

MiniReview

# Nitrogen as a regulatory factor of methane oxidation in soils and sediments

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## Abstract

The oxidation of methane by methane-oxidising microorganisms is an important link in the global methane budget. Oxic soils are a net sink while wetland soils are a net source of atmospheric methane. It has generally been accepted that the consumption of methane in upland as well as lowland systems is inhibited by nitrogenous fertiliser additions. Hence, mineral nitrogen (i.e. ammonium/nitrate) has conceptually been treated as a component with the potential to enhance emission of methane from soils and sediments to the atmosphere, and results from numerous studies have been interpreted as such. Recently, ammonium-based fertilisation was demonstrated to stimulate methane consumption in rice paddies. Growth and activity of methane-consuming bacteria in microcosms as well as in natural rice paddies was N limited. Analysing the available literature revealed that indications for N limitation of methane consumption have been reported in a variety of lowland soils, upland soils, and sediments. Obviously, depriving methane-oxidising bacteria of a suitable source of N hampers their growth and activity. However, an almost instantaneous link between the presence of mineral nitrogen (i.e. ammonium, nitrate) and methane-oxidising activity, as found in rice soils and culture experiments, requires an alternative explanation. We propose that switching from mineral N assimilation to the fixation of molecular nitrogen may explain this phenomenon. However, there is as yet no experimental evidence for any mechanism of instantaneous stimulation, since most studies have assumed that nitrogenous fertiliser is inhibitory of methane oxidation in soils and have focused only on this aspect. Nitrogen as essential factor on the sink side of the global methane budget has been neglected, leading to erroneous interpretation of methane emission dynamics, especially from wetland environments. The purpose of this minireview is to summarise and balance the data on the regulatory role of nitrogen in the consumption of methane by soils and sediments, and thereby stimulate the scientific community to embark on experiments to close the existing gap in knowledge.

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## 1. Introduction

Despite its low atmospheric mixing ratio ( $1.7 \mu\text{l l}^{-1}$ ) and short atmospheric residence time (about 10 years),  $\text{CH}_4$  is considered as the most potent greenhouse gas after carbon dioxide [1]. This is due to the higher effectiveness (20–30 times) at absorbing long-wave radiation in comparison to  $\text{CO}_2$ , and due to the involvement of  $\text{CH}_4$  in chemical reactions leading to the formation of ozone [2]. Because  $\text{CH}_4$  concentration in the atmosphere has more than

doubled in the post-industrial era, much of the research efforts have been expended to identify sources and sinks of methane, and to estimate their strengths. Methane flux measurements have demonstrated that soils are the most important biological sources and sinks of atmospheric methane (cf. [3]). The balance between the production of methane by methanogenic bacteria under anoxic conditions and the consumption of methane by methanotrophic bacteria under oxic conditions determines whether a particular soil is a net source or a sink of atmospheric methane.

Submerged wetland soils (e.g. swamps, bogs, rice paddies) are regarded as the most important source of atmospheric methane. The contribution of these ecosystems to the annual global methane emission has been estimated at 55% [3]. Non-flooded upland soils (e.g. forests, grassland,

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Abbreviations: MMO, methane monooxygenase; FD, ferredoxin

arable) are regarded as the only biological sink of atmospheric methane and are responsible for 6% of the global methane consumption [3]. In both wetland and upland soils, obligate aerobic methanotrophic bacteria use molecular oxygen to oxidise methane to CO<sub>2</sub> and cell carbon [4]. In wetland soils these bacteria are active in the surface layers and in the rhizosphere of oxygen-releasing plants (cf. [5]), where they substantially reduce the potential amount of emitted methane [5]. Factors that limit or even inhibit the activities of methanotrophic bacteria have major effects on the global methane budget. Since the finding that nitrogenous fertilisation represses methane consumption in forest soils [6], more than a decade of research has focused on elucidating this aspect (cf. [3,7]). However, recently it was found that ammonium-based fertilisation stimulated growth and activity of methane oxidisers in the rhizosphere of rice [8,9]. Moreover, upon depletion of mineral nitrogen in natural rice paddies, methane oxidation decreased to zero (cf. [10]). A restudy of literature revealed a high number of articles already in 1993 and onwards showing the same effect and providing data on the importance of mineral nitrogen for the consumption of methane in soils. However, their implications have never been included in the discussions about global methane budgets. This minireview will summarise the results from these early and from the recent studies, in order to firmly establish that mineral nitrogen can be one of the limiting factors for growth of methanotrophic bacteria in soils and sediments and can even be a prerequisite for methane-oxidising enzyme activity in these ecosystems.

## 2. Nitrogen as an inhibiting factor of methane consumption in soils: 'one side of the coin'

To fully comprehend the potentially inhibitory effects of nitrogenous fertilisers on methane oxidation we have to bear in mind that two types of kinetics have been encountered with respect to the consumption of methane in soils and sediments. The first kinetic pattern of methane oxidation, known as 'low-affinity' methane consumption, is observed in all methane-producing soils (e.g. wetland, peat, landfill) and is carried out by 'conventional' type I and II methanotrophs that display  $K_m$  values in the  $\mu\text{M}$  range.

The second type, known as 'high-affinity' methane oxidation [11] occurs in soils that receive methane only by diffusion from the atmosphere (e.g. forest soil). These soils have methane concentrations in the nM range and display low apparent  $K_m$  values for methane uptake. Since the apparent  $K_m$  values of the monooxygenase enzyme systems of all cultured bacteria able to oxidise methane (methanotrophic bacteria and nitrifiers) are an order of magnitude higher, as yet uncultured organisms with novel variants of methane monooxygenase (MMO) are believed to be responsible for the high-affinity consumption. One study demonstrates that a normal *Methylocystis* strain can

display high-affinity activity under certain conditions, and that the above assumption needs not to be true [12]. However, molecular data in combination with isotope and radiotracer studies have indicated that bacteria responsible for the process of atmospheric methane consumption in many soils are indeed taxonomically novel and only distantly related to the known type II methanotrophic bacteria [13–15].

