



# Harnessing fungi to mitigate CH<sub>4</sub> in natural and engineered systems

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## Abstract

Methane (CH<sub>4</sub>) is a powerful greenhouse gas emitted from natural and anthropogenic sources, and its emission rates vary among sources as a function of environment, microbial respiration, and feedbacks. Biological CH<sub>4</sub> flux from natural and engineered systems is typically represented simply as generation of CH<sub>4</sub> by methanogens minus oxidation by methanotrophs. In many cases, however, CH<sub>4</sub> flux is modulated by transport and solubility mechanisms that occur before oxidation or other chemical transformation. The ability of fungi to directly oxidize CH<sub>4</sub> remains unclear; however, their hydrophobic growths extending above microbial biofilms can improve surface area and sorption of hydrophobic gases. This can improve overall oxidation rates in a biofilm simply by improving phase transfer dynamics and bioavailability to bacterial or archaeal associates. This indirect facilitation is not necessarily intuitive, but there has been a recent emerging interest in harnessing these fungal abilities in engineering bioreactors and filtration systems designed to capture and oxidize CH<sub>4</sub>. These dynamics may be playing a similar facilitative role in natural CH<sub>4</sub> oxidation, where fungi may indirectly influence carbon mineralization and methanogen/methanotroph communities, and/or directly oxidize and dissolve gaseous CH<sub>4</sub>. This review highlights these unique roles for fungi in determining net CH<sub>4</sub> oxidation rates, and it summarizes the potential to harness fungi to mitigate CH<sub>4</sub> emissions.

**Keywords** Anaerobic digester · Biofilter · Hydrophobin · Methanogen · Methanotroph · Rumen

## Introduction

Methane is a powerful greenhouse gas of significant concern due to the magnitude of its anthropogenic sources and to its potential feedbacks with warming, which may trigger massive natural CH<sub>4</sub> releases. Although it is a trace atmospheric gas, CH<sub>4</sub> is the second most significant greenhouse gas after carbon dioxide (CO<sub>2</sub>), accounting for up to 20% of the global warming effect (IPCC 2014; Xu et al. 2016). The significance of CH<sub>4</sub>-related warming is driven by a potent global warming potential that is ~25 times that of CO<sub>2</sub> over a 100-year period (EPA 2018), and 84–87 times greater over a 20-year period (EPA 2015). This is compounded by the rise in the mean atmospheric concentration of the trace gas from around 0.7 ppm pre-nineteenth century to around 1.845 ppm currently (NOAA 2017). This 2.5 factorial increase has largely been the

result of industrialization and human activity, with natural sources—which originally accounted for ~90% of pre-industrial global CH<sub>4</sub> emissions (Conrad 2009)—now representing only ~30% of the total (Saunois et al. 2016). Today, roughly a third of CH<sub>4</sub> emissions are from enteric fermentation and manure management, while another third are related to the extraction of natural gas and other fossil fuels (EPA 2018).

Many natural fluxes of CH<sub>4</sub> are sensitive to climate change. These include elevated CH<sub>4</sub> releases from permafrost, wetlands, and freshwater systems, as well as the vulnerability of large frozen marine sediment stores, like clathrates (Hamdan and Wickland 2016; Singh et al. 2010). There are also unknown feedbacks between climate and natural terrestrial sources of CH<sub>4</sub> (Campbell et al. 2009). Many of the nuances of CH<sub>4</sub> flux are not yet resolved and, as a consequence, are poorly represented in Earth System Models (James et al. 2016; Wieder et al. 2013). The controls and tipping points of potentially massive CH<sub>4</sub> releases from marine, freshwater, and terrestrial sources remain uncertain.

The significance of human sources, their potential impact on natural CH<sub>4</sub> flux, and the fact that CH<sub>4</sub> has a relatively short (12.4-year) lifespan in atmosphere (IPCC 2014) make

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CH<sub>4</sub> mitigation an important strategy for curbing the near-term effects of climate change (UNEP and WMO 2011). To address this global priority, resolution of the microbial underpinnings of the CH<sub>4</sub> cycle is needed, both to improve models and foster the development of effective CH<sub>4</sub> mitigation strategies (Conrad 2009; Nazaries et al. 2013). To date, most research dealing with microbial CH<sub>4</sub> dynamics have been limited to select prokaryotes known to generate and oxidize CH<sub>4</sub>, despite the fact that these microbial groups rarely exist in solitude and instead form complex community networks (Konopka 2009). There is also new evidence from natural (Ivarsson et al. 2016b) and engineered systems (Lebrero et al. 2016) that eukaryotic fungi have an underappreciated yet integral role in the CH<sub>4</sub> cycle. In the subsequent sections, this mini-review will summarize our understanding of the indirect and direct roles fungi play in oxidizing and generating CH<sub>4</sub>, and will highlight the potential utility of these eukaryotes in CH<sub>4</sub> mitigation strategies.

