Methane emissions from the East Siberian Arctic Shelf

Production and removal pathways

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1. Introduction

1.1. The methane budget

Methane (CH₄) is an important greenhouse gas, contributing to past and present global climate change. Emissions originate from anthropogenic (e.g. fossil fuel burning, agriculture, waste management) and natural sources (e.g. wetlands, geological sources, termites). The most important sink is the reaction of CH₄ with the hydroxyl radical (OH) in the troposphere, though a significant amount of methane is removed by methanotrophic bacteria in oceans and soils and never reaches the atmosphere. Since pre-industrial times, the atmospheric methane concentration has increased from approximately 700 nmol mol⁻¹ to 1790 nmol mol⁻¹ (IPCC, 2007), mainly due to anthropogenic emissions from the fossil fuel industry and agriculture combined with strong climatic feedbacks. The different components of the methane budget are constrained within a factor of 2 (Dlugokencky *et al.*, 2011), and large uncertainties remain concerning the response of feedback mechanisms of natural sources to the on-going climate changes.

1.2. The Arctic methane source

In the Arctic regions, climate change is strongly coupled to the carbon cycle. During cold climatic episodes atmospheric carbon dioxide accumulates as biomass in permafrost soils. The flux reverses upon warming; thawing permafrost can turn into boreal wetland and carbon is released partially in the form of methane, providing a strong positive feedback to the Earth's radiative balance.

The Arctic has been through several warming episodes – the Dansgaard-Oeschger events - in the late Quaternary. Atmospheric methane increases associated with these warmings have been attributed to emissions from the vast boreal carbon pools through stable isotope analysis of methane from Greenland ice cores (Fischer *et al.*, 2008, Bock *et al.*, 2010). Another feedback process is associated with gas hydrate destabilization triggered by warming of Arctic waters. Based on early measurements of methane hydrate isotopic composition and isotope data from ice cores, Sowers (2006) and Bock *et al.* (2010) preclude a dominant hydrate contribution to Pleistocene warmings, suggesting that instead these warmings were fuelled by boreal wetland methane emissions. However, the isotopic composition of hydrate methane is still very uncertain and appears to include a wetland isotopic signature as well (Milkov, 2005) and rapid paleoatmospheric methane fluctuations induced by hydrate dissociation may be unrecognizably smoothed in ice core records (Bock *et al.*, 2012).

Present-day Arctic methane sources are dominated by boreal wetlands in summer and leaks associated with industrial gas extraction in spring and winter, when wetlands are frozen (Fisher *et al.*, 2011). As Arctic warming continues, large uncertainties exist about the strength of the Arctic methane feedback, of individual sources and sinks and the response of its vast carbon reservoirs.

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The Arctic harbours one of the world's largest active carbon pools. Terrestrial and subsea permafrost may store over 3000 Pg C, ranking second before the biosphere (2160 Pg C) and after the ocean (38.000 Pg C) (Tarnocai *et al.*, 2009). We can distinguish two main carbon reservoirs (Fig.1): subsea permafrost (1400 Pg C, Shakhova *et al.*, 2010b) and terrestrial permafrost - underlying tundra and taiga soils (1672 Pg C, Tarnocai *et al.*, 2009), of which 400 Pg carbon is stored in Yedoma (ice-bearing) deposits (Zimov *et al.*, 2006). The subsea permafrost carbon reservoir consists of inundated terrestrial permafrost soil (500 Pg C, Shakhova *et al.*, 2010b), methane hydrates (540 Pg C, Shakhova *et al.*, 2010a) and free gas pockets (360 Pg C, Shakhova *et al.*, 2010b, Soloviev *et al.*, 1987). These size estimates remain subject to on-going research. The global atmospheric carbon pools of carbon dioxide (760 Pg C) and methane (3.5 Pg C) could be significantly affected by carbon release from thawing permafrost.



Figure 1 - Size of different carbon pools in the Arctic in Petagram C. The left chart represents the total Arctic carbon reservoir, divided into a terrestrial and a submarine component (right chart). The atmospheric methane concentration is included for comparison. Sources are listed in text.

Permafrost thaw, induced by rising Arctic temperatures, has increased the re-mobilization of stored carbon and potential outgassing to the atmosphere. An expert assessment conducted by Schuur *et al.* (2013) stated that if warming continues at its current pace, by 2100 terrestrial permafrost alone could release 155±35 Pg C, 7-14% of the terrestrial permafrost pool. Despite the estimation that only 2.3% of this carbon would be released as methane (±44 Tg C/yr, 9% of global methane emissions), the 100 year global warming potential of thawing permafrost soils is ±220 Pg CO₂ equivalent (Schuur *et al.*, 2013). It should be noted that the gas hydrate and sub-sea permafrost carbon pools were not part of the assessment.

1.3. The case of Siberian permafrost

The East Siberian Arctic Shelf (ESAS) is an area of particular interest because it holds the potential to release huge amounts of carbon to the atmosphere (see previous section). Since the Holocene transgression (5-12 kyr ago, Bauch *et al.*, 2001), relatively warm water temperatures have subjected terrestrial permafrost to extensive thaw and anoxic conditions (Nicolsky *et al.*, 2012), and continuous coastal erosion provides a steady source of organic carbon to the marine environment (Vonk *et al.*, 2012). Continued thaw of subsea permafrost could lead to widespread dissociation of methane hydrates beneath it. Extensive methane venting has been observed from thawing subsea permafrost, emitting up to 7.98 ± 1.7 Tg C / yr to the atmosphere – comparable to the combined emissions of the World oceans (Shakhova *et al.*, 2010a). Even though this estimate is supported by multi-year observations, it is subject to substantial uncertainties associated with upscaling sparse measurements from a highly dynamic heterogeneous environment.

Though the magnitude of the ESAS methane flux appears significant, little is known about the methane sources, sinks and transport processes in the ESAS. The role of subsea permafrost thaw and potential methane hydrate instability also remains to be better understood (Shakhova *et al.*, 2013).

1.3.1. Terrestrial permafrost

Permafrost is defined as soil or rock that remains at or below 0 °C for at least two consecutive years. It covers roughly 25% of the Earth's land surface and underlies large parts of the Arctic shelf seas. The climate in Arctic permafrost regions is characterized by low mean annual temperatures (-8 to -15 °C) and precipitation (90 to 370 mm). As a result, growing seasons are short and vegetation is sparse (French, 2007). Wetland methane production is largely confined to the short Arctic summer, when part of the surface sediments thaw (Fisher *et al.*, 2011). This seasonally thawed surface layer is called the *active layer*, and plays an important role in permafrost geochemistry, as it supports microbial activity as well as redistribution of water, nutrients and dissolved organic matter (Wagner & Liebner, 2010). The physical and chemical boundary between active layer and perennially frozen ground is called the *permafrost table*. The thickness of the perennially frozen layer is determined by the average winter temperature and the geothermal gradient (between permafrost table and base), and measures up to 1500 meters in areas with continuous permafrost and harsh winters (French, 2007).

The fast response of the Arctic wetland methane feedback is based on easily degradable carbon accumulated in permafrost soils (Zimov *et al.*, 1997, Walter *et al.*, 2006). In the active layer of seasonally frozen soils, limited decomposition rates promote accumulation of high quality carbon. High quality carbon consists mostly of dissolved organic carbon (DOC) and is mineralized fast – it is highly *biolabile* (Alling *et al.*, 2010). However, in permafrost soils anoxic conditions and low temperatures impede microbial degradation; methanogenic anoxic processes are up to 5 to 10 times

slower than oxic respiration because of lower energy yields (Bethke *et al.*, 2011). Below the permafrost table even the high quality dissolved carbon is preserved (Knoblauch *et al.*, 2013): Vonk *et al.* (2013) identified 34±1% of ice-complex DOC as biolabile compounds. Low quality substrate that remains can be loosely defined as particulate organic carbon (POC), long organic chains which mineral-OC bonds ballast the OC and make it resilient against degradation and dissolution (Sánchez-García *et al.*, 2011). The latter type of carbon is transported with sediment by rivers and deposited on the ESAS seafloor (Vonk *et al.*, 2010). Transport of terrestrial OC from the oxic (terrestrial) to anoxic (marine) environment could initiate a marine feedback mechanism fuelled by seafloor methanogens.

1.3.2. Subsea permafrost

In Northern Siberia the Holocene transgression submerged extensive parts of the continental shelf, forming the shallow East Siberian Seas (Romanovskii et al., 2005). As a result the sub-sea permafrost has been continuously exposed to increasing seawater temperatures, salt and anoxic conditions. Continuous permafrost becomes discontinuous and punctured by deepening taliks (layers of unfrozen ground underneath rivers or lakes), which may provide pathways for gaseous and dissolved methane escape from the marine sediment to the atmosphere (Nicolsky & Shakhova, 2010). Warming of the bottom water layers of the shelf seas – accelerated by present-day decreasing seaice coverage - could invoke further degradation (Dmitrenko et al., 2011, Nicolsky et al., 2012). A key question is how the thawing of this permafrost "lid" affects gas hydrate stability zones and gas pockets: sudden release of methane hydrates could initiate a catastrophic climate runaway process, faster and stronger than the wetland methane feedback. A recent model study found no indication of large scale methane hydrate dissociation in the Siberian Arctic (Nicolsky et al., 2012), though localized methane plumes or hotspots have been discovered in the Laptev and East Siberian Shelf seas and along the West Spitsbergen continental margin (Shakhova et al., 2010a, Westbrook et al., 2009). As warming and thawing continued throughout the Holocene, the available Pleistocene carbon has fuelled methanogenic communities up to 58 meters below the seafloor (Koch et al., 2008). Sub-sea permafrost is a source of methane to the atmosphere, though the relative magnitude of the different pathways has yet to be determined.

1.4. The marine Arctic carbon cycle

From the perspective of potential climate change, the key question concerns the amount, type and rate of thawing permafrost carbon transferred to the atmosphere from terrestrial and marine pools (Schuur *et al.*, 2013). Outlined in figure 2 is the redistribution of carbon through the different reservoirs and conversion between different carbon species in what constitutes the *permafrost carbon cycle*. To determine the amount, rate and type of carbon released into the atmosphere it is useful to distinguish its components:

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Atmospheric carbon enters the terrestrial reservoir through uptake of CO_2 by plants and subsequent breakdown of plant material in soils. Soil organisms gain energy for growth through cellular respiration – a series of catabolic redox reactions breaking down large organic molecules into smaller ones. Figure 3 illustrates several of these reactions relevant to methane cycling. Under aerobic conditions (in the ocean water column, seabed oxic surface sediment, or oxygen saturated streams) aerobic respiration and oxidation of methane takes place, releasing carbon dioxide. Under anaerobic conditions, methanogenesis takes place in the presence of sufficient substrate and absence of competition by other bacteria. The final net reactions, triggered by methanogenic archaea, are fermentation of acetate to CH_4 and carbonate and reduction of inorganic carbon (CO_2) to CH_4 , deriving electrons from oxidation of H_2 or formate (Lessner, 2009):

 $CH_3CO_2^- + H_2O \rightarrow CH_4 + HCO_3^-$

 $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

These anaerobic processes are much slower than oxidative respiration (Wagner & Liebner, 2010). The final mineralization products CH₄ and CO₂ either escape to the atmosphere or remain dissolved in seabed pore water, as gas pockets or in the form of methane hydrates depending on seawater temperature, salinity and pressure (Sloan, 2003).

If sulfate is present, methane can be oxidized back to HCO₃ through anaerobic methane oxidation (AOM) by a consortium of methanotrophic archaea (ANaerobic MEthanotrophs, or ANME's) and sulfate reducing bacteria (Boetius *et al.*, 2000). Microbial mats found near gas hydrate deposits and seepage hotspots in the Black Sea have been shown to locally limit the global warming potential of the methane emitted (Treude *et al.*, 2007). Methanogens and -trophs are closely related; methanogenesis and oxidation can occur simultaneously in terrestrial and marine sediments (Seifert *et al.*, 2006). In addition to methane, sulfate reducing bacteria consume acetate and H₂, and are able to outcompete methanogens over these "competitive substrates" (Lessner, 2009).



fault zone



fault zone

Figure 2 - The Arctic Marine Carbon Cycle in winter (top panel) and summer (bottom panel) – transport of terrestrial organic carbon and subsequent degradation to methane and carbon dioxide, shelf edge gas hydrate dissociation and escape of gaseous methane through open taliks (orange arrows). Oxidation of methane (circular arrows) may take place in marine sediment and in the water column (at depth or below the sea ice). White arrows indicate transport of terrestrial carbon and swirls microbial degradation. Areas bordered yellow and white represent layers of subsea permafrost and the gas hydrate stability zone respectively. Image inspired by Karlsson (2012, Figure 1), with permission of the author.

