



Biotic Interactions in Microbial Communities as Modulators of Biogeochemical Processes: Methanotrophy as a Model System

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Microbial interaction is an integral component of microbial ecology studies, yet the role, extent, and relevance of microbial interaction in community functioning remains unclear, particularly in the context of global biogeochemical cycles. While many studies have shed light on the physico-chemical cues affecting specific processes, (micro)biotic controls and interactions potentially steering microbial communities leading to altered functioning are less known. Yet, recent accumulating evidence suggests that the concerted actions of a community can be significantly different from the combined effects of individual microorganisms, giving rise to emergent properties. Here, we exemplify the importance of microbial interaction for ecosystem processes by analysis of a reasonably well-understood microbial guild, namely, aerobic methane-oxidizing bacteria (MOB). We reviewed the literature which provided compelling evidence for the relevance of microbial interaction in modulating methane oxidation. Support for microbial associations within methane-fed communities is sought by a re-analysis of literature data derived from stable isotope probing studies of various complex environmental settings. Putative positive interactions between active MOB and other microbes were assessed by a correlation network-based analysis with datasets covering diverse environments where closely interacting members of a consortium can potentially alter the methane oxidation activity. Although, methanotrophy is used as a model system, the fundamentals of our postulations may be applicable to other microbial guilds mediating other biogeochemical processes.

Keywords: microbial interaction, microbial network, methanotrophy, methane oxidation, ecosystem functioning

INTRODUCTION

Natural microbial communities are characterized by complex networks of microbial populations forming intricate relationships of synergistic, antagonistic, and/or neutral nature. Accumulating evidence stresses the relevance of microbial interactions and their role in altering microbial mediated processes, referred here as community/ecosystem functioning (Murase and Frenzel, 2008; Comolli, 2014; Daebeler et al., 2014; Ho et al., 2014; Abrudan et al., 2015; Amin et al., 2015; Fiegna et al., 2015; Willett et al., 2015). Moreover, emergent properties may arise when microorganisms

interact (e.g., interaction-induced production of metabolites; Watrous et al., 2012; Tyc et al., 2014; Abrudan et al., 2015), leading to altered community functions otherwise absent in the case of non-interacting individual cells. Therefore, biotic interactions can be important modulators in community functioning, steering the community composition and dynamics. As such, determining significant relatedness between responses of microbial communities to specific environmental cues, and/or linking the diversity (evenness and richness) and abundance to process rates, without taking underlying biotic interactions into account, may lead to misguided views on causal relationships as well as an incomplete understanding of ecosystem functioning.

Interdependent relationships between microorganisms due to nutritional reliance between community members are well known (e.g., symbiotic phototrophic consortia comprising green sulfur bacteria and members of *Betaproteobacteria*; Müller and Overmann, 2011). In contrast, exemplified by aerobic methane-oxidizing bacteria (MOB), we focused on microorganisms which are regarded as being able to function as individuals without relying on any interacting partners for growth (see review by Semrau et al., 2010 for MOB metabolism). However, it has been shown *in vitro* that the presence of other microorganisms may still significantly alter process rates (i.e., methane oxidation as the functional response variable; Iguchi et al., 2011; Ho et al., 2014; Jeong et al., 2014; Oshkin et al., 2014), prompting us to hypothesize that microbial interactions in complex communities modulate process rates, and may account for observed variability in biogeochemical processes. Here, we find evidence for the relevance of microbial interaction in modulating biogeochemistry by reviewing the literature for close associations of MOB with their biotic neighbors which may lead to altered methane oxidation rates. Support for possible consistent metabolic interactions in methane-fed communities spanning multiple habitats was inferred using co-occurrence network analysis of selected datasets where methane-derived carbon incorporation into MOB and non-MOB community members was performed by combining DNA-based stable isotope probing (SIP), and high throughput sequencing (Table 1). The coupling of ^{13}C - CH_4 labeling to the network analysis allows the assessment of associations of actively interacting microorganisms, sharing carbon derived from a single relevant biogeochemical process (Dumont et al., 2011). However, our co-occurrence network analysis comes with a caveat; we cannot assess the spatial and temporal dynamics of the interaction as the DNA-SIP studies were only performed at a particular point in space and time. Moreover, these interactions may be affected by edaphic properties of the soil/sediment from the different environments. Phylogenetic assignment and relative abundance of community members in these datasets were determined *de novo* using a standardized pipeline (see Supplementary Information), enabling the comparison and interpretation of networks built for different environments revealing (in) consistent associations of MOB with other microbes. We argue that incorporating mechanistic knowledge on biotic interactions in community functioning is a step forward in linking microbial diversity and abundance to ecosystem functioning,

facilitating predictions of ecosystem functioning under disturbance.

MOB FORM CLOSE ASSOCIATIONS WITH THEIR BIOTIC ENVIRONMENT

Aerobic methane-oxidizing bacteria co-exist with other (micro)organisms, and actively interact to form tight associations with their biotic environment. A mutually beneficial interaction occurs in ombrotrophic peatlands where MOB-*Sphagnum* moss interaction is thought to drive carbon sequestration by *Sphagnum*, while mitigating methane emission (Putkinen et al., 2012; Larmola et al., 2014; Vile et al., 2014). Diazotrophic MOB seemingly form a symbiotic relationship with *Sphagnum*; in return for molecular oxygen, the MOB provide the moss with additional carbon in the form of CO_2 derived from the respired methane, as well as being a source for assimilable nitrogen by MOB nitrogen fixation (Raghoebarsing et al., 2005; Larmola et al., 2014; Vile et al., 2014; Kox et al., 2016). Given the close proximity of the MOB being localized in the hyaline cells of the *Sphagnum* and that nitrogen fixation is energetically costly, it has recently been proposed that a more mutually beneficial partnership yielding a higher return on investment (e.g., reducing equivalents needed in methane oxidation) for the MOB may occur (Ho and Bodelier, 2015). Therefore, the MOB are suggested to be closely associated to the *Sphagnum*, which forms the base of the food web in peat ecosystems in an inter-play inherent to the carbon and nitrogen cycles in peatlands. Similarly, in a stratified lake (Lago di Cadagno, Switzerland) where light penetrated to the anoxic zone, aerobic methane oxidation was fuelled by molecular oxygen produced *in situ* by photosynthetic algae (Milucka et al., 2015). Not only were gammaproteobacterial MOB found to form the active population assimilating methane in this environment, these microorganisms were also expressing the *nifH* gene, a subunit of the gene encoding for the nitrogenase enzyme, indicating their likely contribution to the carbon and nitrogen cycles (Halm et al., 2009; Milucka et al., 2015). In a partnership of microalgae and MOB, the microalgae were also found to fuel aerobic methane oxidation under oxygen-limiting conditions (Van der Ha et al., 2011). Indirect interaction with invertebrates can be seen in a termite mound where the termites engineer their immediate environment, shaping the MOB community composition and significantly stimulated methane oxidation (Ho et al., 2013a). Likewise, in the marine environment, MOB act as epibiont/endsymbiont of benthic invertebrates around hydrothermal vents, a hotspot for methane cycling. For instance, gammaproteobacterial MOB were found to be part of the active epibiotic community in the setae of *Shinkaia crosnieri*, a deep sea dwelling crab found around hydrothermal vents (Watsuji et al., 2014). In a stable isotope labeling study, ^{13}C derived from ^{13}C - CH_4 to $\text{H}^{13}\text{CO}_3^-$ (bicarbonate) could be retrieved from tissue of the *S. crosnieri*, showing that carbon derived from MOB or other epibionts was assimilated into the crab, and provided evidence that epibionts may also nutritionally support their host, a role

TABLE 1 | Studies considered for the network analysis, including site information and incubation/experimental conditions.