## Methanogens and methanotrophs

Traditionally, biologically generated (biogenic) CH<sub>4</sub> has been attributed solely to methanogens, while methanotrophs have been considered exclusive oxidizers of CH<sub>4</sub>. Methanogens are archaea that belong to the phylum *Euryarchaeota*, and are classified into two groups based on their fastidious mechanism of CH<sub>4</sub> generation. One type—the hydrogenotrophs—generates CH<sub>4</sub> almost exclusively from the reduction of CO<sub>2</sub>, with H<sub>2</sub> used as the typical electron donor. Other methanogens can generate CH<sub>4</sub> by degrading acetate or other simple, methylated organic compounds (Costa and Leigh 2014). Methanogens can be thought of as terminal organisms whose metabolism is highly dependent upon the supply of simple carbon metabolites generated by other microbes.

Most methanotrophs belong to the gamma- (type I methanotrophs) or alpha-*Proteobacteria* (type II) and are differentiated by their morphologies and mechanism of carbon assimilation. These bacteria generate energy by oxidizing CH<sub>4</sub> with O<sub>2</sub> to form methanol, formaldehyde, formate, and finally CO<sub>2</sub>. CH<sub>4</sub> monooxygenase (MMO), a critical enzyme that catalyzes the initial oxidation of CH<sub>4</sub>, is indicative of methanotrophs and exists in two forms—particulate (pMMO) or solubilized (sMMO)—the latter restricted to type II methanotrophs (Hanson and Hanson 1996). More recently, a third group of methanotrophs has been identified that uniquely couple carbon and nitrogen cycles, bacteria capable of nitrite-dependent anaerobic methane oxidation (N-DAMO) in consortia with archaea (Raghoebarsing et al. 2006) and in pure culture (Ettwig et al. 2008). While N-DAMO methanotrophs may be more important in inland lakes and rivers where nitrate runoff occurs (Shen et al. 2015), methanotrophs, regardless of type, typically reside in anoxic/

oxic interphase habitats, co-localized near methanogens, where they can effectively reduce the CH<sub>4</sub> emissions produced by methanogenesis (Conrad 1996).

## CH<sub>4</sub> transport limitations and the potential influence of fungal hyphae

The flux of biogenic CH<sub>4</sub> is governed by gas transport mechanisms through solid-phase environments such as soil in nature or biofilter media in engineered bioreactors—it is not simply the interplay of methanogens and methanotrophs (Nazaries et al. 2013; Scheutz et al. 2009). CH<sub>4</sub> is a hydrophobic, relatively insoluble gas at ambient conditions (solubility = 0.024 g CH<sub>4</sub> L<sup>-1</sup> water at 20 °C, 1 atm), with limited mass transport through water (dimensionless Henry's law constant = 30) estimated to be 10,000 times slower than through air (Castro et al. 1995; López et al. 2013). Consequently, in the case of well-studied soil systems, CH<sub>4</sub> moves more readily through aerated, coarse soils than through saturated sediments and wetted fine loams (Dorr et al. 1993). With transport rate limiting, CH<sub>4</sub> generation and oxidation are highly dependent on the soil/sediment properties, with oxidation rates increasing with diffusivity, porosity, and organics content (Ball et al. 1997a, b) and decreasing when air is displaced by moisture (Bhullar et al. 2013; Maier et al. 2017).

In these microhabitats in soils and other similar environments, fungi are unique regulators of transport and agents of mass transfer from gas to aqueous phases (Treseder and Lennon 2015). Many fungi secrete hydrophobic proteins that self-assemble on their surfaces, and enable their hyphae to escape the surface tension of water films and extend into soil pore space (Fricker et al. 2017). For this purpose, fungi essentially bridge inter-colloidal airspace, form a conduit for water and nutrient transport (Linder 2009), and effectively alter the soil/sediment structure and its permeability (Ritz and Young 2004). These hydrophobic proteins, called hydrophobins (Wosten 2001), have also been shown to facilitate fungal adsorption of hydrophobic gases (Vergara-Fernández et al. 2006; Vigueras et al. 2008), and physical chemistry research suggests there are unique interactions between the surfaces of hydrophobin monolayers and CH<sub>4</sub> (Patel and Garde 2014; Ley et al. 2015).

The aerial growths of fungi are also a well-recognized point of attachment for bacterial biofilms (Hogen et al. 2007). It is thus plausible that the methanotrophs co-localize on the growing hyphae of CH<sub>4</sub>-capturing fungi where gas transfer limitations would be minimal, although little is known about these interactions. One study verified with molecular approaches that methanotrophic bacterium from the genus *Methylobacterium* had successfully colonized the surface of the soil fungus *Glomus intraradices* (Scheublin et al. 2010). Another study presented scanning electron microscopy (SEM)

images that showed the hyphae of an arbuscular mycorrhizal fungus co-located, spatially, with *Methylobacterium oryzae* on plant roots (Kim et al. 2010). While the CH<sub>4</sub> oxidizing potential of *Methylobacterium* is debated (Dedysh et al. 2004), these studies do suggest that bacteria known to grow on one-carbon compounds like formate, formaldehyde, and methanol can utilize soil fungi as a point of attachment. There is also evidence that CH<sub>4</sub> can trigger simultaneous growth of fungi and methanotrophs in agricultural (Arif et al. 1996) and landfill soils (Watzinger et al. 2008).

