Microbial CH₄ and N₂O consumption in acidic wetlands

Stefan Kolb* and Marcus A. Horn

Department of Ecological Microbiology, University of Bayreuth, Bayreuth, Germany

INTRODUCTION

Microbial communities of acidic wetlands are responsible for emissions of the potent greenhouse gases methane (CH₄), and nitrous oxide (N₂O). Consumption of both atmospheric gases has been observed in various acidic wetlands, but information on the microbial mechanisms underlying these phenomena is scarce. A substantial amount of CH₄ is consumed in sub soil by aerobic methanotrophs at anoxic–oxic interfaces (e.g., tissues of Sphagnum mosses, rhizosphere of vascular plant roots). Methylopestis-related species are likely candidates that are involved in the consumption of atmospheric CH₄ in acidic wetlands. Oxygen availability regulates the activity of methanotrophs of acidic wetlands. Other parameters impacting on the methanotroph-mediated CH₄ consumption have not been systematically evaluated. N₂O is produced and consumed by microbial denitrification, thus rendering acidic wetlands as temporary sources or sinks for N₂O. Denitrifier communities in such ecosystems are diverse, and largely uncultured and/or new, and environmental factors that control their consumption activity are unresolved. Analyses of the composition of N₂O reductase genes in acidic wetlands suggest that acid-tolerant Proteobacteria have the potential to mediate N₂O consumption in such soils. Thus, the fragmented current state of knowledge raises open questions concerning methanotrophs and denitrifiers that consume atmospheric CH₄ and N₂O in acidic wetlands.

Keywords: Peat, bog, fen, CH₄ cycle, nitrogen cycle, greenhouse gas sink, soil microbiology

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Table 1 | Acidic wetlands exhibiting consumption of atmospheric CH₄ or N₂O, and associated methanotroph and denitrifier communities if such data were available.

| Wetland                          | Methanotrophs                  | Denitrifiers                                                                 | Consumption of atmospheric CH₄ | N₂O          | Reference                          |
|---------------------------------|--------------------------------|------------------------------------------------------------------------------|-------------------------------|--------------|------------------------------------|
| Fen (Germany) pH 4.2            | Methylocystis, Methylocella    | Fen clusters one to five [nosZ] [next cultivated relatives: Bradyrhizobium  | −5.1 μmol gₛₒᵢₙ DW⁻¹ h⁻¹     | −19.0 to    | Wieczorek et al. (2011), Palmer et al. (2010) |
|                                 | (Methylcococcus)² (pmoA, mmoX) | japonicum, Achromobacter xylosoxidans, Azospirillum lipoferum, Azospirillum |                  | −105.0 nmol h⁻¹ gₛₒᵢₙ DW⁻¹ |                      |
|                                 |                                | ikarense)                                                                    |                               |              |                                    |
| Sovetskii raised bog (Russia) pH 3.8–4.2 | n.a.                          | n.a.                                                                         | −1.0 nmol gₛₒᵢₙ DW⁻¹ h⁻¹ F | n. a.  | Dedysh and Panikov (1997)           |
| Cryoturbated and unturbated tundra peat soil (Russia) pH 3–4 | n. a.                          | 11 operational taxonomic units of nosZ [next cultivated relatives: Mesorhizobium sp. D187B, Bradyrhizobium sp. BTAi1, Achromobacter xylosoxidans, Pseudomonas lini, Ralstonia solanacearum] |                               |              |                                    |
| Peat swamp forest (Indonesia) pH 3.3 | n.a.                          | n.a.                                                                         | −6.3 μmol m⁻² h⁻¹ D           | n. a.  | Jauhiainen et al. (2005)            |
| Boreal Wetland (Alaska, USA) pH < 4.0 | n.a.                          | n.a.                                                                         | −2.3 μmol m⁻² h⁻¹ D           | n. a.  | Whalen and Reeburgh (2000)          |
| Boreal Fens (Finland) pH < 4.5 | n.a.                          | n.a.                                                                         | −0.2 nmol gₛₒᵢₙ DW⁻¹ h⁻¹ E    | n. a.  | Kettunen et al. (1999)              |
| Tropical peatland (Kalimantan, Indonesia) pH 3–4 | n.a.                          | n.a.                                                                         | n.c. C                       | −0.3 to   | Inubushi et al. (2003)              |
| Neleger forest–alas ecosystem (Russia) pH 6.3 | n.a.                          | n.a.                                                                         | −1 to                        | −0.1 to   | Takakai et al. (2008)               |

