**Interactions between methane and the nitrogen cycle in light of climate
change**

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Abstract

Next to carbon dioxide, methane is the most important greenhouse gas which predominantly is released from natural wetlands and rice paddies. Climate change predictions indicate enhanced methane emission from global ecosystems under elevated CO₂ and temperature. However, the extent of this positive feedback is far from clear and depends on factors modulating microbial responses of microbes involved in methane cycling in various ecosystems. Nitrogen input by atmospheric deposition or fertilizer additions, is such a factor with a range of possible effects on microbial methane production and consumption. In this paper we discuss the crucial lacks in knowledge preventing a better understanding and predictions of climate change effects on global methane emissions.

Introduction

Climate change, processes, ecosystems

The recent, 5th assessment report of the IPCC (Intergovernmental Panel on Climate Change) [1] states that it is very likely that climate change phenomena observed during the last decades are caused by human action. It is unequivocal that since the 1950s, the atmosphere and the oceans have warmed, the amounts of snow and ice have diminished, sea level has risen and concentrations of greenhouse gasses (GHGs) have increased in a way unprecedented for decades to millennia. The main driving factors determining anthropogenic radiative forcing are emitted greenhouse gases. CO₂, CH₄ and N₂O together comprise more than 80% of the total radiative forcing (i.e. global warming effect) [2] and their current concentrations and rate of increase exceed what has been observed in the past 800.000 and 20.000 years, respectively [2]. While CO₂ is by far the most abundant GHG in the atmosphere (390 ppm; not taking H₂O into account), CH₄ (1.804 ppm) and N₂O (0.324 ppm) have a much higher

warming potential which shifted research efforts and possible mitigation strategies towards these non-CO₂ GHGs[3].

Methane, contributing 17% to radiative forcing is increasing again in concentration since 2007 after a period of stabilization, which is currently being attributed to climate-induced changes in methane emissions from natural wetlands [2]. Although methane can be formed thermogenically, the largest part of methane emitted to the atmosphere is produced microbiologically (see next section) in anoxic habitats like wetland soils and sediments, lakes, the digestive tract of ruminants and insects and in anthropogenically created habitats like landfills and other waste systems (see Figure 1). The single largest source of atmospheric methane is global wetlands, including rice cultivation, where anoxic conditions and the availability of large amounts of plant-derived carbon promote microbial methane formation which can escape through plant roots and stems into the atmosphere [4]. Global sinks for methane are microbiological consumption (see next section) and chemical degradation by OH radicals in the troposphere and stratosphere (see Figure 1).

[Insert Figure 1]

Anthropogenic nitrogen input by cultivation of N₂-fixing crops and via reactive nitrogen produced via the Haber-Bosch process, has more than doubled the N-input into global ecosystems [5] with associated detrimental effects on atmospheric chemistry and eutrophication of global soils, sediments and water bodies.

Nitrogen being one of the crucial elements of life is intimately linked with the carbon and other elemental cycles by modulating plant, animal and microbial activities. Assessing climate change effects and feedbacks through general circulation models (GCM) indicate that interactions between carbon and nutrient cycles will be determinative for the nature of these feedbacks and that more detailed knowledge on these interactions is required to make better

predictions [2,5,6]. One of these areas to be explored is the interactions between methane and nitrogen cycling, which are strongly modulated by microbial processes and by the biotic and abiotic environment (see next section and Figure 2) [4]. To complicate matters, recently new processes and organisms were discovered, which adds to the complexity of methane-nitrogen interactions [7]. This paper will focus on the methane-nitrogen interactions as influenced by the most important climate change drivers (see Figure 2), confined to the most important ecosystems in terms of global microbial-mediated methane emission (Figure 2). We will focus on microbial interactions and possible consequences of climate change for methane emission, including possible avenues leading to the highly needed mechanistic information.