## Oxidation of CH<sub>4</sub> by aerobic fungi

In addition to providing space for gas capture and methanotroph attachment, there are reports of fungi in the genus *Graphium* oxidizing CH<sub>4</sub>, directly. Using batch biodegradation tests, Lebrero et al. (2016) demonstrated that a *Graphium* culture, verified not to be contaminated with methanotrophic bacteria, was able to sustain CH<sub>4</sub> oxidation, providing methanol supplementation. Methanol has also been shown to promote CH<sub>4</sub> oxidation in forest soils (Benstead et al. 1998), and like *Graphium* sp., some pure culture methanotrophs similarly co-metabolize CH<sub>4</sub> and methanol (Jensen et al. 1998). *Graphium* is a small genus, but has widespread distribution on varied substrates. It is noteworthy that the fungal isolate used by Lebrero et al. (2016) was originally isolated from natural gas plumbing in CH<sub>4</sub>-enrichment cultures (Curry et al. 1996). How widespread this ability in fungi is, or how prevalent fungi are in CH<sub>4</sub>-rich environments, is not known.

## Harnessing fungi for CH<sub>4</sub> biofiltration

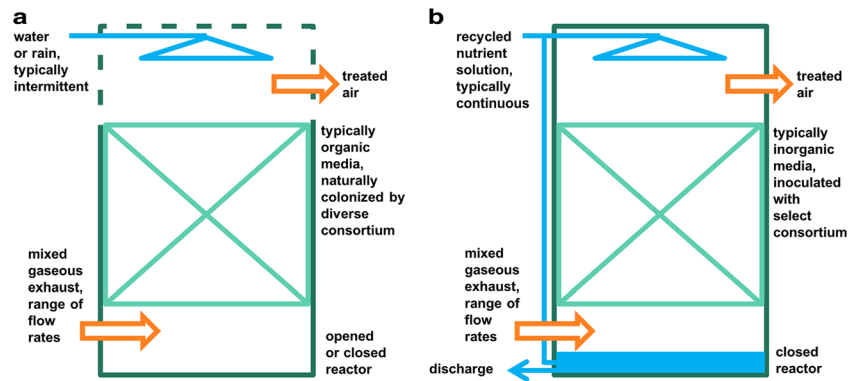
Based on their potential to facilitate CH<sub>4</sub> capture and oxidation, fungi are of growing interest for their applications to biotechnologies designed to mitigate anthropogenic CH<sub>4</sub> emissions, a field long dominated by prokaryotic-centric research. While high-concentration CH<sub>4</sub> emissions (> 30% v/v) can be harnessed for energy production, and flaring is a cost-effective technology to mitigate lower concentration CH<sub>4</sub> emissions (> 20% v/v) if emitted at sufficient flow rates (> 10 m<sup>3</sup> h<sup>-1</sup>), fugitive emissions from landfills, coal mines vents, manure pits, and long-term storages typically do not meet these requirements (Menard et al. 2012). This presents a problem, globally, as it is estimated that most anthropogenic CH<sub>4</sub> is emitted at concentrations less than 3% (Avalos Ramirez et al. 2012). Potential solutions are gas-phase biofilters (Fig. 1a) and biotrickling filters (BTF, Fig. 1b), both harnessing microbial catalysts that are immobilized on the media of packed-bed reactors. These designs can treat low-concentration contaminants in gaseous effluent emitted at a

range of flow rates (Delhomenie and Heitz 2005), and are suitable for many anthropogenic CH<sub>4</sub> emissions.

CH<sub>4</sub> biofiltration was first recognized in soils covering a retired landfill (Whalen et al. 1990). In the decades following that research, biofilters harnessing the action of methanotrophs have been engineered and applied to landfill emissions (Menard et al. 2012), coal mine (Limbri et al. 2014), and livestock facility ventilation exhausts (Girard et al. 2011). While methanotrophic biofilters can routinely achieve removal efficiencies > 80% from passive-flow landfill exhausts (Chanton et al. 2011; Obulisamy et al. 2016), CH<sub>4</sub> capture is less efficient in biofilters used to treat high flow rate exhausts. When ventilation rates are rapid, as is typical of coal mine (Limbri et al. 2013) and livestock facility ventilation systems (Veillette et al. 2012), the low solubility of CH<sub>4</sub> creates mass transfer limitations in biofilters (Ferdowsi et al. 2017) and BTFs (Estrada et al. 2014). In BTFs, the use of liquid sorbents or innovative reactor configurations has been demonstrated by Estrada et al. (2014) to improve mass transfer and CH<sub>4</sub> removal rates. To improve biofilter CH<sub>4</sub> capture, larger reactors can be used to increase empty bed residence time (EBRT = volumetric flow rate [m<sup>3</sup> min<sup>-1</sup>] / volume of reactor media [m<sup>3</sup>]), but this is reasonable only for some applications like large coal mining facilities where coal can be used as an inexpensive reactor media (Limbri et al. 2014). In other applications like livestock facility ventilation, which are responsible for 14.5% of global CH<sub>4</sub> (Gerber et al. 2013), space and cost restraints limit the feasibility of large biofilters.