²Taxon with little abundance (<5%).
³n.a., Not analyzed.
⁴n.c., No consumption.
⁵Calculated based on values of the respective study.
⁶Calculated based on reported first order rate constant (i.e., −0.03 h⁻¹ gₛₒᵢₙ DW⁻¹) at 1.8 ppmv (Assumption: water content 50%, 1.8 ppmv = 2.5 nM; thus, dissolved CH₄ concentration is 5 pmol gₛₒᵢₙ DW⁻¹).
⁷This rates are calculated from first order rate constants of CH₄ consumption (i.e., based on K₉₅ [43 nM; or ∼3.6 nmol gₛₒᵢₙ wet-weight⁻¹] and V₉₅ [38.5 nmol gₛₒᵢₙ wet-weight⁻¹] values as reported in the reference) at a headspace concentration of 1.8 ppmv. The water content was about 96%.
in water-logged soils and the wealth of information on denitrifiers of agricultural and pH-neutral upland soils, studies addressing acid-tolerant denitrifiers in acidic wetlands are just emerging and under-represented in current literature. Evidence is accumulating that soils including acidic wetlands can act as temporary sinks for N₂O (Inubushi et al., 2003; Hadi et al., 2005; Chapuis-Lardy et al., 2007; Goldberg et al., 2010). Indeed, negative fluxes of up to \(-1.7 \, \mu\text{mol N}_2\text{O} \, \text{m}^{-2} \, \text{h}^{-1}\) were observed in situ for acidic wetlands (Table 1). Thus, the review intends to delineate the current understanding of microbial communities in acidic wetlands for CH₄ and N₂O consumption and to highlight research needs.

**METHANOTROPHS OF ACIDIC WETLANDS**

CH₄ fluxes of acidic wetlands are highly variable due to non-linear interactions of environmental factors that control methanogenesis, methanotrophy, and CH₄ gas transport (Whalen, 2005; Spahni et al., 2011). One reason of high spatial and temporal variability of CH₄ exchange with the atmosphere might be a mitigation of net flux by consumption of atmospheric CH₄ (Wieczorek et al., 2011). Hot spots of activity of methanotrophs in acidic wetlands are anoxic–oxic interfaces, where CH₄ and oxygen gradients overlap (Sundh et al., 1995; Kettunen et al., 1999). Thus changes in oxygen availability alter both CH₄ consumption, and production, and hence, reduce or increase net flux of CH₄ from soil into the atmosphere (Kettunen et al., 1999; Whalen, 2005).

Prevalent methanotrophs of acidic wetlands belong to Alphaproteobacteria (Chen et al., 2008a; Dedysh, 2009; Wieczorek et al., 2011). Total cell numbers of methanotrophs of acidic wetlands range from \(10^5\) to \(10^8\) cells per gram of wet weight (Sundh et al., 1995; Dedysh et al., 2001, 2003; Dedysh, 2002, 2009; Belova et al., 2011). Methanotrophic pure cultures have primarily been isolated from Sphagnum-covered wetlands, and are acid-tolerant strains of *Methylocystis* (Methylocystaceae), *Methylocella*, and *Methylodacpsa* (both Beijerinckiaceae; Dedysh et al., 2001, 2003; Dedysh, 2002, 2009; Kip et al., 2011). *Methylocella* species, and some species of *Methylocystis* and *Methylodacpsa* utilize also simple monocarboxylic compounds, such as acetate (Dedysh et al., 2005; Theisen et al., 2005; Dunfield et al., 2010; Belova et al., 2011). Fluctuations of soil-internal methanogenesis may be caused by varying exudation of plants and redox conditions due to water table changes that can be consequences of drought periods, or heavy rainfall (Knorr et al., 2008; Knorr and Biodau, 2009, Dorodnikov et al., 2011). Acetate-utilizing methanotrophs are largely independent of such CH₄ deficits in acidic wetlands.

*Methylocystis* strain H2s apparently represents a key methanotroph in acidic wetlands (Belova et al., 2011; Wieczorek et al., 2011). Strain H2s-related 16S rRNA genes ubiquitously occur in northern wetlands, and contribute with 18–58% (i.e., \(0.4 \times 10^7\) to \(3.4 \times 10^8\) cells g\(^{-1}\) of wet weight) to the alphaproteobacterial methanotrophs in such wetlands (Belova et al., 2011). Uncultivated species that are phylogenetically related to *Methylocystis*, and *Methylodacpsa* are associated with hyaline cells of submerged mosses of Sphagnum. These *Sphagnum* methanotroph associations have been found in various northern wetlands (Kip et al., 2010). Methanotrophs apparently contribute up to 30% to carbon assimilation of the moss (Raghoebarsing et al., 2005; Kip et al., 2010; Larmola et al., 2010; Parmentier et al., 2011). The association is, based on current observations, not specific since different methanotroph-free Sphagnum species can be colonized by methanotrophs that were provided from the same Sphagnum individual (Larmola et al., 2010). The physiological basis of the interaction between methanotroph and moss has not been fully resolved.