1.5. Sources of carbon in the ESAS

Carbon enters the Arctic marine environment by thawing subsea permafrost, riverine transport from modern terrestrial reservoirs and by primary production. The East Siberian Arctic Shelf is an area of particular interest. Bordered by a vast coastline of Yedoma cliffs (carbon-rich permafrost consisting of 50-90% ice by volume (French, 2007)), its shallow shelf seas provide a discharge basin for the Great Siberan Rivers (see section 2.1 on the study area).

Concentrations in the ESAS water column average 160 μ M DOC (Alling *et al.*, 2010), 5.68 μ M POC (Sánchez-García *et al.*, 2011) and 1400 μ M DIC (Alling *et al.*, 2012). By comparison, methane concentrations in the Laptev Sea range from 0.1 μ M at background locations and 5 μ M over hotspots (Shakhova *et al.*, 2010a).

1.5.1. Pleistocene carbon activation

The bulk of organic carbon stored in Siberian permafrost accumulated there during the Pleistocene (2.5-0.01 Ma BP), before the onset of the warmer Holocene epoch, 11.4 ka BP (French, 2007). Methanogenesis from Pleistocene carbon begins with transport of organic material from the thawing (subsea) permafrost. There are four key physical mechanisms that release carbon from thawing permafrost: active layer deepening, thermokarst development, coastal erosion and gas hydrate decomposition (O'Connor *et al.*, 2010).

The rate and type of greenhouse gas production depends on carbon type and quality, temperature as well as the moisture content and availability of nutrients and electron acceptors. Often decomposition rates are slow because only low quality carbon is available and Arctic summers are short, keeping annual average temperatures low. In dry uplands, such as the Lena river catchment area, carbon is exposed to aerobic conditions and is released as CO_2 that can be again assimilated in living biomass. In waterlogged lowlands, such as the Lena delta, mobilized carbon is either slowly decomposed by microbes - producing CH_4 and CO_2 - or transported in meltwater streams to thaw lakes and shelf seas (Schuur *et al.*, 2013).

Decay of Yedoma DOC already starts in meltwater streams originating from thawing terrestrial permafrost (Vonk *et al.*, 2013): Bussmann (2013) found methanogenic bacteria are present in meltwater streams along the Lena delta riverbank, generating methane concentrations of up to 1500 nM. During transport, the oxygen-rich freshwater enhances oxidation of terrestrial organic carbon (OC_{ter}) and outgassing of CO₂. Lena River dissolved methane concentrations drop from 200 nM to background concentrations (5-20 nM) once the river reaches its headwaters in the Laptev Sea (Semiletov *et al.*, 2011). The main sink of riverine CH₄ is likely diffusion to the atmosphere, as Bussman (2013) found isotopic evidence of a low methane oxidation potential in the Lena River.

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Microorganisms in sediment and water actively mineralize Pleistocene carbon delivered to the ESAS marine environment – a potential methane source. In the western ESAS (west of 160°E) annually 35 ± 8 Tg C of POC_{ter} is deposited with sediments on the shelf seabed. Approximately 57% of this resilient carbon originates from thaw of Yedoma coasts, 16% from surface soils and 26% from primary production (Vonk *et al.*, 2012). The ESAS DOC budget is dominated by Lena river input (5.4 Tg C /y). ESAS waters can be oxygen saturated in spring and summer (Savel'eva *et al.*, 2012) and in nutrient-rich turbid waters, most terrestrial organic material remains suspended and is aerobically mineralized to dissolved inorganic carbon (DIC) (Anderson *et al.*, 2011, Alling *et al.*, 2012). Degradation of DOC (6.5 Tg C/y, Alling *et al.*, 2010) and POC (2.5 Tg C/y, Sánchez-García *et al.*, 2011) approximately add up to the ±10 Tg of excess DIC that annually outgasses as CO₂ from the western ESAS (Anderson *et al.*, 2009). A large fraction (>75%) of Pleistocene POC_{ter} is buried in the seafloor of the continental shelves. This thesis explores the pathways by which Pleistocene carbon can contribute to the large methane fluxes observed in the region today.

1.5.2. Modern carbon

"Modern" organic carbon was incorporated in biomass after the era of nuclear bomb testing in the early 1960's. It is therefore enriched in ¹⁴C, which makes it a traceable component in the Arctic carbon cycle (Alling *et al.*, 2012). Modern carbon originates from tundra and riverbank topsoil and is transported to the ESAS by meltwater channels and rivers (Karlsson *et al.*, 2011). Owing to a high microbial accessibility, in the Laptev Sea most dissolved organic carbon (DOC) compounds have a lifetime of several years (Alling *et al.*, 2010), and all DOC transported by the Lena River is modern (Raymond *et al.*, 2007). An estimated 20±10% of the carbon that is buried on the shelf seafloor originates from topsoil and is of modern age (Vonk *et al.*, 2012, Feng *et al.*, 2013). Though less significant in the sediment, modern carbon represents up to $63\pm10\%$ of the water column POC budget in the Laptev Sea (Karlsson *et al.*, 2011). This carbon consists of highly biolabile buoyant humic compounds that are mineralized to CO₂ in the oxic ESAS surface waters (Alling *et al.*, 2012). Because modern carbon is likely to remain at the surface, any greenhouse gasses derived from it diffuse rapidly to the atmosphere in summer (Semiletov *et al.*, 2011).

1.5.3. Gas hydrate destabilization

Hydrates in the Arctic shallow shelves have a significant global warming potential, as opposed to deep ocean hydrates (Kvenvolden, 1988). They hold huge quantities of carbon, most of which is methane that is released the instant the hydrate dissociates. Studies on hydrate stability in seafloor sediment indicate that the warming since the Last Glacial Maximum is causing the formation of methane seepages in the continental margin of West-Svalbard (Tatcher *et al.*, 2013) and the Beaufort Sea (Paull *et al.*, 2011), but these emissions occur at depths of <300m and may not reach the

atmosphere (Fisher *et al.*, 2011). In the ESAS, however, the overlying water layer may be too shallow to allow significant methane oxidation; even in ice-covered periods methane escapes through the atmosphere through areas of open water (flaw polynyas) (Shakhova *et al.*, 2010b, Kort *et al.*, 2012). These shallow shelf hydrates are stabilized by low temperature rather than high pressure – making them vulnerable to the shelf's 17°C Holocene temperature rise. In addition, this type of hydrates requires only a third of the thermal energy deep ocean hydrates need to convert to free gas and also has a much greater pore occupancy (20-100% vs 1-2%). Decrease of permafrost impermeability and hydrate dissociation has been hypothesized to cause extensive methane venting from the East Siberian Arctic Shelf seafloor (Shakhova *et al.*, 2010b). We use the isotopic composition of methane to estimate the relative importance of different carbon sources outlined above.

1.6. Methane isotope analysis

Stable isotope ratio measurements of methane are used to identify its sources and sinks, thus its involvement in the carbon cycle. The usefulness of isotopes in process characterization is grounded in their distinct chemical and physical properties, expressed in diffusive transport and phase transitions that discriminate against heavier or lighter isotopologues.

The influence of molecular mass differences on chemical pathways is due to generally slightly stronger chemical bonds of the heavy isotopologues compared to the lighter ones, resulting in lower reaction rate constants. This *kinetic isotope effect* is most pronounced if the isotopic element is part of the chemical bond that is involved in the reaction, and if the relative mass change is large. Deuterium replacing hydrogen results in a mass change of 100%, compared to 8% for ¹³C replacing ¹²C; the associated reaction rates for C-H / ¹²C-¹²C bonds are typically 6-10 / 1.04 times faster compared to C-D / ¹²C-¹³C bonds respectively (Melander & Saunders, 1980). This is why the majority of isotope research focuses on small molecular compounds such as CH₄, H₂, O₂ and CO₂.

The isotopic composition of a substance is defined by its molar isotope ratio, R = [heavy]/[light]. The δ -notation is commonly used, expressing isotope ratios relative to a standard in units of per mill (parts per thousand):

$\delta = (R_{SAMPLE}/R_{STANDARD} - 1)$

Isotope fractionation results in different δ -values for substrate and product. Isotope discrimination can be expressed by the fractionation factor, defined as the ratio of reaction rate constants (k) for different isotopologues; $\alpha = k_{HEAVV}/k_{LIGHT}$. Alternative constants that are commonly used to express isotope fractionation are the "kinetic isotope effect" – KIE = $1/\alpha$ – and "isotopic fractionation": $\varepsilon = \alpha$ –1. Isotopic fractionation can be the result of breaking or forming a chemical bond, or of different

diffusion rates of isotopologues in a medium. Fractionation constants are often used to identify chemical pathways. For example, microbial oxidation of dissolved methane has a relatively strong isotope effect while removal through diffusion has a smaller isotope effect; the two processes can be distinguished through their very different KIE-values. An overview of fractionation factors relevant to Arctic environments from peer-reviewed literature can be found in table 1 and figure 3.



Figure 3 – Isotopic fractionation associated with aerobic and anaerobic processes that govern methane production and oxidation (scheme after Holler *et al.*, 2009). In this figure isotope fractionation (*KIE*, sources listed in table 1), are expressed as ε =KIE-1 in per mill. Coloured arrows represent biochemical pathways involved in methane cycling and substrate competition.

1.6.1. The isotopic signatures of methane

In figure 4, the isotopic composition (δD and $\delta^{13}C$) of methane from different sources is plotted. The isotopic composition of methane has been identified with field measurements (e.g. Quay *et al.*, 1999, Fischer *et al.*, 2008). In case of biogenic methane, laboratory studies have been able to identify the isotope fractionation between precursor and product (e.g. Whiticar, 1999, Conrad, 2005). In these studies, the source signatures of methane depends primarily on the precursor isotopic composition (δ_{PREC}) and the fractionation factor associated with the methanogenic pathway (table 1):

$$(\delta_{\text{SOURCE}} + 1) = (\delta_{\text{PREC}} + 1) \times 1/\text{KIE}$$

Methane derived from CO₂ appears depleted in ¹³C but enriched in D with respect to acetoclastic methane, while thermogenic methane is enriched in both isotopes with respect to biogenic methane (Whiticar, 1999). Measured isotopic compositions that differ from a source signature can result from fractionation processes or linear mixing between sources or a background concentration (e.g. an enrichment of a biogenic source can be caused by oxidation or mixing with a thermogenic source).

In the two sections that follow we calculate the value of δ_{SOURCE} using measured values of δ_{PREC} and α . Conservative estimates of methanogenic fractionation factors were taken from a frequently cited literature study by Whiticar (1999). These estimates fall within a much wider range of KIE-values found in the literature (table 1), reflecting a wide range of temperatures under which different microorganisms can produce methane.

1.6.1.1. Carbon isotopic composition of microbial gas

Both biogenic methane production pathways involve separation of stable carbon isotopes (¹³C / ¹²C). In methyl-type fermentation processes the methyl group of a methylated compound, such as acetate, is converted to methane. This conversion is accompanied by an isotope separation of ca. ε_c =-50±10‰ (Whiticar, 1999) between the precursor plant material (δ^{13} C = -26‰) and acetoclastic methane (δ^{13} C=-60±10‰). In the specific case of acetate fermentation, the carboxyl group joins the bicarbonate pool, which in alkaline conditions can serve as an additional methane source (Conrad, 2005). In the case of methanogenesis from CO₂, the isotopic composition of inorganic carbon is assumed to vary from -8‰ (atmospheric, Keeling *et al.*, 1995) to -26‰ (derived from C_{org}), higher values have been measured in the ESAS water column because of primary production ($\delta^{13}C_{co2}$ = +1‰ Alling *et al.*, 2012). An isotope separation between CO₂ and CH₄ of ca. ε_c =-75±15‰ (Whiticar, 1999) constrains the signature for hydrogenotrophic methane in the ESAS to $\delta^{13}C$ =-90±30‰.

1.6.1.2. Hydrogen isotopic composition of microbial gas

For hydrogen isotopes, source identification is complicated because the precursor signature is not fixed, but instead depends on location and time. The precursor signature is given by $[\delta D_{PREC} = (1-f)^* \delta D_{ORG} + f^* \delta D_{H2O}]$. The hydrogen for acetate-derived methane originates mainly (f = 0.25, Whiticar, 1999) from its depleted methyl group ($\delta D_{ORG} = -100\pm20\%$, Whiticar, 1999), whereas the CO₂-derived methane receives all H-atoms from environmental water (f = 1, Chanton *et al.*, 2006). Using Whiticar's estimate of isotope fractionation and precursor signatures of $\delta D_{ORG} = -100\%$ and $\delta D_{H2O} = 0\%$, we obtain $\delta D = -200\pm50\%$ and $\delta D = -340\pm60\%$ for hydrogenotrophic and acetoclastic methane respectively. Note that we assume no isotope effect for the formation of H₂ from H₂O in the acetate fermentation process (see Valentine *et al.*, 2004 for a discussion on this topic). Estimates of the hydrogen isotopic composition are subject to great uncertainty because very few laboratory experiments have been conducted (Valentine *et al.*, 2004) and CH₄ signatures depend largely on that of highly variable precursor material, resulting in more negative δD_{CH4} than depicted in figure 4.

