8

'involved' in what

Comment: We added... 'microorganisms involved in the turnover of CH4 within' ... and

replaced 'microbes' by 'microorganisms'.

It now reads: "In addition to these perturbations, the concentration of methane also influences

the abundance of the microorganisms involved in the turnover of CH<sub>4</sub> within the redox zones

of both deeps."

- line 10

replace 'within' by 'of the'

**Comment:** replaced

- lines 11-14

sentence should be streamlined for better readability; replace 'studies' by 'study'; indicate depth

ranges for lower redox zones

Comment: 'studies' replaced by 'study'; we stated the water depths to the corresponding CH<sub>4</sub>

concentrations

It now reads: "In conjunction with our study, this would imply that in comparison to the GD

the higher methane concentrations within the redox zone of the LD (GD: 200 nM CH<sub>4</sub>, 139 m

and LD: 799 nM CH<sub>4</sub>, 124 m) promote microbial methane oxidation and the growth of the

methanotrophic community."

- lines 15-18

How does the assumption of microbial methane oxidation correspond to the fact that no pmoA

genes could be amplified for samples from GD?

**Comment:** Our DNA analyses are not used to support active methane oxidation in the GD.

For the argument of active methane oxidation we use the expression of the pmoA gene. The

fact that no pmoA genes could be amplified in the GD is related to the lower concentration of

*pmoA* genes and thus a lower abundance of the total methanotrophic assemblages in the GD.

Furthermore, pmoA genes do not represent a direct link to active methane oxidation (see also

comment to Referee #1, page 12262, line 23 and the changed text in the manuscript).

- line 18

'positive products' correct expression?

**Comment:** We removed 'positive'

- lines 19-21

remove 'pelagic'; specify 'assess the methane oxidation'; because processes specifically taking

place in the chemocline ('transition to anoxic conditions') were not highlighted in the preceding

chapters, this statement is somewhat confusing here

**Comment:** We removed 'and further assess the methane oxidation in the pelagic chemocline'

and 'pelagic'

It now reads: "To determine the kinetic fractionation factor ( $\alpha$ ) of microbial methane

oxidation within the redox zones we created a plot of  $\delta^{13}$ C CH<sub>4</sub> versus 1/CH<sub>4</sub> (Figure 5).

- line 21

exemplify 'side effects'

Comment: e.g. lateral input of methane with a different d<sup>13</sup>C CH<sub>4</sub> signature. We changed the

text.

It now reads: "To avoid any side effects in these calculations which could be caused by lateral

intrusions (i.e. input of CH<sub>4</sub> from other regions with a different  $\delta^{13}$ C CH<sub>4</sub> signature), we used

in a first step the dataset obtained in the LD which is compared to the GD characterized by a

less disturbed intermediate water layer (Dellwig et al., 2012)."

- lines 24-25

specify 'methane concentration and isotope patterns' (see also my comment on relatively constant

d13C-CH4 signatures in redox zone at LD

**Comment:** We specified 'methane concentration and isotope patterns'.

It now reads: "Motivated by the apparent restriction of methane oxidation within the redox

zone, as observable from the methane concentration gradient and <sup>13</sup>C CH<sub>4</sub> enrichment in the

LD redox zone (Figure 3B), we applied a closed system Rayleigh fractionation approach

(Coleman et al., 1981)."

- line 29

remove 'could be'

**Comment:** removed

Referee:

# Page 12266

#### - line 1

statement of a stronger extent of disturbance at the GD compared to LD requires reference

**Comment:** We added the reference '(Dellwig et al., 2012)', which shows the stronger disturbance in the GD. See also added reference to comment line 21.

### - lines 4-5

remove 'identified

**Comment:** removed

#### - lines 8-9

reference 'Mau et al., 2012' is not correct at this place because that study considered seepage off Costa Rica

**Comment:** We replaced the reference (Mau et al., 2012) to page 12265 line 21.

It now reads: "To determine the kinetic fractionation factor ( $\alpha$ ) of microbial methane oxidation within the redox zones we created a plot of  $\delta^{13}$ C CH<sub>4</sub> versus 1/CH<sub>4</sub> (Figure 5) according to (Mau et al., 2012)."

