REVIEW ARTICLE

Microbial syntrophy: interaction for the common good

Brandon E.L. Morris[1], Ruth Henneberger[2], Harald Huber[3] & Christine Moissl-Eichinger[3]

[1]Microbiology, Institute for Biology II, University of Freiburg, Freiburg, Germany; [2]Institute for Biogeochemistry and Pollutant Dynamics, ETH Zuerich, Zuerich, Switzerland; and [3]Institute for Microbiology and Archaea Center, University of Regensburg, Regensburg, Germany

Correspondence: Christine Moissl-Eichinger, Institute for Microbiology and Archaea Center, University of Regensburg, Universitaetsstr. 31, 93053 Regensburg, Germany. Tel.: +49 941 943 4534; fax: +49 941 943 1824; e-mail: christine.moissl-eichinger@ur.de

Received 3 June 2012; revised 25 February 2013; accepted 27 February 2013. Final version published online April 2013.

DOI: 10.1111/1574-6976.12019

Editor: Hauke Hennecke

Keywords
metabolic association; electron transfer; nutrient exchange; degradation; ecological niche; microbial cooperation.

Abstract

Classical definitions of syntrophy focus on a process, performed through metabolic interaction between dependent microbial partners, such as the degradation of complex organic compounds under anoxic conditions. However, examples from past and current scientific discoveries suggest that a new, simple but wider definition is necessary to cover all aspects of microbial syntrophy. We suggest the term 'obligately mutualistic metabolism', which still focuses on microbial metabolic cooperation but also includes an ecological aspect: the benefit for both partners. By the combined metabolic activity of microorganisms, endergonic reactions can become exergonic through the efficient removal of products and therefore enable a microbial community to survive with minimal energy resources. Here, we explain the principles of classical and non-classical syntrophy and illustrate the concepts with various examples. We present biochemical fundamentals that allow microorganism to survive under a range of environmental conditions and to drive important biogeochemical processes. Novel technologies have contributed to the understanding of syntrophic relationships in cultured and uncultured systems. Recent research highlights that obligately mutualistic metabolism is not limited to certain metabolic pathways nor to certain environments or microorganisms. This beneficial microbial interaction is not restricted to the transfer of reducing agents such as hydrogen or formate, but can also involve the exchange of organic, sulfurous- and nitrogenous compounds or the removal of toxic compounds.

Introduction

When complex, difficult jobs have to be done, it is wise to divide the work into smaller, simpler tasks and to include specialists to ensure a positive and worthwhile outcome for all team members. This principle of labor division is also true for microorganisms that act in the framework of the microbial food chain, degrading (complex) organics or cycling carbon-, nitrogen-, and sulfur-containing compounds (e.g. Costa *et al.*, 2006). In the last few years, 'syntrophy' has increasingly become a buzzword for cooperation between microorganisms (Fig. 1) and is sometimes used synonymously with symbiosis or commensalism, causing ambiguity in the definition of the term.

The term 'syntrophy' was previously used to describe microbial cross-feeding, and dates back to at least the mid-twentieth century. In 1956, *Escherichia coli* mutants deficient in tryptophan production were grown in co-culture with *Salmonella typhi* to investigate whether the mutant could survive using tryptophan from the *S. typhi* strain (Fildes, 1956). Selwyn & Postgate (1959) also used the term during the study of *Desulfovibrio* spp. that utilize metabolic products provided by other acetate- and butyrate-degrading bacteria during sulfidogenesis. They attempted to isolate 'syntrophs' during this work but were unsuccessful at the time (Selwyn & Postgate, 1959). Later, the term was used for the observed exchange of sulfur compounds between phototrophs and sulfur-reducing bacteria (Biebl & Pfennig, 1978).

However, what is now thought of as the classical syntrophic relationship can be illustrated by the activity of '*Methanobacillus omelianskii*' (Barker, 1939). This system, under further scrutiny, turned out to be a co-culture of two microbial partners in close metabolic association (Bryant *et al.*, 1967). The 'S organism' fermented ethanol to acetate and hydrogen, while *Methanobacterium bryantii*

FEMS MICROBIOLOGY REVIEWS

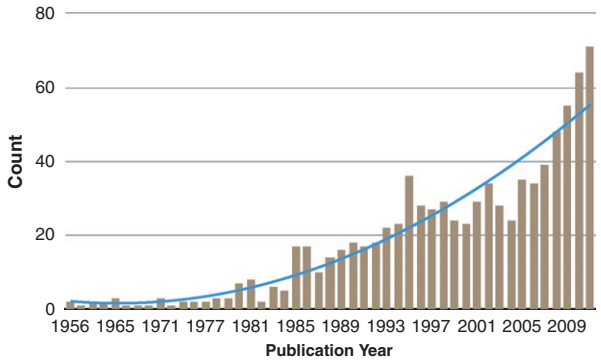

Fig. 1. Scientific articles with 'Syntrophy' or 'Syntrophic' as a topic published from 1956 to 2011. The data were obtained from Web of Knowledge™ on 1 June 2012. Only articles from English-language journals are included. The blue trendline is provided as a reference.

strain M.o.H. used the produced hydrogen to reduce CO_2 to CH_4. Interestingly, the conversion of ethanol to acetate and hydrogen is an endergonic reaction, yet under low hydrogen partial pressures the overall metabolic process becomes exergonic (i.e. favorable). The activity of the methanogenic archaeon (or another hydrogen- or formate-using microorganism) permits the other partner to metabolize the substrate by effectively maintaining inhibitory metabolic end products at very low concentrations (McInerney *et al.*, 1981, 2008). These basic principles are reflected in the classical definitions of syntrophy, explained as:

(1) 'Cooperations in which both partners depend on each other to perform the metabolic activity observed and in which the mutual dependence cannot be overcome by simply adding a cosubstrate or any type of nutrient' (Schink, 1997).

(2) 'Tightly coupled mutualistic interactions', proving 'essential for global carbon cycling in anaerobic environments' (McInerney *et al.*, 2011).

(3) A 'thermodynamically interdependent lifestyle where the degradation of a compound such as a fatty acid occurs only when degradation end products, usually hydrogen, formate, and acetate, are maintained at very low concentrations' (McInerney *et al.*, 2009).

(4) A 'nutritional situation in which two or more organisms combine their metabolic capabilities to catabolise a substrate that cannot be catabolised by either one of them alone' (Stams & Plugge, 2009).

(5) 'Relationships in which both partners depend on each other for energetic reasons and perform together a fermentation process that neither one or both could run on its own' (Schink, 2002).

Since all of these definitions need to be addressed and known examples of syntrophy need to be included (e.g. sulfur-compound exchange), in this review we define syntrophy simply as 'obligately mutualistic metabolism'. This definition of syntrophy continues to focus on the set of chemical reactions that occur from the microbial cooperation (i.e. the metabolism), but is expanded to include information about the ecology of the interdependence.

Syntrophy therefore represents a facet of symbiosis, defined as a long-term stable relation of organisms which can be either beneficial or unbeneficial (i.e. mutualism, commensalism and parasitism, respectively; Smith & Douglas, 1987; Moissl-Eichinger & Huber, 2011). In contrast to syntrophy, symbiotic relationships are not necessarily based on metabolism but, for instance, on protection against chemical or mechanical stress (Steward, 2002).

The mutualism that occurs during syntrophy can most often be defined as a *resource-service* type (Bronstein, 1994), with one partner providing a chemical compound that is consumed by the other in exchange for a reward. In all cases, syntrophic activity produces a set of chemical outcomes that are different from what could occur when each microbe acts separately, and the benefits of this metabolic interaction often come at the cost of low energetic yields and slower growth rates.

Although it is assumed that the overwhelming majority of *Bacteria* and *Archaea* interact on a metabolic basis, our understanding of these interactions is restricted due to a limited number of syntrophic microorganisms available in pure culture (Orphan, 2009). The number of cultures representing 'obligately syntrophic' relationships is even lower (e.g. *Pelotomaculum schinkii*, *Syntrophomonas zehnderi* and *Pelotomaculum isophthalicicum*; McInerney *et al.*, 2008). In fact, one could argue that obligately syntrophic microorganism do not exist and should rather be considered as 'facultatively syntrophic partners'. Under artificial laboratory conditions, with supplementation of select substrates, these syntrophic bacteria can most likely grow axenically (McInerney *et al.*, 2008).

Nevertheless, bacteria involved in syntrophy often seem to be highly adapted to a cooperative lifestyle, containing reduced genomic inventories and unique multiple-membrane complexes (McInerney *et al.*, 2007; Orphan, 2009). For example, syntrophic microorganisms can contain multiple copies of specific reductases, acyl-CoA synthases, and hydrogen or formate-evolving/producing dehydrogenases. Depending on environmental conditions, these bacteria may be able to grow partner-free by fatty acid fermentation or disproportionation, or partner-dependent by production of reduced electron carriers (i.e. hydrogen and formate; McInerney *et al.*, 2008).

Still, these adapted, syntrophic microorganisms most likely fulfill a unique niche in nature and play an important role in carbon cycling under anoxic conditions. Organisms, by their very existence, change the

environments around them and these changes will differ depending on whether or not the overall metabolic process is performed singly or syntrophically. For example, acid fermentation may alter the pH of a system while methane emission has consequences for the atmospheric warming potential. Despite the above indications that syntrophic microorganism are not constrained to an interactive lifestyle, many anaerobic *processes* are indeed obligate with respect to their need for combined metabolic efforts and are therefore considered 'syntrophic'.

Syntrophy based on the degradation of carbon compounds is mainly observed under anoxic conditions, involving one or two steps of fermentation and subsequent methanogenesis. Therefore, in many cases, syntrophy is an inter-phylum action. It is also a phenomenon observed in a broad variety of ecosystems, both natural and man-made, and under a range of different pH and temperature regimes. The educts and products of syntrophy can appear in different physical states, from solid to gaseous, and can be bound or unbound to carriers (McInerney *et al.*, 2008). Yet, each microbe must perform (ecologically important) chemical reactions that help drive the overall favorability of the metabolic process. Chemical cycling can be partially driven by abiotic processes, but microorganism act as the keystone of nutrient cycling processes by interaction with biotic and abiotic constituents in the ecosystem.

As each example described in this review will show, microorganisms are in need of partners and – over the course of evolution – have developed specialized biochemical mechanisms to allow them to adapt to their specific environment(s). Here, we summarize the basic principles of syntrophy and highlight specific examples of metabolic cooperation in natural systems and artificial laboratory cultures. The partnerships presented reflect metabolic cooperation on different levels, following the classical principle of syntrophy, or representing other types of metabolically-based relationships.

When these and other examples are understood scientifically, it will allow microbial processes to be better engineered to, for example, treat wastewater or realize the proposed recovery of methane gas from entrenched petroliferous resources, and will support a new understanding of how nature copes with energetic constraints via unique biochemical mechanisms.

Principles behind the classical concept of syntrophy

The classical concept of syntrophy is based on a close association of microorganisms under anoxic conditions and energy constraints. In the following, we will highlight the basic principles, identify strategies for electron transfer, and emphasize ecological, environmental, and phylogenetic aspects of syntrophic relationships.

The general process

The anaerobic degradation of (complex) chemical compounds is usually a two- or three-step process. Polysaccharides, proteins, nucleic acids, and lipids are primarily fermented to simple educts for methanogenesis (hydrogen, formate, acetate, CO_2) and to smaller organic compounds (like lactate, ethanol, propionate, butyrate, fatty acids; Fig. 2). In environments that lack external electron acceptors, these intermediate products are further degraded by secondary fermentation processes resulting

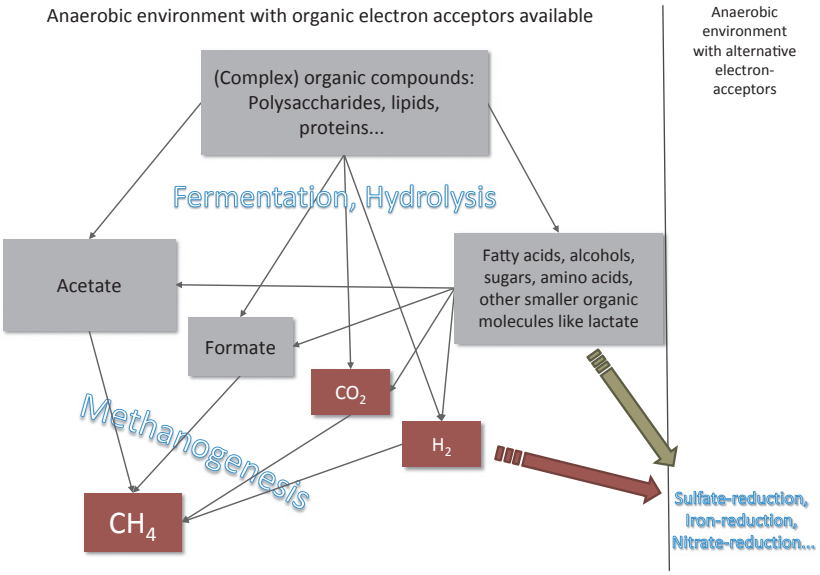

Fig. 2. Schematic drawing of 'classical' syntrophy depending on the environmental conditions (methanogenic or non-methanogenic environment). Gaseous products are shown in red.

in the production of substrates that can feed directly into methanogenic pathways (Fig. 2; Schink, 2002; McInerney *et al.*, 2008, 2011).

Methanogenesis is certainly a key process in carbon cycling, leading to the formation of methane from small carbon compounds (C1, C2). Syntrophic microorganisms very much depend on the activity of methanogenic archaea, who are primarily responsible for the efficient removal of hydrogen and formate – major electron carriers – in the absence of other terminal electron acceptors. On the other hand, methanogens need the fermenting microorganism for the production of their substantial metabolic educts.

Homoacetogens typically use CO_2 and hydrogen to form acetate. However, when methanogens are present, they tend to produce acetate and hydrogen by fermentation of organic compounds and form cooperative relationships (Stams & Plugge, 2009). Since methanogenic archaea generally have hydrogen K_m values two orders of magnitude lower than homoacetogens (Kotsyurbenko *et al.*, 2001), direct competition between both microbial groups appears impossible.