In the Arctic, we may need to factor in a different δD_{H20} precursor signature. Most incubation experiments were performed with standard water ($\delta D_{H20} \approx 0\%$), whereas Arctic Ocean water can have a δD_{H20} of -20‰ due to depleted river runoff, precipitation and melting sea ice (Friedman *et al.*,

1964). This precursor effect is most pronounced in thermokarst and glacial lakes, where CO₂-reducing methanogens use depleted permafrost meltwater ($\delta D_{H20} = -135\pm25\%$, Chanton *et al.*, 2006).

Process	KIE (¹³ C)	KIE (D)	Source			
Methanogenesis						
- CO ₂ reduction	1.021-1.071	1.160-1.430	¹³ C: Conrad, 2005; D: Valentine, 2004			
- CO ₂ reduction	1.049-1.095	1.160-1.180	Whiticar, 1999			
 Methyl-type fermentation 	1.007-1.094	1.260-1.420	¹³ C: Conrad, 2005; D: Chanton, 2006			
 Methyl-type fermentation 	1.024-1.077	1.300-1.377	Whiticar, 1999			
Anaerobic Methane Oxidation						
- Mar. sed. and water column	1.002-1.014		Whiticar & Faber, 1986			
- Marine Sediments – Baltic Sea	1.011-1.013	1.100-1.140	Martens et al., 1999			
- Marine Sediments - Alaska	1.008-1.010	1.134-1.180	Alperin <i>et al.,</i> 1988			
- Marine Sediments - Hydrate	1.012	1.109-1.115	Holler <i>et al.,</i> 2009			
Ridge, Pacific Ocean						
 Marine Sediments - Mud 	1.019-1.023	1.144-1.180	Holler <i>et al.,</i> 2009			
Volcano, Mediterranean Sea						
 Microbial mat – Black Sea 	1.035-1.039	1.281-1.315	Holler <i>et al.,</i> 2009			
 Water column – Black Sea 	1.016-1.024		Reeburgh <i>et al.,</i> 2006			
- Water column – Black Sea	1.020-1.022	1.181-1.221	Kessler <i>et al.,</i> 2006			
- Incubation, nitrite-driven AOM	1.027-1.032	1.214-1.241	Rasigraf et al., 2012			
Aerobic Methane Oxidation						
- Soil uptake	1.017-1.018	1.066-1.099	Snover & Quay, 2000			
- Seep field offshore CA, USA	1.022-1.030	1.156-1.320	Kinnaman <i>et al.,</i> 2007			
- Laboratory incubation	1.013-1.025	1.097-1.350	Coleman <i>et al.</i> , 1981			
- Laboratory incubation	1.015-1.028	1.110-1.232	Feisthauer et al., 2011			

Table 1 – Overview of KIE-values $(1/\alpha)$ for methane-related processes as determined by in-situ measurements or incubation studies. Note: in the cited publications the term "isotopic fractionation factor" is often used to describe a KIE value.

1.6.2. Isotopic composition of methane hydrates

Methane hydrates constitute a reservoir for methane of thermogenic and biogenic origin (Sloan, 2003). The signature of hydrate methane is therefore almost indistinguishable from that of its primary sources: a small deuterium isotope fractionation of ca. -10% is associated with hydrate formation (Hachikubo *et al.*, 2007). Milkov (2005) constructed a global dataset of the isotopic composition (D and ¹³C) of marine hydrate methane and reported a broad range of isotopic compositions: $\delta^{13}C = -42$ to -75% and $\delta D = -115$ to -242%. These values fall within the range of thermogenic ($\delta^{13}C=-35\pm15\%$, $\delta D=-200\pm100\%$) and CO₂-derived methane ($\delta^{13}C=-90\pm30\%$, $\delta D=-200\pm50\%$) reported by Whiticar (1999): an acetoclastic signature is an unlikely find in sulfate-rich marine sediment, because sulfate-reducing bacteria may outcompete methanogens for methylated substrates (Lessner, 2009). Hydrates containing methane with a distinctive acetate-fermentation signature were found below the fresh waters of Lake Baikal, Russia (Kida *et al.*, 2006).



Figure 4 - The isotopic composition of methane from different sources - data from Whiticar, 1999, Quay *et al.*, 1999, Milkov, 2005, Fisher *et al.*, 2011 and Dlugokency *et al.*, 2011. Rhombuses indicate sources we expect to find in the Arctic sediment and water column. Colours represent source classification used throughout this thesis, with their isotopic boundaries outlined by shaded areas. Adjustments to the range of ¹³C and D signatures are made according to non-conservative fractionation factors for biogenic methane (dotted lines, see table 1 and Whiticar, 1999). The isotopic composition of methane hydrates is included here (Milkov, 2005), although strictly they are not a source, but a reservoir.

1.6.3. Tools in isotope analysis

To establish the source and predominant fractionation process of a compound three analysis tools are often used: the Keeling, Miller-Tans and Rayleigh fractionation plots. Figures 5 and 6 show an example of the Keeling and Miller-Tans plots and Rayleigh plot respectively. Furthermore, the Rayleigh plot can be used to distinguish isotope fractionation from two-reservoir mixing (figure 7).

1.6.3.1. Keeling plot

The Keeling plot shows the isotopic composition against the inverse of the concentration, assuming a linear correlation between the two. We have to assume a mixing between a / several source(s) and a background concentration in a well-mixed closed system, so that the highest concentrations have been subject to the least dilution. The source signature is then found at the intercept between the δ -axis and the linear regression line (Keeling, 1958, 1961).

1.6.3.2. Miller-Tans plot

An alternative source-identification method developed by Miller & Tans (2003) is effectively a nonlinear transformation of the Keeling plot. One plots the δ -value against δ -value × concentration, so that the slope of the linear regression line corresponds to the source value. Kayler *et al.* (2010) analysed the precision of both plotting techniques for δ^{13} C analysis of CO₂ samples. Both methods provide very comparable results ($\Delta\delta$ (‰) < 0.87) as long as the difference between source and background concentrations is sufficiently large (Δ [CO₂] (ppm) > 1000). Choice of method is therefore based on robustness against outliers. As a linear regression method, we used Geometric Mean Regression; the frequently used Ordinary Least Square method is inadequate because both measured variables are expected to contain errors (Miller & Tans, 2003).

For atmospheric samples the Miller-Tans plot has shown to be less sensitive to high isotope ratio variance found in our low concentration samples (Kayler *et al.*, 2010). In this thesis the Miller-Tans plot will be compared to the Keeling plot.



Figure 5 – Examples of the Keeling (left panel) and Miller-Tans (right panel) plots for carbon isotopes of an artificial atmospheric dataset (right table). The data (open circles) describe an atmospheric background concentration (δ^{13} C=-47.25‰) mixed with a boreal wetland source (δ^{13} C=-65‰). A typical natural variability of ±0.1‰ was added to the δ^{13} C values (Fisher *et al.*, 2011). The intercept (Keeling) and slope (Miller-Tans) of the linear regression lines provide an estimate of the source signature. Both methods correctly identify the source signature within 0.3‰.

1.6.3.3. The Rayleigh plot

The Rayleigh plot is used to calculate the characteristic partitioning of isotopes as one of two reservoirs decreases in size. Figure 6 shows two common applications of the Rayleigh plot. In the left panel, this technique used to derive the fractionation factor α that is unique for different removal pathways. It is derived from the Rayleigh distillation equation:

$$(R/R_0) = (C/C_0)^{\varepsilon}$$

that describes a non-replenished depletion of a compound with initial concentration C_0 and isotope ratio R_0 . Paired measurements of R and C can then be used in a linear transformation of the Rayleigh distillation equation:

$$\ln(R/R_0) = \varepsilon \ln(C/C_0)$$

The slope ε of the regression line is equal to $\alpha - 1$ or 1/KIE-1, measured with the ordinary least square method. Plotting the methane remaining fraction (*C*/*C*₀) against the isotopic composition - figure 6 (right panel) - we can compare the data with idealized Rayleigh curves using different KIE-values.



Figure 6 – A Rayleigh fractionation plot (left panel) shows the correlation between $ln(C/C_0)$ and $ln(R/R_0)$ for an artificial dataset representing anaerobic methane oxidation in a water column (open circles). The slope of the linear regression line (ϵ =-0.1071) reveals the AOM KIE-value of 1.12 (KIE=1/(ϵ +1)). In the right panel three theoretical Rayleigh distillation curves are plotted (solid lines) using different KIE-values for AOM (table 1). The theoretical mixing curve (dotted line) shows the isotopic composition of a mixture between a reservoir with a high methane concentration and one with a lower concentration.

A major issue associated with this method is the assumption of a closed system, putting the emphasis on the initial concentration by forcing the regression line through the origin (Scott *et al.*, 2004). Especially in systems where production and removal of methane take place simultaneously the Rayleigh plot does not produce meaningful results (Kessler *et al.*, 2006).

1.6.3.4. The theoretical mixing curve

The Rayleigh plot can also be used to distinguish between a fractionation process and conservative mixing between two reservoirs. Figure 7 shows mixing between a reservoir (A) with isotopically light methane (δD =–325‰, e.g. a biogenic source) with a reservoir (B) with a different concentration of isotopically heavy methane (δD =–180‰, e.g. a thermogenic source) in a fractional mixing plot (left panel) and a Rayleigh plot (right panel). The theoretical signature of methane in the mixed reservoir can be calculated with the methane concentration and isotope ratio in each reservoir:

$$\delta_{MIX} = (C_A f_A \delta_A + C_B f_B \delta_B) / (C_A f_A + C_B f_B)$$

where C_A and C_B and δ_A and δ_B represent the methane concentration and δ -value in reservoir A and B respectively, f_A and f_B represent the fraction of each reservoir the mixture ($f_A + f_B = 1$), and δ_{MIX} the mixture isotopic composition.



Figure 7 – The isotopic composition ($\delta D_{CH4,MIX}$) of a mixture between two reservoirs (A,B) with unique methane concentrations ([CH_{4,A}], [CH_{4,B}]) and isotopic composition ($\delta D_{CH4,A}$ =-325‰, $\delta D_{CH4,B}$ =-180‰). The labelled curves represent initial reservoir methane concentration ratios: [CH_{4,B}]:[CH_{4,A}] = 1:1, 1:2, 1:10 and 1:100. The left panel shows the resulting isotopic composition as a function of the reservoir A mixed fraction f_A . In the right panel $\delta D_{CH4,MIX}$ is plotted against the 'methane remaining fraction' ([CH₄,A]/[CH_{4,A}]) as in figure 6.

The shape of the theoretical mixing lines depends on the initial methane concentration difference between reservoirs. Mixing between a source and a background concentration ($[CH_{4,B}]$: $[CH_{4,A}]$ = 1:100 in figure 7) results in a nonlinear curve, whereas a concentration ratio of 1:1 results in a linear curve.

The use of mixing curves becomes more complicated with a real dataset. When plotting a mixing curve one assumes that the highest and lowest of concentrations measured are representative of different reservoirs, thus this method may not resolve mixing between reservoirs with even higher and lower concentrations. Furthermore, in the artificial dataset we have assumed a mixture between two sources, while a real dataset may reflect multiple sources and/or a combination of mixing and a removal process.

2. Study area

The East Siberian Arctic Shelf (ESAS) is the the world's largest (2.1×10⁶ km²) and shallowest (<50m) continental shelf (Shakhova *et al.*, 2010a). It can be roughly divided in the Laptev Sea region and the East Siberian Sea region, which are seperated by the Dmitri Laptev Strait and the Novosibirsk Islands. To the west the ESAS is bordered by the Russian part of the Chukchi sea. Figure 8 displays our study area, which covers most of the ESAS.



Figure 8 - International Bathymetric Cart of the Arctic Ocean (IBCAO). The study area is highlighted in red. (Source: NOAA)

The shelf has been subject to a series of regressions and transgressions coupled to the Pliocene-Pleistocene eustatic cycles - the most recent inundation occured 5-12 kyr ago (Romanovskii *et al.*, 2005). During the Last Glacial Maximum (19 kyr ago), the sealevel was 100 m lower and the Arctic Ocean coastline was located ±1000 km up north. Continuous subzero air temperatures enabled deep (1500m) and continuous permafrost formation in the entire Arctic coastal plain at that time. The ESAS has been submerged in relatively warm seawater (ΔT = +12°C) during most of the Holocene, and as a result the subsea permafrost has started to thaw (Nicolsky *et al.*, 2012).