[Insert Figure 2]

Organisms and Interactions

Methane production

The production of methane is carried out by microbes belonging to the phylum *Euryarchaeota* within the domain of the *Archaea*. The phylogeny, physiology as well as ecology of this group of archaea has been extensively described and for more detailed information the reader is referred to the following excellent reviews [8-10]. Methanogens obtain energy by forming methane from H_2+CO_2 (hydrogenotrophic), from acetate (acetotrophic) or from methanol and methylamines (methylotrophic). These substrates are the end product of organic matter degradation in anoxic ecosystems (e.g. wetlands, sediments, permafrost, landfills), catalyzed by a network of microbes hydrolyzing polymers to monomers which subsequently can be fermented. Methane formation in general is controlled and affected mainly by concentrations of oxygen and alternative electron acceptors (e.g. NO_3^- , NO_2^- , Fe^{3+} , SO_4^{2-}), temperature, and amount and type of organic matter, which in turn are all regulated by physical factors (such as

water table/flooding in wetlands) or other microbes or plants [11,12]. Nitrogen is generally regarded as an inhibitory factor for methanogenesis, either as oxidant (NO_3^- , NO_2^-) for denitrifiers that can outcompete methanogens for substrate, or by the denitrifying intermediates (NO_2^- , N_2O and NO) which are toxic [4,7] (Figure 3). However, methanogens also need nitrogen as nutrient which they either can acquire by fixation of N_2 or by uptake of NH_4^+ or NO_3^- . For the latter two they have to compete with other bacteria (e.g. denitrifiers) and plants (see Figure 3), an interaction largely uninvestigated.

[Insert Figure 3]

Methane consumption

Aerobic microbial methane oxidation is carried out by bacteria predominantly in soils and sediments, either by consuming internally produced methane (e.g. wetlands, lake sediments), or methane diffusing from the air into soils. Processes and organisms involved have been extensively studied and for detailed information we refer the reader to the following reviews (aerobic [7,13-16], anaerobic [9,17-19]). Most aerobic methane-oxidizing bacteria (MOB) utilize methane as energy as well as carbon source, (α and γ -proteobacterial MOB), while methanotrophs belonging to the phylum Verrucomicrobia fix CO_2 [20]. In contrast to MOB in methane producing habitats (“low methane affinity”) there are no cultivated representatives available yet of MOB oxidizing atmospheric methane (“high affinity”) diffusing into aerobic soils. Growth on atmospheric methane has only been associated to the uncultivated lineages $\text{USC}\alpha$ and $\text{USC}\gamma$, of which the ecology and physiology is still unclear. It has been recently shown that not all members of both low and high affinity methane oxidizers are obligatory methanotrophic and can grow on or assimilate small non- C_1 compounds like acetate [21-24]. Methane oxidation is controlled by a wide range of environmental factors (methane, oxygen,

moisture, pH, nitrogen, temperature, grazing, deforestation, plants, agricultural practice etc.).

The links between methane consumption and nitrogen cycling are strong (see Figure 3).

When nitrogen is limiting methanotrophic activity, many but not all methanotrophs are capable of fixing atmospheric nitrogen [25,26]. The extent of methanotrophic N-fixation in the environment and its influence on the N-cycle is unclear. However, transcripts of MOB-related *nifH* sequences have been detected in a range of natural environments [27], which implies that methanotrophic N-fixation is common in natural habitats, probably restricted by their differential sensitivity of the nitrogenase enzyme for oxygen (see [28]) .