Wetland methanotroph communities are usually dominated by Alphaproteobacteria, but gammaproteobacterial methanotrophs may also be present (Morris et al., 2002; Chen et al., 2008a,b; Wieczorek et al., 2011). Some of them (*Methylomonas*, *Methylosoma*-related strains) have been isolated, which represent the first acid-tolerant members of the Methyllococcaceae (Kip et al., 2011). All cultured methanotrophs of acidic wetlands are acid-tolerant and have growth optima below pH 6 (Dedysh, 2009; Kip et al., 2011). Most information on the identities and physiologies of methanotrophs of acidic wetlands are derived from *Sphagnum*-associated microbial communities, but there is a lack of information on vascular plant-associated methanotrophs in acidic wetlands.

**SPECIALIZED METHANOTROPHS MEDIATE CH₄ SINK ACTIVITY OF ACIDIC WETLANDS**

Acidic wetlands may temporarily turn into net sinks of atmospheric CH₄, which has been documented in various studies, or have the capability to utilize atmospheric CH₄ concentrations (Table 1; Figure 1; Dedysh and Panikov, 1997; Kettunen et al., 1999; Whalen and Reeburgh, 2000; Jauhiainen et al., 2005; Elberling et al., 2011; Wieczorek et al., 2011). Many wetland sites have annual emission rates of less than 3.8 mol CH₄ ha\(^{-2}\) year\(^{-1}\) (Dalal and Allen, 2008) indicating not necessarily only low CH₄ production, but also a certain proportion of consumption of atmospheric CH₄ over the year.

A very limited number of studies analyzed the composition of methanotroph communities of acidic wetlands, and measured the potential of the respective wetland to consume atmospheric CH₄ (Table 1). *Methylocystis* species that were frequently detected in acidic wetlands are closely related to the solely known methanotroph strains that are capable of consumption of atmospheric CH₄, i.e., strains LR1, SC2, and DWT (Dunfield et al., 1999, 2002; Kolb et al., 2005; Baani and Liesack, 2008; Dedysh, 2009; Wieczorek et al., 2011). Nonetheless, *pmoA2* genes (that encode for the hydroxylase subunit of a high affinity CH₄ mono-oxygenase) that would indicate the capability of consumption of atmospheric CH₄ (Dunfield et al., 2002; Yimga et al., 2003; Ricke et al., 2004; Baani and Liesack, 2008) have only been detected at low relative abundances (<5%; Wieczorek et al., 2011). To warrant consumption of atmospheric CH₄ in wetlands a small fraction (about \(10^6\) cells per gram dry weight in forest soils that consume atmospheric CH₄; Kolb et al., 2005; Degelmann et al., 2010) of the total cell number of present methanotrophs (about \(10^8\) cells per gram dry weight) need presumably be active to allow for consumption of atmospheric CH₄ at rates that were observed in acidic wetland sites. The major proportion of detectable *pmoA* (encodes the beta-subunit of a low affinity membrane bound CH₄ mono-oxygenase) and *mmoX* (encodes the hydroxylase subunit of a low affinity cytoplasmic bound CH₄ mono-oxygenase) genes in an acidic wetland soil that exhibited the capability of consumption of atmospheric levels
of CH₄ (i.e., 82 and 64%, respectively), affiliated with genes of *Methylocystis* (Wieczorek et al., 2011). Some *Methylocystis* species have the capability to utilize atmospheric CH₄ concentrations. The abundant *Methylocystis* strain H2s harbors as well pmoA2 indicating that this methanotroph may consume atmospheric CH₄ (Dedysh, personal communication). Thus, *Methylocystis* species of acidic wetlands likely are capable of atmospheric CH₄ consumption (Figure 1), and may have the advantage to survive more likely periods of CH₄ deficiency, may be periodically the case in minerotrophic fans, and at oxygen-saturated surface-near soil layers.

Recently described thermoacidophilic methanotrophs of the *Methylacidiphilaceae* (Op den Camp et al., 2009) as well as anaerobic methanotrophs *Candidatus* *Methyloirabilis oxyfera* (Ettwig et al., 2010) have not been detected in acidic wetlands.