Habitat	Location (sampling time)	Methodology	Incubation conditions					References	Placement of network analysis and OTU table
			Incubation period (d)	Temperature (°C)	Headspace methane (% v/v)	Treatment			
Sediment from geothermal springs	Hot springs across Canada (2009–2012)	SIP coupled to 16 s rRNA gene sequencing	7	22–45 (<i>in situ</i> temperature)	5–10	Un-amended incubations.	Sharp et al., 2014	Figure 1, Table S1	
Sediment from a freshwater lake [#]	Lake Qalluuraq, Alaska, USA (July, 2009)	SIP coupled to 16 s rRNA gene sequencing	212–248	4	10	Un-amended incubation of sediments (0–1 and 15–20 cm from surface).	He et al., 2012a	Figure S2, Table S2	
			144–212	10					
			55–74	21					
			188	4	10	Un-amended incubation of sediment (0–1 cm from surface).	He et al., 2012b		
			38–212	4	10	Un-amended incubation of water column and sediment (0–25 cm from surface).	He et al., 2012c		
Grassland soil	Grændalur Valley, Iceland (August, 2012)	SIP coupled to 16 s rRNA gene sequencing	28	25	1	Un-amended and amended oxic incubations with 15 and 150 $\mu\text{g NH}_4^+ - \text{N g dw}^{-1}$.	Daebeler et al., 2014	Figure S3, Table S3	
Rice paddy soil	Jiangsu Province, China (January, 2009)	SIP coupled to 16 s rRNA gene sequencing	19	28	0.9–1	Amended oxic incubations with CH_4 , $\text{CH}_4 + \text{urea}$, and $\text{CH}_4 + \text{urea} + \text{CO}_2$.	Zheng et al., 2014	Figure S4, Table S4	
Surface water of oilsands tailing pond	Fort McMurray, Alberta, Canada (2010–2011 at 3 months intervals)	SIP coupled to 16 s rRNA gene sequencing	6–10	23	1	Oxic incubation with CO_2 adjusted to 10 % $_{\text{v/v}}$	Saidi-Mehrabad et al., 2013	Figure S5, Table S5	

[#]The network analysis was derived from three studies of the same environment (by the same main authors).

that was so far only evident in endosymbionts (Watsuji et al., 2010). Among the epibiotic community in the hydrothermal shrimp *Rimicaris exoculata*, gammaproteobacterial MOB were found to be localized in the gill chamber of the shrimp (Zbinden et al., 2008). Specific localization of methanotrophic epibionts was also demonstrated for a hydrothermal vent mussel *Bathymodiolus puteoserpentis* where higher *pmoA* gene expression was detected in areas where methane was transported into the mussel by water flow (Wendeberg et al., 2012); *pmoA* gene expression was higher in the frontal regions of the gill, and decreased toward the anterior. Although the exact role of MOB in many of these interactions require further mechanistic probing, it is clear that MOB form significant

relationships with their biotic components in widespread environments.

Moreover, MOB may benefit from interaction with other prokaryotes. In a microbial community enriched from a forest soil, specific heterotrophs (*Rhizobium* sp.) are thought to provide MOB with essential nutrients (Iguchi et al., 2011). Co-culturing MOB along with some *Rhizobium* sp. increased the growth of *Methylovulum miyakonense*, an alphaproteobacterial MOB. Analyzing the filtered spent medium from the co-culture identified the growth-stimulating factor to be cobalamin, an essential trace nutrient the MOB are incapable of synthesizing intracellularly, and thus rely on an external source; the *Rhizobium* seemingly provides these MOB with cobalamin, stimulating

growth (Iguchi et al., 2011). Similarly, other microorganisms may promote growth of MOB (e.g., *Cupriavidus taiwanensis*; Stock et al., 2013; *Sphingopyxis* sp; Jeong et al., 2014). In the MOB—*Sphingopyxis* sp co-culture, significant stimulation of methanotrophic activity and growth was attributable to higher gene transcription of the enzymes involved in methane catabolism (Jeong et al., 2014). Conversely, MOB are known to sustain entire isolated ecosystems and act as a primary producer in methane-driven environments (e.g., Movile cave, Romania; Hutchens et al., 2004; aquatic ecosystems; Agasild et al., 2014), as well as in enrichments with methane as the sole carbon and energy source (Beck et al., 2013; Oshkin et al., 2014). In these enrichments, however, specific accompanying microorganisms (e.g., methylotrophic species; Beck et al., 2013; Kerckhof et al., 2014; Oshkin et al., 2014; Yu et al., 2016) were observed to co-enrich along with the MOB even after successive transfers in independent studies, indicating a reciprocal selection of MOB and non-methanotrophic interacting partners. In a soil and lake sediment from the Arctic region, the relative abundance of methanotrophs and methylotrophs was directly correlated, suggesting a close association of these groups of microorganisms, likely as a consequence of a direct exchange of metabolites (Martineau et al., 2010; He et al., 2012a). The nature of the interaction remains enigmatic, but is worthy of speculation (see below). Besides, recently discovered versatility in the MOB metabolism shows that *Methylomicrobium alcaliphilum*, an obligate gammaproteobacterial MOB may directly exude carbon-based compounds (e.g., acetate, succinate) under oxygen limitation (Kalyuzhnaya et al., 2013), suggesting yet another means by which MOB can support heterotrophic microorganisms. Although these studies suggest that MOB interact with specific microorganisms, MOB interacting partners may not be necessarily exclusive (Ho et al., 2014). On the contrary, Ho et al. (2014) showed the relevance of having a diverse microbial community, regardless of the community members, to significantly stimulate methanotrophic activity. Collectively, these studies demonstrate a close-knit association of MOB and other biotic components across multi-trophic levels.

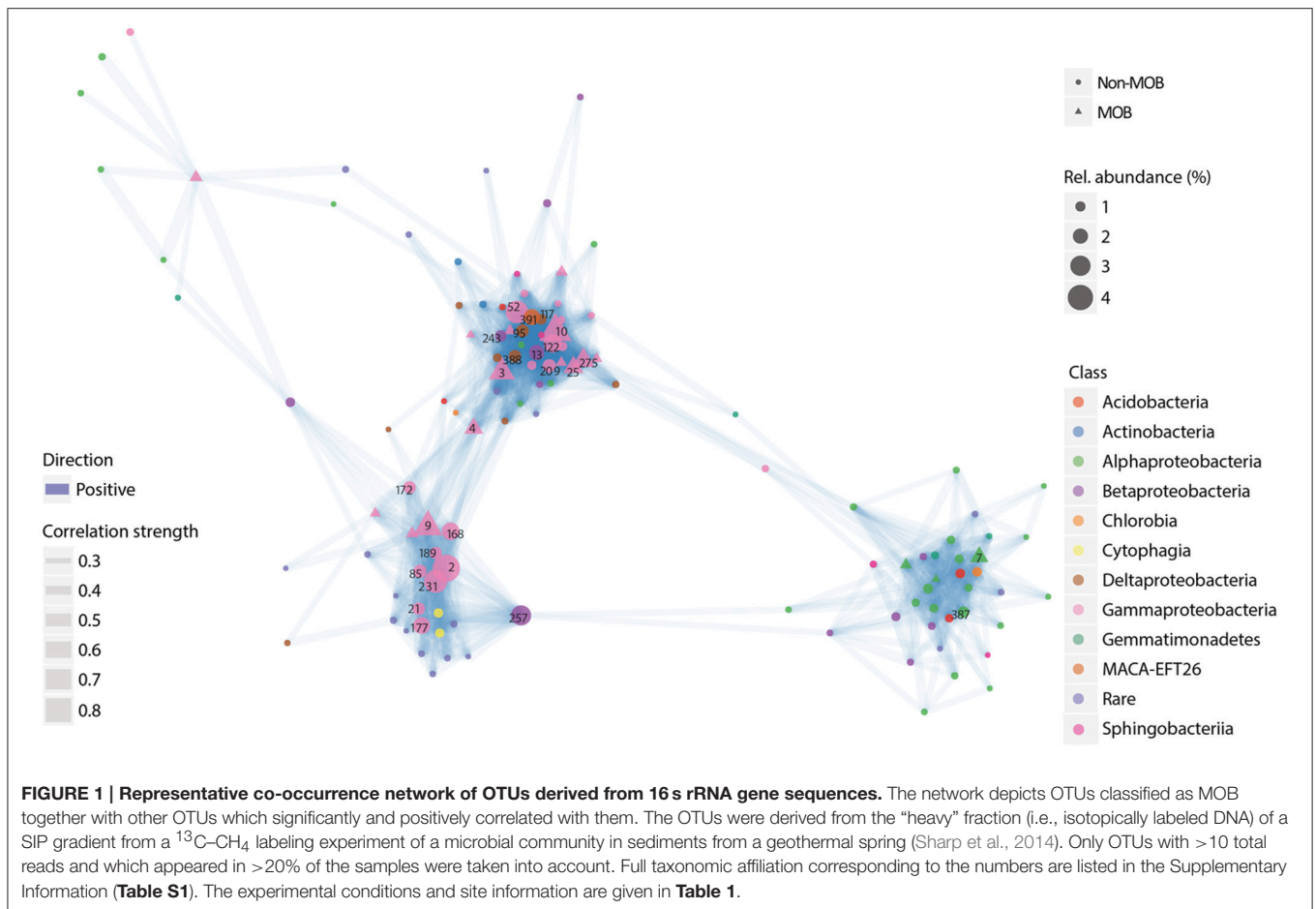
Not all interactions are cooperative/synergistic endeavors (Oliveira et al., 2014). Antagonistic biotic interactions can be represented by a predator-prey relationship between MOB and protists where gammaproteobacterial MOB were found to be preferentially grazed than alphaproteobacterial ones (Murase and Frenzel, 2008). Protist grazing caused a shift in the soil bacterial community composition, including the MOB (Murase et al., 2006; Murase and Frenzel, 2007), and is thought to affect methane oxidation as a consequence of an indirect effect through enhanced nitrogen mineralization following grazing (Murase and Frenzel, 2007). Similarly, a predatory relationship had been suggested for the interaction between the MOB *Methylocapsa acidiphila*, and the white rot fungus *Hypholoma fasciculare* (De Boer and van der Wal, 2008). The presence of *H. fasciculare* in beech wood coincided with the reduction of wood-inhabiting bacteria due to bactericidal effects induced by the fungus. Among the bacteria still detected at a relatively high proportion in the