Biofilters that utilize fungi as biocatalysts in concert with methanotrophs may provide a strategy to improve CH<sub>4</sub> mass transfer without major changes to the reactor size or configuration. Fungi offer many advantages in biofiltration, including their ability to improve capture of hydrophobic emissions (Kennes and Veiga 2004). To our knowledge, Girard et al. (2012) conducted the first experiments that tested the ability of fungi to improve CH<sub>4</sub> biofiltration. Using lab-scale biofilters packed with inorganic media and designed for a 4.2-min EBRT, they compared four fungal strains against a non-inoculated control and the influence of swine manure irrigation. Although elimination capacities up to 19 g CH<sub>4</sub> m<sup>-3</sup> h<sup>-1</sup> were obtained, by the end of the experiment, the fungal inoculations were outcompeted by competitive colonizer microbes entering the system via the semi-continuous manure irrigation. Lebrero et al. (2016) were able to maintain a fungal inoculant (*Graphium* sp.) in a biofilter designed for a 20-min EBRT, and achieved an elimination capacity of 39 g m<sup>-3</sup> h<sup>-1</sup> (removal efficiencies of ~90%). Although this residence time would be too long (i.e., required reactor size would be too large) for many anthropogenic CH<sub>4</sub> emission sources, this study demonstrated that fungal-inoculated biofilters had more stable and higher performance than comparable methanotroph-only biofilters.

**Fig. 1** Simplified schematic of a biofilter (a) and a biotrickling filter (b)



In lab-scale biofilters designed for a 25-s EBRT (more representative of a livestock facility ventilation system), we previously showed certain inoculums of wood decay fungi could also improve capture of  $\text{CH}_4$  from manure storage emissions (Oliver and Schilling 2016), although elimination capacities were low ( $5 \text{ g m}^{-3} \text{ h}^{-1}$ ) at these high flow rates. Utilizing the same multi-staged biofilters and a chromatographic isotherm, it was also demonstrated that capture of  $\text{CH}_4$  was proportional to the amount of fungal biomass. It is noteworthy, that this isotherm-tested dormant fungal spores, not living hyphae, but like hyphae, these spores are similarly coated in hydrophobin proteins (Klimes and Dobinson 2006). This ability of fungal biomass to capture  $\text{CH}_4$  has subsequently been supported by batch isotherm work (Liew and Schilling, *paper in review*). To our knowledge, no one has explored the ability of fungi to capture  $\text{CH}_4$  in a BTF, although Palanisamy et al. (2016) have used fungi to improve the performance of a BTF-treating chloroform, a single-carbon compound.

Biofilters offer a useful model system for studying these dynamics in nature, as the microbial communities in biofilters exist in a discrete system and inputs and outputs can be easily controlled and monitored (Cabrol and Malhautier 2011). Collectively, biofilter research findings suggest a new conceptual model for  $\text{CH}_4$  biofiltration that may be useful for ecologists and engineers (Fig. 2). Originally,  $\text{CH}_4$  biofilters were modeled as a two-phase system (gaseous and aqueous biofilm) on solid media, where advection, mass exchange (from gas to liquid), absorption, diffusion, and biofilm concentrations were all driven by methanotrophic biodegradation

reactions and limited by absorption and diffusion of  $\text{CH}_4$  in aqueous biofilm (Nikiema et al. 2009).

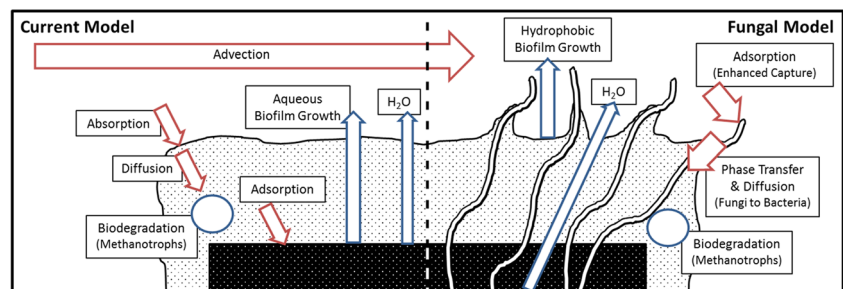
When fungi are included in the model, however, new processes and limits must be considered. First, fungi can dwell inside the solid media and extend into the air space, increasing their desiccation tolerance and increasing the effective surface area of the biofilm (Oliver et al. 2016). Second, the biofilms infiltrated by filamentous fungal hyphae are no longer exclusively aqueous, as these hyphal protrusions are hydrophobic and better suited for capture of insoluble  $\text{CH}_4$ . Methanotrophs co-localized along these fungi may also create preferential diffusion gradients for  $\text{CH}_4$  that reduce rate limits to the biofiltration process. As research and modeling efforts advance, we may one day be able to fine-tune fungal and methanotroph communities in biofilters to optimize mitigation of anthropogenic  $\text{CH}_4$  emissions.