## - lines 20-22

statement relevant for this study? If the authors intend to keep the sentence it should be amended by information on the chemical nature of the respective Black Sea water body inhabiting such microbes.

**Comment:** We changed the text in line 18-20, to give additional information on the chemical nature of the Black Sea water body.

It now reads: "The restricted diversity of type I methanotrophs in the central Baltic Sea is in agreement with studies conducted by Schubert et al. (2006c) in the Black Sea, who identified type I methanotrophic bacteria as the most important methane consumer in the redox zone. However, other studies conducted in the oxic zone as well as in the redox zone of the Black Sea also proved the existence of type II and X methanotrophs (Durisch-Kaiser et al., 2005; Blumenberg et al., 2007)."

# - lines 22-25

statement is repetition of pages 12262 (lines 20-23) and 12263 (lines 18-19)

**Comment:** We removed the last part of the sentence to avoid a repetition. However, the first

part represents a phylogenetically comparison between GD and LD and is the basis for the

following discussion.

It now reads: "The methanotrophic bacteria identified in the GD and LD is restricted to the

phylotype Uncultured GotDeep pmoA1 (Schmale et al., 2012)."

- lines 26-27

indicate subject (microbial population, water column?) for which structural stability is

considered; indicate subject for which lateral intrusions (water mass?) is assumed. In my opinion

the relation between lateral water intrusion and low diversity of microbial population requires

some more detailed explanation.

Comment: The relevant references and mechanisms have been shown in this study.

Furthermore, Schmale et al. (2012) refer also to the assumed relation between microbial

diversity and the toxicity of sulfide.

Referee:

Page 12267

- line 1

citations are incorrectly positioned because at present state they appear to support own

interpretation

Comment: We changed the text at page 12266, line 25-28 as well as at 12267, line 1-2

It now reads: "We assume that the reduced diversity in the Baltic Sea redox zone is related to

an overlap of sulfide- and oxygen-containing waters as a result of lateral intrusions (Dellwig

et al., 2012). The influence of sulfide containing waters on the microbial diversity has been

shown by (Labrenz et al., 2010). Based on this relation it is assumed that the toxicity of

sulfide to many organisms may inhibit the activity of other methanotrophs than the detected

phylotype (Schmale et al., 2012)."

- line 3

'disturbed' in respect to what?

**Comment:** We changed the text.

It now reads:

"However, a higher diversity of active aerobic methanotrophic bacteria in the compared to the

GD less disturbed redox zone of the LD could not be detected."

- lines 8-9

calculation of individual sulfate concentrations from continuous water salinity profiles requires

better explanation

**Comment:** We changed the text.

It now reads: "From a theoretical point of view, sulfate-depending methane oxidation (AOM)

in the anoxic water layer should be possible as the ambient sulfate concentrations (LD: 0.81 g

kg<sup>-1</sup> and GD: 0.93 g kg<sup>-1</sup>; derived from the averaged salinity in the anoxic water layer,

calculated after Bruland, 1983) would enable this process (Reeburgh, 2007).

- lines 11-16

section bears potential for condensation

**Comment:** See changed text to comment line 12

- line 12

specify 'affected by errors'

**Comment:** affected by errors like underestimation due to the tracer amount. We changed the

text.

It reads now:

"However, the determined oxidation rates are significantly affected by high standard

deviations and a potential underestimation due to the chosen <sup>14</sup>CH<sub>4</sub> tracer amount. The

method developed for this study was designed to analyze microbial methane oxidation within

the oxic and redox zone, so that these oxidation rates cannot provide a clear evidence for the

existence of AOM processes."

- line 19

title might be amended by the matter that is mixed

Comment: We would like to leave the title. "Vertical mixing" is a common oceanographic

term.