beech wood was *M. acidiphila*, an acidophilic MOB capable of N-fixation, which can utilize both methane and methanol as substrates (Dedysh et al., 2002). Hence, this may seem like a mutualistic interaction where the MOB provide nitrogen in return for methanol (a side-product of ligninolytic activity), but the authors also suggest a predatory interaction (induced bactericidal effect to gain assimilable nitrogen from lysed cells; De Boer and van der Wal, 2008). Further support for possible antagonistic interactions between MOB and fungi can be found in a correlative study showing spatial and/or niche separation between MOB and fungal abundances (Burke et al., 2012). Therefore, both synergistic and antagonistic interactions determine MOB distribution and prevalence in the environment, with possible consequences for environmental methane oxidation.

BIOTIC INTERACTION MODULATING MOB ACTIVITY

Metabolites are the currency of microbial interaction (Morris et al., 2013; Beliaev et al., 2014; Amin et al., 2015; Audrain et al., 2015; Schmidt et al., 2015; Zelezniak et al., 2015). In broad terms, metabolites encompass (by) products of microbial metabolism, including (non) volatile compounds which can be secreted into the environment, inducing interaction with synergistic, antagonistic, and neutral outcomes. Elucidating the link between microbial interaction in natural communities and biogeochemical processes is made challenging by the complexity of the potential metabolic networks between co-occurring microorganisms. Moreover, it is not trivial to pinpoint the source of a particular metabolite given the versatility in microbial metabolism which confers a high level of redundancy to a single process. Therefore, we focused on methane-driven environments to find evidence for putative, and possibly, consistent interactions, as represented in single resource driven communities. Accordingly, we surveyed the literature for ^{13}C - CH_4 labeling studies (Table 1), and mined datasets derived from these studies to perform network analyses on the microbial communities (derived from the 16S rRNA gene) incorporating the ^{13}C (Figure 1, Figures S2–S5; see Supplementary Information for details on network construction). In contrast to previous work inferring interaction *via* coexistence of microbial communities in DNA-based studies (excluding SIP), the coupling of ^{13}C - CH_4 labeling to a network analysis provides a direct link through a shared substrate and cross-feeding between interacting microorganisms. Hence, while previous work provided direct evidence for interaction-induced modulation of methane oxidation in simplified ecosystems (i.e., synthetic communities; Iguchi et al., 2011; Stock et al., 2013; Ho et al., 2014; Jeong et al., 2014), our present approach combining DNA-SIP and a co-occurrence network analysis provides a first insight into the MOB interactome of naturally-occurring complex communities.

Based on the literature survey, we selected seven datasets (Table 1) covering widespread terrestrial methane-cycling



environments (i.e., arctic lake, geothermal springs, oilsands tailings ponds, grassland, and rice paddy), and with adequate sequencing coverage to obtain sequences which could be affiliated to microorganisms at a high phylogenetic resolution (genus level). Co-occurrence networks were constructed using the OTU tables based on SparCC correlation coefficients (Friedman and Alm, 2012) and calculated in R (V3.2.2; R Core Team, 2014) using igraph (V1.0.1; Csardi and Nepusz, 2006). Briefly, the OTU tables were generated from raw sequence reads using Mothur (Schloss et al., 2009) and the UPARSE pipeline (Edgar, 2013), and classified against the SILVA NR 99 database (V119; Pruesse et al., 2007; see Supplementary Information). Besides, we only considered the ^{13}C -labeled OTUs which represent >1% relative abundance assuming these to be the ones increased in abundance as a consequence of methane-derived carbon transfer.

The network analysis revealed a methane-derived food web in the study sites, and that, with the exception of the methylotrophs, the accompanying non-MOB community (taxonomic hierarchy: family and genus) was rather site-specific and not consistent; habitat-overarching set of genera was associated with the MOB. Interestingly, in some study sites (geothermal springs and arctic lakes; Figure 1, Figure S2), the network analysis revealed clustering of gammaproteobacterial

and alphaproteobacterial MOB, and their associated non-MOB communities, suggesting that MOB species have distinct associated communities. This may point to differences in amount and composition of metabolites exchanged by MOB species. The clustering according to MOB subgroups is not obvious in the grassland soil (Figure S3), but a higher connectivity (degree of connectedness) was observed among microorganisms associated to the alphaproteobacterial MOB, indicating more complex routes of transfer of metabolites or a higher diversity of MOB “compatible” microorganisms in this habitat. Similarly, there were no obvious clusters in the rice paddy soil, but a higher connectivity was observed among the gammaproteobacterial MOB (Figure S4). The dominant non-MOB community (>1% relative abundance) associated to either the alphaproteobacterial or gammaproteobacterial MOB appears to be distinct within the study sites with the exception of *Xanthomonadaceae* in the grassland soil, albeit different genera were associated to the different MOB subgroups (e.g., Figures S1B,C). Between the study sites, families *Anaerolineaceae* and *Caulobacteraceae* (represented by different genera) were found to be associated to both MOB subgroups (e.g., Figures S1B,C). Hence, *in lieu* of lower taxonomic ranks (family/genus level), we focused on the communities which appear to be specific to the gammaproteobacterial and alphaproteobacterial MOB at the

order level where some members seemingly converged after incubation (**Figure S1**). For instance, in the arctic lake sediment and grassland soil, OTUs affiliated to *Rhizobiales* were associated with *Methylosinus* (alphaproteobacterial MOB), whereas the non-MOB community associated with gammaproteobacterial MOB was more diverse with *Pseudomonadales* occurring in both sites. Although the accompanying non-MOB community differs, this trend was consistent in the sediment from the geothermal spring where *Rhodospirillales* was the only dominant order (>1% relative abundance) associated to *Methylosinus* (**Figure 1**, **Table S1**). *Rhodospirillales* was also consistently found to be associated with the alphaproteobacterial MOB in other sites (**Figure S1**). Given that only gammaproteobacterial MOB were predominantly active in the oilsands tailing ponds, a clustering based on MOB subgroups was not observed (**Figure S5**). Admittedly, considering higher taxonomic ranks will render considerable overlaps in the community composition. Yet, out of 27 dominant OTUs (order level), only *Xanthomonadales* was found in all sites with the exception of the arctic lake sediment, indicating that the occurrence of the methane-fueled community was site-and MOB-species specific. The site-to-site variation of the methane-driven community is not unexpected considering the different soil/sediment edaphic characteristics and environmental conditions of the study sites spanning across three continents (Knief, 2015). However, we cannot completely exclude methodological artifacts inherent to PCR-based studies (arising from high throughput sequencing). Further studies are needed to determine the spatial and temporal dynamics of the interacting partners. Nonetheless, the clustering and association of distinct accompanying microorganisms to the gammaproteobacterial and alphaproteobacterial MOB, more evident in some sites (sediments from the arctic lake and geothermal springs) than others, suggest a selection of interacting partners, possibly through different amounts and/or types of metabolites excreted.