The first report on inhibition of methane oxidation by nitrogenous fertilisers came from soils displaying high-affinity oxidation. In 1989 Steudler and co-workers [6] conducted a study that elucidated factors controlling biological sinks of methane. Their aim was to assess the causes of increasing atmospheric methane concentrations. One of the factors they investigated was the application of nitrogenous fertilisers to mimic the effect of the increasing atmospheric nitrogen deposition in industrialised countries on methane consumption by soils. Application of NH<sub>4</sub>NO<sub>3</sub> to acid forest soils reduced the uptake of atmospheric methane by these soils for up to 33%. The potential implication of this observation for the global methane budget initiated numerous studies assessing the effects of nitrogenous fertilisers on methane consumption in various soils. For a detailed discussion on the literature addressing this aspect, some excellent papers can be consulted [3,7,16,17]. From the studies reviewed in these papers it is evident that the observation of Steudler and co-workers extended far beyond forest soils. Methane consumption in both upland and wetland soils is affected by nitrogenous input, although this generally holds only for ammonium-based additions. Nitrate has been found inhibitory only in very high concentrations, which likely give rise to osmotic effects. Therefore, in this article we will focus on the effect of ammonium only. In Table 1 the results of 53 studies are presented with regard to the type of habitat, the nature of the inhibitory effect, the proposed mechanisms of inhibition and whether the experiments were performed in situ or in vitro. The diversity of effects, the type of habitats, and the number of proposed underlying mechanisms indicate that no generalisations can be made with respect to the effect of ammonium-based nitrogen input on methane oxidation in different soil or sediment ecosystems. The proposed mechanisms operate at the cellular level, community level and at the level of the ecosystem. Immediate, short-term inhibition in soil or slurry incubations can be explained fairly well by the effects at the cellular level, such as competitive inhibition of MMO by ammonia (cf. [33]). Besides the oxidation of methane, the MMO also has the ability to convert ammonia to nitrite, and ammonia will therefore reduce the amount of methane consumed by methanotrophic bacteria in a concentration-dependent way. The intermediates and end products of methanotrophic ammonia oxidation, i.e. hydroxylamine and nitrite, can be toxic to methanotrophic bacteria and will also lead to inhibition of methane consumption [74]. Finally, the addition of high amounts of ammonium salts to lab-

Table 1

Inhibition patterns of methane oxidation following nitrogen-based fertilisation or atmospheric deposition in various soil/sediment habitats and hypothesised underlying mechanisms

Nature of the effect	Habitat	References reporting on in situ or in vitro inhibition of methane oxidation		Proposed mechanisms of inhibition as reported in literature
		In situ	In vitro	
Short-term	Forest soils (boreal as well as temperate)	[18–20]	[21,18,16,22–26]	Full/partial competitive inhibition of the MMO. Nitrite/hydroxylamine toxicity. Osmotic effects due to salt additions.
	Grassland soils		[27]	
	Arable soils		[28–30]	
	Agricultural soils	[31,32]	[21,32,33]	
	Peat	[34]	[34]	
	Tropical pasture soils	[35]		
	Landfill cover soils		[36–39]	
	Wetland sediments	[40,41]	[21,40,42–44]	
	Lake sediment	[45]	[45,46]	
Delayed	Agricultural soils	[47]		Initial protection from ammonium by soil N dynamics (e.g. immobilisation, nitrification). Adsorption of ammonium to soil matrix (cation exchange capacity, CEC). Population shift in the MOB community. Inhibition of cell growth and de novo enzyme synthesis leading to delayed decrease of methane oxidation following natural cell mortality.
	Forest soils	[48]		
Long-term	Forest soils	[6,49–53]	[54]	Damage to MOB due to osmotic stress. Damage due to exposure to nitrite. Cell death due to starvation (NADH limitation). Shifts in the MOB community.
	Agricultural soils	[31,52]	[31]	
	Arable soils	[55]		
	Grassland soils	[52,56]	[57]	
	Alpine meadows	[58]		
No effect	Forest soils (boreal and temperate)	[19,48,50,59–62]	[21,23,43,63]	Plant uptake of added ammonium. MOB active in subsurface layers not reached by the fertiliser. Ammonium-tolerant population. Increase in ammonia oxidisers which oxidise methane. Soil moisture status may vary and subsequent methane diffusion limitation masks inhibition by ammonium.
	Grassland soils	[64]		
	Agricultural soils	[65,66]		
	Arable soil	[31,67–69]	[29]	
	Boreal peat soils	[29,70]	[71]	
	Wetland soils	[72]	[73]	

oratory incubations may also affect methane oxidation due to osmotic stress [22].

The other inhibition patterns (delayed, long-term and no effect) act at the community or ecosystem level and are more difficult to explain. The only mechanism explaining all three patterns would be the changes of the community composition, either by a shift between ammonium-tolerant and ammonium-intolerant methane-oxidising species, or by a relative increase of ammonia oxidisers consuming methane. Generating experimental proof for these hypotheses, taking into account rapid methodological progress in molecular microbial ecology, will be one of the challenges for the researchers in near future. The prerequisites (e.g. presence of plants, cation exchange capacity, soil N dynamics, soil water status, uncoupling of population growth and activity, spatial arrangement of populations in the soil profile) for any of the other proposed mechanisms listed in Table 1 may vary considerably between soil ecosystems. Detailed knowledge on soil phys-

icochemical parameters and on the type of methane oxidisers present in any particular environment is necessary to understand the modes of action of fertiliser application. Also, a consideration of the methodological assessment of fertiliser effects is advisable. Table 1 clearly shows that laboratory incubations (in vitro) mostly result in immediate short-term effects in upland as well as lowland soils, while the majority of the studies performing in situ flux analyses reveal long-term effects or no effect. These discrepancies strongly suggest that a comprehensive investigation of fertiliser effects on methane oxidation should combine in vitro and in situ methodologies.