In anoxic non-methanogenic environments, where alternative electron acceptors are readily available, nitrate, sulfate, iron, manganese, selenate, and arsenate can be respired (Stams *et al.*, 2006). These terminal electron accepting processes ultimately lead to larger energy yields than methanogenesis, and dominate a system until external electron acceptors are exhausted. For example, sulfate reducers usually outcompete methanogenic archaea for hydrogen and acetate (Lovley *et al.*, 1982; Schoenheit *et al.*, 1982), if excess sulfate is available (Robinson & Tiedje, 1984). Therefore, methane production requires efficient metabolic coordination to deal with energetically limited conditions.

Types of extracellular electron transfer

The requirement for syntrophy is ultimately determined by the chemical energy available in the system. In the simplest terms, this energy is conserved and transferred through electron movement from chemical bonds, across biological membranes, or through extracellular electron transfer. In principle, three different types of extracellular electron transfer are possible (Fig. 3; Stams *et al.*, 2006):

(1) Transfer of electrons by (soluble) chemical compounds from one microbe to another in methanogenic and non-methanogenic environments.

(2) Transfer of electrons by organic and inorganic mediators to inorganic materials.

(3) Transfer by direct cell–cell contact or electroconductive cellular appendages (i.e. pili or 'nanowires', Reguera *et al.*, 2005; Summers *et al.*, 2010).

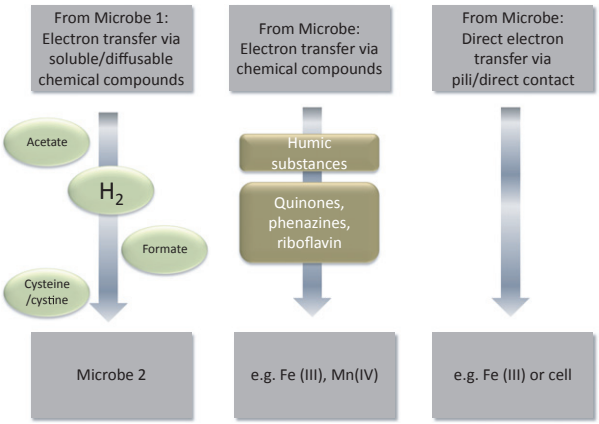

Fig. 3. Schematic drawing visualizing the different possibilities of extracellular electron transfer.

Hydrogen transfer is often considered the 'heart of syntrophy' (McInerney *et al.*, 2011), since many syntrophic relationships rely on an exchange of gaseous hydrogen as an electron shuttle. Molecular hydrogen has many advantages: the molecule is small and can easily diffuse. It can also serve as an efficient carrier for reductive metabolism (Stams *et al.*, 2008) such as methanogenesis, sulfate reduction, or denitrification. Yet hydrogen is a very powerful electron donor under anoxic conditions and is quickly and efficiently removed by metabolic processes (Nedwell & Banat, 1981), maintaining very low steady-state partial pressures.

Formate seems to be the carrier of choice in aqueous environments for several syntrophic processes (Hattori *et al.*, 2001; De Bok *et al.*, 2004). Evidence for formate use as an electron shuttle was discovered during the study of *Syntrophobacter fumaroxidans*, an organism that can grow syntrophically with formate-using methanogens. Other studies have shown that a combination of both hydrogen and formate can be used for interspecies electron transfer (Boone *et al.*, 1989; Dong & Stams, 1995; Stams *et al.*, 2006).

In addition to the two major electron carriers (i.e. hydrogen and formate), other molecules may function as electron shuttles. *Acetate*, the parent compound of both carbon dioxide and methane during aceticlastic methanogenesis, was proven to act as an electron carrier for syntrophic partners (Platen & Schink, 1987; Platen *et al.*, 1994). *Cysteine/cystine* electron shuttles were reported for an artificially established co-culture of *Geobacter sulfurreducens* and *Wolinella succinogenes*, oxidizing acetate while using nitrate as the terminal electron acceptor (Cord-Ruwisch *et al.*, 1998; Kaden *et al.*, 2002). Interestingly, stable *humic substances* can also serve as electron shuttles for microorganisms (e.g. *Geobacter metallireducens*) to reduce ferric iron. Iron reduction is then driven abiotically, thereby

re-oxidizing the humic substances (Lovley *et al.*, 1996). *Methyl sulfides* have been discussed as a possible electron carrier within a consortium capable of the anaerobic oxidation of methane combined with sulfate reduction (AOM; Moran *et al.*, 2008). However, recent studies with these communities could not confirm this hypothesis or the involvement of other electron shuttles and the question remains as to how coupling occurs in AOM communities (Nauhaus *et al.*, 2004; Holler *et al.*, 2011b).

One of the more intriguing possibilities is that direct electron transfer can take place through extracellular, conductive 'nanowires', as observed with *Geobacter* and *Shewanella* species (Reguera *et al.*, 2005; Gorby *et al.*, 2006). The electrons can be transferred from the microbe directly to ferric iron, or perhaps directly to other cells (Gorby *et al.*, 2006; Reguera, 2009). However, the role of electro-conductive pili or 'wires' in syntrophic electron transfer between cells remains speculative, despite evidence provided to support this assertion (Gorby *et al.*, 2006; Summers *et al.*, 2010).

In general, a broad variety of carriers can be envisaged, but teasing out the identity of electron shuttles in the laboratory is difficult. Studying this process experimentally may be further complicated by the possibility that microorganisms may utilize multiple electron shuttles, either alternately or in parallel. Investigations into the nature of electron transfer within syntrophic communities will require creativity and the development of novel assays to address this newly emerging field.

Energetics

Classical syntrophy is a survival strategy arising from energetic constraints of a particular system. By turning endergonic conversions into exergonic reactions, minimal energy resources can be exploited and used to support the survival and proliferation of microbial partners. One partner keeps intermediate products (e.g. hydrogen) at very low concentrations by active consumption, facilitating further degradation by the other. Calculations suggest that very low hydrogen partial pressures allow most syntrophic reactions to reach -20 to -15 kJ mol^{-1} ATP formed under environmental conditions (Schink, 1997; McInerney *et al.*, 2008), with some suggesting that the free energy changes are even lower (Scholten & Conrad, 2000; Adams *et al.*, 2006). These reactions do not produce protons, but instead shuttle electrons using different carrier systems.

When energy yields of single reactions are calculated at standard temperature and pressure for anaerobic methane oxidation, it would seem that the process could not support both methanogens and the sulfate-reducing partners (with a free energy change of -16.67 kJ mol^{-1} under standard conditions, Knittel & Boetius, 2009). Indeed, this process becomes more favorable under *in situ* conditions with high methane partial pressures (Nauhaus *et al.*, 2002), a setting that is very difficult to continuously maintain in the laboratory. Since the energy of a system has to be shared among all microbial partners, slow growth of AOM members and other syntrophic communities seems inevitable – another problematic constraint in cultivation attempts. The AOM example shows that environmental conditions can highly influence the energy yield of syntrophic reactions. For instance, higher temperatures can lead to higher energy gains over biologically feasible ranges, for certain reactions (Holler *et al.*, 2011a).

Conservation of energy by syntrophs is based on both substrate-level phosphorylation and electron transport phosphorylation (Stams *et al.*, 2006). However, by producing H_2 (or formate) from electrons gained during acyl-CoA intermediate oxidation, energy input is required for the reoxidation of reduced electron transfer flavoprotein (ETF) and NADH (Mueller *et al.*, 2009). This chemical energy is conserved by a process called reverse electron transport (McInerney *et al.*, 2007, 2011). Genomic information has made it possible to identify key-genes responsible for syntrophic metabolism, and highlight the importance of 'non-traditional' Rnf-type complexes, ETFs, membrane-bound Fe-S oxidoreductases, and Qrc membrane complexes in reverse electron transport (McInerney *et al.*, 2007; Li *et al.*, 2009; Sieber *et al.*, 2010; Callaghan *et al.*, 2012). It seems that electron confurcation may play a significant role in hydrogen or formate production during methanogenic butyrate oxidation in a manner similar to H_2 formation in the hyperthermophilic bacterium *Thermotoga maritima* (Schut & Adams, 2009). This is the reverse of a process called flavin-based electron bifurcation, now regarded as a major form of energy conservation in anaerobic organisms (Fig. 4). The multienzyme cytoplasmic complexes that catalyze these reactions couple endergonic and exergonic redox reactions through the simultaneous oxidation of the ferredoxin electron donor with a higher potential acceptor (Buckel & Thauer, 2013).

Phylogeny of microorganism associated with syntrophy

Syntrophic metabolism is widespread in nature, generally diverse in phylogenetic organization, and usually composed of a bacterial and archaeal component. In fact, metabolic interactions between representatives from both phyla are increasingly obvious under further scrutiny (e.g. 'string-of-pearls community' or the AOM consortium; Rudolph *et al.*, 2001; Knittel & Boetius, 2009). These interactions are wonderful examples of microbial inter-

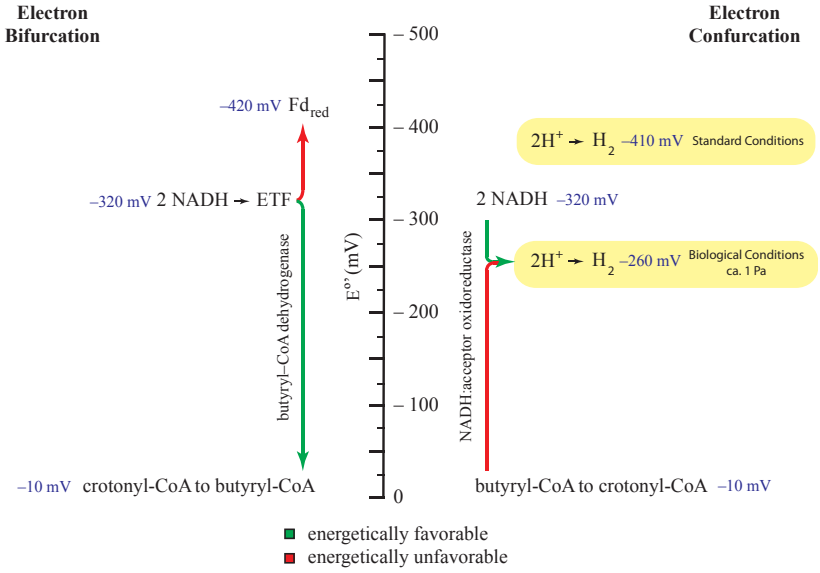

Fig. 4. A comparison of electron bifurcation and electron confurcation. The left side represents the electron bifurcation process as hypothesized by Herrmann *et al.* (2008) and verified by Li *et al.* (2008a, b), where the endergonic reduction of ferredoxin by NADH is coupled to the exergonic conversion of crotonyl-CoA to butyryl-CoA in *Clostridium kluyveri.* The right side illustrates the electron confurcation process during syntrophic butyrate degradation in *Syntrophomonas wolfei* (Mueller *et al.*, 2009). The reactions in yellow are catalyzed by an [FeFe]-type hydrogenase; the produced hydrogen is consumed and maintained at low partial pressures (c. 1 Pa) by a methanogenic partner such as *Methanospirillum hungatei*.

phylum activity, where syntrophy is an important basis for the formation and maintenance of stable microbiological communities (Moissl-Eichinger & Huber, 2011).

In general, classical syntrophic partnerships can be separated into two groups with different tasks: the *syntrophic primary degrader* (responsible for the mineralization of larger chemical compounds into small metabolic products) and the *consumer* (responsible for efficient removal of 'waste' products). Considering syntrophy based on anaerobic carbon degradation, the 'classical' case, the syntrophic primary degraders are typically affiliated with (*Delta-*) *Proteobacteria* (e.g. *Syntrophus*, *Desulfovibrio*) or *Clostridia* (e.g. *Syntrophomonas, Desulfotomaculum*). For a phylogenetic tree see McInerney *et al.* (2008). *Deltaproteobacteria* capable of syntrophic growth are generally strictly anaerobic microorganism with the capacity for fermentation when grown in co-culture; however, many are ecologically widespread sulfate-reducers (e.g. *Desulfuromonadales*, *Desulfovibrionales*) that respire sulfate when it is available. Syntrophic representatives of the *Firmicutes* are generally members of the *Clostridia* or *Bacilli*, as revealed in a novel study by Mueller *et al.* (2008). Members of these clades are strictly or facultatively anaerobic, generally spore-forming bacteria, and include typical syntrophic genera like *Pelotomaculum* and *Syntrophomonas*.

Methanogenic archaea form distinct lineages within the *Euryarchaeota* and are commonly the consuming partner during syntrophic relationships. Methanogens are not monophyletic and includes several clades of uncultivated and cultivated representatives (e.g. *Methanospirillaceae*, *Methanosaetaceae*). These organisms require strictly anoxic conditions for growth in most cases, due to their common inability to deal with reactive oxygen species (Jarrell, 1985) and can be found in oxygen-depleted sites of diverse natural and artificial environments. Many defined syntrophic co-cultures rely upon the activity of acetate- or carbon dioxide-using methanogens to 'pull' the reaction toward completion.

When considering microbial communities that are based on a certain level of nutrient exchange, the phylogeny of the involved microorganism is much more diverse and may include the majority of known and unknown microbial species. Several examples of nutritional synergism are provided in the following sections. For instance, the primary degradation part of the relationship is sometimes performed by the archaeal partner, as in the case of *Ignicoccus hospitalis* (*Crenarchaeota*), *Pyrococcus furiosus,* or the uncultured ANME-archaea. These examples highlight that the consumption task is not only restricted to methanogens. Depending on the environmental conditions, homoacetogens, iron-/sulfate-reducers or representatives of *Epsilonproteobacteria* (e.g. *Wolinella*) can assume this role.

Ecology and environmental conditions for syntrophy

Exchange of metabolic end-products can occur in a broad variety of environments. However, the need for synergistic interactions is reduced or eliminated when oxygen is available, since its use as a terminal electron acceptor results in higher energetic yields and therefore occurs preferentially. Under anoxic conditions, possibilities to yield energy are generally more diverse and pathways more complex. In many cases, interactive metabolism is often required to completely degrade large, complex compounds and to make the stored energy of the system bioavailable.