The Laptev Sea is underlain by a tectonically active rift system believed to result from the ultra-slow spreading of the Gakkel Ridge (Drachev *et al.*, 2003). Formation of open taliks occurs along these fault zones because of the increased geothermal heat flux. On the shelf edge, permafrost is frequently exposed to warm seawater by earthquake-induced slope failure. Rapid permafrost degradation outside fault zones has been hypothesized to result from intrusion of saltwater, increasing the unfrozen water content and thermal properties of the sediment (Nicolsky *et al.*, 2012).

The shelf seas comprise only 1.5% of the global ocean by volume, but receive ±10% of the global river runoff from the Lena, Yana, Indigirka and Kolyma rivers, which together with coastal erosion results in a carbon accumulation rate comparable to that of the entire pelagic area of the world oceans (Stein & Macdonald, 2004). Rivers provide a strong seasonal component in the carbon cycle: in summer the discharge maximum causes turbid waters as it mixes with the colder, anoxic Arctic Ocean waters (Charkin *et al.*, 2011), whereas in winter the relatively warm, oxic freshwater plumes induce strong stratification below the sea-ice (Stein & Macdonald, 2004). The highly dynamic nature of hydrological parameters affecting the Arctic carbon cycle makes it challenging to interpret the seasonal and spatial distribution of ESAS methane as observed by Shakhova *et al.* (2010a).

3. Methods

3.1. Sampling

Figure 9 shows the sampling locations in the ESAS. Water samples were collected during two summer field campaigns on sea (August-September 2011 and 2012) and two winter expeditions (April 2007 and 2011) on the fast ice in the Lena Delta adjacent to the Laptev Sea (table 2).

The majority of water and sediment samples was taken in the proximity of known **hotspot** areas (Shakhova *et al.*, 2010a). These are locations in the western Laptev Sea, Dmitri Laptev Strait and on the shelf edge north of the Lena Delta, where dissolved methane concentrations are higher than 100 nM and methane is actively bubbling from the seafloor.

Water samples from **background** locations were collected on the continental shelf along the Siberian coast. At background locations methane concentrations are relatively low (<100 nM) and the dominant mode of transport to the atmosphere is slow diffusion through the water column. A deep sediment core was drilled at such a background site in the Buor-Khaya Bay in the southern Lena Delta. The original goal of the expedition was to sample a hotspot site further north, but poor ice conditions confined drilling activities to the Buor-Khaya Bay. Surprisingly, the core was entirely unfrozen, providing not only a conduit for dissolved methane, but also enabling potential methanogenesis from Pleistocene carbon. This was recently shown for Lena Delta terrestrial permafrost (Bischoff *et al.*, 2013).

Location	Туре	Period	Reservoir	No. of s.
Lena Delta	Hotspot	Winter 2007	Water	17
Buor-Khaya Bay	Background	Winter 2011	Sediment (one deep core)	52
East Siberian Arctic Shelf	Background	Summer 2011	Water	28
Lena Delta	Hotspot	Summer 2012	Sediment (two shallow cores)	11
Dmitri Laptev Strait	Hotspot	Summer 2012	Sediment (two shallow cores)	9
Laptev sea shelf edge	Hotspot	Summer 2012	Water	13

Table 2 - Overview of locations sampled from 2007 to 2012. The number of samples represents the number of flasks measured for methane stable-isotope analysis. The last column lists the number of samples collected at each site – concentration measurements and measurements of stable isotopes have not been carried out for every sample.



Figure 9 – Map of the East Siberian Arctic Shelf with sampling locations of water (triangles), sediment (rhombuses) samples. An overview of the locations sampled is provided in table 2.

3.1.1. Water samples

Niskin bottle samples were taken from surface, bottom and intermediate layers. Gas was extracted from the water with helium using the headspace method (Johnson *et al.*, 1990). A subset of extracted gas samples was stored in glass flasks for subsequent analysis. Brine was added to inhibit microbial activity prior to sealing with silicon stoppers and tin lids.

3.1.2. Sediment samples

A gravity corer was used to collect the surface sediment (<3m) samples in hotspot areas during a research cruise in the summer of 2012. At the Buor-Khaya Bay background site, a 53 meter sediment core was drilled from a temporary winter base on fast sea ice in April 2011. The core was obtained in segments of 4 meters with a hydraulic drilling rig operated without drilling fluid so as not to contaminate the samples. Because drilling took place below ±12m of sea ice and water column, well tubes and borehole casing were used to avoid sea water infiltration. In all sediment cores samples were taken from different depths below the seafloor; a depth profile of the isotopic composition of methane and concentration will help identify layers of anaerobic oxidation that could affect ascending gaseous methane. For the deep core dissolved gasses - CH_4 , CO_2 , and O_2 - were measured using the headspace method (Johnson *et al.*, 1990). Gasses were stored with brine in sealed glass flasks for further analysis (Semiletov *et al.*, 2011 and Sergienko *et al.*, 2012).

3.2. Continuous Flow-Isotope Ratio Mass Spectrometry system (CF-IRMS)

The isotopic composition of methane was measured with a continuous flow isotope ratio mass spectrometry system at the Institute for Marine and Atmospheric Research (IMAU) in Utrecht, The Netherlands. High precision measurements of δD and $\delta^{13}C$ were obtained by sample preconcentration and subsequent pyrolysis to H₂ or combustion to CO₂ for measurement of either stable isotope, before entering the mass spectrometer. The system is described in detail in Brass & Röckmann, 2010 and Sapart *et al.*, 2011.



Figure 10 - Schematic overview of the methane extraction system (after Brass & Röckmann, 2010). **1.** A sample enters the system via either inlet through the sample loop. **2.** Methane is extracted and focussed in the preconcentration and cryofocus units. **3.** The sample is then pyrolyzed/ combusted to H_2/CO_2 and focussed further before **(4)** entering the gas bench (NAFION and open split) and finally the IRMS.

3.2.1. Measurement procedure

The experimental setup is schematically shown in figure 10. A small subsample is extracted from the sealed flask with a 0.5 mL gas syringe (VICI Pressure-Lok[®]) and enters the system through manual injection in an airtight inlet sealed with a butyl septum. The sample is mixed with the carrier gas helium in a 40mL sample loop, which is evacuated with a vacuum pump prior to injection. The mixture is then transferred via a Valco two-position valve (V1) to the preconcentration unit, where CH₄ is separated from the bulk air. Different components of the gas are trapped in a column of HayeSep D by regulating its temperature: heating the column's stainless steel casing with an electrical current and pumping cold nitrogen gas from a liquid nitrogen (LN2) dewar through a stainless steel spiral coiled around the column. The column is first cooled down to -130°C, flushing

out N₂ and O₂ while trace components including CH₄ remain trapped. Subsequent warming to -85°C releases methane but retains CO₂, H₂O and other condensable gasses on the column. The separation process takes about 4-6 minutes. Without additional focussing the signal amplitude would be too small for isotope ratio measurements. Therefore the CH₄ is cryofocussed in a GC column (Poraplot Q), cooled by evaporation of liquid nitrogen drops in a chamber surrounding the column. Here, methane accumulates before release by first cooling to -156°C and then heating to +50°C, resulting in a narrower IRMS peak (see figure 12). Two Valco two-position valves (V2, V3) direct the flow through the preconcentration and cryofocus units.

In order to measure carbon and hydrogen isotopes CH₄ is converted to either CO₂ or H₂. For ¹³C analysis CH₄ is combusted at 900°C to CO₂+H₂O in an alumina tube containing oxidized Ni-wires that provide a stable source of oxygen. Adding a small amount of oxygen to each sample before it enters the oven restores the oxygen content of the wires. For δ D analysis, methane is pyrolized to elemental carbon + H₂ in a silica tube at 1300°C. The system does not permit simultaneous δ D and δ ¹³C measurements. In case of pyrolysis, further focussing is realised with an additional LN2-cooled GC column (PoraPlotQ). In the δ ¹³C setup, after combustion the sample is led through a GC column to mitigate Kr-interference (PoraPlotQ, operated at 24°C, Schmitt *et al.*, 2013). Any H₂O is removed with a NAFION gas dryer with helium as the dry purge gas. The prepared sample enters the IRMS (ThermoFinnigan MAT Delta^{plus} XL) through an open split interface using a ThermoFinnigan GasBench II unit interface. A run takes approximately 23 minutes after which the system is ready for a new injection.

In addition to manually injected samples, Groningen reference air is measured frequently for system quality control and data correction (see sections 3.2.2 and 3.2.3). Samples of reference air from a pressurized cylinder enter the sample loop through an inlet designed for automated measurements, after which sample preconcentration continues as described above.

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3.2.2. Software and data analysis

The IRMS measurements are analysed with the ISODAT software package. The output signal essentially consists of continuous concentration measurements of the heavy and light isotopes, resulting in a distinctive peak as the ionized sample hits the detector. Integration of the peak areas gives the isotope concentration ratio R_{SAMPLE} , which is then converted to a δ -value referenced to an internal standard. The internal standard is pure H_2 or CO_2 that serves as the working gas for δD and $\delta^{13}C$ measurements respectively. Because the isotopic composition of the working gas is known, the difference between the real and measured peak integrals is used by ISODAT to correct R_{SAMPLE} . A 40mL sample of Groningen (GRO) reference air is measured with a known δ -value ($\delta_{GRO} = R_{GRO}/R_{STANDARD} - 1$) relative to the international standard ($R_{STANDARD}$: δD : V-SMOW, δ^{13} C: V-PDB) in order to calculate $R_{SAMPLE}/R_{STANDARD}$. First, R_{SAMPLE}/R_{GRO} is calculated from the measured sample value δ_{SAMPLE} , eliminating the working gas internal standard R_{WORK} :

$$\frac{R_{SAMPLE}}{R_{GRO}} = \frac{\frac{R_{SAMPLE}}{R_{WORK}}}{\frac{R_{GRO}}{R_{WORK}}} = \frac{(\delta_{SAMPLE} + 1)}{(\delta_{GRO} + 1)}$$

Because we know the ratio of R_{GRO} to the international standard R_{STD} , we can now derive $R_{SAMPLE}/R_{STANDARD}$ and derive the δD -value we are looking for (in the case $R_{STANDARD}=R_{SMOW}$):

$$\delta D \left[vs \ SMOW \right] = \left(\frac{R_{SAMPLE}}{R_{SMOW}} - 1 \right) = \left(\frac{R_{GRO}}{R_{SMOW}} \times \frac{R_{SAMPLE}}{R_{GRO}} - 1 \right)$$

3.2.3. Data quality assessment – system reproducibility

Thorough system evaluations are required in order to assure data quality, particularly after intensive maintenance. A measure of system stability is the reproducibility of measurements, defined as the standard deviation of a predefined number of samples. Such a "sample package" is measured in a period of continuous (sub)system operation without user interference. Two types of 'sample packages' were used to assess system reproducibility and correct for possible bias:

- The first assessment involves only the IRMS. During each run five measurements are carried out of the MS working gas injected directly into the IRMS via the reference open split unit. A sample package consists of five working gas measurements.
- 2. The stability of the integral system, including the preconcentration setup, can be assessed with repeated δ_{GRO} -measurements, taken after every four injections. A series of fully automated reference measurements was carried out after system inactivity or maintenance and as part of routine system checks. A sample package consists of 10-30 δ_{GRO} measurements carried out within a 12 hour period of continuous system operation.

Samples were measured at least twice. The reproducibility of manually injected samples was evaluated by calculating the standard deviation of each sample.

A reproducibility study was done for all δD sample measurements conducted over a three-month period. The results are shown in figure 11. The standard deviation for 90% of reference sample sets (the integral system) is <2.3‰ and for the IRMS only it is <1.5‰. 90% of manually injected sample sets have a standard deviation below 5.2‰. The average standard deviation of reference sample sets (e.g. the average of standard deviations of the δ_{GRO} measurements in each sample package) is 1.5‰ and for the IRMS it is 0.9‰. The sample average standard deviation is 3.0‰.



Figure 11 - Cumulative (left) and regular (right) reproducibility distributions for the IRMS (working gas, red) only, and combined with the sample preparation system in fully automated (GRO reference air, blue) and manual injection mode (samples, green).



Figure 12 – The ISODAT software interface after a typical measurement

A significant source of error is system non-linearity (Brass & Röckmann, 2010); the δ -values depend on the amount of sample injected. Non-linear fractionation can result from sample preconcentration, ion chemistry in the ion source of the IRMS or a mathematical artefact in the data evaluation algorithm. It is of particular importance here, as high CH₄ concentrations make precise manual injection a challenging task. Non-linear effects can be identified by stepwise diluting the amount of sample – either by decreasing filling time for the reference gas into the sample loop or by manually injecting different amounts of a sample. Linearity tests have been carried out regularly after system changes and periods of inactivity, the results of which are plotted in figure 13.



Figure 13 - System non-linearity as shown by 15 linearity-tests done during the measuring period for δD measurements of methane. On the vertical axis the deviation from the IRMS internal standard is shown, with on the horizontal axis the corresponding peak areas. Symbols represent sampling date. Colours represent sampling mode: red: manual injection – blue: automated reference gas measurements. A significant error of –50 to +30 per mill is associated with peak areas of 1 to 30. The orange logarithmic regression line represents the best fit through the ensemble of linearity tests (all data points).