- lines 20-23

separate into two sentences

**Comment:** We separated into two sentences.

It now reads: "The vertical transport of matter (e.g. nutrients, gases, particles) in the central

Baltic Sea is strongly influenced by vertical mixing processes. The different intensities of

mixing directly impact the concentration distribution pattern of chemical species in the water column (Nausch et al., 2008; Holtermann et al., 2012)."

#### - line 24

emphasis should be placed on biogeochemical processes rather than scientific interest

Comment: We replaced 'scientific subject' by 'biogeochemical process'

It now reads: "Especially the transport across the chemocline influence important biogeochemical process as reduced and energy-rich chemical species like  $CH_4$ ,  $H_2S$ , iron  $(Fe^{2+})$  and manganese  $(Mn^{2+})$  are abundant in high concentrations within the deep water and can drive microbial reactions at the redox zone (Labrenz et al., 2005; Dellwig et al., 2012; Schmale et al., 2012)."

# Referee:

# Page 12268

#### - line 3

indicate approx. water depth of 'lower edge of redox zone'

**Comment:** We removed 'lower edge of'.

It now reads: "Between 300 m water depth and the lower edge of the redox zone (130 m) the LD is characterized by a uniform methane profile towards the redox zone whereas the methane concentrations in the GD decrease constantly with decreasing depth."

### - line 4

specify 'this zone'

**Comment:** We removed 'towards this zone'

#### - line 8

processes related to 'boundary effects' deserve some better explanation. Why are boundary effects restricted to water column above 300 m water depth?

### **Comment:**

The reviewer is right. The paragraph line 3–21 includes too many oceanographic terms which are difficult to understand for the target reader without any additional complex explanations. The statements to mechanisms which influence the vertical mixing in both basins (22 -28) are mainly based on assumptions by Axell (1998), which need to be proved by additional oceanographic studies, which were not in the focus of the present work. We generalized the

paragraph to limit oceanographic terms and reduced our discussion to the measured  $K_{\rho}$  values  $(K_{\rho}: GD\ 1.1*10^{-5}\ m^2\ s^{-1}, LD\ 6.2*10^{-5}\ m^2\ s^{-1}$ , annual mean at 150 m water depth, Axell, 1998) and how these values can explain the observed  $CH_4$  trend in both basins. We generalized the text in line 3-21. See chapter 5.2.1 in the manuscript.

#### - line 11

define  $K_p$ 

**Comment:** We changed the text. We defined  $K_{\rho}$  as vertical mixing rate and refer to Eq. 4. See changed text.

## - line 12

'its'?

Comment: See changed text in chapter 5.2.1

### - lines 13-14

'coastal boundary layer' should be illustrated in Fig. 1

**Comment:** We removed 'coastal boundary layer' in the text, because this assumption by (Axell, 1998) cannot be discussed deeply in this study. See the more generalized new text version that excludes the oceanographic terms.

# - lines 13-16

terms 'high-energetic coastal processes' and 'transfer of larger energy flux densities' require some better explanation

**Comment:** We removed these terms. See changed text chapter 5.2.1

## - line 17

give information about distribution/thickness of coastal boundary layer

**Comment:** We removed the term 'coastal boundary layer'. See changed text chapter 5.2.1.

# - line 17

indicate reference parameter for 'increasing'

Comment: See changed text chapter 5.2.1.

#### - line 18

remove 'Towards the bottom'

**Comment:** We removed 'Towards the bottom'. See changed text chapter 5.2.1.

- line 18

in case values for vertical mixing rates are available, they should be stated here

**Comment:** See changed text chapter 5.2.1.

- lines 23-28

terms 'basin interior' and 'basin boundary' require better explanation. Which units assigned for these terms result in an unitless ratio WV/BB? Relation between 'basin interior' and 'water volume (WV)' requires clarification; exact values for WV and BB should be stated for both, GD and LD, along with respective references

**Comment:** We removed the terms. See changed text chapter 5.2.1

- lines 24-25

sentence appears to be incomplete

**Comment:** See changed text.

Referee:

Page 12269

- line 1

unify terminology ('anoxic deep water' vs. 'anoxic zone')

Comment: We replaced 'anoxic deep water' by 'anoxic zone'

- line 3

specify 'our dataset; specify depth interval of 'upper anoxic zone'

**Comment:** 'Our dataset' should be clear by the context in line 3-5 (Eq. 4). We specified the depth interval of the 'upper anoxic zone' and changed the text in line 1-3.