Classical environments where syntrophy occurs are often called 'methanogenic' (Kato & Watanabe, 2010), and lack alternative electron acceptors like sulfate or nitrate. In principle, these are ecosystems in which anaerobic methanogenic archaea can thrive. Anoxic sediments, for example, contain large reserves of carbon educts in various states of degradation and CO_2/hydrogen (or acetate) to support their metabolic activity. Temperatures and pH can range from *c.* 2–100 °C and 3–9, respectively, in anoxic groundwater, yet these ecosystems harbor active methanogenic communities. Methane can be biologically formed in natural biotopes, but may also be present in anthropogenic ecosystems like rice fields, landfills, wastewater systems, hydrocarbon contaminated sites, or anaerobic bioreactors. Natural biotopes include fens, eutrophic bogs, marshes, ruminant digestive tracts, and waterlogged sediments. More extreme biotopes with higher temperature (hot springs) or higher pH (soda lakes) may also become methanogenic (see references in McInerney *et al.*, 2008).

In current intense discussions about global warming, the production of large quantities of methane during syntrophic metabolism is of critical importance. The global warming potential of methane is estimated to be 20–25 times higher than that of CO_2 (Rodhe, 1990), so small changes in methane emissions can have significant impacts on global climate change and ecosystem dynamics. Methanogens produce billions of tons of methane per year and are therefore a major source for greenhouse gas emissions to the atmosphere. Even though aerobic methane-oxidizing bacteria and anaerobic methane-oxidizing consortia can oxidize 20–90% of the microbially generated methane in balanced systems (Reeburgh, 2003), anthropogenic activity and global disturbance can affect this balance and perpetuate larger methane release to the biosphere.

Examples of 'classical' syntrophic microbial communities

In the following section, we will present selected examples from diverse environments of microbial communities involved in syntrophic degradation of complex molecules, according to the 'classic' concept of syntrophy as described above.

Aromatic compound degradation

Early evidence by Nottingham & Hungate (1969) showed that ^{14}C ring-labeled benzoate was converted to carbon dioxide and methane. At the time of this discovery, it was thought that microorganisms could degrade aromatic compounds to methane independently. However, a seminal paper by Ferry & Wolfe (1976) not only demonstrated that multiple organisms cooperate during the methanogenic degradation of benzoate, but also recognized *Methanospirillum* spp. (Ferry *et al.*, 1974) as important players in syntrophic associations. Ferry & Wolfe (1976) showed that benzoate degradation ceased after manual perturbation of cellular associations and that *o*-chlorobenzoic acid caused the benzoate and acetate production to stop while acetate consumption and methane generation continued. Therefore, the metabolic process could not occur due to the action of one organism alone, but in the words of the authors '… appears to involve principles of microbial interaction such as the coupled reaction which occurs between S organism and *Methanobacterium* strain M.o.H.' (Ferry & Wolfe, 1976). Cooperative degradation of aromatic and polyaromatic compounds under anoxic conditions continues to be a dynamic area of modern research (e.g. Fuchs *et al.*, 2011; Berdugo-Clavijo *et al.*, 2012).

Oil-degrading communities

The complete role of individual members in oil-degrading methanogenic or sulfidogenic communities is still unknown. However, it is becoming clear that syntrophic degradation is an important process during aliphatic and aromatic hydrocarbon mineralization in crude oil reservoirs and petroleum-contaminated sediments (Jones *et al.*, 2008). Previous studies have shown that *Smithella* and *Syntrophus* are overwhelmingly enriched during methanogenic alkane degradation and that related organisms comprise a dominant portion of microbial communities from hydrocarbon impacted environments (Gray *et al.*, 2011 and references therein). Hydrocarbons are recalcitrant substrates under anoxic conditions and require specialized biochemical mechanisms for the activation and downstream degradation of these molecules (reviewed in Widdel *et al.*, 2006). This specialized biochemistry may also include syntrophy (e.g. Westerholm *et al.*, 2011). Recently, the first genome sequence of an *n*-alkane degrading, sulfate-reducing bacterium was sequenced (Callaghan *et al.*, 2012). This organism, *Desulfatibacillum alkenivorans* AK-01, was also shown to be capable of complete alkane mineralization in the presence of the hydrogen-/formate-utilizing methanogen *Methanospirillum hungatei* JF-1 and the absence of sulfate. Definition of model systems with two fully sequenced partners, such as this example, will enable researchers to better define the molecular mechanisms of syntrophy.

Reverse electron transport is a defining feature of syntrophic metabolism (McInerney *et al.*, 2009), as discussed in the previous section. In the case of AK-01, the cell lacks a typical hydrogen-evolving (FeFe-type) hydrogenase and the authors therefore proposed that formate was the electron shuttle between the archaeal and bacterial cells in the mixed-culture system during alkane oxidation (Callaghan

et al., 2012). Biochemical studies on these organisms are complicated by low biomass yields, yet these investigations will be necessary to understand the full nature of syntrophic processes. Overall, the degradation of hydrocarbons shares many steps with anaerobic fatty acid oxidation (through CoA intermediates), once the C-H bond is activated (Heider, 2007), and insights into anaerobic aliphatic and aromatic acid metabolism will also facilitate a better understanding of hydrocarbon oxidation by syntrophic communities in the coming years.

Syntrophic degradation of hexoses

The anaerobic degradation of hexoses to acetate, CO_2, and H_2 is an exergonic process. However, this reaction does not yield sufficient energy to support growth unless hydrogen pressure is reduced (Thauer *et al.*, 1977; Schink, 1997). In sulfate-poor anoxic environments, harboring complex microbial communities, methanogens, and homoacetogens are able to maintain hydrogen partial pressures at the level of 10^{-4} to 10^{-5} atm, thereby yielding more energy per mole of substrate for the sugar-fermenting bacteria (Schink & Stams, 2006; Mueller *et al.*, 2008). These greater energy yields facilitate complete fermentation of complex substrates to acetate, CO_2, and H_2 (or formate). These end-products are easily converted to CH_4 or acetate by methanogenic or homoacetogenic partners, respectively. On the other hand, when sugar-fermenting anaerobes are grown in pure culture, fermentation patterns generally shift to the production of butyrate, ethanol, or lactate due to the limited availability of ATP for use in substrate-level phosphorylation reactions (Iannotti *et al.*, 1973; Thauer *et al.*, 1977; Tewes & Thauer, 1980). This shift in fermentation patterns in the absence of a partner has been observed for a range of bacteria such as *Clostridia* spp. or *Ruminococcus albus* (Zeikus, 1983).

Novel sugar-fermenting bacteria from deep lake sediment were found to be incapable of switching to alternative fermentation pathways, but rather depend on a methanogenic partner to promote hexose fermentation (Mueller *et al.*, 2008). Isolation of these bacteria was achieved only in defined co-culture with *M. hungatei*. Growth of the isolated *Bacillus* sp. was slow and inhibited by high substrate concentrations, indicating adaptation to oligotrophic environments. Remaining reports of syntrophic sugar degradation in the literature are few but include work from Krumholz & Bryant (1986) and Dore & Bryant (1990). This unique type of syntrophy seems to be the dominant pathway for sugar degradation in sediments from the deep freshwater lake sampled in the above-described study (Mueller *et al.*, 2008) and may possibly extend to similar anoxic environments experiencing low organic matter input but relative stability with regard to other environmental factors.

Syntrophic growth on formate

Formate is a known electron shuttle in methanogenic communities, especially in aquatic systems (Boone *et al.*, 1989; Stams & Dong, 1995). The potential for anaerobic bacteria to grow by converting formate to hydrogen and bicarbonate was therefore overlooked for many years. However, a recent study reported growth of two different communities on formate (Dolfing *et al.*, 2008). Both communities consisted of a single bacterial strain (*Moorella* sp. strain AMP or *Desulfovibrio* sp. strain AMP) and a hydrogen-consuming methanogenic partner. The bacteria were shown to grow in pure culture on other substrates. However, after the addition of formate as the sole energy and carbon source, biomass increases were only observed in co-culture with the methanogenic partner or when H_2 was scavenged from the system using other means, indicating syntrophic degradation of formate.

In natural systems, formate-metabolizing bacteria will directly compete with methanogens able to use formate as an electron donor to convert CO_2 to CH_4, a reaction that yields more energy than the conversion of formate to H_2 and bicarbonate (Dolfing *et al.*, 2008). This competition is similar to syntrophic growth on acetate, where acetate-oxidizing bacteria compete directly with aceticlastic methanogens (Zinder & Koch, 1984). Even though aceticlastic cleavage is thermodynamically favorable over the oxidation of acetate to CO_2 and H_2, the latter reaction has been shown to take priority in various environmental settings (Nuesslein *et al.*, 2001; Shigematsu *et al.*, 2004). The two-step syntrophic conversion of acetate to methane and CO_2 often occurs under elevated temperatures or lowered pH, as both situations increase the overall energy yields of this metabolic process.

Syntrophic degradation of amino acids

Protein degradation results in a complex mixture of amino acids and small peptides that may be subsequently metabolized by anaerobic microorganisms. Degradation of amino acids (under anoxic conditions) is often accomplished by coupling the oxidation and reduction of amino acids to carboxylic acids, a process known as Stickland fermentation (Schink & Stams, 2006). During this process, hydrogen production occurs if there is a lack of Stickland acceptors (Nisman, 1954). Electrons can be removed to sustain the fermentation activity by hydrogen transfer to sulfate-reducing, homoacetogenic, or methanogenic organisms (Schink & Stams, 2006). Syntrophic growth on amino acids, in particular with methanogenic partners,

has been shown for a range of mesophilic and thermophilic bacteria (Zindel *et al.*, 1988; Tarlera *et al.*, 1997; Baena *et al.*, 1998, 1999, 2000). However, most reactions involved in syntrophic amino acid degradation are not yet understood in detail, and very few related processes have been studied (for a review see Schink & Stams, 2006). Generally, established examples are the oxidative degradation of alanine, valine, leucine, and isoleucine. During initial fermentation, the first step (i.e. the deamination of the amino acid to the corresponding α-keto acid) requires efficient removal of hydrogen to be thermodynamically feasible. Subsequent degradation of the α-keto acid to a fatty acid releases electrons and can result in much higher energy yields depending on the overall partial pressure of hydrogen. Bacteria capable of syntrophic growth on amino acids have been isolated by providing α-keto acids as a carbon source (Schink & Stams, 2006).

Although our understanding of the ecology of anaerobic amino acid degradation is incomplete, Schink & Stams (2006) suggest that pure Stickland fermentation is particularly favored in environments with high amino acid concentrations, while syntrophic degradation dominates in amino acid-poor environments (i.e. where Stickland-acceptor concentrations are low).

Non-classical types of syntrophy

Apart from the 'classical' syntrophic relationships mentioned above, many microbial communities rely on other syntrophic interactions that do not fit the classical definition of syntrophy. In the following section, we will present several examples of unique microbial partnerships that metabolically depend on each other.

Sulfur-syntrophy

Metabolic interdependence based on sulfurous compounds has been studied for several decades. Co-cultures of green sulfur bacteria and sulfate-reducing bacteria (SRB) have been shown to provide stable growth conditions for each other (Biebl & Pfennig, 1978) based on the transfer of elemental sulfur and sulfide. Shaposhnikov isolated one of the first sulfur-based co-cultures in 1960 ('*Chloropseudomonas ethylica* strain 2-K', Shaposhnikov *et al.*, 1960). This culture was initially regarded as a single-species enrichment, but turned out to be composed of two bacterial species, *Prosthecochloris aestuarii* and *Desulfuromonas acetoxidans* (Olson, 1978). In pure culture, *Desulfuromonas*, when grown on acetate, is significantly inhibited by metabolic sulfide-production (from elemental sulfur) long before high cell concentrations are achieved. In contrast, the green sulfur bacteria (e.g. *Chlorobium* spp.) can be grown to high cell numbers when low but constant sulfide concentrations are maintained in the growth medium. Co-cultures of the two organisms are stable and self-sustaining because *Chlorobium* oxidizes sulfide to elemental sulfur which is then used as an electron acceptor by *Desulfuromonas* during acetate oxidation, leading to the regeneration of sulfide. Both sulfurous compounds are kept at non-inhibitory concentrations, allowing the co-culture to thrive (Biebl & Pfennig, 1978; Warthmann *et al.*, 1992).

Sulfur is an essential element for biological building blocks and cofactors, and sulfur-related syntrophy aids in the conversion of organic- to inorganic-forms of sulfur and promotes biogeochemical cycling.

Chlorochromatium aggregatum: a model system for the close interaction between two bacteria

Phototrophic consortia have been known for over 100 years (Lauterborn, 1906). To date, nine different morphotypes have been described (Overmann, 2004). However, '*C. aggregatum*' is the only phototrophic consortium that can currently be cultivated in the laboratory (Mueller & Overmann, 2011). This consortium is clearly structured: green sulfur bacteria (*Chlorobium chlorochromatii* strain CaD) encapsulate the rod-shaped central bacterium related to *Comamonadaceae* (*Betaproteobacteria*; Fig. 5; Froestl & Overmann, 2000). Although the strictly anaerobic, photolithotrophic epibiont has been successfully cultivated in pure culture, all attempts to grow the central bacterium alone have failed. *Chlorobium chlorochromatii* turned out to be a typical representative of its genus, photoassimilating acetate and peptone in the presence of sulfide and hydrogen carbonate (Vogl *et al.*, 2006). However, compared to close phylogenetic neighbors, *C. chlorochromatii* exhibits low cellular concentrations of carotenoids and seems to be unable to produce chlorobactene (Mueller & Overmann, 2011).

Interestingly, *Chlorobia* tend to deposit sulfur globules extracellularly – an observation that led to the assumption that the central bacterium in '*C. aggregatum*' could be a sulfur- or sulfate-reducing organism. However, this hypothesis could not be substantiated and seems unlikely after the discovery that the central bacterium is affiliated with *Betaproteobacteria* (Froestl & Overmann, 2000). Experiments with labeled carbon revealed that organic compounds are transferred from the epibiont to the central bacterium and that this relationship was interrupted when amino acids or 2-oxoglutarate were externally supplemented (Mueller & Overmann, 2011). The latest results hint at a need for, and consumption of, related compounds by the central bacterium.