When correcting for non-linearity one has to assume a reference peak size. A peak size of 10 was chosen because it corresponds for our system to the ideal injection volume for a reference gas in terms of reproducibility and non-linearity:

$$\delta_{CORR} = \delta_{MEAS} - [f(P_M) + f(P_I)]$$

where *f* is the fit function, δ_{CORR} is the corrected value for δ_{MEAS} from ISODAT, P_M and P_I are the measured and ideal peak areas respectively. If we assume a linear correlation this function becomes:

$$\delta_{CORR} = \delta_{MEAS} - (a \times P_M + b) + (a \times P_I + b) = \delta_{MEAS} + a(P_I - P_M)$$

where a and b are the slope and intercept of the linear regression line.

Linear regression analysis was carried out for each individual linearity test. A consistent linear correlation (P_M =7.6±3.1, a=2.5±0.7) was found for peak areas >2; lower concentrations have a bias towards lower δ -values relative to the nonlinearity-corrected δ -values, in addition to a larger standard deviation. No samples had to be excluded as a result of these outcomes, as the smallest measured peak area is >3. The effect of non-linearity weakens for larger peak areas. This has been demonstrated with high-concentration sample linearity tests (P_M =18.5±5.4, a=1.05).

The linearity tests suggest that a non-linear relation could be used in order to increase the accuracy of non-linearity corrections. Indeed, a logarithmic regression shows good ($R^2>0.9$) correlation for all individual linearity sets as well as the ensemble of linearity tests ($R^2=0.91$).

The ensemble logarithmic relation has been used to correct all δ D-measurements for non-linearity. Compared to the uncertainty associated with a normally functioning system the effect of nonlinearity is significant: the average sample peak area is 10.2 with a standard deviation of 5.7, which reflect the range of peak areas that were measured (1-32). It should be noted that δ^{13} C was measured earlier and measurements were corrected with a linear regression function as well.

3.3. Concentration measurements

The CH₄ concentration in all samples was measured on-site with a MicroTech 8160 gas chromatograph with helium as the carrier gas. Concentrations were calculated using the Bunsen solubility coefficient (Wiesenburg & Guinasso, 1979) with the appropriate salinity and equilibration temperature. To assure sample integrity during transport we measured concentrations again, using IRMS peak areas of our samples and of a reference sample with a known concentration. We used reference air (GRO) with a CH₄ concentration of 1.8 ppm. For the summer 2012 water samples, methane concentrations were not measured in-situ, but were instead derived from the methane peak areas using the following equation:

$$C_{sample} = n_{CH4,sample} / n_{gas,sample} = \left[\frac{P_{inj}V_{inj}}{RT}\right] / \left[\frac{n_{CH4,GR0}P_{sample}}{P_{GR0}}\right]$$

where *C* is the calculated methane concentration, *n* represents amount of gas in moles, P_{inj} and V_{inj} the pressure and volume of the injected gas respectively, *R* is the gas constant and *T* the ambient temperature. We make two approximations: (1) there is a linear relationship between the peak area P and amount of methane gas n_{CH4} entering the system, and (2) an injection of 40 mL Groningen reference gas with a methane concentration of 2 ppm results in a peak P_{GRO} of 10 in ISODAT.

4. Results

4.1. Isotopic composition



Figure 14 - Isotopic composition of the ESAS methane samples - δD and $\delta^{13}C$ in per mill versus their international standard ratios. Areas that correspond to known pathways of CH₄ production are indicated by the shaded regions (Whiticar, 1999): **carbonate reduction, methyl-type fermentation** and **thermogenic methane**. Symbols represent reservoir type: water column (triangles) and sediment (rhombuses). Colours represent sampling location: hotspot locations in the Lena Delta, **Dmitri Laptev Strait** and the **Shelf Edge** and **background samples - collected across the ESAS (water)** and in the **southern Buor-Khaya Bay (deep sediment core)**. Open and closed symbols represent samples collected during winter expeditions in 2007 and 2011, and cruises in summer 2011 and 2012, respectively. Note that samples were taken at different depths below the water and sediment surface (see figures 16 and 17).

In figure 14, the water column and sediment isotopic composition is displayed in a double isotope ratio plot. Overall, methane in the sediment samples is depleted with respect to methanogenesis from biogenic and thermogenic sources, with the important exception of two enriched BKB samples closest to the seafloor and three samples from the Lena Delta cores. The most enriched samples are found in the water column: at the shelf edge, followed by the Lena Delta in winter, where methane is more enriched in D than even thermogenic methane. The most depleted samples are found in the sediment: in the Buor-Khaya Bay background deep core and Dmitri Laptev Strait hotspot cores.

In the Lena Delta and DM Laptev Strait, water samples are enriched in both isotopes compared to sediment samples collected at approximately the same location. In the Dmitri Laptev Strait there may be a linear relation between δD and $\delta^{13}C$. Water samples from the shelf edge show no clear relation between isotopes. Though the shelf edge is a known source of thermogenic methane (Cramer & Franke, 2005), only few samples fall directly within its signature range (δD =-190±90‰ and $\delta^{13}C$ =-35±15‰, Whiticar, 1999).

4.2. Methane concentrations at hotspot and background locations

In Shakhova *et al.* (2010a), a clear distinction is made between hotspot and background locations on the basis of the methane concentration in the water column. Here we repeat the analysis for both water column and sediment samples In figure 15 we have plotted all samples after increasing methane concentration, and labelled them according to the hotspot and background areas identified by Shakhova *et al.* (2010a) e.g. all samples collected in the Lena Delta are classified as "hotspot". We then split the population in "background" and "hotspot" samples by fitting two exponential regression lines - each with a different slope – based on the highest correlation coefficient.



Figure 15 - Concentrations of methane in ESAS sediment and water samples, sorted after increasing concentration. Concentrations were measured in ppm with the headspace method and converted to nM using the Bunsen solubility coefficient (Wiesenburg & Guinasso (1979). The colours represent the location where the samples were taken: orange for the **hotspot areas** on the shelf edge, the eastern Lena Delta and the Dmitri Laptev Strait – purple for the **background samples** taken from the Buor-Khaya Bay and across the North Siberian coast.

The majority of sediment samples has a much higher methane concentration than the water samples. All values measured are above atmospheric equilibrium (2-5 nM) but below exsolution concentrations (1-3×10⁶ nM). These values were calculated using the Bunsen solubility coefficient of methane (Wiesenburg & Guinasso, 1979) with the range of temperatures and salinities described for the Laptev sea (Semiletov *et al.*, 2013).

For the water samples, we analysed regression line slopes to distinguish between a background and a hotspot subset, analogue to Shakhova *et al.* (2010a). A clear division can be observed between hotspots and background in water samples between 57 and 109 nM. These concentrations are lower than the 156 nM hotspot threshold concentration in Shakhova *et al.* (2010a). The highest concentrations are found near the shelf edge and show an even steeper slope. Background concentrations were measured mostly outside the Laptev Sea area during a 2011 cruise that covered the entire Northern Siberian coastline.

For the sediment samples the statistical method used by Shakhova *et al.* does not result in a clear hotspot-background separation, despite the low water CH₄ concentrations (20 nM) measured at the BKB background site (Shakhova *et al.*, 2010b). Several samples of the 'hotspot" regions actually show lower concentrations than many of the "background" samples. The highest methane concentrations were measured in a shallow sediment core obtained from the western Lena Delta. Two samples - surface samples of the 53m BKB "background" core - have an extremely low concentration and do not fit the sediment regression line. These samples are considered as outliers e.g. a result from a measurement error or processes significantly different than those affecting the rest of the samples. For convenience we will maintain the hotspot and background classification based on observed ebullition: the Buor-Khaya Bay remains a background site and the Lena Delta and Dmitri Laptev Strait will be classified as hotspots.

4.3. Sediment depth profiles

Figure 16 shows the variation of methane concentration and isotopic composition (δD and $\delta^{13}C$) in ESAS sediment with depth. The average sediment methane concentration is above atmospheric equilibrium levels (3-4 nM) and the water background concentration (±20 nM). Methane concentrations decline rapidly towards the seafloor at all locations. The shallowest samples are located <50 cm (hotspots) and <150 cm (background) below the seafloor. At hotspots, sediment surface concentrations reach minimum values of 6-45 μ M, well above the background water concentrations (20 nM). Methane concentrations increase with depth. Concentration maxima are situated at 1-2.5m depth in the Lena Delta and Dmitri Laptev Strait and at 6m depth in the Buor-Khaya Bay. As expected, high concentrations of 20-100 μ M are found here, but notably also at the **BKB** background site. Concentration maxima are visible at all locations except in subsets LD5 and **DLS3**, where they are not resolved presumably because of a limited sampling depth range (i.e. no samples were taken above or below the maximum zone).



Figure 16 - CH₄ concentration in nM and isotopic composition (δ D and δ ¹³C in per mill) from sediment cores. Colours represent data from four gravity cores collected at hotspot locations in the **Lena Delta** (LD4, LD5) and **Dmitri Laptev Strait** (DLS1, DLS3), and the **deep core at the Buor-Khaya Bay** (BKB) **background site**. Open and closed symbols represent samples collected in winter 2011 and summer 2012 expeditions respectively. The concentrations are measured from sediment pore water using the headspace method and converted to units of nM/µM with solubility coefficients given by Wiesenburg & Guinasso (1979). We assume the headspace pressure was kept at 1 atm. during concentration measurements and the same volume of sediment was extracted for each sample.

In contrast with the concentration profiles the depth profiles for δD and $\delta^{13}C$ show significant variability in the top meters. In the **BKB** and **LD5** samples both δD and $\delta^{13}C$ increase towards the seafloor. In the Dmitri Laptev Strait (**DSL1**) there is depletion in heavy isotopes towards the seafloor. The **LD4** profile shows sharp enrichment in D and ¹³C at 50cm depth, above which methane is becoming more depleted in heavy isotopes. At the **BKB** background site there is enrichment in both heavy isotopes towards the seafloor between 24 and 40 meters.

At the depth of maximum methane concentration for each site the δD values of all samples converge towards $-275\pm25\%$, below which samples are enriched (hotspots) or remain unchanged (background) as concentrations decrease. For $\delta^{13}C$ no such convergence is visible; instead the samples from hotspots are more enriched than the samples from the background location in the Buor-Khaya Bay.

4.4. Water depth profiles

Figure 17 shows methane concentration and isotopic composition at hotspot sites in the Lena Delta (winter and summer), Dmitri Laptev Strait and the Laptev Sea Shelf Edge and from background sites across the ESAS sampled in summer.





The highest concentrations of water column methane were measured near the shelf edge in the Northern Laptev Sea, followed by concentrations in the Lena Delta and DM Laptev Strait. Unlike the sediment samples, no clear depth profile of methane concentration or isotopic composition was observed at these hotspot locations. Water column methane concentrations observed at the shelf edge in summer span four orders of magnitude, representing both the highest ($[CH_4]=21.7 \mu M$) and lowest ($[CH_4]=0.02 \mu M$) concentrations found at any hotspot site. For the inner shelf the highest values were observed in winter 2007 from below the fast ice in the Lena Delta ($[CH_4]=0.4-3.1 \mu M$), and intermediate values in the same area in summer. The lowest water column methane concentrations were measured at background sites east of the Laptev Sea ($[CH_4]=0.006-0.056 \mu M$).

Water samples collected in summer 2012 and winter 2007 at approximately the same location in the Lena Delta reveal a limited seasonal footprint: methane concentrations are generally much higher in winter than in summer because of accumulation below the fast ice. The majority of winter samples appear enriched in heavy isotopes with respect to the summer samples – though the comparison is based on a limited number of samples and is representative of only one hotspot location.

At the shelf edge, an exceptional enrichment in deuterium up to +401±48‰ was measured towards the surface, corresponding to a marked decrease in concentration of 253 to 15 nM. Corresponding high δ^{13} C values of -50 to -40‰ were comparable to earlier measurements from the Laptev Sea shelf edge (Cramer & Franke, 2005), but show no clear relation with depth.

In the winter Lena delta, the two locations where the highest CH₄ concentrations were measured (turquoise open triangles in figure 17) show an isotopic depletion towards the surface, whereas lower concentration samples show an enrichment (blue open triangles in figure 17) or no trend. In summer, inner shelf profiles show no concentration or isotopic signature relation with depth.

4.4.1. Distance from the coast

To test the possibility of methanogenesis from particulate organic carbon from eroding Yedoma coasts in summer, for each summer water sample the distance from the coast was determined using ESRI ArcGIS geoprocessing software. The variation of methane concentration and isotopic composition (δ D) with distance from the coast are plotted in figure 18. In the case of the DM Laptev Strait, the northern coast (of Great Lyakhovsky Island) was used, as coastal erosion is more prevalent there than at the southern coast (Vonk *et al.*, 2012).