## Role of aerobic fungi in community $\text{CH}_4$ dynamics

### In soil systems

Similar to their role in moderating  $\text{CH}_4$  dynamics in engineered systems, fungi may play a similar but overlooked role in the soil  $\text{CH}_4$  cycle. Fungi likely affect  $\text{CH}_4$  fluxes in soils both indirectly (by dictating microbial community assembly and function through metabolite generation and nutrient allocation) and directly (via gas capture and oxidation).

**Fig. 2** New conceptual model of  $\text{CH}_4$  biofiltration that includes the role of fungi





Currently, there is no research exploring the ability of fungi to oxidize  $\text{CH}_4$  in soil, although several studies have suggested their indirect influences. Dissolved organic carbon, inorganic nitrogen, and phosphorus levels strongly influence  $\text{CH}_4$  uptake and oxidation in soils and sediments (Veraart et al. 2015; Wu et al. 2016) with the availability of these dictated in many cases by fungi (Boddy 1999). Fungi degrade lignocellulose in soils, and they maintain growth forms and enzymatic machinery that enable unique acquisition, translocation, and immobilization of dissolved carbon, inorganic nitrogen, and phosphorous (Treseder and Lennon 2015). Fungi also have high assimilation efficiencies and capacities to store carbon (Singh et al. 2010), and in the past 15 years, the contribution of fungal necromass to forest biogeochemical cycles also has been recognized as a significant input into forest carbon and nutrient cycles (Clemmensen et al. 2013; Fernandez and Kennedy 2015; Fernandez et al. 2016).

Fungal regulation of carbon and nutrient cycles in soils also influences the development of soil microbial communities, including methanotrophs (Burke et al. 2012). In a laboratory study of arable soils, following  $\text{CH}_4$  injection, increases in methanotroph populations correlated with increases in fungal biomass (Arif et al. 1996). A similar relationship between fungi and methanotrophs has been observed in landfill soils in response to inputs of  $\text{CH}_4$ -rich biogas (Watzinger et al. 2008). The only study of natural systems found  $\text{CH}_4$ -oxidizing bacteria and fungal biomass/enzymes to be negatively correlated in soils of a mature forest, but attributed these unexpected results to sampling biases which excluded fresh organic soil horizons where soil fungi were likely to reside (Burke et al. 2012).

Despite the potential benefits to Earth System Models from a more complete inclusion of microbial communities, pools, and their impact on the carbon cycle (Todd-Brown et al. 2012; Wieder et al. 2013), fungi have not been given significant attention in the soil  $\text{CH}_4$  cycle (Lai 2009; Mancinelli 1995; Segers 1998; Singh et al. 2010). This connection between fungi and  $\text{CH}_4$  is indirect and not necessarily intuitive; we do not yet understand the interactions of soil fungi with methanogens, methanotrophs, and  $\text{CH}_4$  (Conrad 2009; Nazaries et al. 2013). Resolving these interactions may improve our estimates of  $\text{CH}_4$  budgets, which, not surprisingly, have larger uncertainties in natural systems than for anthropogenic emissions (Kirschke et al. 2013; Saunio et al. 2016).

## In forests

Some wood-degrading fungi may actually facilitate  $\text{CH}_4$  production by providing methanogens with reduced carbon feedstocks derived from the breakdown of lignocellulose. These “mycogenic”  $\text{CH}_4$  emissions were first described by Mukhin and Voronin (2007, 2008) in a series of experiments where wood fragments infected by several species of bracket fungi

were placed into gas-tight jars and the fluxes of  $\text{O}_2$ ,  $\text{CO}_2$ , and  $\text{CH}_4$  were tracked over time. Covey et al. (2012) detected similar  $\text{CH}_4$  emissions in northern hardwoods, with greater emission from tree species more susceptible to heart rot, and postulated that the fungi causing this rot may be supporting the growth of methanogens. The impact of these decay fungi on  $\text{CH}_4$  emission was later confirmed in a study of living Norway spruce trees where those infected by *Armillaria* spp. (fungi with the white rot decay mechanism) had elevated levels of  $\text{CH}_4$  emissions, 3–4 times the levels of healthy uninfected trees (Hietala et al. 2015). With the prevalence of decay fungi like *Armillaria* in forests, the magnitude of these mycogenic emissions could be significant.