Nitrogenous fertilization has various effects on methane oxidation (inhibition and stimulation, both long-term and short-term) of which the mechanisms are far from clear [4,7]. Next to this MOB, ammonia oxidizing bacteria (AOB) and archaea (AOA) have similar enzymes in their primary oxidant metabolism allowing them to oxidize both ammonium as well as methane [13]. Interactions between MOB, AOB, and especially AOA and the role of nitrogen fixation and competition between other bacteria, plants and MOB for nitrogen and the consequences for methane oxidation is largely uninvestigated [7]. Very recently, a novel link in methane-nitrogen cycle interactions has been discovered in the form of well-known MOB that are capable of denitrification to N₂ [28]. Anaerobic methane oxidation is predominantly occurring in marine systems (see section “oceans”), first detected for a syntrophic consortium of archaea (ANME) and sulfate reducing bacteria, performing reverse methanogenesis coupled to hydrogen removal by sulfate reduction (see [9,17]). A recent discovery showed that in principle, specific ANME are capable of performing anaerobic methane oxidation without syntrophic partner, by reducing sulfate to zero-valent sulfur [29] or by using nitrate as electron acceptor [30], the latter again directly linking methane and nitrogen cycling. In addition, methane oxidation with nitrite as electron has been shown to occur widely in freshwater habitats [19] and is carried out by bacteria belonging to the so-called NC10

lineage. The only described culture ca. *Methylobacterium oxyfera* produces its own oxygen by reduction of nitrite to NO and subsequent oxygen dismutation [31]. The oxygen is used for oxidizing methane similar to aerobic MOB [16]. Regarding the regulatory effect of nitrogen on methane fluxes that are mediated by anaerobic methane oxidizers, or their interactions with other organisms in relation to nitrogen, nothing is known yet.

Climate change and interactions between CH₄ and N cycle

Wetlands

Carbon derived from root exudation and dead roots is the source of substrate for methanogenic archaea as well as denitrifiers, while oxygen, released by roots of wetland plants, is the electron acceptor for MOB, AOB and AOA (see Figure 4). Hence, plants are inherently linked to climate change effects on methane emission from wetlands. A number of recent meta-analyses and reviews all support the conclusion formulated by the IPCC, that climate change (elevated CO₂ and temperature) enhances methane emission from wetlands, natural as well as rice paddies [2,32-34], which can substantially offset the assumed moderating effects wetlands can have on global warming potential by enhanced carbon sequestration [32]. The extent to which methane emission will increase is far from certain as was concluded from an inter-comparison of present-day used process models estimating and predicting wetland methane emission [33]. Among the large uncertainties are the extend of increase or decrease of wetland areas, the feedback of anthropogenic nitrogen input, influence of plant species on methane transfer to the atmosphere, influence of soil type and the type of response (linear or otherwise) of the microbial processes to major climate change drivers. Hence, parameterization of many of the locally influenced variables is necessary. In addition, it is highly needed to assess the combined effect of climate change with other anthropogenic

factors [33] to determine the extent of positive or negative feedbacks on methane emission from wetlands.

Atmospheric N-deposition has been shown to increase methane emission from natural wetlands, in conjunction with elevated CO₂ and temperature [35]. Similarly, N-addition enhanced methane emission in combination with elevated CO₂ in a natural marshland invaded by non-native reed [36]. In contrast, in rice paddies where nitrogenous fertilizer application generally increases methane emissions [37], enhanced methane emission due to elevated CO₂ was substantially mitigated by N-addition [38]. A large part of the uncertainty in the effect of N on methane emission can probably be related to varying plant responses, which depend on species, environmental conditions, and on type and amount of fertilizer in agricultural wetlands. The latter gives rise to direct and indirect effects on substrate availability of microbes [4]. It is obvious that the amount of carbon accessible for methane formation is dependent on the N-supply to the plants; many effects of N-fertilization on methanogenesis have been linked to increased C-availability [4,7]. However, what is largely uninvestigated is the role of N as nutrient for methanogenic archaea. There is evidence that methanogens in wetlands can be N-limited (see [4,7]) with consequences for methane emission. However, nothing is known about N-fixation vs. assimilation and on competitive abilities of methanogens in natural environments. As reports on the unexpected oxygen tolerance of some methanogenic species show [39], novel traits of environmental importance may be found, also with respect to N-regulation which may play an important role in the expected methane release from warming permafrost wetlands [40,41]. The large amount of available carbon, in combination with climate change drivers and associated vegetational changes (i.e. mosses to vascular plants) may also here lead to N-limited methanogens. Methane emission from wetlands emerging from permafrost will therefore depend strongly on the characteristics of the methanogenic communities present, as is the case in other wetlands [42].