Figure 18 - Methane concentration and isotopic composition (δ D) of methane versus distance from the coast for water samples from ESAS hotspot- and background locations. Colours represent sampling locations: inner shelf hotspots (Lena Delta and Dmitri Laptev Strait) and background sites across the ESAS. An exponential regression line is fitted through all but one (orange circle) of the hotspot concentration samples ($R^2 = 0.78$, displayed in figure). Error bars are smaller than symbol sizes.

A significant correlation (R²=0.78, P<0.005) was found between methane concentration and distance from the coast at the inner shelf hotspots for all (n=12) but one sample (orange circle in figure 18). This sample was collected closest (4.5 km) to the coast in the Dmitri Laptev Strait. No clear correlation was found for the deuterium isotopic composition or the samples taken outside the hotspot area while for the carbon isotopes a too small number of measurements was made to make a comparison.

4.5. Isotopic composition and concentration

Figure 19 shows the concentration versus the isotopic composition of methane in the sediment and water column in the Lena Delta, Dmitri Laptev Strait and the Laptev Sea shelf edge. Concentrations (in nM) vary over several orders of magnitude in the samples analysed and the isotopic composition covers almost the entire spectrum of known isotopic signatures (figure 14).



Figure 19 - Concentration (in nM) and isotopic composition (δ D and δ ¹³C in per mill versus their international standards) of the ESAS methane samples. Symbols represent reservoir type: water column (triangles) and sediment (rhombuses). Colours represent sampling location: hotspot locations in the Lena Delta, Dmitri Laptev Strait and the Shelf Edge and background samples - collected across the ESAS (water) and in the southern Buor-Khaya Bay (deep sediment core). Open and closed symbols represent samples collected during winter expeditions in 2007 and 2011, and cruises in summer 2011 and 2012, respectively. Note that samples were taken at different depths below the water and sediment surface (see figures 16 and 17). No δ ¹³C measurements were done for the ESAS background water samples.

Sediment methane concentrations are generally higher than those in the water column. Sediment methane concentrations of 0.5-50 μ M were measured at both Lena Delta and Dmitri Laptev Strait hotspots and in the southern Buor-Khaya Bay - perceived as a background site. Furthermore, there is significant variability amongst hotspot sites, as the highest concentrations measured in the Lena Delta sediments hold more than twice the amount of methane found in the Dmitri Laptev Strait.

The isotope data show strong variations, with δD values varying between -340 and +400‰ and $\delta^{13}C$ between -110 and -40‰. Inner shelf water column samples cover an isotopic range of -48< δD <-227‰ and -85< $\delta^{13}C$ <-51‰ and are enriched relative to the sediment samples, while exceptionally enriched samples were observed in the shelf edge water column. The most depleted samples were found in the DM Laptev Strait (δD) and Buor-Khaya Bay ($\delta^{13}C$) sediments. For δD more than for $\delta^{13}C$, there is a clear enrichment in heavy isotopes towards lower concentrations, though only for δD there appears to be a clear correlation between concentration and isotopic composition.

4.6. Hypotheses

Our results indicate that methane production and removal processes may influence the isotopic signature of methane at all measured locations in the ESAS. There could be different explanations for the isotopic signature distribution of methane in water and sediment: a **removal** process (oxidation), **production** from modern or Pleistocene carbon, **transport** from a reservoir, or a **mixture** of the three.

In the deep sediment core from the Buor-Khaya Bay background site we expect a depleted signature as a result of slow, microbial methanogenesis from buried Pleistocene carbon – migration pathways for hydrate or thermogenic methane should be absent here. The strongly depleted deuterium signature could be the result of mixing between hydrogenotrophic and acetoclastic methane, or a hydrogen precursor effect. A strong decrease in methane concentration and heavy isotope enrichment in the top 6 meters of the core may be the result of anaerobic oxidation.

At hotspots we expect to find a biogenic or a thermogenic source originating from methane hydrates or gas pockets (Shakhova *et al.*, 2010a). Increase in methane concentrations closer to the coast suggests methanogenesis from terrestrial organic carbon may take place in the sediment surface and water column. In an oxygenated marine environment such as the Laptev Sea however, this can only be a minor source, because it is more likely that organic material is metabolized to CO_2 (Alling *et al.*, 2012, Semiletov *et al.*, 2013). An alternative hypothesis is that less methane is oxidized closer to the coast because acetate – preferred by sulfate reducing bacteria over methane (Borowski *et al.*, 1996) – is likely to be more abundant where carbon-rich meltwater streams enter the ocean.

Significant oxidation is not expected in the sediment at hotspots, because gas migration pathways are likely to destroy the sulfate reduction zone (Wellsbury & Parkes, 2000). However, a decrease in methane concentrations is observed in all cores and a strong enrichment in heavy isotopes in two. If methane oxidation indeed takes place in our hotspot sediment cores, this means we may not have sampled a gas migration channel and should be careful in our interpretation.

In the water column in summer we observe an enrichment in both isotopes compared to the sediment, presumably because of bacterial oxidation of methane or mixing with a thermogenic sediment source. Our winter water samples were collected in the Lena freshwater plume below the ice, and we may expect bacterial removal as observed in ice-covered Siberian thaw lakes (Boereboom *et al.*, 2012).

We will use three isotope plotting techniques outlined in the introduction to test these hypotheses.

5. Discussion

The previous section describes methane concentration, isotopic composition and spatial variability in the ESAS. To draw meaningful conclusions from our data about processes in the sediment and water column that affect the methane budget, we utilize the three isotopic mixing and distillation models described in the introduction.

5.1. Sources of methane

The dataset presented in the previous chapter provides a unique opportunity to qualitatively identify methane sources in the ESAS sediment and water column. The conventional way to distinguish between the different methane precursors is with a Keeling plot; if the isotope changes are due to production from an unknown source, the intercept of the linear regression lines is equal to the isotope signature of the sources. However, our dataset could violate its two assumptions (Pataki *et al.,* 2003):

- There is simple mixing between only two components: the source and the background
- The isotope ratio of these two components does not change over the observation period

The first assumption effectively requires a closed system, but in the previous section we hypothesized that methane oxidation could be an important removal process in both the sediment and water column. Furthermore, the samples were collected over several years in an environment that likely hosts more than two sources: carbonate reduction, acetate fermentation and a thermogenic source (Cramer and Franke, 2005). In the sections below we will determine for every sample subset whether the Keeling plot can be used to determine the source signature.

5.1.1. Sources of methane in the sediment

In order to determine the sediment source the Keeling plot cannot be used: at hotspots in the Lena Delta and Dmitri Laptev Strait the system is not closed because methane is leaving the sediment reservoir via ebullition. In the Buor-Khaya Bay background site there is no ebullition, but a strong decrease in methane concentration towards the surface is likely the result of anaerobic methane oxidation in the sulfate reduction zone (see section 5.2). Sergienko *et al.* (2012), who carried out a seismic acoustic survey at the drilling location, hypothesized that methane accumulates below a capping layer of marine clays. A layer of high reflectivity was observed at the lithological boundary between Holocene silty-clay sediments and the underlying Pleistocene sands and siltstones, 6 meters below the seafloor. The authors interpreted this layer as a "high gas concentration in sandy interlayers". The lowest methane concentrations at this site can be explained by anaerobic methane oxidation (figure 21). Presumably, methane produced in Pleistocene sediment diffuses slowly through the Holocene layer, where it is partially oxidized. This explains why no ebullition takes place here and why the Buor-Khaya bay is a background site. At hotspots the clay layer may be thinner and

perhaps more permeable, but measurements are scarce: one drilling expedition north of the Dmitri Laptev Strait estimated the thickness of the marine clay layer to be 1-10 meters (Kostyukevich, 1993). It is reasonable to assume here that the isotopic composition of the methane at the depth of the concentration maximum reflects the dominant source(s) in each core.

Table 3 shows the isotopic composition of the methane samples with highest and lowest concentrations in each sample subset. For each subset a maximum methane concentration was determined, above and below which concentrations decreases. At hotspots the depth of maximum concentration was 150±50 cm and in the BKB deep core it can be found at 500-700 cm depth (figure 13). We took an averages of the isotopic composition (D and ¹³C) of samples in this region in order to calculate the isotopic signature of the bulk of the methane in each sample subset.

Sampling location	Concentration	δD at conc.	δD at conc.	δ^{13} C at conc.	δ^{13} C at conc.	
	range (µM)	minimum	maximum	minimum	maximum	
Background/BKB	0.024-29.814	-43.0 ±2.7	-263.7 ±9.2	-69.9±0.1	-100.8 ±2.8	
Hotspot/LD4	0.575-26.871	-124.4 ±0.6	-247.5 ±2.5	-73.0±0.1	-88.3 ±2.2	
Hotspot/LD5	4.517-150.012	-131.4 ±0.9	-267.2 ±1.4	-78.5±0.1	-85.9 ±1.4	
Hotspot/DLS1	0.571-25.450	-322.1 ±0.1	-281.8 ±15.4	-99.9±0.1	-94.1 ±2.2	
Hotspot/DLS3	10.240-20.821	-255.6 ±1.4	-272.6 ±0.1	-77.7±0.1	-79.7 ±0.1	

Table 3 –Characteristic methane concentration and isotopic composition (D and ¹³C) of methane in sediment samples from the Buor-Khaya Bay background site (**BKB**) and four cores drilled at hotspots in the Lena Delta (**LD4**, **LD5**) and Dmitri Laptev Strait (**DLS1**, **DLS3**). The depth of maximum concentration is defined here as a zone of 200 cm in a core above which methane concentrations decrease an order of magnitude from the core maximum concentration. The concentration minima are represented by the sample closest to the seafloor in each core. The error bars represent the standard deviation over all measurements included in the average. The standard deviations are 2 to 10 times larger than measurement errors.

The lowest concentration of methane was measured in the marine clay layer close to the seafloor. This layer may contain high quantities of terrestrial carbon (2-3 wt.% OC, Vonk *et al.*, 2012, Winterfeld *et al.*, 2011) due to erosion of the carbon-rich cliffs and subsequent deposition of particulate organic carbon on the seafloor (Karlsson *et al.*, 2011, Vonk *et al.*, 2012). When meltwater and seawater mix in the Lena estuary, (dissolved) organic carbon compounds coagulate to more biolabile forms before precipitating with sediment to the seafloor (Sholkovitz, 1976, Charkin *et al.*, 2011). This terrestrial OC could be a methane source under anoxic conditions (Bussmann, 2013). Our measurements however do not indicate a strong biogenic source in the marine clay layer: a strong decrease in methane concentration towards the seafloor (figure 16) suggests a removal, rather than a production process, and in three cores (**BKB**, **LD4** and **LD5**) the shallow-sample isotopic composition (table 3) is too enriched in D for a biogenic source ($\delta D \le -150\%$, Whiticar, 1999).

Background sediment sources: Figure 20 shows the isotopic composition (δ^{13} C, δ D) of our sediment samples in a double isotope plot. In the Buor-Khaya Bay sediment, the ¹³C signature of the bulk of methane gas falls within the range of CO₂ reduction (δ^{13} C=–90±30‰, Whiticar, 1999). The deuterium signature however is up to 100 per mill lower than the known range for this source (δ D=–200±50‰, Whiticar, 1999), presumably because the methanogens – when the permafrost thawed – used the depleted permafrost meltwater as a hydrogen source. Measured meltwater signatures of δ D_{H20}=–135±25‰ (Alaskan thermokarst lakes, Chanton *et al.*, 2006) and δ D_{H20}=–220±30‰ (Siberian Pleistocene permafrost, Brosius *et al.*, 2012) explain the difference satisfactorily (the 'precursor effect' was discussed earlier in section 1.5.3). Arctic seawater (δ D_{H20}=–20‰, Friedman *et al.*, 1964), infiltrating in the marine sediment from above, is too enriched to explain the deuterium signature, and our 53 meter core was entirely unfrozen due to high concentrations of salt (Shakhova *et al.*, 2013). This implies that the bulk of methane in our core been produced in-situ with a mixture of fresh- and seawater or has accumulated from deeper (>53m) thawed sediment layers where fresh meltwater is still abundant.



Figure 20 – The isotopic composition (δ^{13} C, δ D) of methane from sediment cores in the Laptev Sea. Symbol colours represent drilling locations: the **Buor-Khaya Bay background site** and hotspots in the **Lena Delta** and **Dmitri Laptev Strait**. Open and closed symbols represent samples collected in winter (2011) and summer (2012) respectively. Coloured areas show the signature of biogenic formation pathways: carbonate reduction (blue) and methyl-type fermentation (green) (Whiticar, 1999). The dotted line shows an example of the hydrogen precursor effect for hydrogenotrophic methane: the depleted signature results from methanogens using depleted meltwater (δ D=–135‰, Chanton *et al.*, 2006).