Taxis of '*C. aggregatum*' toward light and sulfide has been demonstrated (Froestl & Overmann, 1998). It turns

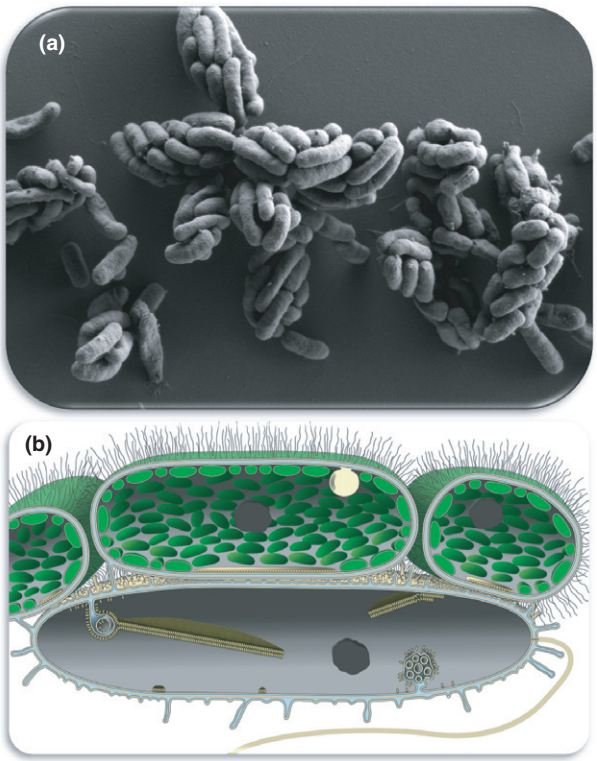

Fig. 5. '*Chlorochromatium aggregatum*': a phototrophic consortium formed by green sulfur bacteria (*Chlorobium chlorochromatii*) and a rod-shaped central bacterium (*Betaproteobacteria*). The syntrophic relationship seems to be based on transfer of organic compounds. (a) Scanning electron micrograph; epibionts are tightly packed on the surface. (b) Schematic drawing of the consortium based on 3D reconstruction. For further details, see Wanner *et al.*, 2008. Figure provided by G. Wanner, Munich.

out that the flagellated, central bacterium is responsible for the mobility of the entire consortium, and chemoreceptor-like structures are found in the betaproteobacterial cells (Wanner *et al.*, 2008; Fig. 5). However, the inner workings and regulation of consortial chemo-/phototaxis are unknown at this time. Still, the '*C. aggregatum*' system is a fascinating example of structured, syntrophic interactions between two bacteria.

Co-cultures of hyperthermophilic archaea

Two hyperthermophilic archaea (*P. furiosus* and *Methanopyrus kandleri*) were chosen for syntrophic growth experiments under laboratory conditions. When co-cultured at 95 °C, a positive effect on the growth of both partners was observed. *Pyrococcus furiosus* ferments organic compounds, whereas *M. kandleri* performs methanogenesis and most likely supports the syntrophic partnership by effective hydrogen removal (Schopf *et al.*, 2008; Fig. 6). In co-culture, these syntrophic partners reach higher cell densities and form a dual-species biofilm on provided surfaces, likely allowing them to interact more closely. However, when *P. furiosus* was brought into contact with other methanogenic archaea, the effect of the other partner on cell density was either positive, neutral, or inhibitory. The highest positive interaction was found during the *P. furiosus* and *Methanocaldococcus villosus* pairing. Compared to single-species cultivation, both partners grew to higher cell densities in co-culture. The lowest cell numbers were observed with the *P. furiosus* and *Methanotorris igneus* culture, and in this case, the partners seemed to prefer independence (Weiner *et al.*, 2012). The authors hypothesized that the symbiotic relationship was based on H_2 transfer. However, conclusive evidence for the exchanged intermediate has not been provided to date. All of these experiments highlight how very little we know about microbial species interactions, cell–cell signaling, and the nature of metabolic intermediate exchange.

The 'intimate association' of *Nanoarchaeum equitans* and *I. hospitalis* is based on a unique interaction between two archaea (Huber *et al.*, 2002; Jahn *et al.*, 2008; Fig. 7). *Ignicoccus hospitalis,* acting as host in this association, can also be grown in pure culture. In contrast, it is a mandatory partner for *N. equitans,* which has not been cultivated independently to date. Cell maintenance and division of *N. equitans* occurs only in direct contact with *I. hospitalis* cells, as shown by live-dead staining (Jahn *et al.*, 2008), and the physiology of *N. equitans* is dependent on the viability of the host cell. In co-culture, doubling times and final cell densities of the host remain unaffected by the presence of *N. equitans*, although it was shown by optical tweezers experiments that *I. hospitalis* cells can no longer divide when three or more *N. equitans* cells are attached to

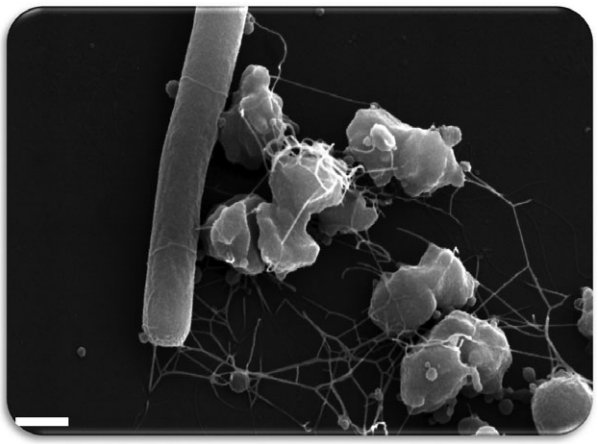

Fig. 6. Co-culture of *Pyrococcus furiosus* (coccus) and *Methanopyrus kandleri* (rod), most likely based on H_2-transfer. The scanning electron micrograph shows the close physical interaction of the two hyperthermophilic *Archaea*. Bar: 1 μm. Provided by S. Schopf, Regensburg.

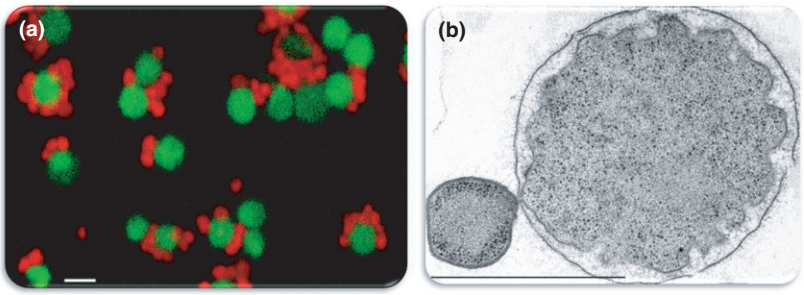

Fig. 7. *Nanoarchaeum equitans and Ignicoccus hospitalis*, an intimate association most likely based on transfer of organic material and possibly ATP. (a) Fluorescence *in situ* hybridization: *N. equitans* (red) and *I. hospitalis* (green), Bar: 2 μm. (b) Thin section of the association, electron micrograph. Bar: 1 μm.

its surface (Jahn *et al.*, 2008). *Ignicoccus hospitalis* is also unique in that it is the only organism known to produce an electrochemical gradient using the *outer* membrane. The ATPase and hydrogen : sulfur oxidoreductase are also located on the outer membrane (Kueper *et al.*, 2010). Further details about this unusual cell structure can be found in a recent review by Huber *et al.* (2012).

Analysis of the *N. equitans* genome (Waters *et al.*, 2003) revealed that the organism lacks essential genes for lipid-, cofactor-, amino acid-, or nucleotide biosynthesis. This fact hints toward a dependency on nutrient and growth factor transfer from *I. hospitalis* to *N. equitans*. In fact, it has been demonstrated that *N. equitans* obtains all of its lipids and amino acids from the host by unknown transport mechanisms (Jahn *et al.*, 2004, 2007). Recently, it has been suggested that this might also be true for ATP, although this has to be proven experientially (Huber *et al.*, 2012).

All of these observations suggest that the relationship between *Ignicoccus* and *Nanoarchaeum* is complicated and may not be a clear case of syntrophy, since *Ignicoccus* is not dependent on the presence of *Nanoarchaeum*. However, this intimate association represents to date the only natural, cultivated community of two archaeal species and a fascinating model for interactive relationships and novel biological processes.

Co-cultures of methanotrophs and *Hyphomicrobium*

The majority of syntrophic relationships described thus far thrive in anoxic environments. However, examples of syntrophy can also be found under aerobic conditions. One such relationship is based on the removal of toxic compounds produced by one partner, rather than for energetic or metabolic advantages. Aerobic methanotrophic bacteria use methane as the sole energy and carbon source, performing the oxidation of methane to methanol, and methanol to formaldehyde as the first two steps during methane oxidation (Hanson & Hanson, 1996). During laboratory enrichment or isolation of methanotrophs, co-enrichment of *Hyphomicrobium* sp. strains has often been observed (e.g. Dedysh *et al.*, 1998; Ferrando & Tarlera, 2009). The latter methylotrophic organisms most likely oxidize methanol, which is excreted to some extent during methane oxidation by the methanotrophs. This removal of methanol from the system prevents the inhibition of methanotrophic growth (Wilkinson *et al.*, 1974; Moore, 1981). It has also been suggested that highly toxic formaldehyde is removed from the system during syntrophic growth of methanotrophs and methylotrophs like *Hyphomicrobium* spp. (Schink, 2002).

Examples of uncultured syntrophic communities

Anaerobic methane oxidation coupled to sulfate reduction

Consortia capable of the AOM are most likely among the best-characterized, uncultured archaeal-bacterial syntrophic communities. This fascinating interaction of SRB and anaerobic methanotrophic (ANME) archaea is responsible for removal of a substantial part (7–25%) of the total global, biologically produced methane (Reeburgh, 2007). In the case of AOM, the archaeal partner has the ability to reverse the normal methanogenic pathway, thereby consuming methane and producing CO_2 as a metabolic end-product. Sulfate reduction by the SRB acts to remove electrons from the system and provides for small energy gains by both microbial partners (Knittel & Boetius, 2009). Interestingly, possible electron shuttles or intermediates for this type of partnership have not been identified and the mechanism of electron transfer between the two partners is still unknown (Knittel & Boetius, 2009). Typical methanogenic substrates (i.e. H_2, formate, acetate, methanol) did not support sulfate reduction in the absence of the methane-oxidizing archaeon (Nauhaus *et al.*, 2002; Widdel *et al.*, 2006). Theories about possible sulfate reduction by the ANME-archaea, or interdependency based on other metabolites, could not be confirmed to date. Tracer experiments using stable carbon isotopes clearly showed that the archaea and bacteria

perform the overall metabolic process together and that the bacteria can grow autotrophically on CO_2 (Wegener *et al.*, 2008). However, the archaea involved in AOM within methane seeps are able to fix nitrogen and to share nitrogenous compounds with the SRB partners. Therefore, the syntrophic relationship between AOM members may not rely on a single dependency (i.e. carbon turnover), but possibly includes sharing of organic nitrogen or other unidentified substrates and compounds (Dekas *et al.*, 2009). AOM-related syntrophy is an exciting avenue for discovery related to this important global process and demonstrates how syntrophy can have large impacts on global carbon cycling. For more information, we refer the reader to an excellent AOM review by Knittel & Boetius (2009).

For the sake of completeness, it shall be mentioned that anaerobic methane oxidation can also be linked to metal-oxide reduction, nitrite dismutation, or disulfide disproportionation (Beal *et al.*, 2009; Ettwig *et al.*, 2010; Milucka *et al.*, 2012) and is not necessarily dependent on syntrophic sulfate reduction. These other types of anaerobic methane oxidation are not dependent on inter-species metabolic activity and are therefore not considered syntrophic. Future work will shed light on the exact processes and when syntrophy is required for this intriguing and biogeochemically important process.

SM1 string-of-pearls community

Growing independently in the subsurface of cold sulfidic springs, the uncultivated SM1 Euryarchaeon forms an association with sulfide-oxidizing bacteria as soon as it is washed up from the deep into oxygenated surface waters (Henneberger *et al.*, 2006; Probst *et al.*, 2013). There, pieces of the SM1 euryarchaeal biofilm are encompassed by filamentous sulfide-oxidizing bacteria (mainly *Thiothrix* or *Sulfuricurvum*); together they form an ordered microbial community that resembles a string-of-pearls (Fig. 8). This community is visible to the naked eye and was originally discovered in a cold spring in Bavaria, Germany (Rudolph *et al.*, 2001; Moissl *et al.*, 2002). The recurrent constellation of SM1 with sulfide-oxidizing bacteria hints at a metabolic interdependency, but the nature of this interaction is still a mystery. Previous studies have assumed that the SM1 euryarchaeon functions as a sulfate-reducing microorganism and suggest that a single 'pearl' is maintaining an internal sulfur-cycle based on the exchange of sulfate and sulfide (Moissl *et al.*, 2002). However, on-going metagenomic analyses, as well as physiological studies of the subsurface SM1 biofilm (using SR-FTIR), refute the hypothesis of sulfate-reducing metabolic activity for SM1 (Probst *et al.*, 2013).

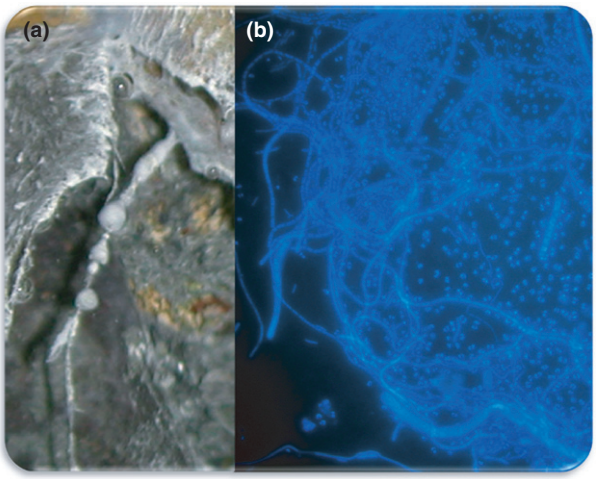

Fig. 8. String-of-pearls community. This consortium represents an uncultivated example of a (syntrophic) microbial association composed of *Archaea* and *Bacteria*. (a) 'Pearls' in their biotope (diameter of a pearl about 3 mm). (b) DAPI-stain of a squeezed pearl. SM1 *Euryarchaea*: cocci; *Thiothrix*: filament-forming.

Although the string-of-pearls community is most assuredly not a random aggregation of *Archaea* and *Bacteria*, the basic principles of this interaction remain to be elucidated. Yet this fascinating community is an example of microbial interactions in nature whose symbiosis or even syntrophy seems obvious at first, but requires deeper analysis using novel and creative techniques to investigate the physiology of these microorganism in more detail. Some of these methods and innovative techniques will be introduced in the following section.