Nevertheless, 15 years after the findings of Studler and co-workers, it is evident that their results cannot simply be extrapolated to various soil ecosystems. Without any doubt nitrogenous fertiliser additions can affect methane consumption, but this is not relevant for every soil or sediment ecosystem. Before starting new studies to assess the impact of ammonium-based N input on methane ox-

idation in soils and to unravel the underlying mechanism, it is advisable to consider whether N-based inhibition is expected to occur in the ecosystem under study. Forest soils with high deposition of atmospheric nitrogen or agricultural soils with high fertiliser input seem to be the most relevant systems for further study in this respect. In pristine, natural soils and also sediments, the role of nitrogen as a limiting factor for methane oxidation may be of more environmental and ecological importance.

### 3. Nitrogen as a stimulating factor of methane oxidation in soils: 'the other side of the coin'

Although the mechanisms of inhibition are still under debate, the studies supporting fertiliser nitrogen as a detrimental factor of methane oxidation are numerous. Potentially large environmental consequences of this trend have led to a rather one-sided research approach with respect to the regulatory role of nitrogen on methane consumption in soils and sediments. Recently, results have been obtained that put this relationship into a different perspective. Bodelier and co-workers [8,9] subjected either planted or unplanted rice soil microcosms to different fertiliser regimes. The activity and the population size of the methanotrophic bacteria in the rice rhizosphere were substantially enhanced by the addition of urea or  $(\text{NH}_4)_2\text{HPO}_4$ , as displayed in Fig. 1. Ammonium in the rhizosphere of unfertilised plants was depleted within 30 days of a total incubation period of 84 days. The absence of mineral nitrogen resulted in an inactive, and probably non-growing methanotrophic community. Using the same microcosm system, Eller and Frenzel [75] showed that in situ rhizospheric methane oxidation, determined by the use of specific inhibitor  $\text{CH}_2\text{F}_2$ , decreased to zero upon depletion of ammonium in the soil. Identical results were found in natural rice paddies [10,76]. Addition of fertiliser nitrogen to these natural rice paddies also led to the stimulation of in vitro and in situ methane consumption [10,76,77].

In order to assess whether this effect was confined to rice paddies, we examined the literature for similar findings in other environments. Table 2 lists studies that measured direct stimulatory effects of ammonium- or nitrate-based fertilisation on methane consumption. The table also lists studies that demonstrated positive correlations between soil mineral nitrogen and methane consumption rates. The latter studies were taken into account because the causal mechanisms may very well be the same as in the studies where fertiliser was actually added. The range of soils in which nitrogen availability seems to be a limiting factor for methane oxidation is as wide as for those soils where inhibition is observed. Consumption of atmospheric methane (i.e. high-affinity methane oxidation) as well as the oxidation of elevated methane concentrations (i.e. low-affinity methane oxidation) can be enhanced by both the addition of ammonium or nitrate.

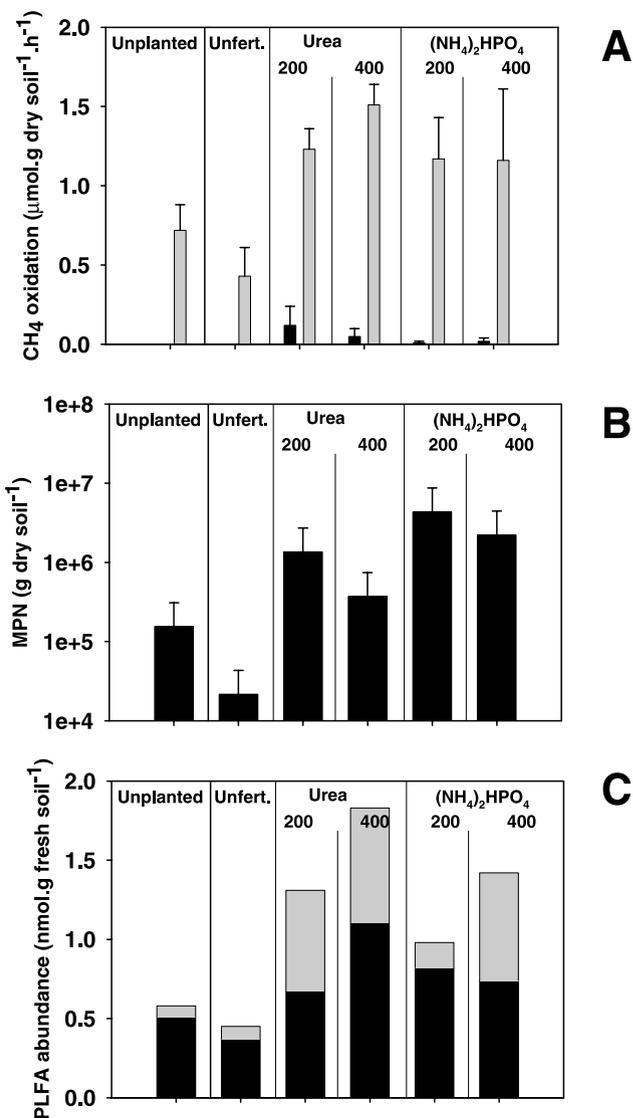


Fig. 1. Methane-oxidising activities (A), most probable numbers (B) and PLFA (C) abundance in rice soil microcosms as determined 68 days after planting. Displayed are the effects of ammonium-based fertilisation and rice plants on the methane-oxidising community in unplanted and in rhizosphere soil from microcosms that were either unfertilised or supplemented with urea (200 or 400 kg ha<sup>-1</sup>) or  $(\text{NH}_4)_2\text{HPO}_4$  (200 or 400 kg ha<sup>-1</sup>). The bars in A represent the arithmetic means of initial (black bars) and induced (grey bars) oxidation rates of four replicate microcosms. The bars in C represent the total abundances of type I specific PLFA (C16:1w8c) (grey bar) and type II specific PLFA (C18:1w8c) (black bar). Data displayed in A and B were analysed using the Kruskal-Wallis test. This test revealed significant ( $P < 0.05$ ) effects of the presence of roots and fertiliser application on methane-oxidising activities (A) as well as on numbers (B).