The majority of the methane produced internally in wetlands (40-60%) is oxidized by MOB before it can escape into the atmosphere through roots and stems of wetland plants [4]. This methane filter function, as was shown for rice soils (see [4]) depends on the availability of mineral nitrogen which is essential for methane oxidation. In fact, the generally observed and predicted increase in methane emission from wetlands under elevated CO₂ and temperature may have significantly been influenced by N-limitation of MOB. Increased plant growth under climate change in combination with higher heterotrophic respiration in the rhizosphere of wetland plants will constrain N-availability for MOB even more. The effect of N-limitation on methane oxidation also depends on the community composition, since MOB subgroups differ in their N-requirements and N₂ fixing abilities (see [4,7,25-27]). However, to what extent the energy-costly nitrogen fixation affects environmental methane oxidation and how proteobacterial MOB can compete and interact with AOB, AOA the recently discovered Verrucomicrobial MOB [20] and the anaerobic methane oxidizers [19,30], too little information is available to speculate on climate change effects.

[Insert Figure 4]

Soils

Non-wetland soils (forest, grassland, agricultural) can be a source as well as a sink for atmospheric methane which strongly depends on a suite of soil-physical parameters (moisture, pH, organic carbon, nutrients etc.) which are directly or indirectly influenced by plants [4]. Recent meta-analyses and reviews on methane emission from soils have revealed many inconsistencies concerning the effect of climate change [10,32,34]. Observed general trends are increased methane emission from upland soils under elevated CO₂ and increased atmospheric uptake due to elevated temperature [32,34], although there are differences between biomes. Increased carbon input by plants (higher methane formation) and dryer soils

in combination with increasing atmospheric methane concentrations (higher diffusion and microbial oxidation) have been assumed to be the acting mechanisms. Confounding factors like precipitation as well as N-deposition and fertilization clearly affect the result of climate drivers as shown for semi-arid grasslands [43] and tropical forest [44], where methane oxidation appeared to be limited by moisture and nitrogen, respectively. Nitrogen clearly interacts with methane consumption in soils displaying atmospheric methane uptake in a dose dependent way, with stimulating effects in applications/deposition below 100kg N.ha⁻¹.y⁻¹ [45]. The inhibitory effects of fertilizer application are well described and discussed (see Figure 3) [4,7]. However, under which conditions atmospheric methane oxidizers will be N-limited, is unknown mainly due to lack of cultivated microbes performing this reaction. From environmental studies, however, it is clear that soil methane uptake (see Figure 3) is clearly dependent on the diversity of these uncultured MOB [46,47]. Combined with recent findings of assimilation of other carbon sources (i.e. acetate) by atmospheric methane oxidizers [22], it is obvious that their traits have to be known and taken into account in any predictions of climate change effects. Growth on acetate may explain the observed positive effects of N-addition on atmospheric consumption, where N would function as nutrient. [44,45]. An alternative explanation comes from the recent discovery that some cultivated proteobacterial MOB (i.e. *Methylocystis* species) are capable of atmospheric methane consumption by expressing high affinity enzymes, which are regulated by the environmental nitrogen concentration [48]. This opens up the possibility that in periods of elevated methane (e.g. when soil moisture increases) these MOB grow, reaching higher cell numbers of potential atmospheric methane consumers. The “flush” feeding strategy has been proposed to predominate in hydromorphic soils with fluctuating internal methane availability [49]. In these soils a complex situation arises with methane coming from above and below enabling both high- and low-affinity MOB to be active. Interaction with nitrogen will largely be as

described for wetlands. Climate-change-induced events, however, affecting moisture (extreme weather events) and plant growth in combination with N-deposition yields a very complicated set of interactions which also makes predictions of methane emissions/uptake in hydromorphic soils uncertain [50]. Recently it was demonstrated that methane consumption in floodplains soils is significantly correlated with the relative abundance a very limited number of MOB species [51], which were also the actively consuming MOB *in situ*, giving rise to possibilities for parameterization of models.