Novel methods to analyze syntrophic relationships of uncultivated and cultivated microorganisms

The metabolic nature of syntrophy and the limited number of available cultures makes it difficult to characterize the microbial interactions in the laboratory, in addition to following the exchange of very low concentrations of metabolites or unknown signaling molecules. The measurement and maintenance of naturally occurring syntrophic relationships under artificial conditions can be challenging: When exchange processes are disrupted by interference, the syntrophic relationship may become unbalanced or is destroyed.

Novel and creative experiments must be developed to study these processes under *in situ* conditions. This section will present some of the methods that we feel hold promise for characterizing syntrophic metabolism in more detail, and identifying key metabolic players within complex microbial communities.

Whole genome analyses and metagenomics

Genomic analyses from a number of cultivated syntrophs have helped to elucidate the specific properties and adaptations necessary for such a lifestyle and provided insight into uncultivated syntrophic communities. For instance, based on genomic studies, it was shown that methanotrophic archaea in AOM communities harbor nearly all of the genes involved in the typical methane production machinery (Hallam *et al.*, 2004). Together with biochemical analyses (Scheller *et al.*, 2010), these findings substantiate the idea of 'reverse methanogenesis' in AOM associations first proposed by Zehnder & Brock (1979, 1980).

Genome-based analyses have also recognized candidatus '*Cloacamonas acidaminovorans*' as a possible amino acid fermenting syntroph in anaerobic wastewater digesters (Pelletier *et al.*, 2008). A recent metagenomic study of a terephthalate-degrading microbial community has identified several syntrophs and novel methanogens that are involved in the syntrophic degradation of this important chemical compound. Based on their results, the authors suggest that a complex microbial community (*Pelotomaculum*, thermotogae, *Syntrophus*, and representatives of candidate phyla OP5) is important for the stability of the degradation process and provide an outline to explore the metabolism of this compound (Lykidis *et al.*, 2011).

These (meta-)genomic approaches (in addition to computational modelling) are powerful tools to analyze and compare cultivated and uncultivated syntrophic microorganism and communities. However, the detection of metabolic pathways on a genomic-level remains theoretical and active transcription and translation of these genes still need to be verified using biochemical and molecular methods. This requires the use of spectrometric and spectroscopic techniques like nanoSIMS (secondary ion mass spectrometry), Raman spectroscopy, or synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy that can follow the chemical-turnover of specific compounds on a cellular level. In addition, stable isotope probing (SIP) or microautoradiography (MAR) can help to elucidate syntrophic activity.

Nano secondary ion mass spectrometry (NanoSIMS)

Investigating syntrophy in detail will truly involve the study of biochemical and biogeochemical processes on a single-cell level. Few techniques offer the possibility to study metabolic activities of single cells better than secondary ion mass spectroscopy (SIMS). NanoSIMS is the evolution of technology (i.e. SIMS) that dates back to the 1940s (Herzog & Viehboek, 1949) and was primarily used to study surfaces in geology, mineralogy, and material science. However, the application of SIMS technology to living or biological tissues is a relatively recent advance, with the foray into microbiology first published in early 2000 (Orphan *et al.*, 2001). Limitations of these early instruments with regard to lateral and mass resolution led to the latest iteration of machines with beam diameters in the range of 50–200 nm, depending on the nature of the ions used. For specifics on the instrumentation and technology behind this approach, we refer the reader to several excellent reviews (Lechene *et al.*, 2006; Boxer *et al.*, 2009; Orphan & House, 2009; Musat *et al.*, 2012). The benefit of SIMS comes from the ability to detect elements in the general ppm range and to rasterise the sample, thereby producing maps of ion intensities at each ion beam position. This means that isotopic and elemental information can be deduced for single cells. NanoSIMS combined with labeled (^{13}C, ^{15}N, ^{18}O) substrates provide the chance to investigate the physiology of individual partners in complex communities (Li *et al.*, 2008a, b; Wagner, 2009). In combination with fluorescence *in situ* hybridization (FISH), SIMS can even link metabolic function to phylogenetic information. For example, the combination of FISH and nanoSIMS (Orphan *et al.*, 2001) has been used to successfully study the interaction of filamentous cyanobacteria (*Anabaena*) and heterotrophic *Rhizobium* sp. (epibiont) in a defined co-culture (Behrens *et al.*, 2008), and to elucidate carbon- and nitrogen-compound transfer within AOM communities (Orphan *et al.*, 2001; Dekas & Orphan, 2011). The *Anabaena* and epibiont were incubated with ^{13}C-bicarbonate and ^{15}N-dinitrogen to investigate the nature of the relationship. Findings indicated that *Anabaena* could fix nitrogen and carbon alone; however, the epibiont only contained labeled, fixed products in co-culture (Behrens *et al.*, 2008). Results such as these highlight that further advances in instrument technology and biological sample preparation will give researchers the potential to visualize cellular processes with remarkable levels of precision and resolution.

Spectroscopy

Raman spectroscopy has been used in combination with FISH (Raman-FISH) and SIP (Huang *et al.*, 2007). Raman is a non-invasive technology to identify biomolecules by their chemical bond reflection patterns after excitation using laser light. Raman-FISH can be used without addition of expensive labeled compounds and is capable of similar optical resolution compared to nanoSIMS. However, the signals obtained are often quite weak and complicated by interfering autofluorescence from cellular components or molecules. Advances in instrumentation and data processing over the past decade have made it

possible to map single cells in well-controlled samples (Schuster *et al.*, 2000). The future of Raman imaging is even brighter with a number of recent technological advancements including surface enhanced Raman spectroscopy of graphene (Schedin *et al.*, 2010). Huang *et al.* (2007) revealed novel insights into microbial naphthalene degradation in groundwater using the aforementioned multi-faceted approach and highlighted the power of combining several methods to understand the ecology and biochemistry of a system (Neufeld & Murrell, 2007).

A recent approach using synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy indicates that a combination of FISH and spectroscopic methods might be unnecessary for certain experiments. For example, Probst *et al.* (2013) demonstrated the general capability of SR-FTIR to differentiate between *Bacteria* and *Archaea* based on the typical lipid and carbohydrate structures. Accordingly, the co-localization of carbonate and organic sulfate compounds with bacterial (and not archaeal) cells was shown for the SM1 euryarchaeal biofilm (Henneberger *et al.*, 2006; Probst *et al.*, 2013). This observation refutes the idea of sulfate-compound turnover by the SM1 euryarchaeon (Probst *et al.*, 2013). In this communication, SR-FTIR has been shown to provide a nucleic-acid independent method to link phylogenetic information with the spatial distribution of chemical compositions and metabolic activities of certain cells. SR-FTIR provides unique advantages; the method is non-invasive, allows molecular imaging without the need for isotopic tracers, uses infrared light to reduce background noise, and permits the observation of living samples under both aerobic and anaerobic conditions (Holman *et al.*, 2009, 2010). In current applications, this technique has a resolution-capability down to 3 μm.

Stable isotope probing

SIP has helped to identify uncultured microorganism involved in the turnover of substrates of interest, and to correlate specific metabolic activity to these microorganisms. Compounds labeled with stable isotopes (^{13}C, ^{15}N, ^{18}O) are provided as substrate and the integration of the tracer is followed into phospholipid fatty acids (PLFA), nucleic acids, or amino acids. These compounds can then be separated by gradient centrifugation, in the case of nucleic acids (Radajewski *et al.*, 2000), or analyzed by isotope ratio mass spectrometry in the case of PLFA (Neufeld *et al.*, 2007) and amino acids (Jehmlich *et al.*, 2010). To date, SIP is mostly applied during *in vitro* laboratory analyses of long-time batch cultures or environmental samples. For instance, SIP was successfully used to demonstrate that a syntrophic community under denitrifying conditions degraded benzene during an 8-year chemostat study (van der Zaan *et al.*, 2012). Dominant benzene degraders related to *Peptococcaceae* were identified in an iron-reducing enrichment culture by analyzing labeled 16S rRNA genes (DNA-SIP). These members of the *Clostridia* oxidize benzene and transfer electrons directly to ferric iron or to syntrophic partners (*Desulfobulbaceae*; Kunapuli *et al.*, 2007).

Proteomics of a methanogenic community growing on uniformally ^{13}C-labeled hexadecane and palmitate demonstrated that both aceticlastic and hydrogenotrophic methanogens were labeled to a similar extent, suggesting that syntrophic acetate oxidation occurs to some extent during mineralization of *n*-alkanes to CO_2 and methane (Morris *et al.*, 2012). Other studies from hydrocarbon impacted environments have also suggested that syntrophic acetate oxidation occurs during methanogenic hydrocarbon degradation (Jones *et al.*, 2008; Gray *et al.*, 2011; Westerholm *et al.*, 2011). SIP experiments have therefore begun to reveal the interaction between primary degraders and their syntrophic partners. Future tracer experiments will help to further highlight the specifics of interspecies cooperation during the degradation of recalcitrant compounds by slow-growing communities.

Application of SIP to natural environments during the study of *in situ* communities is not an easy task. However, several studies have successfully used PLFA-SIP directly in the field, for example, to label methane-oxidizing bacteria in a landfill-cover soil (Henneberger *et al.*, 2013), nitrate- and SRB in a petroleum-contaminated aquifer (Pombo *et al.*, 2002, 2005), or to study carbon fixation by microphytobenthos within intertidal zones (Middelburg *et al.*, 2000). Soil incubated with ^{18}O-labeled water was recently used to investigate ammonia-oxidizing bacteria and archaea activity by following the incorporation of the labeled oxygen into nucleic acids (Adair & Schwartz, 2011). DNA-SIP using a carbon label has also been applied in a field-based study to identify active microbial populations in soil (Padmanabhan *et al.*, 2003). These studies enable microbial potentials to be measured while minimizing the disturbance caused by sampling, transport, and laboratory manipulation.

Microautoradiography

In contrast to the two methods above, MAR relies on the use of radiolabeled substrates and a commercially available autoradiography emulsion to study the activity of single bacterial cells. This is one of the oldest techniques, being in active use for over 50 years (for a review see Nielsen & Nielsen, 2005). The technique was developed by Thomas Brock in the 1960s and first used to study epibionts on marine algae (Brock & Brock, 1966). Spatial resolution is highest when using radiolabeled substrates with weak B-particle emission, such as tritium (^{3}H) or

^{14}C, and is on the order of 0.5–2.0 μm (Okabe *et al.*, 2004). Other radioisotopes used for MAR include ^{33}P (Lee *et al.*, 2002) and ^{35}S (Vila *et al.*, 2004). Although MAR is very sensitive for detecting specific metabolic activity of single cells, the resolution is limited in complex microbial structures and correlating metabolic function with specific microbiological groups was impossible. To address the latter problem, MAR was combined with FISH to merge the activity measurements of MAR with specific, fluorescently-labeled 16S rRNA gene probes to gain phylogenetic information about the metabolically active microorganism (Lee *et al.*, 1999). This technique (MAR-FISH) has been used to study the physiology of uncultured organisms in complex microbial communities such as biofilms (for a review see Wagner *et al.*, 2006). MAR-FISH has even been combined with microelectrode measurements, to quantify dissolved compounds in microenvironments, for example, to measure O_2 consumption and nitrogen turnover in a denitrifying biofilm (Gieseke *et al.*, 2005) or to investigate the O_2 tolerance of uncultured *Chloroflexaceae*-related bacteria associated with a hot spring microbial mat (Nuebel *et al.*, 2002). These examples highlight that unique combinations of methods, such as MAR-FISH and microelectrode measurements, can better address open questions related to substrate turnover rates at a cellular level and help to identify distinct activities in diverse communities.

Syntrophy beyond

Syntrophy, as a co-feeding process, is certainly not restricted to microorganism. Microbial/eukaryotic interactions based on nutrient exchange or waste removal have been reported for marine invertebrates, sponge communities, and even the human gut (Zoetendal *et al.*, 2006). For instance, along hydrothermal vents in the deep ocean, well beyond the euphotic and disphotic zones, light is absent but the sediments are rich in reduced carbon and sulfur compounds. Here, eukaryotic species able to survive the intense pressure and cold temperatures rely upon the activities of endosymbiotic, autotrophic, and chemosynthetic bacteria to produce organic molecules necessary for life.

Riftia species, for example, lack digestive tracts but host sulfur-oxidizing bacteria in specialized organs called trophosomes. The host provides – along with other necessary compounds – CO_2, O_2 and sulfide for the chemoautotroph and creates a safe niche for metabolic functioning (Minic & Herve, 2004). In many cases, these eukaryotic/bacterial symbioses exist for tremendous periods of time, and co-adaptation or even co-evolution with respect to certain metabolic pathways can be observed (e.g. pyrimidine *de novo* synthesis and salvage in *Riftia* spp., Minic & Herve, 2004). For sponges, it has been proposed that co-evolution of associated microorganisms has occurred, since these communities differ significantly from the surrounding, free-dwelling microbiota (Taylor *et al.*, 2007). In addition to bacteria, sponges harbor ammonia-oxidizing archaea affiliated with the newly proposed phylum '*Thaumarchaeota*' (Brochier-Armanet *et al.*, 2008). The eukaryotic host seems to benefit from efficient removal of ammonium, and the archaeal partner most likely benefits from a level of protection and a constant supply of metabolic educts (Radax *et al.*, 2012).

These last examples emphasize again that metabolic interactivity is not at all restricted to carbon turnover, but can also be based on exchange of sulfur- and nitrogen-containing compounds and therefore plays an important role in global cycling of chemicals. Another well-known example is nitrification – an important syntrophy-dependent process in global nitrogen turnover. Metabolic interactions are not restricted to kingdoms, to ecological niches, or to certain types of chemicals, but are omnipresent phenomena that ensure the survival of metabolic partners ranging from microbiological to global scales.

Outlook and conclusion

'There are many, many more examples of this kind [of cooperation] out in nature, and we only have to look at things in such broader terms to widen our eyes for the unexpected'. With these words, Bernhard Schink closed his review entitled 'Synergistic interactions in the microbial world' (Schink, 2002), a highly cited article dealing with syntrophic interactions and cooperative (microbial) systems in general.