The explanations presented in these studies were rather diverse (see Table 2). Some authors did not suggest an explanation, or failed to discuss the findings at all [36,78,83,84]. Others claimed that increased methane consumption by ammonia oxidisers [43,88], changes in community composition of the methanotrophic bacteria [37], general improvement of nutrient availability or methane diffusivity through enhanced development of the vegeta-

Table 2  
Synthesis of studies that demonstrated positive effects of soil/sediment mineral N content and or fertilisation on methane Oxidation

Habitat	High/low CH <sub>4</sub>	Fertiliser	Effect	Explanation proposed by the authors	Reference
Surface layer littoral sediment	high	NH <sub>4</sub> Cl	Positive relation between NH <sub>4</sub> and V <sub>max</sub> .	Artefacts.	[45]
Spruce forest soil	low		High CH <sub>4</sub> consumption with highest NH <sub>4</sub> in soil.	None.	[78]
Native and invaded heath land	low	mix NPK, 56 and 112 kg ha <sup>-1</sup>	Positive relationship between ammonium concentration and CH <sub>4</sub> oxidation in seven heath land sites.	Indirect effect of increased grass vegetation which results in higher nutrient availability.	[79]
Forest soils, urban to rural gradient	low	none	Positive correlation between NH <sub>4</sub> <sup>+</sup> in soil and methane uptake rates in acidic forest soil.	Reduced nutrient availability (N, P) due to slow organic matter degradation in urban areas.	[80]
Meadow cambisol, cultivated cambisol, forest luvisol and paddy soil	high and low	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Shortening of induction times and stimulation of V <sub>max</sub> in short-term assays.	Methanotrophs need nitrogen as an N source.	[81]
Grassland and deciduous forest soil	low	none	Higher methane oxidation in forest soils with high ammonium and nitrate concentrations.	Higher nitrogen turnover in grassland soils leads to higher nitrification and therefore inhibition of methane oxidation.	[82]
Arable and undisturbed woodland and grassland soil	low	none	Higher oxidation rates and numbers in undisturbed woodland and grassland as compared to unfertilised arable soil.	None.	[83]
Arable, forest and set aside soil	low	none	Positive correlation between ammonium and methane oxidation; higher oxidation in fields taken out of production longer.	None.	[84]
Deciduous and spruce forest soil	low	KNO <sub>3</sub> or (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> in vitro	Stimulation of methane oxidation in spruce forest due to fertilisation.	General improvement of living conditions by narrowing the C/N ratio.	[85]
Landfill soil	high	NH <sub>4</sub> Cl and KNO <sub>3</sub>	Lower methane oxidation when N becomes limiting; addition of ammonium and nitrate stimulated methane consumption.	N limitation.	[86]
Landfill soil	high	NH <sub>4</sub> Cl, KNO <sub>3</sub> , lime	Rapid onset of methane oxidation with ammonium and nitrate in soil columns; nitrate also stimulated in fresh landfill soil.	None.	[36]
Coniferous forest soil	low	CaNH <sub>4</sub> NO <sub>3</sub>	Higher methane oxidation in forest soils which have been previously fertilised (2 years).	None.	[87]
Rice field soil	high	NH <sub>4</sub> Cl	Inhibition turns into stimulation by ammonium after several incubations with different methane mixing ratios.	Nitrifiers are stimulated and are therefore consuming more methane.	[43]
Landfill cover soil	high	NH <sub>4</sub> Cl, nitrified sludge, compost	Stimulation of methane oxidation by ammonium of soil exposed to high methane for short period; inhibition increases with exposure time to high methane.	Change in the community composition to N-susceptible methanotrophs.	[37]
Tropical pasture soil	low	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , urea, CaNO <sub>3</sub>	Fertilised pastures had higher methane uptake than traditional and legume pastures.	Higher plant biomass in fertilised pastures results in lower water filled pore space and hence higher diffusion of methane into the soil.	[35]
Rice field soil	high	urea	Stimulation of in vitro methane oxidation.	N-limitation of MOB.	[77]
Natural (forest/prairie) and agricultural soils	low	various regimes	Positive correlation between mineral N of soil and methane consumption at elevated levels.	Increasing contribution of nitrifiers to methane oxidation.	[88]
N-limited forest soil	low	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Higher methane uptake in N-limited soils after initial short-term inhibition.	N limitation of the methanotrophs.	[20]
Landfill cover soil	high	NH <sub>4</sub> Cl, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Stimulation of methane oxidation at high methane (2%) only when sufficient N was present.	Nitrogen limitation of type I methanotrophs.	[38]
Agricultural soil	low	various regimes	High levels of fertilisation (150 kg N ha <sup>-1</sup> year <sup>-1</sup> ) resulted in higher methane uptake of cultivated soils.	Higher growth of methanotrophs; fertilised plots had not been tilled for a long period leading to different soil pore structure and better methane diffusion into the soil.	[89]

Presented are the habitats, whether these habitats are characterised by high or low methane concentrations, which fertiliser was used if any, what the nature of the observed effects was and which explanation the authors gave for the latter.

tion may have been responsible for the observed effects [35,79,85]. Surprisingly, only Bender and Conrad [81], De Visscher et al. [86] and Papen and co-workers [20] argued that methane oxidisers simply need an N source and that the observed effects were a relief of a limitation rather than a stimulation of activity. Already in 1995, Bender and Conrad noted that their results were rather surprising given the fact that many studies reported inhibition only. However, the surprising fact is not that methanotrophic bacteria need nitrogen, but that the observations (summarised in Table 2) have never been followed up experimentally in order to assess environmental consequences of reduced methane consumption under N-limiting conditions. The studies were performed only to investigate the dimensions of the detrimental effect of nitrogen fertilisation on the methane sink function of soils. Yet, one would think that the preservation or even increase of the sink strength due to the relief of a nutrient limitation would be equally important to study in more detail. Very recently, De Visscher and co-workers [38] published the first study that anticipated both inhibitory and stimulatory effects of ammonium on methane oxidation. Nevertheless, experimental proof for a mechanism of nitrogen-based stimulation of methane oxidation in soil is still missing.