Wood decay fungi have been observed co-localized with methanogens in wood biofilms of timbers in an abandoned coal mine, where fungal generation of acetate was suggested to be the specific stimulant for  $\text{CH}_4$  generation (Beckmann et al. 2011). This dynamic is harnessed, purposefully, when white rot fungi are used as selective pretreatments to improve substrate digestibility and biogas production in anaerobic digesters (Rouches et al. 2016). Fungi may be similarly utilized to initiate the remediation of fossil fuel contamination and biomethanization of tar sands (Wong et al. 2015) and coal (Singh et al. 2012).

Some aerobic fungi may also be capable of biomethanization without a methanogenic partner. The first report that organisms other than anaerobic prokaryotes might be able to generate  $\text{CH}_4$  was by Keppler et al. (2006), where there was evidence that plants under aerobic conditions generated  $\text{CH}_4$ . Based on a series of well-controlled experiments, Lenhart et al. (2012) were the first to demonstrate that aerobic decay fungi could independently generate  $\text{CH}_4$ . Using stable-carbon isotopic experiments, methionine was identified as the likely precursor of these  $\text{CH}_4$  emissions (Lenhart et al. 2015). The authors postulated that this methyl sulfide was oxidized by fungi to a sulfoxide, which was then demethylated, and following homolytic bond cleavage, generated methyl radicals that stabilized as  $\text{CH}_4$ . While the mechanistic role of this  $\text{CH}_4$  generation is poorly understood, this may be a widespread stress response of fungi, and mycogenic  $\text{CH}_4$  emissions may be a more significant contribution to global  $\text{CH}_4$  budgets than previously assumed (Liu et al. 2015).

## In termite mounds

Fungi are also associated with termite mounds and may influence  $\text{CH}_4$  fluxes to the atmosphere. Termite mounds are a noteworthy source of  $\text{CH}_4$  which is generated by methanogens residing in the termite gut (Zimmerman et al. 1982; Conrad 2009). These methanogens and their consequential  $\text{CH}_4$  production are strongly influenced by fungal populations, also present in the termite gut, which are the crucial metabolizers of the lignocellulose ingested by the

termites (Brune and Ohkuma 2011). Similar to the biofiltration that occurs in landfill soils, methanotrophs located in the termite mounds will oxidize the CH<sub>4</sub> released by the insects. These methanotroph communities are unique to individual mounds and are shaped by the termite microbiota (Ho et al. 2013). Like the well-recognized leaf-cutting ants, several species of termites also form unique symbioses with fungi (Mueller and Gerardo 2002). In a study to determine the CH<sub>4</sub> emission factors of various termite mounds, Sugimoto et al. (1998) observed appreciable emissions from some mounds, but measured near zero emission factors for mounds that housed termite species that cultivated fungi (*Macrotermes*). While the exact role fungi play in mitigating termite CH<sub>4</sub> emissions has not been investigated, it is plausible that fungi growing in these mounds facilitate methanotrophic activities and CH<sub>4</sub> mitigation and may be suitable candidates for biofilter studies.

### In wetlands and freshwater environments

Wetland soils and the sediments of freshwater environments are very important natural sources of CH<sub>4</sub>. While cycling of CH<sub>4</sub> in these systems has received considerable research attention, there remains significant uncertainty surrounding their response to a changing climate (Natchimuthu et al. 2016; Zhang et al. 2017a). Our ecological understanding of fungi in wetland systems has lagged behind that of prokaryotes (Gutknecht et al. 2006). This is despite knowledge that fungi are the principal litter decay and carbon cycling organisms in freshwater and wetland environments (Baldrian 2017). In rice paddies, for example, the decomposition of plant residues to soil organic matter stimulates the production and release of CH<sub>4</sub> (Kimura et al. 2004). Oxic surface soils of rice paddies also harbor higher numbers of methanotrophs (Kimura et al. 2004) and fungi (Lopes et al. 2011). In an experimental paddy system, it was observed that rice plants inoculated with arbuscular mycorrhizal fungi reduced paddy CH<sub>4</sub> emissions (Zhang et al. 2017b). These authors hypothesized that the fungi increased the soil carbon-to-nitrogen ratio which created nitrogen limitation for methanogens, although the influence of methanotrophs in the system was not explored. Fungi are also found at significant depths (2.5 m) in peatlands (Jackson and Raub 2010), where diverse groups of fungi from diverse guilds (mycorrhizal, saprophytic, pathogenic) make up a significant fraction of the *Sphagnum* microbiome and coexist with methanotrophs in unknown associations (Kostka et al. 2016). There is a need for research on these freshwater fungi and their interactions with methanotrophs and methanogens, to resolve CH<sub>4</sub> dynamics in these environments and improve predictions of their responses to climate change.