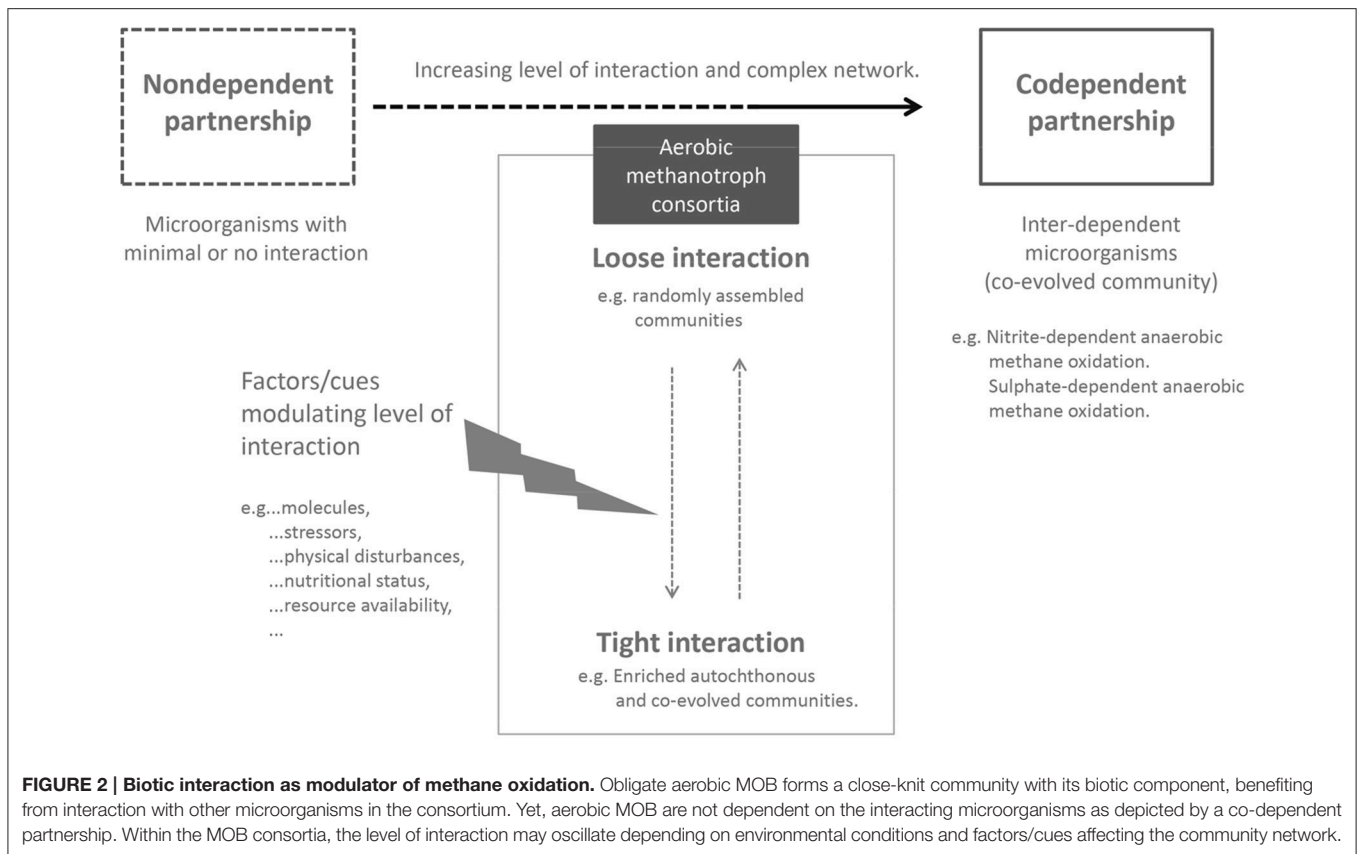
Additionally, despite the different incubation conditions and the length of incubation (**Table 1**), the network analysis revealed the co-occurrence of methanol-oxidizers (e.g., *Methylotenera*, *Methylobacterium*, *Methylobacillus*, *Methylohalomonas*) and MOB in all sites, which is in accordance with a previous study where a high relative abundance (up to 40–50% of total community; Oshkin et al., 2014) of methylotrophs (e.g., *Methylotenera*, *Methylophilus*) was found in a methane-enriched community. It is often hypothesized that the co-detection of methylotrophs and methanotrophs in SIP studies are caused by cross-feeding; the methylotrophs feed on the methanol derived from methane oxidation. While cross-feeding is likely a cause for the co-occurrence of methylotrophs and MOB, the detection of other active microorganisms associated to the different MOB subgroups within each site may not be a stochastic event, but supports the notion of a selection of specific accompanying community members (Oshkin et al., 2014), particularly after several transferring steps (Yu et al., 2016). However, the network analysis was derived from ^{13}C - CH_4 labeling studies representing a snapshot of the active community. Hence, while our meta-analysis lacks in temporal and spatial scales, and is limited by the availability of physico-chemical data, it provides a first insight

into the active MOB interactome. We showed that combining DNA-SIP to a co-occurrence network analysis is a powerful tool to relate interaction of active microorganisms. This approach, when applied to well-designed experimentation in future studies will divulge the robustness of an interacting community as well as the mechanisms of interaction by tracking the labeled metabolites.

MODE OF MOB INTERACTION

Co-occurrence networks in microbial ecology visualize the positive and/or negative correlations between all members of several microbial communities (typically OTUs), and help predicting ecological interactions (Faust and Raes, 2012). The network analysis, however, does not reveal the mode of the interaction. In ^{13}C - CH_4 labeling studies, all non-MOB are positively correlated with the MOB, benefiting from their association with the MOB (*via* cross-feeding), although an antagonistic interaction (nature of the interaction) may also occur (e.g., predation). Biotic interactions can exert a direct and/or indirect effect, modulating process rates (Murase and Frenzel, 2007; Daebeler et al., 2014; Ho et al., 2014), and possibly, structuring the microbial community composition (Murase et al., 2006; Murase and Frenzel, 2007; Yu et al., 2016). In direct interaction, MOB/satellite communities release metabolites which exert a direct response, either mutually benefiting or adversely affecting the interacting partner. Despite their proven ability to synthesize and exude (secondary) metabolites (e.g., acetate, succinate, lactate; Kalyuzhnaya et al., 2013; ectoine: Reshetnikov et al., 2006; Khmelenina et al., 2015; methanobactin: Kim et al., 2004), the role of these compounds in MOB interaction remains largely unexplored. In particular, volatile secondary metabolites, being able to exert an effect even across physical barriers, have yet unknown ways of eliciting a response in community functioning (Schmidt et al., 2015). Indeed, it was only recently that studies began to show the importance of some secondary metabolites expressed and detected only when microorganisms were co-cultured (Watrous et al., 2012; Tyc et al., 2014), suggesting an overlooked interaction-induced mechanism to produce/release compounds.

Conversely, these metabolites may accumulate to prohibitive levels for both the MOB and other microorganisms, such as in the case of hydroxylamine and methanol, intermediary compounds of ammonium and methane oxidation, respectively, which may inhibit methanotrophic activity (Bodelier and Laanbroek, 2004; Poret-Peterson et al., 2008; Bodelier, 2011). “One man’s meat is another man’s poison” (*sic*); because functional traits among MOB vary (Ho et al., 2013b; Hoefman et al., 2014), some MOB and accompanying members of the consortium may consume the inhibitory compounds, thereby relieving toxicity, and facilitate growth and activity of other microorganisms in an indirect interaction. Indeed, a cooperative endeavor to relief inhibition between MOB and methylotrophs, as well as heterotrophs has been inferred in enrichment and co-culture studies (i.e., Beck et al., 2013; Stock et al., 2013; Oshkin et al., 2014), as in our



network analyses where methylotrophs were consistently shown to be dominant members of the accompanying community in all study sites. Therefore, two modes (direct and/or indirect) of interaction underlie community patterns and functioning.