#### 4. Nitrogen-based stimulation of methane oxidation in soil: hypothetical mechanisms

Since none of the studies discussed in the preceding paragraph presents conclusive experimental evidence for the mechanisms underlying the stimulatory effect of nitrogen on methane oxidation, some speculation is necessary. Basically, the addition of nitrogenous fertiliser can act directly on cellular level or it may evoke changes in the soil ecosystem that influence methanotrophic bacteria indirectly. There are three options with respect to the former: (1) the ammonium or nitrate relieves N limitation of cell growth and subsequently increases the activity of methanotrophic community on the long term; (2) N addition interferes more directly with the synthesis of involved enzymes in the methane oxidation pathway of nitrogen-starved cells; (3) the size and activity of the nitrifying population, which also oxidises methane, is increased.

Methanotrophic bacteria have a relatively high nitrogen requirement. For every mole of assimilated carbon 0.25 moles of N have to be taken up [90]. The assimilatory demand for ammonium by methanotrophic bacteria has been demonstrated to even suppress ammonia oxidation in soils [91]. Hence, especially in environments where the molar ratio of methane to nitrogen is higher than 10 (assuming 40% assimilation of every mole of consumed methane), such as in the rhizosphere of wetland plants, in landfill soils and in the upper layer of non-eutrophic sediments, N limitation may occur. Long-term depletion of an N

source will inevitably reduce protein synthesis and growth, leading to a reduction or even cessation of methane consumption. Potentially, this limitation can be compensated for by the fixation of molecular nitrogen in these habitats. Type II and type X methanotrophic bacteria have been demonstrated to fix molecular nitrogen [92], while recently type I methanotrophic bacteria have also been shown to contain genes coding for nitrogen fixation pathway [93]. Hence, the absence of ammonium or nitrate per se does not necessarily imply reduced methane oxidation. However, nitrogen fixation is a costly process in terms of energy and reducing equivalents, and switching from ammonium or nitrate uptake to nitrogen fixation will result in lower growth potential of the methanotrophic bacteria, and possibly lower methane oxidation rates. Additions of ammonium or nitrate to nitrogen-fixing methanotrophic communities in soil could thus lead to stimulated methane oxidation due to the switch to the energetically more favourable consumption of inorganic nitrogen.

Experimental evidence from rice soils [9] but also from culture experiments [94] strongly suggests that a more direct mechanism of stimulation of methane oxidation by inorganic nitrogen compounds might exist than just the relief of nitrogen limitation for growth.

Methane oxidisers from the nitrogen-depleted rhizosphere of rice display a lag in activity of more than 2 days [9]. However, the addition of ammonium leads to immediate methane consumption in methane oxidation assays. Moreover, methane emission from these rice microcosms displayed a very strong inverse relationship with the ammonium availability in the rhizosphere of the plants (Fig. 2), indicating a continuous response of methane consumption to ammonium. Park et al. [94] observed immediate loss of particulate MMO activity upon nitrate depletion in a batch culture of *Methylosinus trichosporium*, while activity was restored again within hours following nitrate application. These observations cannot be ex-

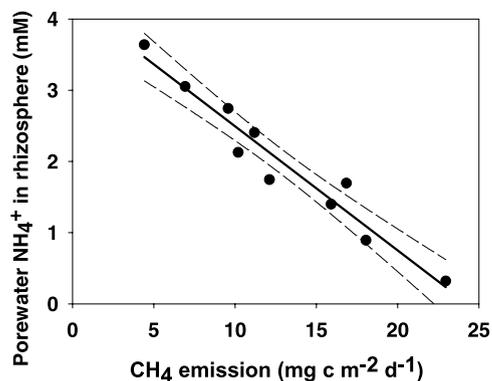


Fig. 2. Relationship between the pore water ammonium concentration in the rhizosphere soil of rice soil microcosms and methane emission from these systems. These microcosms were supplemented weekly after pore water samples had withdrawn with  $(\text{NH}_4)_2\text{PO}_4$  to a total amount of  $400 \text{ kg N ha}^{-1}$ . The emission measurements were analysed weekly for a period of 10 weeks. The dotted line indicates 95% confidence interval of the fitted linear regression.

plained by stimulated growth but only by a more direct effect of ammonium or nitrate on the methane-consuming metabolism itself. Since the immediate stimulation of methane oxidation is mediated by both ammonium and nitrate, a mechanistic explanation must be linked to nitrogen assimilation. The question is how the assimilation of nitrogen is connected so rapidly to the dissimilation of methane. We propose the following mechanism that involves nitrogen fixation. In Fig. 3a the methane oxidation pathway is schematically presented in the case of non-nitrogen-limiting conditions. The first step in this pathway

requires reducing equivalents in the form of  $\text{NADH}_2$ . This reduced compound is derived mainly from the oxidation of formate by formate dehydrogenase [95]. However, the  $\text{NADH}_2$  produced in this step has also been demonstrated to serve as electron donor for methanotrophic nitrogenase activity (cf. [95]). The electron flow is mediated by ferredoxin (FD)- $\text{NAD}^+$  oxidoreductase and FD. This diversion of electron flow towards FD is blocked when ammonium or nitrate is assimilated under nitrogen excess conditions. Switching between inorganic nitrogen assimilation and nitrogen fixation in bacteria can proceed rapidly and is