We indeed have to open our eyes to uncover the unexpected, fascinating microbial capability for metabolic interaction, but we will also have to clarify the definition of syntrophy, as suggested in this review article.

Classical definitions of syntrophy are often descriptive and put their main emphasize on the process, for instance, the degradation of (complex) organic compounds under anoxic conditions (McInerney *et al.*, 2008). However, co-cultures of sulfide-oxidizing and SRB have also been named 'syntrophic mixed cultures' (Biebl & Pfennig, 1978). These mixed definitions put the essence of syntrophy in a predicament. With introducing 'obligately mutualistic metabolism' as a short definition of syntrophy, we hope to cover all aspects of microbial syntrophy and highlight the *overall metabolic process* that benefits all microbial partners involved.

We have taken ecological terms with specific definitions and applied them to syntrophy, bringing together macro- and microecological concepts. In all cases presented here, syntrophy refers to a metabolic interactivity that may be

included as a subset of symbiosis. The difference is that symbiosis does not rely on metabolic interaction while syntrophy does. Under the definition introduced here, it is easy to determine whether a process meets the requirements for being termed syntrophic. First, consider the overall chemical equation of a metabolic process and then decide whether this equation is energetically feasible without the metabolic cooperation of microorganisms. If the answer is no, the process is syntrophic and likely benefits all microbial players by expanding niches and permitting cellular survival under conditions that may be otherwise inhospitable.

In this review, we have presented classical and non-classical types of syntrophic communities based on the transfer of hydrogen, carbon-, sulfur-, or nitrogenous compounds, growth factors, removal of toxic metabolic end products, and also with yet unknown underlying principles (as a summary, please see Concept Box). Our observations suggest that syntrophy is a widespread phenomenon: All living organisms use and produce resources and are part of an omnipresent turnover called the ecosystem. All organisms are dependent on others to assure that life does not stagnate.

Concept Box: Basic principles of non-classical types of syntrophy and presented examples.

Transfer of H_2

The artificial co-culture of *P. furiosus* and *M. kandleri* is most likely based on transfer of H_2. When grown in co-culture, both archaea reach higher cell-densities than compared to single-species cultivation (Schopf *et al.*, 2008; Fig. 6).

Transfer of sulfur compounds

Co-cultures of green sulfur bacteria (e.g. *Chlorobium*) and SRB (e.g. *Desulfuromonas*) are based on transfer of elemental sulfur and sulfide. By the activity of both partners, sulfur-compounds are kept at non-inhibitory concentrations, allowing the co-culture to thrive (Biebl & Pfennig, 1978; Warthmann *et al.*, 1992).

Transfer of carbon-, nitrogen and other compounds

Microbial consortia capable of anaerobic methane oxidation in combination with sulfate-reduction are formed by methanotrophic archaea and SRB. ANME archaea perform the methane-oxidation process, supported by a constant electron removal by sulfatereduction activity of involved *Bacteria*. The transfer of nitrogenous and carbon compounds has been proven; however the electron-shuttle or other transferred compounds – in this particular case – have to date not been identified (Knittel & Boetius, 2009).

Transfer of organic compounds

'*Chlorochromatium aggregatum*': This phototrophic consortium is a physically close association of several cells of *C. chlorochromatii* and a representative of the *Comamonadaceae* (central bacterium). Whereas the central bacterium provides motility, the epibionts seem to feed it with organic compounds, such as amino acids or 2-oxoglutarate (Mueller & Overmann, 2011; Fig. 5).

Transfer of nutrients, growth factors and complex organic compounds

Since *N. equitans* lacks essential genes for lipid-, cofactor-, amino acid and nucleotide biosynthesis, this organism is completely dependent on its host *I. hospitalis,* which provides for instance lipids, amino acids and most likely ATP. *Ignicoccus hospitalis* however, seems to be unaffected by the presence of *N. equitans* (Huber *et al.*, 2012; Fig. 7).

Removal of toxic compounds

Aerobic enrichments of methanotrophic bacteria frequently contain representatives of the genus *Hyphomicrobium*. This microbe seems to remove methanol, which is produced as a by-product during methanotrophy and could inhibit the growth of methane-oxidizing *Bacteria* (Wilkinson *et al.*, 1974; Moore, 1981).

Interesting microbial interactions can seem simple; however, in close spatial microbial associations, syntrophy becomes increasingly complex. Syntrophy is reflected in different levels of metabolic cooperation, from individual cells up to entire communities. The reason(s) that microorganism seek each other, attach to each other, and find interesting possibilities to exchange electrons, nutrients or other compounds, and in turn form highly efficient cooperative metabolic processes, should inspire researchers to expand our understanding of natural systems. While so little has been explored, the examples of close interactions that we know have led to a number of unexpected and amazing insights into the micro-world.

Life evolved under anaerobic conditions; atmospheric oxygen was introduced later by the activity of photosynthetic cyanobacteria *c.* 2.3 billion years ago. Therefore, it is possible that the energetics and principles underlying syntrophy on Earth could drive exploration for life on other planets. Despite oxygenation of the atmosphere, anoxic niches can be found everywhere in nature, including the human body.

In our review, we emphasized that the metabolic interaction of interdependent microorganism is not restricted to carbon compounds, but can involve S-, N-, and C- compounds in a single system. Sulfate reduction coupled to AOM is indeed a fascinating example of how deep an uncultivated system can be studied and analyzed with a combination of high-tech strategies, helping to enlighten ecology, physiology, structure, phylogeny and function of such an unusual, metabolically interacting microbial assembly. AOM also has demonstrated a future direction of how to tackle the questions about the basic principles of syntrophy without necessarily being able to cultivate the microbial players of interest.

For example, choosing microbial systems with a restricted number of bacterial and archaeal representatives can be a good start to understand the role of each partner. For instance, Woyke *et al.* (2006) have studied a microbial community inhabiting the marine oligochaete *Olavius algarvensis*, a worm without a mouth, gut or nephridia. The community mainly comprises two different bacteria, which have been analyzed via metagenomics. By metabolic pathway reconstruction, the physiological capabilities of both microbial partners have been visualized, proposing an internal sulfur-cycle and a syntrophic relationship. This interaction is the basis for the production of biomass and feeding the host, which in turn provides the best growth conditions. Similar scientific approaches have been described earlier (Tyson *et al.*, 2004), leading to the reconstruction of microbial genomes from an environmental, acid mine drainage biofilm with low complexity and an interpretation of the genomic data with respect to physiology. These low-complexity models provide a crucial basis for the next step: To understand environmental microbial processes and turnover, and the links between different microbial communities. Development of representative systems and computer-supported models can certainly be very helpful and will become an essential tool for (microbial) ecologists. However, looking at (meta-)genomic data will not help to fully understand the metabolic process. Gene annotations are in many cases still incomplete and sometimes of low quality, restricting the interpretability of genome data and a possible identification of genes specifically involved in (metabolic) interactions. The full pictures can only be obtained when different methods are combined using advanced instrumentation, the use of isotopic tracers, and clever experimentation to investigate syntrophy on a cellular and sub-cellular level.

In particular, SIP seems to be a promising technical approach that can be combined with additional methods such as cell sorting (Pawelczyk *et al.*, 2011), magnetic bead capture (Miyatake *et al.*, 2013), stable isotope switching (Maxfield *et al.*, 2012), or D_2O-SIP (Wegener *et al.*, 2012) to gain unique insights into complex or slow-growing communities.

Molecular methods evolve fast and are indeed extraordinarily helpful, but approaches to successful cultivation will be the main avenue to many open questions. Novel cultivation techniques for anaerobes will help to obtain more fascinating cultures, as for instance the *Nanoarchaeum/Ignicoccus* system. Only successful cultivation can deliver increased, pure biomass for joint research activities of the ecological community. With these insights, it will be possible to engineer anaerobic processes for wastewater treatment, bioremediation processes, or the conversion of biomass to energy in anaerobic digesters. Microbial (metabolic) cooperation is everywhere, and this alone ensures that microorganisms will survive despite biological and thermodynamic limitations. Microbial interaction serves the community, from subcellular to global processes and therefore ensures survival at each trophic level.

Acknowledgements

We graciously thank our colleagues for helpful discussions and for providing photographs and graphics. We are grateful to Prof. Michael Thomm and Prof. R. Wirth for support and discussion. The work of C. Moissl-Eichinger and H. Huber was supported by the Deutsche Forschungsgemeinschaft (Förderkennzeichen MO1977/3-1 and HU703/2-1). R. Henneberger was funded by the Swiss National Science Foundation (SNSF) under grant no. 31EE30-131170 as part of the European Science Foundation EUROCORES Program EuroEEFG, project MECOMECON, and additional funding was provided by ETH Zurich. We also thank the reviewers of this article for critical input and valuable discussion.

References

Adair K & Schwartz E (2011) Chapter seven – stable isotope probing with ^{18}O-water to investigate growth and mortality of ammonia oxidizing bacteria and archaea in soil. *Methods in Enzymology* (Martin GK, ed.), pp. 155–169. Academic Press, Oxford.

Adams CJ, Redmond MC & Valentine DL (2006) Pure-culture growth of fermentative bacteria, facilitated by H_2 removal: bioenergetics and H_2 production. *Appl Environ Microbiol* **72**: 1079–1085.

Baena S, Fardeau ML, Labat M, Ollivier B, Thomas P, Garcia JL & Patel BK (1998) *Aminobacterium colombiense* gen. nov., sp. nov., an amino acid-degrading anaerobe isolated from anaerobic sludge. *Anaerobe* **4**: 241–250.

Baena S, Fardeau ML, Ollivier B, Labat M, Thomas P, Garcia JL & Patel BK (1999) *Aminomonas paucivorans* gen. nov.,

sp. nov., a mesophilic, anaerobic, amino-acid-utilizing bacterium. *Int J Syst Bacteriol* **49**: 975–982.

Baena S, Fardeau ML, Labat M, Ollivier B, Garcia JL & Patel BK (2000) *Aminobacterium mobile* sp. nov., a new anaerobic amino-acid-degrading bacterium. *Int J Syst Evol Microbiol* **50**: 259–264.

Barker HA (1939) Studies upon the methane fermentation: IV. The isolation and culture of *Methanobacterium omelianskii*. *Antonie Van Leeuwenhoek* **6**: 201–220.

Beal EJ, House CH & Orphan VJ (2009) Manganese and iron-dependent marine methane oxidation. *Science* **325**: 184–187.

Behrens S, Losekann T, Pett-Ridge J, Weber PK, Ng WO, Stevenson BS, Hutcheon ID, Relman DA & Spormann AM (2008) Linking microbial phylogeny to metabolic activity at the single-cell level by using enhanced element labeling-catalyzed reporter deposition fluorescence *in situ* hybridization (EL-FISH) and NanoSIMS. *Appl Environ Microbiol* **74**: 3143–3150.

Berdugo-Clavijo C, Dong X, Soh J, Sensen CW & Gieg LM (2012) Methanogenic biodegradation of two-ringed polycyclic aromatic hydrocarbons. *FEMS Microbiol Ecol* **81**: 124–133.

Biebl H & Pfennig N (1978) Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. *Arch Microbiol* **1**: 9–16.

Boone DR, Johnson RL & Liu Y (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of K_m for H_2 or formate uptake. *Appl Environ Microbiol* **55**: 1735–1741.

Boxer SG, Kraft ML & Weber PK (2009) Advances in imaging secondary ion mass spectrometry for biological samples. *Annu Rev Biophys* **38**: 53–74.

Brochier-Armanet C, Boussau B, Gribaldo S & Forterre P (2008) Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nat Rev Microbiol* **6**: 245–252.

Brock TD & Brock ML (1966) Autoradiography as a tool in microbial ecology. *Nature* **209**: 734–736.

Bronstein JL (1994) Our current understanding of mutualism. *Q Rev Biol* **6**: 31–51.

Bryant MP, Wolin EA, Wolin MJ & Wolfe RS (1967) *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. *Arch Microbiol* **59**: 20–31.

Buckel W & Thauer RK (2013) Energy conservation via electron bifurcating ferredoxin reduction and proton/Na^+ translocating ferredoxin oxidation. *Biochim Biophys Acta* **1827**: 94–113.

Callaghan AV, Morris BEL, Pereira IAC *et al.* (2012) The genome sequence of *Desulfatibacillum alkenivorans* AK-01: a blueprint for anaerobic alkane oxidation. *Environ Microbiol* **14**: 101–113.

Cord-Ruwisch R, Lovley DR & Schink B (1998) Growth of *Geobacter sulfur*reducens with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. *Appl Environ Microbiol* **64**: 2232–2236.

Costa E, Pérez J & Kreft J-U (2006) Why is metabolic labour divided in nitrification? *Trends Microbiol* **14**: 213–219.

De Bok FAM, Plugge CM & Stams AJM (2004) Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Res* **38**: 1368–1375.

Dedysh SN, Panikov NS & Tiedje JM (1998) Acidophilic methanotrophic communities from *Sphagnum* peat bogs. *Appl Environ Microbiol* **64**: 922–929.

Dekas AE & Orphan VJ (2011) Identification of diazotrophic microorganisms in marine sediment via fluorescence *in situ* hybridization coupled to nanoscale secondary ion mass spectrometry (FISH-NanoSIMS). *Methods Enzymol* **486**: 281–305.

Dekas AE, Poretsky RS & Orphan VJ (2009) Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science* **326**: 422–426.

Dolfing J, Jiang B, Henstra AM, Stams AJ & Plugge CM (2008) Syntrophic growth on formate: a new microbial niche in anoxic environments. *Appl Environ Microbiol* **74**: 6126–6131.

Dong X & Stams AJ (1995) Evidence for H_2 and formate formation during syntrophic butyrate and propionate degradation. *Anaerobe* **1**: 35–39.

Dore J & Bryant MP (1990) Metabolism of one-carbon compounds by the ruminal acetogen *Syntrophococcus sucromutans*. *Appl Environ Microbiol* **56**: 984–989.

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S *et al.* (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* **464**: 543–548.

Ferrando L & Tarlera S (2009) Activity and diversity of methanotrophs in the soil-water interface and rhizospheric soil from a flooded temperate rice field. *J Appl Microbiol* **106**: 306–316.

Ferry JG & Wolfe RS (1976) Anaerobic degradation of benzoate to methane by a microbial consortium. *Arch Microbiol* **107**: 33–40.