It now reads:

"Assuming that the flux of methane from the anoxic deep water to the redox zone and the consumption of methane within the redox zone are in steady state, we can use our dataset to calculate vertical mixing rates ( $K_{\rho}$ ) for the upper anoxic zone (GD: 143-200 m, LD: 130-250 m)."

- line 5

'methane gradients' incomplete term

**Comment:** We replaced 'methane gradients' by 'methane concentration gradients'

### - lines 10-11

remove 'observed'

**Comment:** removed

#### - line 13

'though the absolute values ... factor of 4.' incomprehensible

**Comment:** We changed the text.

It now reads:

"This higher vertical mixing rate in the LD is in accordance with the observations of Axell (1998), who reported a 6 times larger  $K_{\rho}$  for the LD than for the GD at the 150 m depth level. However, the mixing rates calculated within the present study are 4 times lower compared to the values reported by Axell (1998).

#### - line 16

title of chapter might be amended by the matter that is intruding

**Comment:** The title should do not only related to the matter that is intruding but should also show the consequence (e.g. perturbations in the redox zone). Furthermore 'vertical mixing and 'lateral intrusions' are common oceanographic terms. On this reason we would like to leave the title as it is.

#### - line 17

turbidity in the water column caused by mineral precipitation is not restricted to stratified water bodies such as in the Baltic Sea. Turbidity was also observed to correlate with oxidation of reduced metal species discharged at hot vents in the open ocean (see e.g. Marbler et al., 2010 and others). Sentence might be re-phrased accordingly.

**Comment:** We changed the text.

It now reads: "The turbidity in anoxic basins is often used as a marker to determine the depth of the chemocline."

# - lines 22-23

clarify relation 'abundance of bacteria' and extent of water column turbidity'; define 'deep pool'

**Comment:** The exactly relation between the abundance of bacteria and the extent of water column turbidity is still not known. It is just known that elevated microbial activity is connected turbidity anomalies (Prokhorenko et al., 1994; Dellwig et al., 2010; Labrenz et al., 2010)

We replaced 'deep pool' by 'anoxic zone'. Furthermore, we added the reference (Labrenz et al., 2010).

#### Referee:

Page 12270

### - lines 3-4

remove 'our data shows that'

**Comment:** removed

# - line 6

specify 'dynamic conditions within the redox zone'

**Comment:** We changed the text.

It now reads: "

Previous studies in the GD indicate, that these turbidity anomalies are related to lateral intrusions into the redox zone (Lass et al., 2003; Dellwig et al., 2012).

#### - line 9

insert 'signal on top of the redox zone'

**Comment:** done

It now reads:

"In contrast, the LD reveals only one pronounced turbidity signal on top of the redox zone with a constant decrease with increasing water depth (Figure 3A)."

### - line 12

'in that transition zone' unclear

**Comment:** We replaced 'in that transition zone' by 'in the LD redox zone'

It now reads:

"Even if this decrease can currently not be explained, the clear trend without turbidity spikes (in contrast to the observation in the GD) point to a more undisturbed situation in the LD redox zone (Dellwig et al., 2012; Kamyshny et al., 2013)."

#### - lines 15-16

use abbreviation 'T-S' introduced in line 13 instead of 'temperature and salinity profiles'

**Comment:** We replaced 'temperature and salinity profiles' by 'T-S profiles'

### - line 22

specify 'different environmental settings'; remove 'pelagic'

**Comment:** We replaced 'different environmental settings' by 'hydrographic conditions'. We removed 'pelagic'

#### - line 27

because there is in my opinion no evidence from this study that the 'hydrographic conditions have no influence on the methanotrophic population' I suggest rephrasing this sentence according to 'hydrographic conditions apparently do not promote development of a higher diversity of methanotrophic communities' or similar

**Comment:** We rephrased the sentence.

It now reads:

"In this intermediate depth interval one potentially active type I methanotrophic bacterium was identified at both sampling sites, indicating that the different hydrographic conditions apparently do not impact the diversity of methanotrophic communities."