## Role of anaerobic fungi in community CH<sub>4</sub> dynamics

### In the rumen

Like the aerobic fungi discussed, anaerobic fungi may also play an important role in anthropogenic and natural cycling of CH<sub>4</sub>. Anaerobic fungi were first detected in the rumen of sheep in the late 1970s and not long after were observed growing in close proximity with rumen methanogens (Bauchop 1981). Mountfort et al. (1982) were the first to suggest that these fungi critically supported methanogenesis based on evidence that CH<sub>4</sub> generation was elevated when cellulose was fermented by a co-culture of rumen methanogens and anaerobic fungi. Anaerobic fungi lack mitochondria and instead utilize hydrogenosomes to produce ATP, H<sub>2</sub>, CO<sub>2</sub>, acetate, formate, and other simple metabolic products, like lactate and ethanol, from the decomposition of lignocellulose (Brul and Stumm 1994; Yarlett et al. 1986). In co-culture, some of these fungal metabolic products are utilized by methanogenic archaea. Interestingly, this relationship between rumen methanogens and anaerobic fungi is more complex than simple cross-feeding, with the metabolic profile of anaerobic fungi able to shift away from lactate or ethanol, and exclusively towards the more reduced and preferred growth substrate of rumen methanogens—formate and acetate (Cheng et al. 2009). Because of this energetically favorable transfer of hydrogen and electrons, fungal degradative enzyme production and the breakdown of ingested lignocellulose in the rumen are also enhanced (Gruninger et al. 2014). Similar syntrophic cooperation between anaerobic fungi and methanogens has recently been demonstrated in coalbeds in China (Guo et al. 2017).

To a farmer, enteric CH<sub>4</sub> is not just a greenhouse gas but also a missed opportunity and production cost, as carbon in feed is lost instead of converted to meat or milk. It is estimated that this inefficiency can represent a 6 to 15% loss of gross energy intake in ruminants (Puniya et al. 2015). Unlike the CH<sub>4</sub> produced in the rumen by methanogens, which is flatulated or belched by rumen animals, the acetate produced by acetogens can be utilized by the animal for growth and production. In the rumen, however, acetogens are less abundant and competitive than methanogens, as the latter require lower levels of H<sub>2</sub> to convert CO<sub>2</sub> and do so via a more thermodynamically favorable reaction (Martin et al. 2009). Anaerobic fungi can increase the level of H<sub>2</sub> in the rumen (Hook et al. 2010), and thus may be able to improve the competitiveness of acetogens.

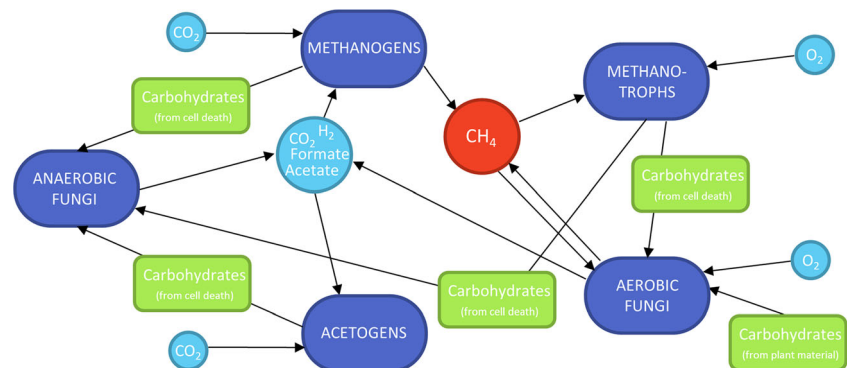
An initial meta-analysis showed that feeding cows anaerobic fungi probiotics only sometimes reduced enteric CH<sub>4</sub> production. However, most of the early fungal strains used as probiotics were selected to improve milk production and not specifically to reduce CH<sub>4</sub> (Sauvant 2005). Since then, others

have provided examples where anaerobic fungi fed as probiotics can specifically enhance acetogenesis and reduce  $\text{CH}_4$  emissions (Puniya et al. 2015). There are at least six unique fungal genera, each representing multiple species, which populate the rumen of a cow (Tapio et al. 2017). Syntrophic interactions between these rumen fungi, bacteria, and archaea are complex, and change with cow diet and maturity (Kumar et al. 2015). While it will take concerted effort to develop strategies that successfully strengthen the interspecies  $\text{H}_2$  transfer from anaerobic fungi to acetogens, this potential to reduce enteric  $\text{CH}_4$  while providing economic value to a livestock producer highlights an important area of research.

### In anaerobic digesters

Manure management is one of the more significant anthropogenic sources of  $\text{CH}_4$  emissions, particularly long-term manure slurry storages. Anaerobic digesters are utilized by many farms to treat organics and manure prior to storage to promote  $\text{CH}_4$  generation where it can be captured and utilized as a renewable energy source (IPCC 2006). The hydrolysis of complex lignocellulose materials like manure is typically the rate-limiting step in anaerobic digestion systems (Donoso-Bravo and Mairé 2012; Ma et al. 2013). Rumen fungi are being applied to these systems for their ability to catalyze lignocellulose breakdown, but unlike research goals in animal production, in anaerobic digesters, the aim is to promote methanogenesis (Cheng et al. 2009). In recent studies, the addition of rumen fungi was able to successfully couple hydrolysis rate limits and the metabolic support of methanogenesis; however, improvements in biogas and  $\text{CH}_4$  production were substrate dependent (Aydin et al. 2017; Yıldırım et al. 2017). While the use of anaerobic fungi in anaerobic digesters still faces many hurdles, such as their survivability and application to industrial scales (Gruninger et al. 2014), additional research into the ecology of these organisms and their function in anaerobic digesters could facilitate both the mitigation of a significant anthropogenic  $\text{CH}_4$  source and the economical production of a renewable energy source (Dollhofer et al. 2015).