Ferry JG, Smith PH & Wolfe RS (1974) *Methanospirillum*, a new genus of methanogenic bacteria, and characterization of *Methanospirillum hungatii* sp. nov. *Int J Syst Bacteriol* **24**: 465–469.

Fildes P (1956) Production of tryptophan by *Salmonella typhi* and *Escherichia coli*. *J Gen Microbiol* **15**: 636–643.

Froestl JM & Overmann J (1998) Physiology and tactic response of the phototrophic consortium "*Chlorochromatium aggregatum*". *Arch Microbiol* **169**: 129–135.

Froestl JM & Overmann J (2000) Phylogenetic affiliation of the bacteria that constitute phototrophic consortia. *Arch Microbiol* **174**: 50–58.

Fuchs G, Boll M & Heider J (2011) Microbial degradation of aromatic compounds – from one strategy to four. *Nat Rev Microbiol* **9**: 803–816.

Gieseke A, Nielsen JL, Amann R, Nielsen PH & de Beer D (2005) *In situ* substrate conversion and assimilation by nitrifying bacteria in a model biofilm. *Environ Microbiol* **7**: 1392–1404.

Gorby Y, Yanina S, McLean J *et al.* (2006) Electrically conductive bacterial nanowires produced by *Shewanella*

oneidensis strain MR-1 and other microorganisms. *P Natl Acad Sci USA* **103**: 11358–11363.

Gray ND, Sherry A, Grant RJ *et al.* (2011) The quantitative significance of *Syntrophaceae* and syntrophic partnerships in methanogenic degradation of crude oil alkanes. *Environ Microbiol* **13**: 2957–2975.

Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, Richardson PM & DeLong EF (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science* **305**: 1457–1462.

Hanson RS & Hanson TE (1996) Methanotrophic bacteria. *Microbiol Rev* **60**: 439–471.

Hattori S, Luo H, Shoun H & Kamagata Y (2001) Involvement of formate as an interspecies electron carrier in a syntrophic acetate-oxidizing anaerobic microorganism in coculture with methanogens. *J Biosci Bioeng* **91**: 294–298.

Heider J (2007) Adding handles to unhandy substrates: anaerobic hydrocarbon activation mechanisms. *Curr Opin Chem Biol* **11**: 188–194.

Henneberger R, Moissl C, Amann T, Rudolph C & Huber R (2006) New insights into the lifestyle of the cold-loving SM1 euryarchaeon: natural growth as a monospecies biofilm in the subsurface. *Appl Environ Microbiol* **72**: 192–199.

Henneberger R, Chiri E, Blees J, Niemann H, Lehmann MF & Schroth MH (2013) Field-scale labelling and activity quantification of methane-oxidizing bacteria in a landfill-cover soil. *FEMS Microbiol Ecol* **83**: 392–401.

Herrmann G, Jayamani E, Mai G & Buckel W (2008) Energy conservation via electron transferring flavoprotein (Etf) in anaerobic bacteria. *J Bacteriol* **190**: 784–791.

Herzog RFK & Viehboek FP (1949) Ion source for mass spectrography. *Phys Rev* **76**: 855–856.

Holler T, Wegener G, Niemann H, Deusner C, Ferdelman TG, Boetius A, Brunner B & Widdle F (2011a) Carbon and sulfur back flux during anaerobic microbial oxidation of methane and coupled sulfate reduction. *P Natl Acad Sci USA*, **108**: E1484–E1490.

Holler T, Widdel F, Knittel K, Amann R, Kellermann MY, Hinrichs K-U, Teske A, Boetius A & Wegener G (2011b) Thermophilic anaerobic oxidation of methane by marine microbial consortia. *ISME J* **5**: 1946–1956.

Holman HY, Wozei E, Lin Z, Comolli LR, Ball DA, Borglin S, Fields MW, Hazen TC & Downing KH (2009) Real-time molecular monitoring of chemical environment in obligate anaerobes during oxygen adaptive response. *P Natl Acad Sci USA* **106**: 12599–12604.

Holman HY, Bechtel HA, Hao Z & Martin MC (2010) Synchrotron IR spectromicroscopy: chemistry of living cells. *Anal Chem* **82**: 8757–8765.

Huang WE, Stoecker K, Griffiths R, Newbold L, Daims H, Whiteley AS & Wagner M (2007) Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence *in situ* hybridization for the single cell analysis of identity and function. *Environ Microbiol* **9**: 1878–1889.

Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC & Stetter KO (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* **417**: 63–67.

Huber H, Kueper U, Daxer S & Rachel R (2012) The unusual cell biology of the hyperthermophilic Crenarchaeon *Ignicoccus hospitalis*. *Antonie Van Leeuwenhoek* **102**: 203–219.

Iannotti EL, Kafkewit D, Wolin MJ & Bryant MP (1973) Glucose fermentation products of *Ruminococcus albus* grown in continuous culture with *Vibrio succinogenes* – changes caused by interspecies transfer of H_2. *J Bacteriol* **114**: 1231–1240.

Jahn U, Summons R, Sturt H, Grosjean E & Huber H (2004) Composition of the lipids of *Nanoarchaeum equitans* and their origin from its host *Ignicoccus* sp. strain KIN4/I. *Arch Microbiol* **182**: 404–413.

Jahn U, Huber H, Eisenreich W, Hugler M & Fuchs G (2007) Insights into the autotrophic CO_2 fixation pathway of the archaeon *Ignicoccus hospitalis*: comprehensive analysis of the central carbon metabolism. *J Bacteriol* **189**: 4108–4119.

Jahn U, Gallenberger M, Paper W, Junglas B, Eisenreich W, Stetter KO, Rachel R & Huber H (2008) *Nanoarchaeum equitans* and *Ignicoccus hospitalis*: new insights into a unique, intimate association of two archaea. *J Bacteriol* **190**: 1743–1750.

Jarrell KF (1985) Extreme oxygen sensitivity in methanogenic archaebacteria. *Bioscience* **35**: 298–302.

Jehmlich N, Schmidt F, Taubert M, Seifert J, Bastid F, von Bergen M, Richnow HH & Vogt C (2010) Protein-based stable isotope probing. *Nat Protoc* **5**: 1957–1966.

Jones DM, Head IM, Gray ND *et al.* (2008) Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature* **451**: 176–180.

Kaden J, Galushko AS & Schink B (2002) Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*. *Arch Microbiol* **178**: 53–58.

Kato S & Watanabe K (2010) Ecological and evolutionary interactions in syntrophic methanogenic consortia. *Microbes Environ* **25**: 145–151.

Knittel K & Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. *Annu Rev Microbiol* **63**: 311–334.

Kotsyurbenko OR, Glagolev MV, Nozhevnikova AN & Conrad R (2001) Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature. *FEMS Microbiol Ecol* **38**: 153–159.

Krumholz LR & Bryant MP (1986) *Syntrophococcus sucromutans* sp. nov., gen nov., uses carbohydrates as electron donors and formate, methoxymonobenzenoids or *methanobrevibacter* as electron-acceptor systems. *Arch Microbiol* **143**: 313–318.

Kueper U, Meyer C, Mueller V, Rachel R & Huber H (2010) Energized outer membrane and spatial separation of metabolic processes in the hyperthermophilic Archaeon *Ignicoccus hospitalis*. *P Natl Acad Sci USA* **107**: 3152–3156.

Kunapuli U, Lueders T & Meckenstock RU (2007) The use of stable isotope probing to identify key iron-reducing microorganisms involved in anaerobic benzene degradation. *ISME J* **1**: 643–653.

Lauterborn R (1906) Zur Kenntnis der sapropelischen Flora. *Allgemeine Botanische Zeitschrift* **12**: 196–197.

Lechene C, Hillion F, McMahon G *et al.* (2006) High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry. *J Biol* **5**: article 20.

Lee N, Nielsen PH, Andreasen KH, Juretschko S, Nielsen JL, Schleifer KH & Wagner M (1999) Combination of fluorescent *in situ* hybridization and microautoradiography-a new tool for structure-function analyses in microbial ecology. *Appl Environ Microbiol* **65**: 1289–1297.

Lee N, la Cour Jansen J, Aspegren H, Henze M, Nielsen PH & Wagner M (2002) Population dynamics in wastewater treatment plants with enhanced biological phosphorus removal operated with and without nitrogen removal. *Water Sci Technol* **46**: 163–170.

Li F, Hinderberger J, Seedorf H, Zhang J, Buckel W & Thauer RK (2008a) Coupled ferredoxin and crotonyl-CoA reduction with NADH catalyzed by the butyryl-Co dehydrogenase/ETF complex from *Clostridium kluyveri*. *J Bacteriol* **190**: 843–850.

Li T, Wu TD, Mazeas L, Toffin L, Guerquin-Kern JL, Leblon G & Bouchez T (2008b) Simultaneous analysis of microbial identity and function using NanoSIMS. *Environ Microbiol* **10**: 580–588.

Li X, Luo Q, Wofford NQ, Keller KL, McInerney MJ, Wall JD & Krumholz LR (2009) A molybdopterin oxidoreductase is involved in H_2 oxidation in *Desulfovibrio desulfuricans* G20. *J Bacteriol* **191**: 2675–2682.

Lovley DR, Dwyer DF & Klug MJ (1982) Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Appl Environ Microbiol* **43**: 1373–1379.

Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP & Woodward JC (1996) Humic substances as electron acceptors for microbial respiration. *Nature* **382**: 445–448.

Lykidis A, Chen CL, Tringe SG *et al.* (2011) Multiple syntrophic interactions in a terephthalate-degrading methanogenic consortium. *ISME J* **5**: 122–130.

Maxfield PJ, Dildar N, Hornibrook ER, Stott AW & Evershed RP (2012) Stable isotope switching (SIS): a new stable isotope probing (SIP) approach to determine carbon flow in the soil food web and dynamics in organic matter pools. *Rapid Commun Mass Spectrom* **26**: 997–1004.

McInerney MJ, Bryant MP, Hespell RB & Costerton JW (1981) *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl Environ Microbiol* **41**: 1029–1039.

McInerney MJ, Rohlin L, Mouttaki H *et al.* (2007) The genome of *Syntrophus aciditrophicus*: life at the thermodynamic limit of microbial growth. *P Natl Acad Sci USA* **104**: 7600–7605.

McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJ, Schink B, Rohlin L & Gunsalus RP (2008) Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Ann NY Acad Sci* **1125**: 58–72.

McInerney MJ, Sieber JR & Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. *Curr Opin Biotechnol* **20**: 623–632.

McInerney M, Sieber J & Gunsalus R (2011) Genomic sequences reveal systems required to produce hydrogen and formate, plus other hallmarks of the syntrophic lifestyle. *Microbe Mag* **6**: 479–485.

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T & Heip CHR (2000) The fate of intertidal microphytobenthos carbon: an *in situ* C^{13}-labeling study. *Limnol Oceanogr* **45**: 1224–1234.

Milucka J, Ferdelman TG, Polerecky L, Franzke D, Wegener G, Schmid M, Lieberwirth I, Wagner M, Widdle F & Kuypers MMM (2012) Zero-valent sulphur is a key intermediate in marine methane oxidation. *Nature* **491**: 541–546.

Minic Z & Herve G (2004) Biochemical and enzymological aspects of the symbiosis between the deep-sea tubeworm *Riftia pachyptila* and its bacterial endosymbiont. *Eur J Biochem* **271**: 3093–3102.

Miyatake T, Macgregor BJ & Boschker HT (2013) Depth-related differences in organic substrate utilization by major microbial groups in intertidal marine sediment. *Appl Environ Microbiol* **79**: 389–392.

Moissl C, Rudolph C & Huber R (2002) Natural communities of novel archaea and bacteria with a string-of-pearls-like morphology: molecular analysis of the bacterial partners. *Appl Environ Microbiol* **68**: 933–937.

Moissl-Eichinger C & Huber H (2011) Archaeal symbionts and parasites. *Curr Opin Microbiol* **14**: 364–370.

Moore RL (1981) The biology of *Hyphomicrobium* and other prosthecate, budding bacteria. *Annu Rev Microbiol* **35**: 567–594.

Moran J, Beal E, Vrentas J, Orphan V, Freeman K & House C (2008) Methyl sulfides as intermediates in the anaerobic oxidation of methane. *Environ Microbiol* **10**: 162–173.

Morris BEL, Herbst FA, Bastida F, Seifert J, von Bergen M, Richnow HH & Suflita JM (2012) Microbial interactions during residual oil and n-fatty acid metabolism by a methanogenic consortium. *Environ Microbiol Rep* **4**: 297–306.

Mueller J & Overmann J (2011) Close Interspecies interactions between prokaryotes from sulfureous environments. *Front Microbiol* **2**: article 146.

Mueller N, Griffin BM, Stingl U & Schink B (2008) Dominant sugar utilizers in sediment of Lake Constance depend on syntrophic cooperation with methanogenic partner organisms. *Environ Microbiol* **10**: 1501–1511.

Mueller N, Schleheck D & Schink B (2009) Involvement of NADH:acceptor oxidoreductase and butyryl coenzyme A dehydrogenase in reversed electron transport during syntrophic butyrate oxidation by *Syntrophomonas wolfei*. *J Bacteriol* **191**: 6167–6177.

Musat N, Foster R, Vagner T, Adam B & Kuypers MM (2012) Detecting metabolic activities in single cells, with emphasis on nanoSIMS. *FEMS Microbiol Rev* **36**: 486–511.

Nauhaus K, Boetius A, Kruger M & Widdel F (2002) *In vitro* demonstration of anaerobic oxidation of methane coupled to sulfate reduction in sediment from a marine gas hydrate area. *Environ Microbiol* **4**: 296–305.

Nauhaus K, Treude T, Boetius A & Krueger M (2004) Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. *Environ Microbiol* **7**: 98–106.

Nedwell DB & Banat IM (1981) Hydrogen as an electron donor for sulfate-reducing bacteria in slurries of salt marsh sediment. *Microb Ecol* **7**: 305–313.

Neufeld JD & Murrell JC (2007) Witnessing the last supper of uncultivated microbial cells with Raman-FISH. *ISME J* **1**: 269–270.

Neufeld JD, Dumont MG, Vohra J & Murrell JC (2007) Methodological considerations for the use of stable isotope probing in microbial ecology. *Microb Ecol* **53**: 435–442.