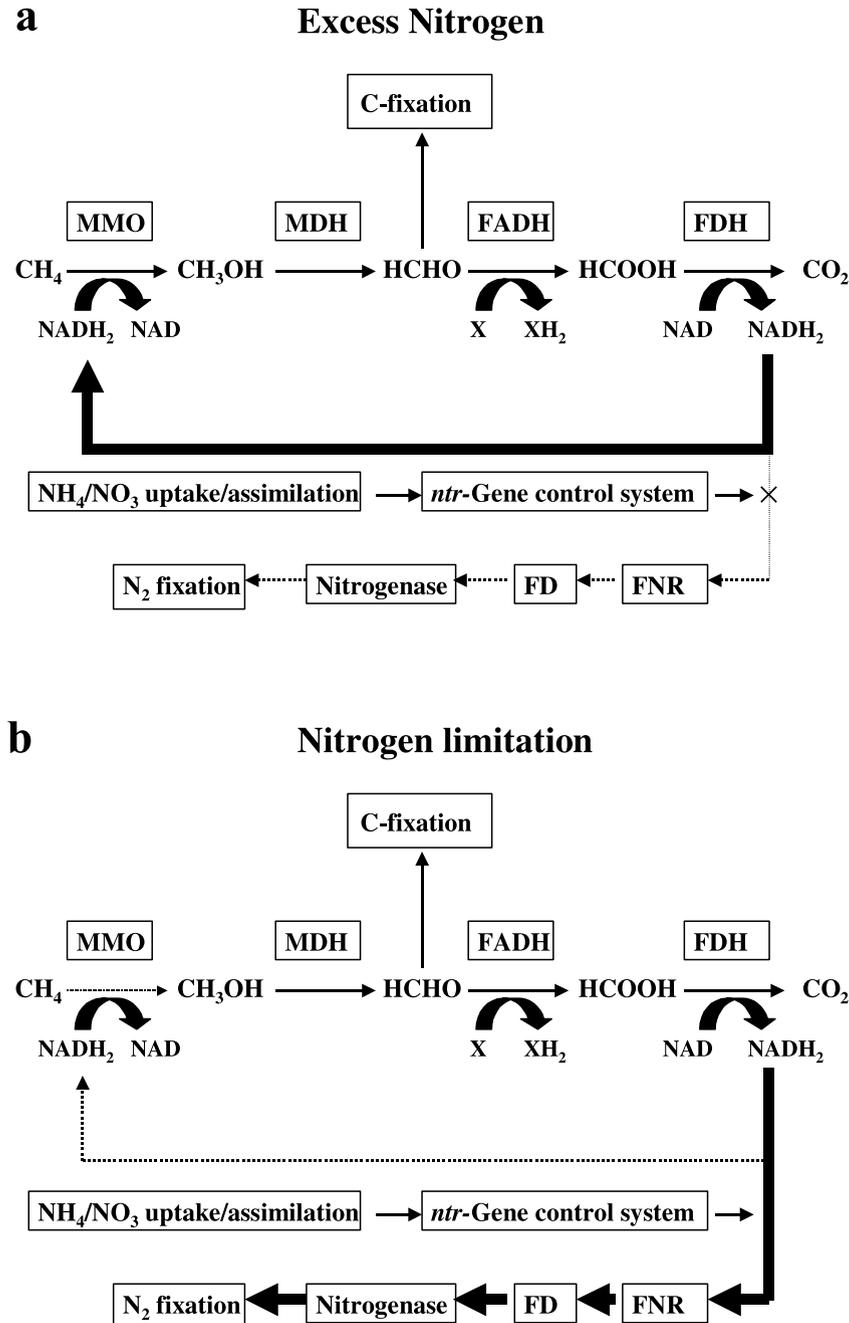


Fig. 3. Schematic presentation of the hypothetical mechanism explaining the immediate stimulation of methane oxidation by ammonium or nitrate addition to soils or sediments. a: The flow of  $\text{NADH}_2$  when nitrogen is not limiting.  $\text{NADH}_2$  is diverted to the MMO reaction. The *ntr* gene control system prevents the use of  $\text{NADH}_2$  for the nitrogenase reaction. b: The situation when inorganic nitrogen is available for assimilation. The *ntr* gene control system diverts  $\text{NADH}_2$  to enable nitrogen fixation thereby reducing or even diminishing the  $\text{NADH}_2$  flow to the methane oxidation reaction.

under control of *ntr* gene control systems [96], which regulates nitrogen assimilation and nitrogen fixation at the transcriptional level. In *Methylococcus capsulatus*, nitrogen fixation was switched off 5 min after addition of the ammonia [97]. Glutamine was the possible internal regulatory molecule, indicating that an *ntr* type of control was involved [96]. When no ammonium or nitrate is available (Fig. 3b) the *ntr* system will allow the nitrogenase system to be active, thereby diverting NADH<sub>2</sub> to nitrogen fixation. Combined with the fact that methylotrophs have a high NADH<sub>2</sub> requirement for C assimilation [98], this could lead to a limited supply of NADH<sub>2</sub> to the monooxygenase. Consequently, the oxidation of methane proceeds at a level that is often below the detection limit of the common methane consumption assays. This hypothetical mechanism would explain the rapid responses of methane oxidation to ammonium and nitrate addition that are observed in cultures, soil incubations and field studies. This mechanism is most likely to occur when methane consumption and hence NADH<sub>2</sub> generation are low, and there is a limited availability of nitrogen. Also, elevated C assimilation relative to respiration could lead to a limited supply of NADH<sub>2</sub> to the monooxygenase enzyme [88,98]. The rhizosphere of wetland plants or nitrogen-limited upland and landfill soils are the environments where this mechanism could operate. However, the proposed mechanism is only one hypothetical possibility. Of course other modes of connection between the nitrogen assimilation pathways and the monooxygenase enzyme machinery, including gene transcriptional regulation, have to be considered.

Since ammonia-oxidising bacteria also have the potential to consume methane, the stimulatory effect of nitrogen additions could also be related to enhanced populations of nitrifiers, and subsequent enhanced methane oxidation by these organisms. However, the phenomena as observed in the rice microcosm experiments are most definitely not related to ammonia oxidisers. First of all the numbers of methanotrophic bacteria increased due to the fertilisation (see also Fig. 1), indicating that they have been utilising methane while the ammonia-oxidising bacteria did not increase [9]. Secondly, using data from culture experiments [99], it would have required between 10<sup>8</sup> and 10<sup>9</sup> cells per g of dry soil to account for the observed methane oxidation rate. Most probable number counts in these rice soils yielded between 10<sup>5</sup> and 10<sup>6</sup> ammonia oxidisers per g [9]. Moreover, the stimulatory effects of inorganic nitrogen additions in high methane environments like landfill soils were also demonstrated to occur with nitrate [86], ruling out an involvement of ammonia oxidisers. This also appears to be the case for atmospheric methane uptake. The observed positive relationship between ammonium content of soils and methane consumption (Table 2) would require an unrealistically high number of cells, if it were caused by ammonia oxidisers [100].