### Referee:

# Page 12271

# - lines 1-2

specify 'considerable differences of microbial methane turnover in redox zones in both deeps'

**Comment:** We specified and changed the text.

It now reads:

"In contrast, the microbial turnover of methane in the redox zones reveals considerable differences with lower methane oxidation rates in the Gotland Deep compare to the Landsort Deep."

# - line 3

give idea on location of 'deep methane pool'

**Comment:** We replaced 'deep methane pool' by 'anoxic zone'. Furthermore, in line 7 we replaced 'deep pool' by 'deep anoxic zone'

It now reads:

**Line 3:** "The intensity of lateral intrusions and the vertical transport rate of methane from the anoxic zone into the redox zone are different between both deeps, and seem to represent the key-processes which control the turnover of methane within the redox zone."

**Line 7:** "Our results confirm that pelagic microbial methane oxidation within redox gradients represent an efficient methane sink that prevents the escape of methane from the deep anoxic zone into the atmosphere."

#### Table 1

Reference to Table 1 is missing in text

**Comment:** We added the reference at page 12263 line 22 to refer to the Table 1.

It now reads:

"Our physical, chemical and microbiological results (summarized in Table 1) show considerable differences between both deeps."

# **Figures**

### Figure 1

- Fig. might be enlarged to full page size

**Comment:** We will provide the enlarged Figure to the "Biogeosciences" journal and ensure that the Figure will be published in full page size.

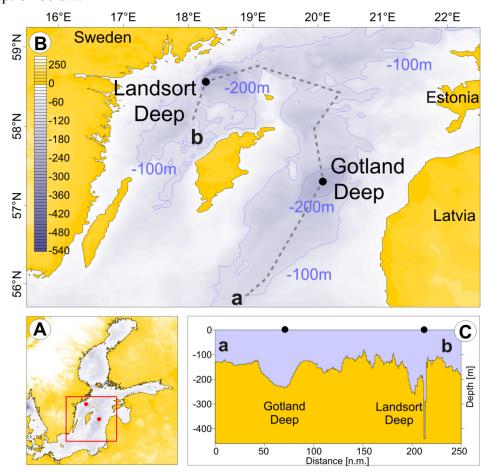
- Position of numbers for longitude misleading (see Figs. 1A and 1C)

**Comment:** We separated Figure 1 in three individual illustrations. Furthermore, we shifted the longitude scale to the top of Figure 1B. See changed Figure 1.

- Orientation of profile in 1C might be illustrated in Fig. 1B; direction of Fig. 1C might be mirrored;

**Comment:** The orientation of profile 1C is illustrated in Fig. 1B by the letters (a) and (b) that are connected with a dashed line. To highlight the cross section of 1C we increased the letter size and changed the color of (a) and (b) as well as the thickness of the dashed line. In

addition, we added the letters (a) and (b) to the figure caption. See Figure 1 and changed figure caption below.



**Figure 1.** (**A**) Sampling sites in the central Baltic Sea (red dots). (**B**) Bathymetric map of the central Baltic Sea with the Gotland Deep in the eastern and the Landsort Deep in the western Gotland Basin. (**C**) Cross section from the eastern (**a**) to the western Gotland Basin (**b**).

- Flow directions of water masses mentioned in the text at several places should be illustrated in Fig. 1B

**Comment:** Our intention of Figure 1 is the illustration of the two sampling sites. Therefore, we restricted the explanation of inflowing water masses to the text. The illustration of specific flow directions (oceanographic characteristics) might be overload Figure 1 and will may cause confusion.