Nielsen JL & Nielsen PH (2005) Advances in microscopy: microautoradiography of single cells. *Method Enzymol* **397**: 237–256.

Nisman B (1954) The Stickland reaction. *Bacteriol Rev* **18**: 16–42.

Nottingham PM & Hungate RE (1969) Methanogenic fermentation of benzoate. *J Bacteriol* **98**: 1170–1172.

Nuebel U, Bateson MM, Vandieken V, Wieland A, Kuhl M & Ward DM (2002) Microscopic examination of distribution and phenotypic properties of phylogenetically diverse *Chloroflexaceae*-related bacteria in hot spring microbial mats. *Appl Environ Microbiol* **68**: 4593–4603.

Nuesslein B, Chin KJ, Eckert W & Conrad R (2001) Evidence for anaerobic syntrophic acetate oxidation during methane production in the profundal sediment of subtropical Lake Kinneret (Israel). *Environ Microbiol* **3**: 460–470.

Okabe S, Kindaichi T & Ito T (2004) MAR-FISH – An ecophysiological approach to link phylogenetic affiliation and *in situ* metabolic activity of microorganisms at a single-cell resolution. *Microbes Environ* **19**: 83–98.

Olson JM (1978) Confused history of *Chloropseudomonas ethylica* 2K. *Int J Syst Bacteriol* **28**: 128–129.

Orphan V (2009) Methods for unveiling cryptic microbial partnerships in nature. *Curr Opin Microbiol* **12**: 231–237.

Orphan VJ & House CH (2009) Geobiological investigations using secondary ion mass spectrometry: microanalysis of extant and paleo-microbial processes. *Geobiology* **7**: 360–372.

Orphan VJ, House CH, Hinrichs KU, McKeegan KD & DeLong EF (2001) Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* **293**: 484–487.

Overmann J (2004) Phototrophic consortia: a tight cooperation between non-related eubacteria. *Symbiosis – Mechanisms and Model Systems*, Vol. 4 (Seckbach J, ed.), pp. 241–255. Kluwer Academic Publishers, New York, Boston, Dordrecht, London, Moscow.

Padmanabhan P, Padmanabhan S, DeRito C, Gray A, Gannon D, Snape JR, Tsai CS, Park W, Jeon C & Madsen EL (2003) Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. *Appl Environ Microbiol* **69**: 1614–1622.

Pawelczyk S, Bumann D & Abraham WR (2011) Kinetics of carbon sharing in a bacterial consortium revealed by combining stable isotope probing with fluorescence-activated cell sorting. *J Appl Microbiol* **110**: 1065–1073.

Pelletier E, Kreimeyer A, Bocs S *et al.* (2008) "Candidatus *Cloacamonas acidaminovorans*": genome sequence reconstruction provides a first glimpse of a new bacterial division. *J Bacteriol* **190**: 2572–2579.

Platen H & Schink B (1987) Methanogenic degradation of acetone by an enrichment culture. *Arch Microbiol* **149**: 136–141.

Platen H, Janssen PH & Schink B (1994) Fermentative degradation of acetone by an enrichment culture in membrane-separated culture devices and in cell suspensions. *FEMS Microbiol Lett* **122**: 27–32.

Pombo SA, Pelz O, Schroth MH & Zeyer J (2002) Field-scale C-labeling of phospholipid fatty acids (PLFA) and dissolved inorganic carbon: tracing acetate assimilation and mineralization in a petroleum hydrocarbon-contaminated aquifer. *FEMS Microbiol Ecol* **41**: 259–267.

Pombo SA, Kleikemper J, Schroth MH & Zeyer J (2005) Field-scale isotopic labeling of phospholipid fatty acids from acetate-degrading sulfate-reducing bacteria. *FEMS Microbiol Ecol* **51**: 197–207.

Probst AJ, Holman HYN, DeSanits TZ, Andersen GL, Bechtel HA, Sonnleitner M, Venkateswaran K & Moissl-Eichinger C (2013) Tackling the minority: sulfate-reducing bacteria in an archaea-dominated subsurface biofilm. *ISME J* **7**: 635–651.

Radajewski S, Ineson P, Parekh NR & Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. *Nature* **403**: 646–649.

Radax R, Hoffmann F, Rapp HT, Leininger S & Schleper C (2012) Ammonia-oxidizing archaea as main drivers of nitrification in cold-water sponges. *Environ Microbiol* **14**: 909–923.

Reeburgh WS (2003) Global methane biogeochemistry. *Treatise on Geochemistry* (Holland HD & Turekian KK, eds), pp. 65–89. Elsevier, New York.

Reeburgh WS (2007) Oceanic methane biogeochemistry. *Chem Rev* **107**: 486–513.

Reguera G (2009) Are microbial conversations being lost in translation? *Microbe Mag* **4**: 506–512.

Reguera G, McCarthy K, Mehta T, Nicoll J, Tuominen M & Lovley D (2005) Extracellular electron transfer via microbial nanowires. *Nature* **435**: 1098–1101.

Robinson J & Tiedje J (1984) Competition between sulfate-reducing and methanogenic bacteria for H_2 under resting and growing conditions. *Arch Microbiol* **2**: 6–32.

Rodhe H (1990) A comparison of the contribution of various gases to the greenhouse effect. *Science* **248**: 1217–1219.

Rudolph C, Wanner G & Huber R (2001) Natural communities of novel archaea and bacteria growing in cold sulfurous springs with a string-of-pearls-like morphology. *Appl Environ Microbiol* **67**: 2336–2344.

Schedin F, Lidorikis E, Lombardo A, Kravets VG, Geim AK, Grigorenko AN, Novoselov KS & Ferrari AC (2010) Surface-enhanced Raman spectroscopy of graphene. *ACS Nano* **4**: 5617–5626.

Scheller S, Goenrich M, Boecher R, Thauer RK & Jaun B (2010) The key nickel enzyme of methanogenesis catalyses the anaerobic oxidation of methane. *Nature* **465**: 606–608.

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* **61**: 262–280.

Schink B (2002) Synergistic interactions in the microbial world. *Antonie Van Leeuwenhoek* **81**: 257–261.

Schink B & Stams AJ (2006) Syntrophism among Prokaryotes. *The Prokaryotes* (Dworkin M, Falkow S, Rosenberg E, Schleifer K-H & Stackebrandt E, eds), pp. 309–335. Springer, New York.

Schoenheit P, Kristjansson JB & Thauer RK (1982) Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. *Arch Microbiol* **132**: 285–288.

Scholten JC & Conrad R (2000) Energetics of syntrophic propionate oxidation in defined batch and chemostat cocultures. *Appl Environ Microbiol* **66**: 2934–2942.

Schopf S, Wanner G, Rachel R & Wirth R (2008) An archaeal bi-species biofilm formed by *Pyrococcus furiosus* and *Methanopyrus kandleri*. *Arch Microbiol* **190**: 371–377.

Schuster KC, Urlaub E & Gapes JR (2000) Single-cell analysis of bacteria by Raman microscopy: spectral information on the chemical composition of cells and on the heterogeneity in a culture. *J Microbiol Methods* **42**: 29–38.

Schut GJ & Adams MW (2009) The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. *J Bacteriol* **191**: 4451–4457.

Selwyn SC & Postgate JR (1959) A search for the *rubentschikii* group of *Desulphovibrio*. *Antonie Van Leeuwenhoek* **25**: 465–472.

Shaposhnikov VN, Kondratieva EN & Fedorov VD (1960) A new species of green sulfur bacteria. *Nature* **187**: 167–168.

Shigematsu T, Tang Y, Kobayashi T, Kawaguchi H, Morimura S & Kida K (2004) Effect of dilution rate on metabolic pathway shift between aceticlastic and nonaceticlastic methanogenesis in chemostat cultivation. *Appl Environ Microbiol* **70**: 4048–4052.

Sieber JR, Sims DR, Han C *et al.* (2010) The genome of *Syntrophomonas wolfei*: new insights into syntrophic metabolism and biohydrogen production. *Environ Microbiol* **12**: 2289–2301.

Smith DC & Douglas AE (1987) *The Biology of Symbiosis*. Hodder Arnold (Publishers) Ltd, London.

Stams AM & Dong X (1995) Role of formate and hydrogen in the degradation of propionate and butyrate by defined suspended cocultures of acetogenic and methanogenic bacteria. *Antonie Van Leeuwenhoek* **68**: 281–284.

Stams A & Plugge C (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Microbiol* **7**: 568–577.

Stams A, de B, Plugge C, van E, Dolfing J & Schraa G (2006) Exocellular electron transfer in anaerobic microbial communities. *Environ Microbiol* **8**: 371–382.

Stams AJM, Lens PNL, Buisman CJN, Janssen AJH & Muyzer G (2008) Microbiology and application of biological sulfate reduction. *Microbes and the Environment: Perspective and Challenges* (Liu SJ & Drake HL, eds), pp. 97–106. Science Press, Beijing.

Steward PS (2002) Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol* **292**: 107–113.

Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS & Lovley DR (2010) Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science* **330**: 1413–1415.

Tarlera S, Muxi L, Soubes M & Stams AJ (1997) *Caloramator proteoclasticus* sp. nov., a new moderately thermophilic anaerobic proteolytic bacterium. *Int J Syst Bacteriol* **47**: 651–656.

Taylor MW, Radax R, Steger D & Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol Rev* **71**: 295–347.

Tewes FJ & Thauer RK (1980) Regulation of ATP synthesis in glucose fermenting bacterial involved in interspecies hydrogen transfer. *Anaerobes and Anaerobic Infections* (Gottschalk G, Pfennig N & Werner H, eds), pp. 97–104. G. Fischer, Stuttgart, Germany.

Thauer RK, Jungermann K & Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* **41**: 100–180.

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS & Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428**: 37–43.

van der Zaan BM, Saia FT, Stams AJ, Plugge CM, de Vos WM, Smidt H, Langenhoff AA & Gerritse J (2012) Anaerobic benzene degradation under denitrifying conditions: *Peptococcaceae* as dominant benzene degraders and evidence for a syntrophic process. *Environ Microbiol* **14**: 1171–1181.

Vila M, Simo R, Kiene RP, Pinhassi J, Gonzalez JM, Moran MA & Pedros-Alio C (2004) Use of microautoradiography combined with fluorescence *in situ* hybridization to determine dimethylsulfoniopropionate incorporation by marine bacterioplankton taxa. *Appl Environ Microbiol* **70**: 4648–4657.

Vogl K, Glaeser J, Pfannes K, Wanner G & Overmann J (2006) *Chlorobium chlorochromatii* sp. nov., a symbiotic green sulfur bacterium isolated from the phototrophic consortium "*Chlorochromatium aggregatum*". *Arch Microbiol* **185**: 363–372.

Wagner M (2009) Single-cell ecophysiology of microbes as revealed by Raman microspectroscopy or secondary ion mass spectrometry imaging. *Annu Rev Microbiol* **63**: 411–429.

Wagner M, Nielsen PH, Loy A, Nielsen JL & Daims H (2006) Linking microbial community structure with function: fluorescence *in situ* hybridization-microautoradiography and isotope arrays. *Curr Opin Biotechnol* **17**: 83–91.

Wanner G, Vogl K & Overmann J (2008) Ultrastructural characterization of the prokaryotic symbiosis in "*Chlorochromatium aggregatum*". *J Bacteriol* **190**: 3721–3730.

Warthmann R, Cypionka H & Pfennig N (1992) Photoproduction of H_2 from acetate by syntrophic cocultures of green sulfur bacteria and sulfur-reducing bacteria. *Arch Microbiol* **157**: 343–348.

Waters E, Hohn MJ, Ahel I *et al.* (2003) The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. *P Natl Acad Sci USA* **100**: 12984–12988.

Wegener G, Niemann H, Elvert M, Hinrichs K-U & Boetius A (2008) Assimilation of methane and inorganic carbon by microbial communities mediating the anaerobic oxidation of methane. *Environ Microbiol* **10**: 2287–2298.

Wegener G, Bausch M, Holler T, Thang NM, Prieto Mollar X, Kellermann MY, Hinrichs KU & Boetius A (2012) Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water and 13C-bicarbonate. *Environ Microbiol* **14**: 1517–1527.

Weiner A, Schopf S, Wanner G, Probst A & Wirth R (2012) Positive, neutral and negative interactions in cocultures between *Pyrococcus furiosus* and different methanogenic archaea. *Microbiol Insight* **5**: 1–10.

Westerholm M, Dolfing J, Sherry A, Gray ND, Head IM & Schnurer A (2011) Quantification of syntrophic acetate-oxidizing microbial communities in biogas processes. *Environ Microbiol Rep* **3**: 500–505.

Widdel F, Boetius A & Rabus R (2006) Anaerobic biodegradation of hydrocarbons including methane. *The Prokaryotes* (Dworkin M, Falkow S, Rosenberg E, Schleifer K-H & Stackebrandt E, eds), pp. 1028–1049. Springer, New York.

Wilkinson TG, Topiwala HH & Hamer G (1974) Interactions in a mixed bacterial population growing on methane in continuous culture. *Biotechnol Bioeng* **16**: 41–59.

Woyke T, Teeling H, Ivanova NN *et al.* (2006) Symbiosis insights through metagenomic analysis of a microbial consortium. *Nature* **443**: 950–955.

Zehnder AJB & Brock TD (1979) Methane formation and methane oxidation by methanogenic bacteria. *J Bacteriol* **137**: 420–432.

Zehnder AJB & Brock TD (1980) Anaerobic methane oxidation: occurrence and ecology. *Appl Environ Microbiol* **39**: 194–204.

Zeikus JG (1983) Metabolic communication between biodegradative populations in nature. *Soc Gen Microbiol Symp*, Vol. 34 (Slater JH, Whittenbury R & Wimpenny JWT, eds), pp. 423–462. Cambridge University Press, Cambridge, UK.

Zindel U, Freudenberg W, Rieth M, Andreesen JR, Schnell J & Widdel F (1988) *Eubacterium acidaminophilum* sp. nov., a versatile amino acid-degrading anerobe produzing or utilizing H_2 or formate:description and enzymatic studies. *Arch Microbiol* **150**: 254–266.

Zinder SH & Koch M (1984) Nonaceticlastic methnogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. *Arch Microbiol* **138**: 263–272.

Zoetendal EG, Vaughan EE & de Vos WM (2006) A microbial world within us. *Mol Microbiol* **59**: 1639–1650.