The overview in Table 2 offers some explanations of

nitrogen-based stimulation of methane oxidation that acts indirectly through changes in the habitat of the methanotrophic bacteria. Fertilisation leads to enhanced development of the vegetation. The subsequent increased evapotranspiration lowers the soil water-filled pore space, leading to higher diffusion of methane and oxygen into the soil [35,89]. Of course, the studies proposing this explanation have been performed in upland soils. In wetlands this would be a highly unlikely mechanism. It has been suggested that enhanced biomass of wetland vegetation can lead to higher soil oxygen input from the roots of these plants, which combined with higher nitrogen availability stimulates methane oxidation [101,102].

Fertiliser-induced effects on the vegetation can also lead to a general improvement of soil fertility, creating improved conditions for microbial growth. Higher mineralisation rates will lead to higher carbon, nitrogen and possibly micronutrient availability [79,80,85]. The improved availability of carbon may be especially important for atmospheric methane consumers, which have been demonstrated to profit from and may even depend on additional carbon sources besides methane [103,104]. Changes in composition of the vegetation can also lead to altered soil texture around the new root systems, which may result in improved diffusivity of gases into the soil.

It is evident that nitrogenous fertiliser additions may promote methane uptake in both upland and lowland soils directly or indirectly. However, direct experimental evidence substantiating or ruling out any of the mechanisms described above is missing. It will be challenging to unravel the relationships between nitrogen availability and methane consumption, and to identify involved bacteria. For the time being nitrogen has to be treated as a potentially inhibitory and as a beneficial factor for methane consumption in soils and sediments.

## 5. Environmental aspects of the N prerequisite of methane consumption in soils and sediments

The reduced methane consumption activity under N-limiting conditions can have major environmental consequences. The methane source strength of soils and sediments can be enhanced while the sinks of atmospheric methane may lose part of their methane mitigating potential. However, this will depend on the natural nitrogen dynamics and also on the anthropogenic nitrogen input into soils and sediments through fertilisers and industry-derived atmospheric deposition.

### 5.1. Nitrogen regulation of methane emission from rice paddies and natural wetlands

#### 5.1.1. Rice paddies

Rice paddies are among the most prominent methane sources on earth [3]. The contribution of rice paddies is

believed to increase in future as the consequence of increased use of nitrogenous fertiliser in order to increase crop yield. More fertiliser will inhibit methane oxidation and enhance emission; at least that was the general idea. However, experimental evidence assessing this issue has been contradictory. Lower as well as higher methane emissions have been found following fertiliser application (cf. [9]). The unexpected reduction of methane emission after fertiliser application has often been explained by inhibition of methanogenesis. However, the observations of reduced emission may also be explained by a stimulation of methane consumption due to the elimination of the N-limiting conditions for the methanotrophic bacteria. Overlooking this possibility may be the result of the applied methodology for assessing methane oxidation in these systems. Often inhibitors of methane oxidation were used to estimate the percentage of methane oxidised in the rhizosphere of rice plants that also inhibited methanogenesis and led to overestimation of methane consumption. As already outlined in Section 3, Krüger and co-workers [10,76] employed for the first time a 'truly' selective inhibitor (difluoromethane;  $\text{CH}_2\text{F}_2$ ) of methane oxidation, and therefore their measurements were the first reliable estimates of the actual methane oxidation in natural rice fields. These studies indicated that increased use of fertiliser would lead to lowering of the methane emission, a fact that has to be considered in global methane emission models. A very recent study using  $^{13}\text{C}$ -labelled  $\text{CH}_4$  in rice microcosms confirmed this [105].

Variability of methane fluxes in relation to environmental dynamics or agricultural practices (e.g. fertilisation) have generally been assessed top-down in an ecosystem fashion, with methane emission or consumption as the response variable. The underlying microbial processes, in particular the characteristics and ecology of involved microorganisms, have seldom been taken into account when explaining observed variability in global methane budgets. The microcosm experiments performed by Bodelier and co-workers [8] even indicated that the diversity of the methanotrophic bacteria might play a major role in this issue. Genera belonging to the type I methanotrophic bacteria were preferentially stimulated by the addition of N, indicating that the characteristics of involved organisms have to be taken into account when explaining global fluxes. Therefore, to better understand regulation of methane oxidation by nitrogen in rice paddies, in situ oxidation technique of Krüger and co-workers should be generally applied, and combined with the physicochemical characterisation of the soil and community analyses of methane oxidisers.

### 5.1.2. Natural wetlands

Similarly as in rice paddies, the dimension and dynamics of methane emission in natural wetlands (e.g. bogs, fens, swamps, marshes) are of high environmental importance [3]. The consequences of anthropogenic disturbances re-

ceive a lot of attention. Rhizospheric methane oxidation in natural wetlands has been demonstrated to fluctuate during the growing season of wetland plants and also among plant species [101,106]. High oxidation rates were detected early in the plant growth cycle and low rates when plants matured. The gradually diminishing nitrogen pool for methane oxidation, caused by increased nitrogen uptake by the growing plants, may well explain these observations. Likewise, differences in rhizospheric methane oxidation among plant species may be explained by different nitrogen demands of the plants. The decrease of ammonium in pore water during the growing season has been documented for rice paddies [9,76], and there is no reason to assume that this will be different for natural wetlands that are normally unfertilised.

The distinctly enhanced deposition of atmospheric nitrogen over the past decades, which is still increasing, can be regarded as nitrogen fertilisation. Especially in nutrient-poor mires and peat lands this could result in a shift to more productive plant species, leading to higher methane production and emission from these systems. However, Granberg and co-workers [102] found a strong negative effect of nitrogen ( $\text{NH}_4\text{NO}_3$ ) additions on methane emission from boreal mire. This effect was found only when the cover of plants (*Carex* sp.) was high. The high plant density probably provides sufficient oxygen to the methanotrophic bacteria to profit from the nitrogen additions. In northern peat lands, Updegraff [107] found a strong correlation between methane flux and nitrogen retention. Methane emissions from fens were much lower than from bogs, a trend that the authors attributed to the higher nitrogen availability in the fens due to the lower plant productivity. The authors concluded that stimulation of methane oxidation by nitrogen was the most likely explanation for the differences in methane emissions between bogs and fens.