DO DENITRIFIERS CONSUME N₂O IN ACIDIC WETLANDS?

One of the most remarkable features of denitrifiers is their capacity to produce and consume N₂O (Table 1; Figure 1; Zumft, 1997). Such organisms are widely distributed in various environments and highly diverse (e.g., Zumft, 1997; Braker and Conrad, 2011). Not all denitrifiers known to date are capable of N₂O consumption (Zumft and Kroneck, 2007). However, N₂O is a common intermediate during denitrification and serves as sole electron acceptor for supporting growth of many denitrifiers (Zumft, 1997; Strohm et al., 2007). DNA based stable isotope probing with ¹³C-labeled succinate as electron donor and N₂O as electron acceptor and cultivation-based studies demonstrated growth of putative denitrifiers by N₂O reduction in rice field soil (Ishii et al., 2011). These findings are in line with a ΔG° of N₂O reduction to N₂ that approximates −340 kJ mol⁻¹ under standard conditions, indicating a highly exergonic process (Zumft and Kroneck, 2007). Thus, it might not be surprising that N₂O consumption by soil communities is a more common phenomenon than previously thought (Table 1; reviewed in Holtan-Hartwig et al., 2006; Chapuis-Lardy et al., 2007).

N₂O reductases of denitrifiers catalyze the efficient reduction of N₂O to N₂ and are copper-dependent metallo-enzymes that exhibit *K₉* values in the lower micromolar range for N₂O, highlighting the potential of denitrifiers to consume low concentrations of N₂O (Zumft, 1997). Abiotic conversion of N₂O at ambient temperatures might occur with (reduced) transition-metal complexes (which occur in the active site of N₂O converting enzymes) and metal amides (Banks et al., 1968; Zumft and Kroneck, 2007). Metallo-enzymes like carbon monoxide dehydrogenase and cobalamin-dependent methionine synthase are also capable of reducing N₂O to N₂, while nitrogenase converts N₂O to NH₃; bacterial nitrite cytochrome c reductase transforms N₂O to an unknown product (Jensen and Burris, 1986; Bannerjee and Matthews, 1990; Lu and Ragsdale, 1991; Drummond and Matthews, 1994; Stach et al., 2000). Such enzymes are hosted by diverse organisms including non-denitrifiers (see references in Zumft, 1997; Zumft and Kroneck, 2007). However, the conversion of N₂O by these aforementioned enzymes is regarded as a non-physiological reaction. Low *K₉* values and high specific activities for N₂O (as were demonstrated for N₂O reductases of denitrifiers) have not been demonstrated for such enzymes, suggesting a minor role, if any, for the consumption of atmospheric N₂O concentrations (319 ppb, equivalent to app. 8 nmol N₂O per liter soil solution; Conrad, 1996) in soils (Zumft, 1997; Stach et al., 2000; Zumft and Kroneck, 2007). In this context, dissimilatory reducers of nitrate producing ammonia of the *Enterobacteriacea* that were shown to consume N₂O in the laboratory displayed a *K₉* value of 3 mM for N₂O, likewise suggesting that the N₂O reduction is
a non-physiological reaction and probably of minor importance in situ (Kaldorf et al., 1993). Moreover, assimilatory reduction of \( \text{N}_2 \text{O} \) that might theoretically proceed via nitrogenase action was not detected in soils (Vieten et al., 2008). Thus, denitrifiers hosting \( \text{N}_2 \text{O} \) reductases deserve primary attention in the context of \( \text{N}_2 \text{O} \) consumption in soils.