# Figure 2 and 3

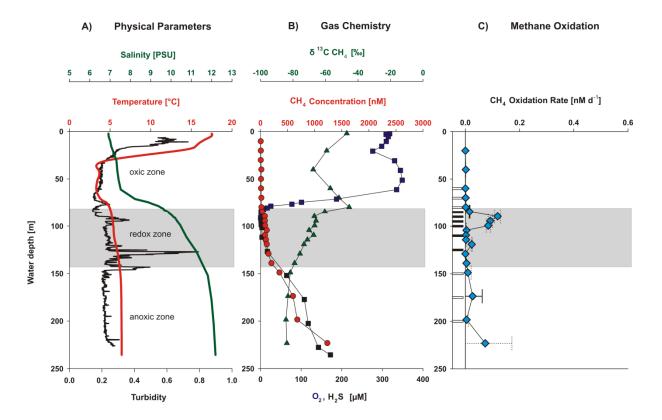
annotate oxic, suboxic and anoxic zones in figure

**Comment:** We annotated oxic, redox zone and anoxic zone in Figure 2A and 3A. See changed figures below.

color coding (blue, green, black) used in Figs. 2B and 3B, respectively are difficult to differentiate in the printout version; use different symbol shapes at least

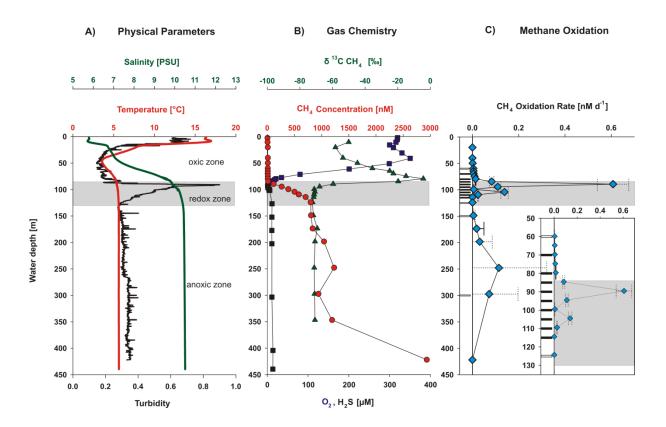
**Comment:** We changed the symbol shapes in the Figs. 2B and 3B and changed the figure captions accordingly. See changed figures 2 and 3 below.

color coding used for gene expression analysis in Figs. 2C and 3C, respectively, cannot be **Comment:** We changed the color coding to black and white symbols. See changed figures 2C and 3C below.



**Figure 2.** Gotland Deep. **(A)** Vertical profiles of salinity (green), temperature (red), and turbidity (black); **(B)** Oxygen (blue squares), hydrogen sulfide (black squares), methane (red circles), and  $\delta^{13}$ C values of methane (green triangles); **(C)** Methane oxidation rates (light blue diamonds), and sampling depths of *pmoA* gene expression analysis (black bars denote the occurrence and white bars the absence of active type I methanotrophs). To obtain the standard deviation (s) for methane oxidation rates, triplicates were taken in three water depths (70, 85,

175 m). The solid error bars indicate the standard deviation from these triplicates whereas the dashed error bars show the standard deviation calculated from these triplicates for the single water samples. The redox zone is indicated by the gray shaded area.



**Figure 3.** Landsort Deep. **(A)** Vertical profiles of salinity (green), temperature (red), and turbidity (black); **(B)** Oxygen (blue squares) and hydrogen sulfide (black squares), methane (red circles), and  $\delta^{13}$ C values of methane (green triangles); **(C)** Methane oxidation rates (light blue diamonds), and sampling depths of *pmoA* gene expression analysis (black bars denote the occurrence and white bars the absence of active type I methanotrophs). To obtain the standard deviation (*s*) for methane oxidation rates, triplicates were taken in three water depths (70, 80, 175 m). The solid error bars indicate the standard deviation from these triplicates whereas the dashed error bars show the standard deviation calculated from these triplicates for the single water samples. The redox zone is indicated by the gray shaded area. The insert in **C)** illustrates the depth interval of the redox zone in higher vertical resolution.

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