Hence, the understanding of the dynamics of methane fluxes from rice paddies and natural wetlands, especially in response to anthropogenic disturbances, has been misguided by the strong focus on nitrogen inhibition aspects on the methane consumption side and by the inability to assess the in situ activity of methanotrophic bacteria. Future studies assessing the effect of more intensive fertiliser use, enhanced atmospheric N deposition, elevated temperatures and  $\text{CO}_2$  on methane emission from wetlands should also assess these questions bottom-up determining the in situ functioning and diversity of methanotrophic bacteria. The latter will include the combined use of specific inhibitors together with the techniques that make use of incorporation of stable isotopes ( $^{13}\text{CH}_4$ ) into phylogenetically relevant markers (phospholipid-derived fatty acid (PLFA), DNA, RNA) [108–110].

### 5.2. Nitrogen regulation of sink strength of upland soils

The consumption of atmospheric methane by upland

soils can be affected by a vast array of environmental factors, as has been extensively investigated and reviewed [3,7,17]. Atmospheric nitrogen deposition in forest soils has received a lot of attention and is still a matter of great concern. As seen in Table 2, there are several studies on forest soils that contradict the generally found suppressing effect. Hence, even in the case of forest soils, nitrogen has to be taken into account as a possible stimulus for methane oxidation. It is hard to imagine, however, that atmospheric methanotrophic bacteria are actually limited by N. Methane concentrations in upland soils are in the nM range and up to date it is still not clear which bacteria are responsible for the process and whether growth on atmospheric methane alone is possible (cf. [111]). It has been demonstrated though that the consumption of atmospheric methane in soils can be promoted by non-methane substrates like methanol, formate and acetate [102,103,111]. If methane is not the primary source of carbon and energy for these microorganisms, then nitrogen input in upland soils may facilitate mixotrophic or even heterotrophic growth of bacteria capable of atmospheric methane consumption. The resulting elevated cell numbers will cause higher consumption of atmospheric methane. This would be a possible explanation for the positive correlation between ammonium content and atmospheric methane consumption in upland soils. Another explanation for the latter involves the assumption that the atmosphere is the only source of methane for high-affinity methanotrophic bacteria, a matter that is still not resolved. All upland soils may become partially anoxic following high precipitation events and start producing methane. The methanotrophic bacteria can profit from this enhanced methane flux and grow when sufficient nitrogen is present. Thereby the nitrogen status of upland soils may allow for a population increase of microorganisms, which, after drying of the soil, retain a higher potential for atmospheric methane consumption.

Hence, the sink strength of upland soils for atmospheric methane can be regulated by nitrogen. However, whether this effect is negative or positive, has to be assessed on a case-wise basis. There are numerous physical, chemical and management aspects that make comparisons between soils in light of one single factor (fertiliser or N deposition) difficult to interpret. It is clear, however, that nitrogen is a potential regulating factor that has to be investigated in more detail if we are to properly assess the role of upland soils in the global methane budget.

### 5.3. Nitrogen regulation of the biofilter function of soils

Landfills are prominent sources of methane, emitting 10–70 Tg of CH<sub>4</sub> per year (cf. [86]) globally. Covering the landfills with an aerobic soil layer containing methanotrophic bacteria reduces methane emission. The effect of nitrogenous additions to these cover soils has also been the subject of numerous studies, again bearing in mind the

possible reduction of methane consumption and subsequent enhanced emission. In recent years it has been shown that in landfill cover soils the addition of ammonium or nitrate can stimulate methane oxidation [36,37,86]. The rapid stimulation found in these studies points to an enzymatic effect rather than a growth-related response. This could be mediated through the proposed mechanisms listed in Fig. 3. Nevertheless, the management of landfill cover soils in order to reduce methane emission as effectively as possible has to take into account the nitrogen requirement of methanotrophic bacteria. However, landfill cover soils are normally vegetated, which can result in nitrogen-limiting conditions and hence, higher methane emissions. Bare landfill cover soils are not favoured since the plants are required for the prevention of erosion of the cover soil. Therefore, fertilisation strategies need to be developed in order to ensure optimal methane oxidation.

The top soil layer of C-rich wet soils can also act as a biofilter for methane. Kammann and co-workers [69] demonstrated a high methane production potential for managed grassland after anoxic incubation, which may occur after wetting of the soil. The methane concentration in these soils increased substantially after autumn rainfall. Nevertheless, no methane was released due to the very active methane-oxidising community in the top soil layer. Nitrogen limitation of methane oxidation in the top layers of wet soils may turn these systems into a source of methane and therefore also these soil systems are an object for future investigations on the regulatory role of N in methane consumption.

## 6. Conclusion

The important role of methanotrophic bacteria in the global methane budget, and hence in our present and future climate, is evident. This realisation has resulted in an immense amount of studies on the reactions and fluxes that these bacteria catalyse, as well as on the factors that control, regulate and affect this process. Nitrogenous fertilisers have generally been regarded as inhibitory to methane consumption by soils and sediments. This paradigm, combined with traditional 'top-down' ecosystem approach used in global methane flux studies, has led to erroneous interpretation of data by ignoring the ecological characteristics of involved organisms. With respect to methanotrophic bacteria the knowledge is still far from complete. The essential role of mineral nitrogen availability for these microorganisms and the process they mediate, have been largely neglected. Mineral nitrogen seems to be a prerequisite for the occurrence of methane consumption and might even initiate and stimulate the enzymatic machinery in a yet unknown way. The stimulation differentially affects methanotrophic species, which demonstrates the essence of a 'bottom-up' approach in global flux stud-

ies. These facts place methane oxidation in soils and sediments into a new perspective. New research approaches are required to link nitrogen availability with methanotrophic bacteria. Soil physicochemical and biological factors (e.g. competition for N with plants and other microorganisms) are potential areas for new research. However, we first have to define new concepts and research questions that will have to integrate ‘top-down’ ecosystem studies with ‘bottom-up’ approaches considering microbial populations and cellular characteristics.

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