Denitrification rates and the ratio of \( \text{N}_2 \text{O} \) to total nitrogen gas products are regulated by oxygen availability, \( \text{pH} \), temperature, carbon to nitrogen ratio, the availability of substrates, and electron acceptors, as well as by the denitrifier community composition (van Cleemput, 1998; Dörsch et al., 2011). Anoxia (as indicated by a water filled pore space of greater than 60%), a carbon to nitrogen ratio greater than 30, and \( \text{pH} \)-neutral conditions favor complete denitrification to \( \text{N}_2 \) in soils including acidic wetlands (Conrad, 1995, 1996; Klemedtsson et al., 2005; Cuhel et al., 2010; Braker and Conrad, 2011). A \( \text{pH} \) value below 5 is often encountered in peatlands, and it is hypothesized to impair \( \text{N}_2 \text{O} \) reduction and to increase the product ratio of \( \text{N}_2 \text{O} \) to \( \text{N}_2 \) (Simek and Cooper, 2002; Cuhel et al., 2010). However, acidic wetlands host acid-tolerant denitrifiers capable of \( \text{N}_2 \text{O} \) reduction and complete denitrification (Palmer et al., 2010, 2011). Moreover, evidence of active denitrification-associated \( \text{N}_2 \text{O} \) production and consumption, (including consumption of atmospheric \( \text{N}_2 \text{O} \) concentrations) was obtained by compound specific stable isotope analyses of \( \text{N}_2 \text{O} \) in one acid wtland (Goldberg et al., 2008, 2010). In that specific wetland, \( ^{15} \text{N} \) values indicated that \( \text{N}_2 \text{O} \) produced in deep soil layers was partially consumed upon its diffusion to the soil surface (Goldberg et al., 2008, 2010), suggesting that denitrifiers might contribute to the mitigation of \( \text{N}_2 \text{O} \) emissions from acidic wetlands. Denitrifiers that exhibit the genetic potential to consume \( \text{N}_2 \text{O} \) were detected in acidic wetlands by the analysis of nos\( Z \) genes. Detected nos\( Z \) genes in acidic wetlands mostly affiliated with nos\( Z \) from uncultured soil bacteria or were only distantly related to known genes, suggesting a wealth of uncultured denitrifier diversity in acidic wetlands (Palmer et al., 2010, 2011). Phylogenies of nos\( Z \) and 16S rRNA genes from the same organisms are basically congruent (Jones et al., 2008; Palmer et al., 2009), allowing to speculate on the identity of organisms hosting the detected nos\( Z \). Thus, putative \( \text{N}_2 \text{O} \) reducers in acidic wetlands include Alphaproteobacteria (Azospirillum, Brady–, and Mesorhizobium), Betaproteobacteria (Achromobacter, Ralstonia), and Gammaproteobacteria (Pseudomonas). However, it remains to be determined whether the detected \( \text{N}_2 \text{O} \) reductase genes were expressed, i.e., the detected organisms reduce \( \text{N}_2 \text{O} \) in situ (Figure 1).

Analyses of other genes associated with denitrification corroborate that acidic wetlands harbor diverse and new denitrifiers (Palmer et al., 2010, 2011). However, given the low number of studies addressing \( \text{N}_2 \text{O} \)-consuming denitrifier communities in acidic wetlands, and the selectivity of PCR-based gene marker analyses, the present studies have to be regarded as providing a minimal estimate of denitrifier diversity capable of \( \text{N}_2 \text{O} \) consumption (Throback et al., 2004; Green et al., 2010; Heylen et al., 2011; Jones et al., 2011). The fact that high affinity respiratory \( \text{N}_2 \text{O} \) reduction occurs in non-denitrifying organisms that reduce nitrate but lack nitrite reductase activity, and that nitrofiers were shown to have the capability to consume \( \text{N}_2 \text{O} \) and produce \( \text{N}_2 \) further complicates matters (Yoshinari, 1980; McEwan et al., 1985; Zumft, 1997; Schmidt et al., 2004). Nevertheless, the relevance of \( \text{N}_2 \text{O} \) consumption for mitigation of \( \text{N}_2 \text{O} \) emissions, and eventually a temporary sink function of acidic wetlands for \( \text{N}_2 \text{O} \) is emerging, although the physiology and taxonomic identity of “microbial catalysts” have not been sufficiently resolved. Furthermore, the regulation of \( \text{N}_2 \text{O} \) reduction in situ and on cellular level, and thus the potential of the soil to reduce \( \text{N}_2 \text{O} \) to \( \text{N}_2 \), and the diffusivity of \( \text{N}_2 \text{O} \) within the soil profile will impact the sink functions of wetland soils for \( \text{N}_2 \text{O} \) but are likewise only poorly resolved.

**FUTURE PERSPECTIVES**

Few studies addressed the role of methanotrophs and denitrifiers in consumption of atmospheric \( \text{CH}_4 \) and \( \text{N}_2 \text{O} \) in acidic wetlands. Potential consumption activities have been measured in laboratory-scale experiments, and first evidence for denitrification-associated \( \text{N}_2 \text{O} \) consumption in situ is available. Systematic in situ flux measurements combined with measurements of environmental parameters, consumption potentials, stable isotope signatures of carbon and nitrogen in \( \text{CH}_4 \) and \( \text{N}_2 \text{O} \) pools, and community analyses are needed to improve our understanding of environmental factors that trigger \( \text{CH}_4 \) and \( \text{N}_2 \text{O} \) consumption in acidic wetland soils.

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