The presence of seawater also offers an explanation for the absence of acetoclastic methane, despite an abundance of organic material: with the seawater sulfate enters the marine sediment (Henrichs & Reeburgh, 1987), providing sulfate reducing bacteria with the electron acceptor they need to outcompete methanogens for acetate (Lessner, 2009). CO₂ remains as the only non-competitive substrate for methanogens. Carbon dioxide measurements from the same core further support the isotopic evidence of a strong carbonate reduction source; high CO₂-concentrations ($\pm 100 \mu$ M) follow the profile of methane concentrations ($\pm 6 \mu$ M) (Shakhova *et al.*, in preparation). A similar methaneto-substrate ratio was found in incubation experiments of Pleistocene permafrost soil by Knoblauch *et al.* (2013), which resulted in production of dissolved inorganic carbon (66-94%) and methane (6-33%). Our data shows that degradation of organic carbon in thawing subsea permafrost is an important source of methane in the ESAS background sediment. However, no measurements of acetate, sulfate or H₂ were available to the author, so a more detailed analysis is beyond the scope of this thesis.

An alternative explanation for the depleted deuterium signature is mixing of two biogenic production pathways – methyl-type fermentation and CO_2 reduction. Whereas methanogenesis from acetate may be prevented by competition from sulfate-reducers, non-competitive substrates like methanol or methylated amines might be readily available. Koch *et al.* (2008) studied methanogenesis in a subsea permafrost core in the Eastern Laptev Sea, where a zone of maximum methane concentration (284 nmol g⁻¹) was observed in an unfrozen layer 52-62 meter below the seafloor, together with a maximum in TOC content (8.7% wt.) and high sulfate concentrations. DNA-analysis revealed a community of cold and sulfate-tolerant methanogenic archaea facilitating in-situ methanogenesis. Still, the relatively low concentration means that production rates for methyl-type fermentation are likely very low in the presence of sulfate (Ferdelman *et al.*, 1997), and cannot explain much higher methane concentrations (29814 nM, and even higher in units of nmol g⁻¹) in our background sediment samples.

Hotspot sediment sources: In the Lena Delta and Dmitri Laptev Strait – where methane is actively bubbling from the seafloor – the bulk of methane has an isotopic composition between that of hydrogenotrophic and acetoclastic methane. In general, methane at hotspots is 5-25‰ more enriched in ¹³C than at the Buor-Khaya Bay background site (table 3). Like in the Buor-Khaya Bay, the methane depleted in deuterium and has a signature close to that of acetoclastic methane (δD =-335±40‰, Whiticar (1999)).

Similar to the Buor-Khaya Bay background site the depleted deuterium content in our hotspot samples could be the result of carbonate reduction in combination with a depleted hydrogen source.

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It is likely that at hotspot locations seawater has infiltrated the sediment as well. From this assumption it follows that a mixture of methyl-type fermentation and carbonate reduction production pathways is unlikely, albeit measurements of sulfate or salinity at these sites would be necessary to confirm this.

Hydrogenotrophic methane could be produced in-situ as seawater percolates downwards in the sediment, but production rates may be too slow - due to low temperatures or competition for H₂ by sulfate reducers (Senior *et al.*, 1982) - to explain the observed bubbling. Instead, methane could originate from hydrate reservoirs which are destabilized by the lowering permafrost table or warming by a geothermal heat source from below (Shakhova *et al.*, 2010b). Compared to marine hydrates, methane in our sediment samples is depleted in deuterium (Milkov, 2005). However, in the ESAS we do not expect marine hydrate formation because the shallow water column does not provide high enough pressure. Shallow hydrates are hypothesized to have formed in terrestrial permafrost before the Holocene inundation (Kvenvolden, 1988), and the enclosed methane gas may have been produced with depleted hydrogen. This process would explain the depleted deuterium content in our samples. It should be noted that the existence of shallow hydrates in the ESAS and their isotopic composition remain to be established.

The enrichment in ¹³C relative to the background site could be the result of mixture with a thermogenic (hydrate) source (Milkov, 2005), a methanogenic community with a different carbon kinetic isotope effect or anaerobic methane oxidation. Oxidation in the zone of maximum methane concentration is unlikely however, and an isotopic fractionation of ε_{c} =–2-22‰ and ε_{D} =–98-153‰ (Whiticar & Faber, 1986, Holler *et al.*, 2009) from the BKB background signature (δ^{13} C=–101±3‰, δ D=–264±9‰) would lead to the observed values for ¹³C but not for D.

5.1.2. Sources of methane in the water column

For the water column samples, isotope mixing plots can be applied on selected subsets only. Deriving the source signature is complex, because the most prominent source - methane bubbling from the sediment – has a wide range of isotopic signatures. Furthermore, the assumption of a closed system may not be applicable everywhere. Methane escapes the water column via diffusion and ebullition in summer and outgassing may take place in winter via open leads in the sea-ice cover (Kort *et al.*, 2012). In addition, methanotrophic archaea may remove methane from the water column. In this section we plot our water column isotope data in the Keeling and Miller-Tans plots to test their applicability in our study area.



Figure 21 - Keeling (top) and Miller-Tans plots (bottom) of the ESAS water column methane samples for D (left panels) and ¹³C (right panels). Colours represent sampling locations: hotspots in the Lena Delta, Laptev Sea (inners shelf), Shelf Edge and background sites across the ESAS. Open and closed symbols represent samples collected during winter (2007) and summer (2011 and 2012) respectively. Secondary axes (top and right) apply to the Lena Delta samples. The equations and correlation coefficients of the geometric mean regression lines are displayed in the figure; colours match those of each subset. The intercepts (Keeling) and slopes (Miller-Tans) represent the source signatures (see section 1.41). Signature standard deviations are omitted for figure legibility and are provided in table 4. Error bars are smaller than symbol sizes.

In figure 21, the water column samples from the Lena Delta (summer and winter), Dmitri Laptev Strait and the shelf edge are plotted in Keeling- and Miller-Tans plots. Table 4 lists the source signatures derived with both methods. The highest correlation coefficients were obtained for samples from the shelf edge (δD =-170±47‰, $\delta^{13}C$ =-46±5‰) and the Lena Delta in winter (δD =-239±32‰, $\delta^{13}C$ =-71±6‰). Both the inner shelf hotspot and ESAS background summer samples have no clear signature: the Keeling plot analysis shows that the isotopic signature and methane concentration are not well correlated. At these locations the isotope signature could not be derived for $\delta^{13}C$ due a low number (≤4) of measurements. For further analysis we will instead use the average isotopic composition of methane at inner shelf hotspots (δD =-208±17‰, $\delta^{13}C$ =-72±9‰) and ESAS background samples (δD =-154±31).

Location	δD	R ²	δ ¹³ C	R ²	δD	R ²	δ ¹³ C	R ²
Shelf edge	-170±47	0.68	-46±5	0.52	-170±1	0.99	-47±1	0.99
Lena Delta (winter)	-239±32	0.67	-71±6	0.31	-232±9	0.98	-71±2	0.99
Inner shelf	-268±88	0.04	х	х	-182±12	0.96	x	x
Background/ESAS	-209±38	0.26	х	х	-200±29	0.87	x	x

Table 4 - Source signatures (δD and $\delta^{13}C$ in per mill vs their respective international standards) of methane from ESAS waters with their standard deviation and correlation coefficient – Keeling (left) and Miller-Tans method (right, italic). Standard deviations are equal to the standard error of the intercept (Keeling) and slope (Miller-Tans).

The most enriched signature – measured at the shelf edge – is close to that of thermogenic methane $(\delta D=-190\pm90\%, \delta^{13}C=-35\pm15\%, Whiticar, 1999)$, and confirms the observations of Cramer and Franke (2005) who measured a methane carbon isotopic composition of $-40\pm1\%$ in the same region. The Lena Delta and inner shelf methane isotopic composition is much closer to that of carbonate reduction ($\delta D=-200\pm50\%, \delta^{13}C=-90\pm30\%$, Whiticar, 1999). An acetoclastic signature ($\delta D=-335\pm40\%, \delta^{13}C=-60\pm25\%$, Whiticar, 1999) was not observed in any of the water samples.

5.1.2.1. Evaluation of the isotopic mixing models

Isotopic mixing models are used frequently for atmospheric samples, where the fit between model and data is good (e.g. Fisher *et al.*, 2011 and Kayler *et al.*, 2010). Our water column samples show a marked difference between the source signatures predicted by each model as well as the errors associated with the estimate. Where the Miller-Tans plot shows a near perfect fit (R²>0.98) between data and model, in the Keeling plot the correlation coefficients do not exceed 0.7. This is a consequence of the linear transformation in the Miller-Tans plot, which forces the points onto a line. The artificial good fit makes the residuals small and the standard slope error low. For our dataset the Keeling plot may be more applicable, because the intercept standard error better reflects the variety of processes that may affect the isotope signature, such as non-perfect mixing (Cramer & Franke, 2005) or a removal process.

5.1.2.2. Carbon sources of water column methane

These results show first and foremost that we are in presence of a highly heterogeneous environment where methane originates from a mixture of sources. Production processes cannot explain the enriched signature of methane in the shelf edge and Lena Delta (winter) samples. It is likely that some of the water samples are affected by removal processes, which are discussed in section 5.3. Here we will consider two potential carbon sources:

- Methanogenesis from terrestrial carbon supplied by the Lena River and coastal erosion.
 - Production in the water column from dissolved organic compounds
 - Production in the seafloor sediment from particulate organic carbon
- A Pleistocene sediment source: organic carbon degradation or hydrate dissociation

Water column production hypothesis: In the Lena Delta during winter, the methane deuterium isotopic signature (-239±32‰) is more depleted than in summer (-208±17‰). Competition of sulfate reducers for acetate is unlikely: elevated methane concentrations were found exclusively in the Lena freshwater plume (figures in Shakhova et al. (2010b) and Semiletov et al. (2013)). These observations suggest contribution from riverine production from buoyant or dissolved organic material. Raymond et al. (2007) measured dissolved organic carbon concentrations in the Lena River - 850km upstream of the Arctic Ocean - throughout the year. Though the river discharge varies orders of magnitude with season, there is only a doubling of DOC concentrations from (6.7 to 14.8 mg/L) during the spring freshet, while high Δ^{14} C (+90±25‰) indicates modern carbon reaches the river throughout the year. The reason only modern DOC is found is because DOC is highly biolabile and degrades rapidly to CO₂ and CH₄ in meltwater streams (Vonk et al., 2013). High concentrations of methane (±200 nM) were observed in the Lena headwaters in summer (Semiletov et al., 2011). A winter ice cover - as well as providing low-oxygen conditions (Cauwet & Sidorov, 1996) - prevents diffusion to the atmosphere, allowing for methane production and accumulation in the river and its estuary. However, the isotope signature of our samples is not exclusively acetoclastic and in the sampled area strong ebullition indicates a sediment, rather than a water column source (Shakhova et al., 2013): riverine carbon may not be more than a minor source in the ESAS methane budget.

In summer, methane leaves the Lena River through diffusion (Bussmann, 2013) and concentrations to drop to background levels once the river reaches the Laptev Sea (Semiletov *et al.*, 2011). Storms mix marine and fresh waters (Shakhova *et al.*, 2013), and oxygen or sulfate reducing bacteria inhibit water column methanogenesis from terrestrial organic carbon. Instead, decomposition of terrestrial carbon is the most important source of carbon dioxide in the ESAS water column (Alling *et al.*, 2012).

Marine sediment production hypothesis: terrestrial OC that is buried in anoxic sediment can act as a methane source under anoxic conditions (Walter *et al.*, 2006). Our measurements indicate that the concentration of methane generally increases towards the coast (figure 18), in accordance with the distribution of Yedoma-derived organic carbon in ESAS sediment – particulate OC is transported by meltwater streams and settling close to the coast (Vonk *et al.*, 2012). Coagulation of dissolved organic carbon in particulate OC and subsequent deposition in marine sediment could be another source of biolabile carbon, albeit the significance of coagulation processes has not been quantified in the Arctic Ocean (Sholkovitz, 1976, Charkin *et al.*, 2011). Up to 75% of carbon in the Laptev seafloor is of terrestrial origin (Vonk *et al.*, 2012). In water column and (hotspot) sediment isotope data we find no evidence of a strong acetoclastic sediment source. Some methane may be produced in anoxic marine sediment, but sulfate reducing bacteria are likely to limit production rates (Senior *et al.*, 1982, Ferdelman *et al.*, 1997), albeit no samples were collected from the topmost sediment layer that would help estimate the significance of this process.