Although, obligate MOB may not be solely dependent on other microorganisms, current understanding clearly shows their reliance on other interacting partners to facilitate survival and growth. To this end, our network analyses provide support for the potential selection of interacting partners specific to the different MOB subgroups. We postulate that MOB in interaction with their satellite microorganisms represent a close-knit association, but are not exclusive. Close cooperation (e.g., as a result of metabolic inter-dependencies) between microorganisms drives their co-occurrence (Fiegna et al., 2015; Zelezniak et al., 2015), which may evolve to become a co-dependent relationship (Figure 2; Morris et al., 2012). Exemplifying a potentially co-dependent interaction, the nitrite-dependent anaerobic methane oxidizer, *Candidatus Methyloirabilis oxyfera* has so far resisted purification but could be highly enriched (Ettwig et al., 2010). The gene cluster encoding the enzymes catalyzing the reduction of nitric oxide to molecular nitrogen and oxygen, a key pathway in the proposed scheme to self-oxygenate in *Ca. M. oxyfera* was undetected in the microorganism (Ettwig et al., 2010). Consequently, the authors suggest that the missing catalytic activity may be complemented by the action of other interacting partners in the enrichment. Such co-dependent interaction is true for

another specialized process i.e., sulfate-dependent anaerobic methane oxidation (see reviews by Valentine and Reeburgh, 2000; Stams and Plugge, 2009). It stands to reason that a division of labor by splitting complex metabolic pathways or exchanging intermediate products (e.g., electron transfer; McGlynn et al., 2015) between multiple participants is a practical solution to overcome a lack of metabolic capacity and energetically demanding processes. An exception to the rule is cheater-microorganisms that exploit the cooperative interaction by imposing a cost on the cooperating partners, while benefiting themselves. The role of cheaters in the social behavior of microorganisms is recognized (Crespi, 2001), but is not yet firmly established in interacting MOB communities. On the other hand, the methane oxidation rate was significantly stimulated in co-cultures containing a high diversity of interacting partners although these accompanying microorganisms were randomly selected, and had not co-evolved with the methanotroph (Ho et al., 2014). Similarly, the combinations of methanotrophs and heterotrophs in a study showing a higher growth response in some co-cultures were randomly selected from a culture collection (Belgian Coordinated Collections of Microorganisms/Laboratory of Microbiology-Gent University, Gent, Belgium; Stock et al., 2013). Hence, synergistic microbial interactions may not necessarily be exclusive and restricted to co-evolved communities, which questions the predominant modes of interaction in different (co-evolved) communities.

CONCLUDING REMARKS

With emerging evidence, biotic interactions are gaining more recognition as important modulators of biogeochemical processes. However, similar to other attributes of microbial communities (e.g., diversity, traits), this “parameter” is not as well integrated in biogeochemical models designed to predict ecosystem functioning as well as processes (van de Leemput et al., 2011; Bouskill et al., 2012). Incorporation of explicit microbial traits into biogeochemical models, for example decomposition or greenhouse gas emission models, is starting to develop increasing predictive power (Treseder et al., 2012; Wieder et al., 2013, 2015; Wang et al., 2015) compared to traditional models. However, parameterizing of these models will require detailed knowledge on the breadth of trait responses and trade-offs in various microbial groups and processes which will very likely be strongly dependent on microbial interactions. The lack of this knowledge on traits and the role of biotic interactions in combination with the absence of a unifying framework to assess and determine when biotic interaction becomes relevant are the most important inadequacies, hindering integration of “biotic interaction” as a parameterized input in existing biogeochemical models. The association of ecosystem functioning with community diversity, traits, and abundances has been assessed *in vitro* based on experimental manipulation studies in the laboratory (Bell et al., 2005; Wertz et al., 2006; Wittebolle et al., 2009; Ho et al., 2011). Admittedly, microbial interactions underlie an array of relationships, shaping community composition, and although microbial interactions have been shown to be relevant controls of ecosystem functioning (Stock et al., 2013; Daebeler et al., 2014; Ho et al., 2014; Jeong et al., 2014), it remains a challenge to disentangle community interaction from other relationships (e.g., biodiversity-ecosystem functioning). Hence, microbial interaction is an integral component, often confounded, but seldom explicitly tested in complex communities particularly in the context of biogeochemical cycles. This reverberates previous calls for a more integrated approach, including microbial interaction when elucidating the response of community composition to environmental cues (Comolli, 2014; Lupatini et al., 2014). In complex environments, interaction may well be a key neglected determinant, if not as important as diversity and community abundance, driving ecosystem functioning. Hence, there is a need to move beyond our current understanding of relating biodiversity (richness and evenness) and abundance to ecosystem functioning in environmental studies; “biotic interaction” as a modulator of ecological processes warrants further attention.

Disentangling biotic interaction from other environmental parameters altering process rates is challenging. Nevertheless, this challenge may be partly circumvented by experimental setups capitalizing on artificially assembled communities (De Roy et al., 2013, 2014; Stenuit and Agathos, 2015). A synthetic community provides a well-defined biotic environment, allowing the assembly of communities comprising well-characterized microorganisms with available genomes, to reduce complexity in interaction. Therefore, synthetic communities facilitate understanding of the underlying mechanism of the interaction

(e.g., bacteria–fungal interaction: Schneider et al., 2010; microbe–microbe interaction: Beliaev et al., 2014). However, because of the reduced complexity, it is not entirely surprising that community functioning in synthetic communities may not reflect on the behavior of naturally-occurring communities in the environment (Yu et al., 2016). Although, general compositional dynamics of the methanotrophs in synthetic communities have been shown to resemble dynamics of natural communities, the similarities were not observed at the species level (Yu et al., 2016). Nevertheless, knowledge gained from synthetic community studies may help predict community response. For instance, as shown by Stock et al. (2013), microbe–microbe interaction models can be “trained” (cross-validation techniques in supervised learning of predictive models; Hastie et al., 2009) to predict co-culture response. The input of the predictive model was derived from the growth response of a subset of MOB and heterotroph combinations, which was subsequently used for predicting all possible combinations of MOB and heterotrophs. *In lieu* of determining the growth response of all combinations of the co-cultures, the values were inferred using the predictive model (Stock et al., 2013).

Accordingly, microbial interaction may become important under certain conditions (Figure 2). For instance, a more complex microbial network may arise as a response to limiting substrate availability, forcing metabolic exchange and increase co-occurrence (Zelezniak et al., 2015). Moreover, there is a myriad of secondary (volatile) compounds secreted by microorganisms to the environment. These compounds may act as signaling molecules and have yet unknown ways of modulating process rates (Schulz-Bohm et al., 2015). For example, some secondary compounds (resuscitating-promoting factors; Lennon and Jones, 2011), as well as a shift in temperature (Ho and Frenzel, 2012; Ho et al., 2016) may awaken the dormant population, effectively contributing to the active members of a community. Only when we understand the mechanisms of interaction, can we predict the response of community functioning which calls for a strong focus on mechanistic studies using representative microbes, catalyzing a relevant biogeochemical process.

AUTHOR CONTRIBUTIONS

AH, PB conceived the review and analysis. RA performed the analyses with input from AD, AH, and PB. AH wrote the initial manuscript. RA, AV, AD, ZJ, SK, FK, NB, and PB critically revised and approved the manuscript. All authors are accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmicb.2016.01285>

Figure S1 | Venn diagrams showing co-occurring non-MOB community (order level) in all environments (A), as well as the accompanying non-MOB community associated to the alphaproteobacterial (B), and gammaproteobacterial (C) MOB where relevant. MOB and unclassified microorganisms are not included in the Venn diagrams. Blue, green, orange, gray, and turquoise denote samples collected from a geothermal spring sediment, grassland soil, arctic lake sediment, oilsands tailing ponds sediment, and rice paddy soil, respectively.

Figure S2 | Network analysis of 16s rRNA gene sequences derived from the “heavy” fraction of a $^{13}\text{C}\text{-CH}_4$ labeled community in sediments from an arctic lake (He et al., 2012a,b,c). The correlating OTUs with >1% relative abundance are given in the figure, and the corresponding taxonomic affiliation are listed in the Supplementary Information (Table S2).

Figure S3 | Network analysis of 16s rRNA gene sequences derived from the “heavy” fraction of a $^{13}\text{C}\text{-CH}_4$ labeled community in a grassland soil (Daebeler et al., 2014). The correlating OTUs with >1% relative abundance are given in the figure, and the corresponding taxonomic affiliation are listed in the Supplementary Information (Table S3).