**Fig. 3** Interactions of aerobic and anaerobic fungi with methanotrophs, methanogens, and  $\text{CH}_4$ . Modified from Ivarsson et al. (2016b)



### In the deep sea

A unique and poorly explored environment where anaerobic fungi may also play a critical role in the  $\text{CH}_4$  cycle is the deep sea (Ivarsson et al. 2016a). Though fungi were first reported from the deep sea by Roth in 1964, the ecology of fungi in marine environments has received considerably little attention. It is now known that fungi are ubiquitous in ocean water and sediments, around hydrothermal vents, cold- $\text{CH}_4$  seeps (Nagano and Nagahama 2012), and even in the ocean crust (Ivarsson et al. 2016b), and that these fungi are both genetically and biologically diverse (Le Calvez et al. 2009; Nagano and Nagahama 2012). The ecological role of deep sea fungi is still mysterious (Calvez et al. 2009), but their association with certain environmental gradients, like carbonates, suggests that as on land, fungi in the sea are underpinning carbon cycles (Pasulka et al. 2016). While many of these fungi are extremophiles, some like the *Malassezia* are hyperdiverse and common in many different terrestrial and aquatic habitats (Amend 2014). Other species like *Exophiala* which are associated with hydrothermal vents (Le Calvez et al. 2009) and basalt ocean crust (Hirayama et al. 2015) are also found and used in biofilters to treat hydrophobic gaseous emissions (Cox et al. 1997; Estévez et al. 2005). Ivarsson et al. (2016a) have suggested that the same feedbacks formed between anaerobic fungi, methanogens, and methanotrophs in other systems may underpin oceanic  $\text{CH}_4$  fluxes and other crucial processes in the deep biosphere. Understanding this process in greater detail is a critical need in understanding  $\text{CH}_4$  emissions from both deep and shallow marine environments (Borges et al. 2016; James et al. 2016). At least one study has also found anaerobic fungi in freshwater lakes and landfill soils (McDonald et al. 2012), suggesting their importance may extend beyond the ocean.

### Outlook

Aerobic and anaerobic fungi play an integral, often underappreciated, role in environmental and anthropogenic  $\text{CH}_4$  fluxes and mitigation (Fig. 3). In soils, forests, and wetlands,

aerobic fungi initiate the breakdown of lignocellulose and other carbon stores, releasing carbon as CO<sub>2</sub> or CH<sub>4</sub> as well as feeding methanogen associates. These fungi in soils can constitute a major fraction of organic matter, including dead mycelial necromass, and their ability to capture hydrophobic CH<sub>4</sub> may play an underappreciated role in CH<sub>4</sub> emission rates. Their control of carbon and domination of transport processes also guides microbial community assembly in soils, forests, and wetlands. Anaerobic fungi can also regulate anthropogenic CH<sub>4</sub> emissions through their control of carbon allocation and the support of acetogenic and methanogenic communities. As these cross-feeding relationships are better resolved, there is opportunity to both reduce enteric CH<sub>4</sub> emissions and facilitate the generation of CH<sub>4</sub>—and the subsequent capture and utilize of this renewable energy source—in anaerobic digesters. Anaerobic fungi in shallow and deep sea environments also appear integral to the terminus of carbon cycling and CH<sub>4</sub> flux. With the recent recognition of these vast unexplored fungal communities, the potential for novel organisms with applicability to engineered CH<sub>4</sub> mitigation systems is significant.

Improving our understanding of fungal interactions with methanotrophs, methanogens, and CH<sub>4</sub> in these various natural and human environments is a critical next step to bolster the predictive power of Earth System Models and to develop the biological technologies and strategies to minimize anthropogenic CH<sub>4</sub> emissions. Critical research areas include resolving the roles and mechanisms (1) of fungal/hydrophobin sorption of CH<sub>4</sub>, (2) of co-localization of methanotrophs on fungal scaffolds, (3) of syntrophic cooperation between fungi, methanotrophs, and methanogens in natural environments, (4) of independent fungal oxidation and degradation of CH<sub>4</sub>, (5) of fungal biocatalysts in CH<sub>4</sub> biofilters, rumen function, and anaerobic digesters, and (6) of fungi in underexplored natural environments such as peatlands and the deep sea. These efforts will help us understand and harness fungi for their important, but often indirect and overlooked, role in the CH<sub>4</sub> cycle.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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