Lakes

Natural and man-made lakes cover less than 1% of the earth's surface, but are considered to be major contributors to methane fluxes to the atmosphere (92 Tg CH₄ yr⁻¹ according to a recent estimation in which also man-made lakes are taken into account [52]). Deep lakes display thermal stratification resulting in methane dynamics distinctly different from shallow lakes and wetlands. During summer stratification in temperate lakes, and the all-year stratification in tropical lakes, the epilimnion (i.e. the upper, warmer water layer) becomes depleted in nutrients, whereas the hypolimnion (i.e. the lower, colder layer) becomes depleted in oxygen, leading to methane production in the anoxic sediments (see [53]) which can be attenuated under anoxic conditions by methanotrophic members of the NC10 phylum, which couple denitrification to methane oxidation [31] and have been detected in top-layers of sediments in deep-water sites [54,55]. While the lake is stratified, methane that escapes the NC10 bacteria accumulates in the hypolimnion and can only be oxidized by aerobic MOB. However, in the epilimnion MOB face nitrogen limitation due to uptake by phytoplankton and have to fix N₂ to meet their N demands. Although many methanotrophs are capable of N fixation (see [7]), it has rarely been shown that N₂ is fixed *in situ*. However, the in lakes often dominating MOB [56] typically occur where low oxygen conditions prevail [57,58], which gives a strong clue that the MOB are actively fixing N, as the oxygen sensitive nitrogenases

are not inhibited. The oxycline MOB prevent high epilimnetic methane concentrations, even for lakes with high methane concentrations in the hypolimnion [59]. However, methane can also be produced in fully oxygenated surface water of lakes and oceans [58,60], which in some cases can only be explained by *in situ* microbial methane production. Methanogenesis in surface layers may occur in anoxic micro-niches (e.g., in particles or the guts of animals; [60]). In addition, potential methanogenic archaea have been shown to be attached to photoautotrophic cells [58] which can produce H₂ [61] forming a substrate for methanogens. Due to the absence of methanotrophic bacteria in the N-limited epilimnion, this methane source is likely to contribute to the atmospheric budget. Lake methane dynamics change during periods of overturning, when the methane- and ammonium-rich waters from the hypolimnion are mixed with the oxygenated epilimnion waters, resulting in both a sudden increase of methanotrophic activity and higher emission of methane to the atmosphere [62].

Climate change will have a whole repertoire of effects on lakes, which have recently been reviewed by Moss [63]. Dissolved oxygen concentrations will generally decrease due to temperature increase, an increase in the period of stratification, and an increase in floating plant cover at higher temperatures (restricting oxygen diffusion into the water) [see 63]. The effects of climate change are likely to be exacerbated by eutrophication, as the biomass derived from eutrophication-associated phytoplankton blooms causes oxygen depletion in the hypolimnion. In turn, eutrophication is exacerbated by climate change due to increased runoff from catchment areas of lakes by a changed precipitation pattern [64]. It is not clear what the combined effect of climate change and eutrophication will be on methane release from lakes. However, nitrogen as nutrient for both methanogens as well as methanotrophs will be an important modulating factor which has to be addressed in future research.

Oceans

In contrast to the methane flux from lakes, the flux of marine methane to the atmosphere is small considering the large surface of oceans on Earth (estimated at 2% – 10% of the total atmospheric input; for comprehensive reviews on marine methane biogeochemistry see [60,65,66]. The rates of methanogenesis are generally lower in marine than in freshwater sediments due to the high sulfate concentration of seawater. Other sources of marine methane include submarine volcanoes, hydrothermal vents, and cold seeps, where methane of geologic origin is vented into the water column. The dissolution of methane gas hydrates due to global warming can be a potentially large source of methane to the oceans. However, most of the methane from these diverse sources is oxidized in sediments and in the water column, thereby preventing oceans from being a large contributor to the atmospheric methane budget.