REFERENCES

- Abrudan, M. I., Smakman, F., Grimbergen, A. J., Westhoff, S., Miller, E. L., van Wezel, G. P., et al. (2015). Socially mediated induction and suppression of antibiosis during bacterial coexistence. *Proc. Natl. Acad. Sci. U.S.A.* 112, 11054–11059. doi: 10.1073/pnas.1504076112
- Agasild, H., Zingel, P., Tuvikene, L., Tuvikene, A., Timm, H., Feldmann, T., et al. (2014). Biogenic methane contributes to the food web of a large, shallow lake. *Freshwat. Biol.* 59, 272–285. doi: 10.1111/fwb.12263
- Amin, S. A., Hmelo, L. R., van Tol, H. M., Durham, B. P., Carlson, L. T., Heal, K. R., et al. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522, 98–101. doi: 10.1038/nature14488
- Audrain, B., Farag, M. A., Ryu, C.-M., and Ghigo, J.-M. (2015). Role of bacterial volatile compounds in bacterial biology. *FEMS Microbiol. Ecol.* 39, 222–233. doi: 10.1093/femsre/fuu013
- Beck, D. A. C., Kalyuzhnaya, M. G., Malfatti, S., Tringe, S. G., del Rio, T. G., Ivanova, N., et al. (2013). A metagenomic insight into freshwater methane-utilizing communities and evidence for cooperation between the *Methylococcaceae* and the *Methylophilaceae*. *Peer J.* 1:e23. doi: 10.7717/peerj.23
- Beliaev, A. S., Romine, M. F., Serres, M., Bernstein, H. C., Linggi, B. E., Markillie, L. M., et al. (2014). Inference of interactions in cyanobacterial-heterotrophic co-cultures via transcriptome sequencing. *ISME J.* 8, 2243–2255. doi: 10.1038/ismej.2014.69
- Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., and Lilley, A. K. (2005). The contribution of species richness and composition to bacterial services. *Nature* 436, 1157–1160. doi: 10.1038/nature03891
- Bodelier, P. L. E. (2011). Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. *Curr. Opin. Environ. Sust.* 3, 379–388. doi: 10.1016/j.cosust.2011.06.002
- Bodelier, P. L. E., and Laanbroek, H. J. (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol. Ecol.* 47, 265–277. doi: 10.1016/S0168-6496(03)00304-0
- Bouskill, N. J., Tang, J., Riley, W. J., and Brodie, E. L. (2012). Trait-based representation of biological nitrification: model development, testing, and predicted community composition. *Front. Microbiol.* 3:364. doi: 10.3389/fmicb.2012.00364
- Burke, D. J., Smemo, K. A., Lopez-Gutierrez, J., and DeForest, J. L. (2012). Soil fungi influence the distribution of microbial functional groups that mediate forest greenhouse gas emissions. *Soil Biol. Biochem.* 53, 112–119. doi: 10.1016/j.soilbio.2012.05.008
- Comolli, L. R. (2014). Intra- and inter-species interactions in microbial communities. *Front. Microbio.* 5:629. doi: 10.3389/fmicb.2014.00629
- Crespi, B. J. (2001). The evolution of social behavior in microorganisms. *Trends Ecol. Evol.* 16, 178–183. doi: 10.1016/S0169-5347(01)02115-2
- Csardi, G., and Nepusz, T. (2006). *The Igraph Software Package for Complex Network Research*. InterJournal Complex System. Available online at: <http://igraph.sf.net>
- Daebeler, A., Bodelier, P. L. E., Yan, Z., Hefting, M. M., Jia, Z., and Laanbroek, H. J. (2014). Interactions between *Thaumarchaea*, *Nitrospira* and methanotrophs modulate autotrophic nitrification in volcanic grassland soil. *ISME J.* 8, 2397–2410. doi: 10.1038/ismej.2014.81
- De Boer, W., and van der Wal, A. (2008). “Interactions between saprotrophic and basidiomycetes and bacteria,” in *Ecology of Saprotrophic and Basidiomycetes*, eds L. Boddy, J. C. Frankland, and P. van West (London: Elsevier Academic Press), 143–153.
- Dedysh, S. N., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Semrau, J. D., Liesack, W., et al. (2002). *Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and dinitrogen-fixing acidophilic bacterium from Sphagnum bog. *Int. J. Syst. Evol. Microbiol.* 52, 251–261. doi: 10.1099/00207713-52-1-251
- De Roy, K., Marzorati, M., Negroni, A., Thas, O., Balloi, A., Fava, F., et al. (2013). Environmental conditions and community evenness determine the outcome of biological invasion. *Nat. Commun.* 4:1383. doi: 10.1038/ncomms2392
- De Roy, K., Marzorati, M., Van den Abbeele, P., Van de Wiele, T., and Boon, N. (2014). Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environ. Microbiol.* 16, 1471–1481. doi: 10.1111/1462-2920.12343