Pleistocene sediment hypothesis: observations of ebullition at hotspots suggests a strong sediment source. Gas migration pathways through marine clays have been observed at hotspots north of the Lena Delta using seismic imaging (Shakhova *et al.*, 2010b) and indicate that bubbles originate from the zone of maximum methane concentration. However, dissolved methane in the water column is enriched in both isotopes compared to the methane produced in (Pleistocene) sediment, especially near the shelf edge. To connect this isotopically light sediment source with enriched values observed in the water column, it would require enrichment in the water column or sediment surface. There could be mixing with a thermogenic source (Cramer & Franke, 2005). Furthermore, bubbles transporting CO₂-derived methane from the zone of maximum concentration to the atmosphere may only release a small amount of methane in the shallow ESAS water column, depending on bubble size, release intensity and rise velocity (Leifer & Patro, 2002): gas migration pathways could facilitate mixing between depleted methane in the deep sediment and enriched methane in the water column. To establish the significance of removal pathways, we will look at isotopic fractionation associated with methane oxidation in the next section.

The isotopic variability of methane in the ESAS water column is likely caused by the mixing of several sources with different signatures (e.g. CO₂-derived from sub-sea permafrost, acetate-derived from terrestrial OC and thermogenic from the deep sediment).

5.2. Removal processes

Removal processes may explain the enrichment and decrease in concentration we observe in sediment cores from the Lena Delta and Buor-Khaya-Bay background location (figure 16), as well as the generally heavy isotope signature of methane in the water column compared to the sediment. Methane removal processes observed in oxygen-saturated waters are aerobic methane oxidation and outgassing to the atmosphere (Buor-Khaya Bay, Bussmann, 2013). Methane removal processes in the sediment are limited to anaerobic oxidation and diffusion to the water column.

Here we use a Rayleigh plot to discern different removal processes. First, the methane isotope ratios and concentrations of different subsets were plotted in a linear transformation of the Rayleigh plot:

$$\ln(R/R_0) = \varepsilon \ln(C/C_0)$$

where R and C indicate the measured methane isotope ratio and isotopic composition respectively, and the subscript indicates their initial values. We assume here that the initial concentration C_0 is equal to the maximum concentration in the sample subset, and take the corresponding isotope ratio as R_0 . The slope of the linear regression line, $\varepsilon = 1/KIE-1$. was found using a geometric mean regression, from which the kinetic isotope effect (KIE) was easily devised.

The results of this analysis are displayed in regular Rayleigh fractionation plots in figures 22 and 23. We plotted the isotope ratio (δ D and δ^{13} C in per mill) against the residual concentration (C/C₀) of each sample subset. The KIE-values and correlation coefficients from the linear regression analysis are listed in each plot. We used our isotope data to plot three idealized Rayleigh models (i.e. nonreplenished depletion of a reservoir by a single removal process):

$$(R/R_0) = (C/C_0)^{\varepsilon}$$

initialized with identical R_0 and C_0 but with different fractionation factors. Here we show fractionation due to methane oxidation by methanotrophic archaea. The oxidation uncertainty envelope bordered by dotted lines are given by the KIE minima and maxima for marine environments (table 1): KIE_{D,MIN}=1.100 (Martens *et al.*, 1999), KIE_{D,MAX}=1.221 (Kessler *et al.*, 2006), KIE_{C,MIN}=1.008 (Alperin *et al.*, 1988), KIE_{C,MAX}=1.024 (Reeburgh *et al.*, 2006). Outgassing carries negligible carbon isotope effect (KIE_C=1.0008, Prinzhofer *et al.*, 1997) and is not shown in our plots. The solid lines represent idealized fractionation with sample KIE's displayed in each plot.

5.2.1. Removal processes in the water column

In figure 22 the water column methane remaining fraction and isotopic composition are plotted in Rayleigh plots together with idealized models of oxidation. The pink solid line shows theoretical mixing between the highest- and lowest concentration samples.



Figure 22 – Rayleigh fractionation plots of water samples from the Lena Delta in winter (left), inner shelf in summer (middle) and shelf edge in summer (right). Open and closed symbols represent samples collected during winter (2007) and summer (2011 and 2012) respectively. Solid and dotted lines represent idealized Rayleigh fractionation models of the different removal processes. R² refers to data linearity in the linear Rayleigh model described in the method section.

From figure 22 it is evident that isotopic composition of our water samples cannot be explained by removal processes alone. Overall, KIE-values for water column methane are lower than those for aerobic and anaerobic oxidation found in the literature (table 1). This is consistent with observations; at all hotspot locations: isotopically light methane from the sediment continuously enters the water column.

In the Lena Delta (left panel), methane accumulates below the ice cover, and accumulation rates exceed removal rates. Mixing with a depleted source directly below the ice could explain why only the deep and enriched samples fall within the KIE-boundaries of methanotrophic oxidation. Despite the absence of a clear oxidation pattern in the Rayleigh plots however, it is likely that oxidation takes place in the entire water column in winter, because no known process or source (e.g. thermogenic methane, $\delta D \le -100\%$, Whiticar, 1999), can otherwise explain the high δD -values at this location.

In the samples collected in the **inner shelf** and **shelf edge** water column in summer (center and right panel), neither oxidation or mixing between two water masses (pink line) can explain the observed isotopic composition. This heterogeneity may be the result of sampling of different water masses (Cramer & Franke, 2005), each containing a mixture of (partially oxidized) thermogenic and biogenic methane. This is no surprise, as thorough mixing of the water column as a result of storms is common in the ESAS (Shakhova *et al.*, 2013). In that case, the Rayleigh plot may not be the appropriate tool to identify removal processes, because methane is added to the system by a sediment source and removed by outgassing, violating the assumption of a closed reservoir.

5.2.2. Removal processes in the sediment

Removal in the sediment may be observed as an enrichment toward lower concentrations in the sediment depth profiles (Borowski *et al.*, 1996). In figure 23 we have plotted residual methane and isotopic composition of samples from the top 6 meters of the Buor-Khaya Bay core (background site) and from both Lena Delta gravity cores (hotspot) in a Rayleigh plot. In the Dmitri Laptev Strait a concentration decrease was observed without isotopic enrichment or depletion (figure 16). The mixing curves in figure 23 were calculated using the highest and lowest methane concentrations and their respective isotopic composition in each surface sediment subset (see section 1.6.3.4). We used two deep samples in the hotspot subset to investigate mixing with a minor enriched source at depth.



Figure 23 - Rayleigh fractionation plots of sediment samples from the Buor-Khaya Bay background site (left), and Lena Delta hotspot (right). Open and closed symbols represent samples collected during winter 2011 and summer 2012 expeditions respectively. Solid and dotted lines represent idealized Rayleigh fractionation models of the different removal processes. "Surface sediment" refer to the 2-3 shallowest samples that have significantly lower methane concentrations than the next deeper sample. "Deep sediment" refers to all samples at depths greater than the "surface sediment" samples. R² refers to data linearity in the linear Rayleigh model described in the method section.

In the **Buor-Khaya Bay** background site only the two sediment surface samples appear to be subject to oxidation. We find little enrichment of methane towards lower concentrations and samples do not follow the oxidation line (dotted lines in figure 23). It is likely that while methane is oxidized by a consortium of methanotrophic archaea and sulfate reducing bacteria in the marine sediment, methane from zone of maximum concentration is continuously supplied from below. This would violate the closed-system assumption of the Rayleigh plot.

In the **Lena Delta** sediment, we find a similar pattern: surface samples are depleted in heavy isotopes with respect to the ideal Rayleigh fractionation curve for oxidation. Presumably isotopically light methane is supplied continuously from the sediment below. Because of the similarities in the hotspot and background Rayleigh plots, especially the low concentration of methane in the sediment surface, it is likely that the Lena Delta core does not represent an actual ebullition spot. A unique feature at this location is that beneath the zone of maximum methane concentration there is strong concentration decrease associated with a depletion in ¹³C and an enrichment in D (figure 16). The pink theoretical mixing line indicates mixing between a large reservoir (δD =–267‰, $\delta^{13}C$ =–86‰, table 3) of CO₂-derived methane (produced with D-depleted meltwater in the deep sediment) and a smaller reservoir (δD =–148‰, $\delta^{13}C$ =–92‰), which could be bacterial carbonate reduction with H₂ from Arctic seawater (δD =–20‰, Friedman *et al.*, 1964). Low production rates of methane from carbon dioxide in the presence of sulfate have been observed in salt marshes in the United Kingdom (Senior *et al.*, 1962).

Oxidation was not observed in the **Dmitri Laptev Strait**, where the highest concentrations of methane were observed and a distinct isotope depth profile is absent – despite sampling very near the seafloor. The lack of a clear oxidation profile could relate to vigorous bubbling of methane at these locations: ebullition can destroy the sulfate reduction zone where methane oxidation normally takes place (Wellsbury & Parkes, 2000). Still, a strong concentrations decrease in the top sediment was observed. This could be explained by bubbling-induced mixing of water and sediment, releasing interstitial methane gas that would otherwise be trapped in the impermeable clays.

Removal could also be limited by introduction of acetate in the seafloor carbon cycle. In the ESAS heavy coastal erosion introduces acetate in the marine environment as a final reaction product of organic carbon decomposition. Once settled in marine sediment layers, acetate provides not only substrate for methanogens, but also for sulfate reducing bacteria, and they prefer energetically favorable acetate to methane-derived H₂ as electron donor (Bethke *et al.*, 2011). Methanotrophs now lack a recipient of the electrons freed by the redox reaction of methane to CO₂ and H₂, and as a result anaerobic oxidation of methane is inhibited. Other AOM-limiting factors are availability of

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sulfate, nitrite or other oxidizing agents (Joye, 2012). This observation and the absence of a clear acetoclastic signature in surface sediments (section 5.1.1) suggests that the decrease of water column methane concentrations with distance from the coast (figure 18) may actually result from inhibited methane oxidation in the marine sediment layer.

6. Conclusion

Methane is an important greenhouse gas. Large quantities of methane have been observed bubbling from the seafloor in the East Siberian Arctic Shelf (ESAS). We measured the concentration and isotopic composition (δ D and δ^{13} C) of methane in samples collected from the sediment and water column of the ESAS during several winter (ice drilling) and summer (cruise) campaigns between 2007 and 2012 using continuous flow isotope ratio mass spectrometry. Depth profiles and three isotopic mixing models were used to identify key formation and removal pathways of methane in the ESAS.

Several methane sources have been proposed for the ESAS, ranging from dissociating methane hydrates, production from Pleistocene carbon and seepage of thermogenic methane. Stable isotope analysis carried out in this thesis provides a first qualitative insight in the relative importance of these pathways in the ESAS methane cycle. Our analysis identified a mixture of formation and removal pathways that appear to greatly influence methane concentrations observed, and can be summarized in three key findings:

- Methane is produced from Pleistocene organic material via the CO₂-reductin pathway in thawing subsea permafrost at background sites, where we do not observe any ebullition. It is likely that thermokarst meltwater is used as a hydrogen source in the production process.
- 2. Methane is removed by oxidation in the capping marine sediment in the seafloor surface at background sites, which shows that very little methane reaches the water column. At hotspots there is no clear oxidation pattern, which supports the hypothesis that ebullition can destroy the sulfate reduction zone.
- An isotopic enrichment from sediment to water column cannot be explained by oxidation alone: methane in ESAS waters is most likely a mixture between thermogenic and biogenic methane, possibly from gas hydrates.

Our dataset indicates that the methane formation and oxidation pathways in the East Siberian Shelf form a complex and dynamic system that should be seen separate from the Arctic carbon cycle.

Future work in the East Siberian Arctic Shelf could further deepen and expand our knowledge by addressing some of the limitations and uncertainties associated with this primary dataset. First, samples collected in different years and at different locations made it difficult to compare sediment and water samples or to resolve seasonal variability. Second, an improved spatial and depth resolution in sediment samples could enable an estimation of the fraction of methane oxidized in the sediment before reaching the water column. Future sampling efforts could focus on all three reservoirs: sediment, water column and atmosphere, taking high resolution vertical profiles at a number of hotspot and background locations in summer and winter. Such an approach would include

an estimate of the relative contribution of methane production and removal pathways to the atmospheric carbon pool. Flux measurements would then help estimate how much methane produced in sediment ends up in the atmosphere.

Isotope research is essential to advance our knowledge further. Stable isotopes analysis of methane precursors – H_2 , CO_2 , methyl-type compounds - could help identify key methanogenic pathways and show whether methane is produced in-situ or transported with subsurface flow (groundwater) from terrestrial permafrost or deeper sediment layers. In addition to stable isotopes, future research could include analysis of $\Delta^{14}C$ of methane. This way, the role of Pleistocene and modern carbon as methane precursors can be better understood.

Methane release from the ESAS is intimately connected to larger geophysical systems – thawing subsea permafrost, coastal erosion, the diminishing sea-ice cover, the carbon cycle – that continue to evolve as global warming progresses. An urgent call now goes out to researchers from many disciplines – microbiologists, atmospheric chemists, permafrost modelers and even a bubble expert – to expand our knowledge.

7. References

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