Under anoxic conditions in or near sediments, methane can be oxidized by methanotrophic archaea [67,68] during which sulfate (or potentially nitrate or oxidized iron or manganese; [30,31,69]) is reduced. It is estimated that anaerobic oxidation of methane (AOM) can oxidize approximately 80% of seep methane before it enters the water column [60]. As a result of the high methane concentrations at vent and seep sites, microbial communities can become N-limited. Even though the energy-yield of methane oxidation coupled to sulfate reduction is one of the lowest known for microbial metabolism [70] and N-fixation is a very costly process, anaerobic methane oxidizing (ANME) archaea from these habitats have been shown to fix nitrogen [71,72]. However, it is not yet known how much N-fixation by ANME archaea contributes to the input of reactive nitrogen to the oceans [73], which is especially interesting in the light of climate-change-induced hydrate dissolution and subsequent methane oxidation by ANME archaea. In addition, although the process of N-fixation has been shown not to decrease methane oxidation rates for existing ANME archaeal communities [71], N-limitation may limit community growth and thereby the AOM potential to oxidize methane before it reaches the atmosphere.

Marine methane concentrations are lowered even further under oxic conditions. Deep-water methane concentrations are generally under-saturated with respect to the atmosphere. It is not yet known which organisms are responsible for this oxidation to very low methane concentrations (approximately 0.5 nM; [60]); if they are obligate methanotrophs then they should be adapted to chronic energy limitation due to the low methane concentrations and long turn-over times of oceanic deep water [66]. Another possibility for the low methane concentrations is co-metabolism of methane by ammonium oxidizers [66].

Surprisingly, methane concentrations in the fully oxygenated surface mixed layer are generally over-saturated with respect to the atmosphere [60]. The source of this methane has been reason for speculation; methanogenesis in anoxic microniches is a plausible explanation. Recently, two related alternative explanations for the mixed layer methane paradox have been offered, in which methane is produced during microbial degradation of methylated compounds present in the water (Methylphosphonates [74]; DMSP – dimethylsulfoniopropionate [75]). High N-availability will favor the degradation of methylphosphonates to relieve P-limitation [74]. In this way, the presence of nitrogen-fixing organisms in the mixed layer can contribute to oceanic methane emission.

Anthropogenic effects on the biogeochemistry of oceanic methane include eutrophication of coastal areas, increasing temperature leading to a reduction of sea-ice cover (which can prevent methane from diffusing to the atmosphere [76]), and the dissolution of methane hydrates. All of these factors can potentially lead to a higher methane emission to the atmosphere. In general, however, the present-day small contribution of marine methane to the atmospheric budget is not expected to increase significantly due to anthropogenic forcing [1,77]. Even though large amounts of methane can enter the oceans from hydrates and because of increased methane production due to coastal eutrophication, most of this methane is likely to be oxidized in the water column.