- Dumont, M. G., Pommerenke, B., Casper, P., and Conrad, R. (2011). DNA-, rRNA- and mRNA-based stable isotope probing of aerobic methanotrophs in lake sediment. *Environ. Microbiol.* 13, 1153–1167. doi: 10.1111/j.1462-2920.2010.02415.x
- Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998. doi: 10.1038/nmeth.2604
- Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M., et al. (2010). Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 464, 543–548. doi: 10.1038/nature08883
- Faust, K., and Raes, J. (2012). Microbial interactions: from networks to models. *Nat. Rev. Microbiol.* 10, 538–550. doi: 10.1038/nrmicro2832
- Fiegna, F., Moreno-Letelier, A., Bell, T., and Barraclough, T. G. (2015). Evolution of species interactions determines microbial community productivity in new environments. *ISME J.* 9, 1235–1245. doi: 10.1038/ismej.2014.215
- Friedman, J., and Alm, E. J. (2012). Inferring correlation networks from genomic survey data. *PLoS Comput. Biol.* 8:1002687. doi: 10.1371/journal.pcbi.1002687
- Halm, H., Musat, N., Lam, P., Langlois, R., Musat, F., Peduzzi, S., et al. (2009). Co-occurrence of denitrification and nitrogen fixation in a meromictic lake, Lake Cadagno (Switzerland). *Environ. Microbiol.* 11, 1945–1958. doi: 10.1111/j.1462-2920.2009.01917.x
- Hastie, T., Tibshirani, R., and Friedman, J. (2009). *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. Berlin: Springer.
- He, R., Woller, M. J., Pohlman, J. W., Quensen, J., Tiedje, J. M., and Leigh, M. B. (2012a). Shifts in identity and activity of methanotrophs in arctic lake sediments in response to temperature changes. *Appl. Environ. Microbiol.* 78, 4715–4723. doi: 10.1128/AEM.00853-12
- He, R., Woller, M. J., Pohlman, J. W., Quensen, J., Tiedje, J. M., and Leigh, M. B. (2012c). Diversity of active aerobic methanotrophs along depth profiles of arctic and subarctic lake water column and sediments. *ISME J.* 6, 1937–1948. doi: 10.1038/ismej.2012.34
- He, R., Wooller, M. J., Pohlman, J. W., Catranis, C., Quensen, J., Tiedje, J. M., et al. (2012b). Identification of functionally active aerobic methanotrophs in sediments from an arctic lake using stable isotope probing. *Environ. Microbiol.* 14, 1403–1419. doi: 10.1111/j.1462-2920.2012.02725.x
- Ho, A., and Bodelier, P. L. E. (2015). Diazotrophic methanotrophs in peatlands: the missing link? *Plant Soil* 389, 419–423. doi: 10.1007/s11104-015-2393-9
- Ho, A., de Roy, K., Thas, O., De Neve, J., Hoefman, S., Vandamme, P., et al. (2014). The more, the merrier: heterotroph richness stimulates methanotrophic activity. *ISME J.* 8, 1945–1948. doi: 10.1038/ismej.2014.74
- Ho, A., Erens, H., Mujinya, B. B., Boeckx, P., Baert, G., Schneider, B., et al. (2013a). Termites facilitate methane oxidation and shape the methanotrophic community. *Appl. Environ. Microbiol.* 79, 7234–7240. doi: 10.1128/AEM.02785-13
- Ho, A., and Frenzel, P. (2012). Heat stress and methane-oxidizing bacteria: effects on activity and population dynamics. *Soil Biol. Biochem.* 50, 22–25. doi: 10.1016/j.soilbio.2012.02.023
- Ho, A., Kerckhof, F.-M., Luke, C., Reim, A., Krause, S., Boon, N., et al. (2013b). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environ. Microbiol. Rep.* 5, 335–345. doi: 10.1111/j.1758-2229.2012.00370.x
- Ho, A., Lüke, C., and Frenzel, P. (2011). Recovery of methanotrophs from disturbance: population dynamics, evenness and functioning. *ISME J.* 5, 750–758. doi: 10.1038/ismej.2010.163
- Ho, A., Lüke, C., Reim, A., and Frenzel, P. (2016). Resilience of (seed bank) aerobic methanotrophs and methanotrophic activity to desiccation and heat stress. *Soil Biol. Biochem.* 101, 130–138. doi: 10.1016/j.soilbio.2016.07.015
- Hoefman, S., van der Ha, D., Boon, N., Vandamme, P., De Vos, P., and Heylen, K. (2014). Niche differentiation in nitrogen metabolism among methanotrophs within an operational taxonomic unit. *BMC Microbiol.* 14:83. doi: 10.1186/1471-2180-14-83
- Hutchens, E., Radajewski, S., Dumont, M. G., McDonald, I., and Murrell, C. (2004). Analysis of methanotrophic bacteria in Movile Cave by stable isotope probing. *Environ. Microbiol.* 6, 111–120. doi: 10.1046/j.1462-2920.2003.00543.x
- Iguchi, H., Yurimoto, H., and Sakai, Y. (2011). Stimulation of methanotrophic growth in cocultures by cobalamin excreted by Rhizobia. *Appl. Environ. Microbiol.* 77, 8509–8515. doi: 10.1128/AEM.05834-11
- Jeong, S.-Y., Cho, K.-S., and Kim, T. G. (2014). Density-dependent enhancement of methane oxidation activity and growth of *Methylocystis* sp. by a non-methanotrophic bacterium *Sphingopyxis* sp. *Biotechnol. Rep.* 4, 128–133. doi: 10.1016/j.btre.2014.09.007
- Kalyuzhnaya, M. G., Yang, S., Rozova, O. N., Smalley, N. E., Clubb, J., Lamb, A., et al. (2013). Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. *Nat. Commun.* 4, 2785–2790. doi: 10.1038/ncomms3785
- Kerckhof, F.-M., Courtens, E. N. P., Geirnaert, A., Hoefman, S., Ho, A., Vilchez-Vargas, R., et al. (2014). Optimized cryopreservation of mixed microbial communities for conserved functionality and diversity. *PLoS ONE* 9:99517. doi: 10.1371/journal.pone.0099517
- Khmelenina, V. N., Rozova, O. N., But, S. Y., Mustakhimov, I. I., Reshetnikov, A. S., Beschastnyi, A. P., et al. (2015). Biosynthesis of secondary metabolites in methanotrophs: biochemical and genetic aspects (Review). *Appl. Biochem. Microbiol.* 51, 150–158. doi: 10.1134/S0003683815020088
- Kim, H. J., Graham, D. W., DiSpirito, A. A., Alterman, M. A., Galeva, N., Larive, C. K., et al. (2004). Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305, 1612–1615. doi: 10.1126/science.1098322
- Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. *Front. Microbiol.* 6:1346. doi: 10.3389/fmicb.2015.01346
- Kox, M. A. R., Lüke, C., Fritz, C., van den Elzen, E., van Alen, T., Op den Camp, H. J. M., et al. (2016). Effects of nitrogen fertilization on diazotrophic activity of microorganisms associated with *Sphagnum magellanicum*. *Plant Soil*. doi: 10.1007/s11104-016-2851-z. [Epub ahead of print].
- Larmola, T., Leppänen, S. M., Tuittila, E.-S., Aarva, M., Merilä, P., Fritze, H., et al. (2014). Methanotrophy induces nitrogen fixation during peatland development. *Proc. Natl. Acad. Sci. U.S.A.* 111, 734–739. doi: 10.1073/pnas.1314284111
- Lennon, J. T., and Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* 9, 119–130. doi: 10.1038/nrmicro2504
- Lupatini, M., Suleiman, A. K. A., Jacques, R. J. S., Antoniolli, Z. I., Ferreira, A. S., Kuramae, E. E., et al. (2014). Network topology reveals high connectance levels and few key microbial genera within soils. *Front Environ. Sci.* 2:10. doi: 10.3389/fenv.2014.00010
- Martineau, C., Whyte, L. G., and Greer, C. W. (2010). Stable isotope probing analysis of the diversity and activity of methanotrophic bacteria in soils from the Canadian high arctic. *Appl. Environ. Microbiol.* 76, 5773–5784. doi: 10.1128/AEM.03094-09
- McGlynn, S. E., Chadwick, G. L., Kempes, C. P., and Orphan, V. J. (2015). Single cell activity reveals direct electron transfer in methanotrophic consortia. *Nature* 526, 531–535. doi: 10.1038/nature15512
- Milucka, J., Kirf, M., Lu, L., Krupke, A., Lam, P., Littmann, S., et al. (2015). Methane oxidation coupled to oxygenic photosynthesis in anoxic waters. *ISME J.* 9, 1991–2002. doi: 10.1038/ismej.2015.12
- Morris, B. E. L., Henneberger, R., Huber, H., and Moissl-Eichinger, C. (2013). Microbial syntrophy: interaction for the common good. *FEMS Microbiol. Rev.* 37, 384–406. doi: 10.1111/1574-6976.12019
- Morris, J. J., Lenski, R. E., and Zinser, E. R. (2012). The black queen hypothesis: evolution of dependencies through adaptive gene loss. *MBio* 3, 00036–00012. doi: 10.1128/mBio.00036-12
- Müller, J., and Overmann, J. (2011). Close interspecies interactions between prokaryotes from sulfurous environments. *Front. Microbiol.* 2:146. doi: 10.3389/fmicb.2011.00146
- Murase, J., and Frenzel, P. (2007). A methane-driven microbial food web in a wetland rice soil. *Environ. Microbiol.* 9, 3025–3034. doi: 10.1111/j.1462-2920.2007.01414.x
- Murase, J., and Frenzel, P. (2008). Selective grazing of methanotrophs by protozoa in a rice field soil. *FEMS Microbiol. Ecol.* 65, 408–414. doi: 10.1111/j.1574-6941.2008.00511.x
- Murase, J., Noll, M., and Frenzel, P. (2006). Impact of protists on the activity and structure of the bacterial community in a rice field soil. *Appl. Environ. Microbiol.* 72, 5436–5444. doi: 10.1128/AEM.00207-06
- Oliveira, N. M., Niehus, R., and Foster, K. R. (2014). Evolutionary limits to cooperation in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 111, 17941–17946. doi: 10.1073/pnas.1412673111
- Oshkin, I. Y., Beck, D. A. C., Lamb, A. E., Tchesnokova, V., Benuska, G., McTaggart, T. L., et al. (2014). Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. *ISME J.* 9, 1119–1129. doi: 10.1038/ismej.2014.203
- Poret-Peterson, A. T., Graham, J. E., Gulledege, J., and Klotz, M. G. (2008). Transcription of nitrification genes by the methane oxidizing