Synthesis and outlook

Current predictions on climate-change effects on global methane emission, based on experimental work as well as global circulation and process-based modeling, show consensus on increasing emissions from wetlands, but contradictory results and high uncertainties in soils and aquatic ecosystems. Next to this, the extent to which methane emission will increase is difficult to predict mainly due to interactive effects of local modulating factors and their feedbacks, which are difficult to parameterize in models. The availability of nutrients (N,P) for the microbes catalyzing the processes that collectively lead to the emission of methane from ecosystems is one of the crucial confounding factors. With respect to methane, nitrogen has particular importance given the many modes of action (nutrient, toxicity, inhibition, competition) potentially affecting the functioning and the interaction of microbes involved in methane cycling (archaea, ANME, NC10, MOB (high and low affinity), AOB, AOA). Increasing atmospheric deposition of N as well as fertilizer use affects microbial communities globally, both in terms of abundance [78] as well as in functionality, affecting e.g. the production of extracellular enzymes with increasing N and lowering degradation of recalcitrant carbon [79], which can affect methane formation. These recent meta-analyses elucidating global relationships between microbial communities and soil physical parameters are crucial for modeling and parameterization. However, they do not account for the actual process rates under influence of environmental change and also not for interaction between biota. To understand and deal with non-linear and often idiosyncratic processes, it will be crucial to single out the active species and take their traits into account to obtain deeper mechanistic understanding of climate change effects on ecosystem functions, which is lacking at this moment for methane cycling microbes. The consequences of varying availability of N for methane production, oxidation and subsequent ecosystem uptake or emission, especially regarding strategies dealing with limitation (costly N₂ fixation, competition with other

microbes and plants), are not well understood and need urgent attention. Next to this, persistent efforts of clever culturing combined with genomic techniques has revealed a number of novel organisms and processes of importance for methane cycling in various ecosystems [7,9,30,31]. For these novel organisms the role of methane-N interactions is far from clear, let alone effects of climate change. Methanogenesis under fully oxic conditions in lakes and oceans is maybe the most remarkable example which is not conceptually covered in any model [58,74]. Hence, the way forward in many environmental studies is to indentify the biogeochemically active microbes and assess their traits and characteristics either by cultivation or by applying cultivation independent methods in combination with e.g. stable isotope tracing (see [4,7]). For a group of organisms (ammonia oxidizing bacteria) where available cultures (and associated eco-physiological traits) cover the environmental diversity, a trait-based modeling framework was constructed which successfully predicted a number of functions (i.e. ammonia oxidation, N₂O emission) in published datasets in various environmental gradients [80]. But, on top of species traits, interactions with other organisms will be another important next step in elucidating methane nitrogen interactions. Besides the basic ecophysiological knowledge which we have to obtain, the coupling of traits and environmental responses may be strongly facilitated by the use of large scale genomic and proteomic screenings, where the validation of relationships between genetic and ecophysiological information is an important step [81]. But, in addition to this “back to basics” strategy, the most important research priority will be to up-scale our microbial knowledge (by using replicated, full-factorial-design experiments) to find the causal, mechanistic basis for the interactions between climate change and N in regulating global cycles.

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Figure legends

Figure 1: Sources and sinks of atmospheric methane (in Tg CH₄ yr⁻¹). Adapted from table 6.8 in [2] and ref. EPA 2010. Note that all sources are net fluxes of methane to the atmosphere; methane production in most systems is attenuated by biological methane oxidation.

617

618 **Figure 2:** Schematic representation of the scope of this paper. The most important, climate
619 change associated drivers of microbial interactions in CH₄ and N cycling are temperature,
620 CO₂, precipitation and derived effectors like ocean anoxia and land-use change. The
621 interactions on the microbial level are mediated by (i) toxic effects of anthropogenically
622 added N, and intermediates produced via denitrification on methane formation, (ii) production
623 of oxidants for methane oxidizing bacteria by nitrification or denitrification, (iii) stimulation
624 or limitation by N conversions or uptake of N by e.g. plants, (iv) competition for
625 methanogenic substrates induced by nitrogenous oxidants. These interactions and possible
626 consequences for GHG emission is discussed in context of the most relevant global sources of
627 atmospheric methane.

628

629 **Figure 3:** Schematic overview of interactions between methane and nitrogen cycles and
630 organisms involved. The possible effects of nitrogen on methane consumption are shown in
631 the top half of the figure, effects on methane production in the lower half. Aerobic and
632 anaerobic processes are shown on the left- and right-hand side of the figure, respectively.
633 Methane is formed anaerobically by methanogenic archaea which transform end products of
634 microbial degradation of complex carbon compounds into methane. Acetogenic methanogens
635 (i.e. utilizing acetate) can also obtain substrate directly from plants by root exudates. In the
636 presence of strong oxidants like NO₃⁻ or NO₂⁻ methanogens can be outcompeted by
637 denitrifiers, utilizing e.g. acetate as substrate, thereby effectively suppressing methane
638 formation. Intermediates of denitrification (NO₃⁻, NO₂⁻, NO, N₂O) can also be toxic to
639 methanogens. Aerobically, methane can be formed as the consequence of a chemical reaction
640 in plants or fungi. However, nothing is known yet to what extent these processes are linked to

641 nitrogen cycling. The degradation of methane is predominantly carried under aerobic
642 conditions by methane oxidizing bacteria (MOB), belonging to phyla of α - and γ -
643 proteobacteria and Verrucomicrobia. These organisms have a very wide environmental
644 distribution but thrive especially in methane producing habitats where oxygen and methane
645 availability overlap, which is typically in the surface mm's of sediments (marine as well as
646 freshwater), around oxygen-releasing roots of wetland and aquatic plants, or in landfill covers
647 soils. MOB in these habitats are usually referred to as "low affinity", being active at elevated
648 methane concentrations displaying affinities for methane in the μM range. Atmospheric
649 methane is only consumed in aerobic upland soils (i.e. grasslands, forests) by as yet
650 uncultivated groups of bacteria (high affinity, consuming methane below 1.8 ppmv,
651 displaying affinities for methane in nM range). The best studied lineages, based on
652 environmental DNA assignment, are the so-called upland clusters USC α and USC γ , based on
653 the phylogenetic affiliation of the pmoA (particulate methane monooxygenase) gene to the
654 nearest α - and γ -proteobacterial MOB. USC α seems to predominate forest soils while USC γ
655 seems to be more prevalent in agricultural soils. Both, low- and high-affinity MOB can be
656 inhibited by nitrogenous fertilizers mainly due to competitive inhibition of the key-enzyme
657 (methane monooxygenase) by NH_4^+ . The methane monooxygenase oxidizes both methane
658 and ammonium, which also makes MOB nitrifiers. Also NO_3^- and NO_2^- can be inhibitory to
659 methane oxidation by as yet unclear mechanisms. Nitrogen is also an important nutrient for
660 methane oxidation as in many habitats it has been shown that N-addition can stimulate
661 methane oxidation. Hence, interaction with other organisms also utilizing mineral nitrogen
662 (i.e. competition) is an important regulator of methane oxidation. In this respect, ammonia
663 oxidizers, bacterial (AOB) as well as archaeal (AOA) will interact with methane oxidation
664 because of the mutual requirement for ammonium, oxygen and maybe also methane. AOB
665 and AOA use ammonia-monooxygenase to oxidize ammonia to nitrite, an enzyme which also

can oxidize methane. Interactions between methane oxidation and nitrification in natural habitats have hardly been investigated.

Figure 4: Schematic representation of climate change effects on methane emission in wetlands. The main climate change drivers in wetlands are elevated CO₂, temperature and precipitation and associated hydrology. In general, elevated CO₂ and temperature leads to higher methane emission from wetlands due to higher plant biomass and carbon introduction into wetland soil, fuelling carbon mineralization and the production of methanogenic substrates. However, plants will also consume more nutrients (N,P) which are important regulators of methane production as well as oxidation which can give a negative feedback on methane emission. The effect of climate change on wetland methane emissions and the nature of the feedback will be determined by the interactions between the microbial groups involved (see also Figure 3). The input of nitrogen via atmospheric deposition or via fertilization is an important modulator of climate change effects on wetland methane emission since it both influences plant growth as well as activities of methanogens and methanotrophs. In general, this should give a positive feedback to wetland methane emission, however, the extent to which is very unclear and an important research priority. Precipitation and associated hydrology can decrease or increase wetland areas modulating the climate change effects on methane emission by changing the vegetation and associated effects.