- bacterium, *Methylococcus capsulatus* strain Bath. *ISME J.* 2, 1213–1220. doi: 10.1038/ismej.2008.71
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., et al. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35, 7188–7196. doi: 10.1093/nar/gkm864
- Putkinen, A., Larmola, T., Tuomivirta, T., Siljanen, H. M., Bodrossy, L., Tuittila, E.-S., et al. (2012). Water dispersal of methanotrophic bacteria maintains functional methane oxidation in *Sphagnum* mosses. *Front Microbiol.* 3:15. doi: 10.3389/fmicb.2012.00015
- Raghoebarsing, A. A., Smolders, A. J. P., Schmid, M. C., Rijpstra, W. I. C., Wolters-Arts, M., Derksen, J., et al. (2005). Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature* 436, 1153–1156. doi: 10.1038/nature03802
- R Core Team (2014). *R: A language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Reshetnikov, A. S., Khmelenina, V. N., and Trotsenko, Y. A. (2006). Characterization of the ectoine biosynthesis genes of haloalkalotolerant obligate methanotroph “*Methylomicrobium alcaliphilum* 20Z”. *Arch. Microbiol.* 184, 286–297. doi: 10.1007/s00203-005-0042-z
- Saidi-Mehrabad, A., He, Z., Tamas, I., Sharp, C. E., Brady, A. L., Rochman, F. F., et al. (2013). Methanotrophic bacteria in soils and tailings ponds of northern Alberta. *ISME J.* 7, 908–921. doi: 10.1038/ismej.2012.163
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. doi: 10.1128/AEM.01541-09
- Schmidt, R., Cordovez, V., de Boer, W., Raaijmakers, J., and Garbeva, P. (2015). Volatile affairs in microbial interactions. *ISME J.* 9, 2329–2335. doi: 10.1038/ismej.2015.42
- Schneider, T., Gerrits, B., Gassmann, R., Schmid, E., Gessner, M. O., Richter, A., et al. (2010). Proteome analysis of fungal and bacteria involvement in leaf litter decomposition. *Proteomics* 10, 1819–1830. doi: 10.1002/pmic.200900691
- Schulz-Bohm, K., Zweers, H., De Boer, W., and Garbeva, P. (2015). A fragrant neighborhood: volatile mediated bacterial interactions in soils. *Front. Microbiol.* 6:1212. doi: 10.3389/fmicb.2015.01212
- Semrau, J. D., DiSpirito, A. A., and Yoon, S. (2010). Methanotrophs and copper. *FEMS Microbiol. Rev.* 34, 496–531. doi: 10.1111/j.1574-6976.2010.00212.x
- Sharp, C. E., Martínez-Lorenzo, A., Brady, A. L., Grasby, S. E., and Dunfield, P. F. (2014). Methanotrophic bacteria in warm geothermal spring sediments identified using stable-isotope probing. *FEMS Microbiol. Ecol.* 90, 92–102. doi: 10.1111/1574-6941.12375
- Stams, A. J. M., and Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat. Rev. Microbiol.* 7, 568–577. doi: 10.1038/nrmicro2166
- Stenuit, B., and Agathos, S. N. (2015). Deciphering microbial community robustness through synthetic ecology and molecular systems snycology. *Curr. Opi. Biotechnol.* 33, 305–317. doi: 10.1016/j.copbio.2015.03.012
- Stock, M., Hoefman, S., Kerckhof, F.-M., Boon, N., de Vos, P., de Baets, B., et al. (2013). Exploration and prediction of interactions between methanotrophs and heterotrophs. *Res. Microbiol.* 10, 1045–1054. doi: 10.1016/j.resmic.2013.08.006
- Treseder, K. K., Balsler, T. C., Bradford, M. A., Brodie, E. L., Dubinsky, E. A., Eviner, V. T., et al. (2012). Integrating microbial ecology into ecosystem models: challenges and priorities. *Biogeochemistry* 109, 7–18. doi: 10.1007/s10533-011-9636-5
- Tyc, O., van den Berg, M., Gerards, S., van Veen, J. A., Raaijmakers, J. M., de Boer, W., et al. (2014). Impact of interspecific interactions on antimicrobial activity among soil bacteria. *Front. Microbiol.* 5:567. doi: 10.3389/fmicb.2014.00567
- Valentine, D. L., and Reeburgh, W. S. (2000). New perspectives on anaerobic methane oxidation. *Environ. Microbiol.* 2, 477–484. doi: 10.1046/j.1462-2920.2000.00135.x
- van de Leemput, I. A., Veraart, A. J., Dakos, V., de Klein, J. M. J., Strous, M., and Scheffer, M. (2011). Predicting microbial nitrogen pathways from basic principles. *Environ. Microbiol.* 13, 1477–1487. doi: 10.1111/j.1462-2920.2011.02450.x
- Van der Ha, D., Bundervoet, B., Verstraete, W., and Boon, N. (2011). A sustainable, carbon neutral methane oxidation by a partnership of methane oxidizing communities and microalgae. *Water Res.* 45, 2845–2854. doi: 10.1016/j.watres.2011.03.005
- Vile, M. A., Wieder, R. K., Zivkovic, T., Scott, K. D., Vitt, D. H., Hartsock, J. A., et al. (2014). N₂-fixation by methanotrophs sustain carbon and nitrogen accumulation in pristine peatlands. *Biogeochemistry* 121, 317–328. doi: 10.1007/s10533-014-0019-6
- Wang, G. S., Jagadamma, S., Mayes, M. A., Schadt, C. W., Steinweg, J. M., Gu, L. H., et al. (2015). Microbial dormancy improves development and experimental validation of ecosystem model. *ISME J.* 9, 226–237. doi: 10.1038/ismej.2014.120
- Watrous, J., Roach, P., Alexandrov, T., Heath, B. S., Yang, J. Y., Kersten, R. D., et al. (2012). Mass spectral molecular networking of living microbial colonies. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1743–1752. doi: 10.1073/pnas.1203689109
- Watsuji, T. O., Nakagawa, S., Tsuchida, S., Toki, T., Hirota, A., Tsunogai, U., et al. (2010). Diversity and function of epibiotic microbial communities on the Galatheid Crab, *Shinkaia crosnieri*. *Microb. Environ.* 25, 288–294. doi: 10.1264/jisme2.ME10135
- Watsuji, T. O., Yamamoto, A., Takaki, Y., Ueda, K., Kawagucci, S., and Takai, K. (2014). Diversity and methane oxidation of active epibiotic methanotrophs on live *Shinkaia crosnieri*. *ISME J.* 8, 1020–1031. doi: 10.1038/ismej.2013.226
- Wendeborg, A., Zielinski, F. U., Borowski, C., and Dubilier, N. (2012). Expression patterns of mRNAs for methanotrophy and thiotrophy in symbionts of the hydrothermal vent mussel *Bathymodiolus puteoserpentis*. *ISME J.* 6, 104–112. doi: 10.1038/ismej.2011.81
- Wertz, S., Degrange, V., Prosser, J. I., Poly, F., Commeaux, C., Freitag, T., et al. (2006). Maintenance of soil functioning following erosion of microbial diversity. *Environ. Microbiol.* 8, 2162–2169. doi: 10.1111/j.1462-2920.2006.01098.x
- Wieder, W. R., Bonan, G. B., and Allison, S. D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nat. Clim. Change* 3, 909–912. doi: 10.1038/nclimate1951
- Wieder, W. R., Grandy, A. S., Kallenback, C. M., Taylor, P. G., and Bonan, G. B. (2015). Representing life in the Earth system with soil microbial functional traits in the MIMICS model. *Geosci. Mod. Develop.* 8, 1789–1808. doi: 10.5194/gmd-8-1789-2015
- Willett, J. L. E., Gucinski, G. C., Fatherree, J. P., Low, D. A., and Hayes, C. S. (2015). Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways. *Proc. Natl. Acad. Sci. U.S.A.* 112, 11341–11346. doi: 10.1073/pnas.1512124112
- Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., et al. (2009). Initial community evenness favors functionality under selective stress. *Nature* 458, 623–626. doi: 10.1038/nature07840
- Yu, Z., Krause, S. M. B., Beck, D. A. C., and Chistoserdova, L. (2016). A synthetic ecology perspective: How well does behavior of model organisms in the laboratory predict microbial activities in natural habitats? *Front. Microbiol.* 7:946. doi: 10.3389/fmicb.2016.00946
- Zbinden, M., Shillito, B., Le Bris, N., de Montlaur, C. D., Roussel, E., Guyot, F., et al. (2008). New insights on the metabolic diversity among the epibiotic microbial community of the hydrothermal shrimp *Rimicaris exoculata*. *J. Exp. Mar. Biol. Ecol.* 359, 131–140. doi: 10.1016/j.jembe.2008.03.009
- Zelezniak, A., Andrejev, S., Ponomarova, O., Mende, D. R., Bork, P., and Patil, K. R. (2015). Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 112, 6449–6454. doi: 10.1073/pnas.1421834112
- Zheng, Y., Huang, R., Wang, B. Z., Bodelier, P. L. E., and Jia, Z. J. (2014). Competitive interactions between methane- and ammonia-oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil. *Biogeosciences* 11, 3353–3368. doi: 10.5194/bg-11-3353-2014

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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