Life at the oxic–anoxic interface: microbial activities and adaptations

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Received 26 May 2000; received in revised form 17 August 2000; accepted 18 August 2000

Abstract

Molecular oxygen is one of the most important reactants in biogeochemical cycles. Due to its low solubility in water, the consumption of oxygen leads to the development of oxic–anoxic interfaces, which separate aerobic from anaerobic processes in virtually all environments, ranging in scale from oceanic sediments to the fecal pellets of a small soil invertebrate. Three case studies were selected to illustrate the basic situation and the specific characteristics of oxic–anoxic interfaces: sediments, the rhizosphere of aquatic plants, and the intestinal tract of insects. Each system is governed by the same general principles, but striking differences arise from, e.g., the nature of the major microbial activities and the mechanisms controlling metabolite fluxes. Also scale and dimensional differences as well as the consequences of temporal fluctuations are of fundamental importance. Recent developments in microbial ecology, which often combine traditional and modern approaches, have significantly furthered our understanding of the specific microniches and the metabolic and behavioral adaptations of microorganisms to life at the oxic–anoxic interface. New concepts help to define the targets of future studies: the spatial organization of microbial populations, their microenvironments and in situ activities, and the functional interactions within structured microbial communities. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Oxic–anoxic interface; Gradient; Sediment; Bioturbation; Rhizosphere; Termite gut

Contents

1. Introduction ........................................................................... 692
   1.1. Gradients as a key feature of the biosphere ......................... 692
   1.2. Role of oxygen ................................................................ 692
   1.3. Scope of the review ...................................................... 692
2. Sediments ........................................................................... 693
   2.1. General aspects .......................................................... 693
   2.2. Oxygen flux into the sediment ......................................... 693
   2.3. Electron fluxes in the sediment ........................................ 694
   2.4. Microbial communities at the oxic–anoxic interface ............ 694
   2.5. Physiological adaptations .............................................. 694
   2.6. Bioturbated sediments .................................................. 696
3. Plant roots ........................................................................... 697
   3.1. Oxygen gradients ........................................................ 698
   3.2. Methane oxidation and methanogenesis ............................ 699
   3.3. Nitrogen metabolism .................................................... 699
   3.4. Linking N and C cycles ............................................... 700
4. Insect guts ........................................................................... 701
   4.1. Radial oxygen gradients ................................................. 701
   4.2. Impact of oxygen influx ............................................... 702

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PII: S0168-6445(00)00054-1

FEMSRE 705 3-11-00
1. Introduction

1.1. Gradients as a key feature of the biosphere

The biosphere is generally not physicochemically homogeneous, and patchiness is a general feature of all ecological systems. In structured environments, the spatial differences in the distribution of a given compound are maintained by a physical separation of sources and sinks. Consumption of resources and formation of metabolic products by spatially separated microbial populations are the driving forces for the formation of gradients. When fluxes along such gradients control the supply of a limiting resource, they determine in turn the activity of the microbiota, which usually leads to a situation best described as a dynamic steady state.

Microbial activities are often limited by more than one compound. If the limiting resources are supplied from different directions, microorganism accumulate at the interface and establish opposing gradients of two chemical reactants; quite often, metabolic rates are so high that the chemical species hardly overlap.

With increasing slope of the gradients, the fluxes and the corresponding microbial activities also increase. A microbial mat that is only a few millimeters thick can fix about as much carbon per area as a tropical rain forest [1,2]. Jørgensen [1] has provided an impressive comparison about as much carbon per area as a tropical rain forest

A. Brune et al. / FEMS Microbiology Reviews 24 (2000) 691–710

1.3. Scope of the review

This review will focus on the gradients and the corresponding microbial activities at oxic–anoxic interfaces and on microbial adaptations to this situation. Three typical examples will be discussed:

1. In aquatic sediments, which cover a large part of the Earth, the position of the oxic–anoxic interface relative to the sediment surface is influenced by the sedimentation rates and the organic input. In undisturbed sediments, the main transport mechanism is molecular diffusion, but bioturbation and water movement strongly influence the depth of oxygen penetration. The effect of cycles. However, it reacts spontaneously only with a limited number of reductants. Within the temperature range allowing life, the oxidation of organic matter depends mainly on enzymatic catalysis. A few inert compounds, such as alkanes, aromatic compounds, and ammonia, are directly oxidized by O2 with the help of mono- or dioxygenases. In most cases, however, oxygen only functions as the electron acceptor of aerobic respiration and is reduced to water by terminal oxidases. By this route, also most of the electrons from the anaerobic oxidation of organic matter to CO2 that are initially transferred to alternative electron acceptors are finally transferred to oxygen by aerobic lithotrophic bacteria.

The availability of oxygen has a tremendous impact not only on the redox potential of the environment, but also on the energetic situation of the microorganisms. The oxidation of NADH (E°' = −320 mV) coupled with oxygen reduction to water (E°' = +818 mV) has a free energy change (ΔG°') of −220 kJ mol⁻¹, whereas the same reaction coupled with CO2 reduction to CH4 (E°' = −244 mV) yields a free energy change of merely −15 kJ mol⁻¹ [3].

The solubility of oxygen in water is quite low. At atmospheric pressure and a temperature of 25°C, air-saturated water contains about 250 μmol O2 per liter; this value decreases further with increasing temperature and salinity [4]. Oxygen is therefore rapidly depleted at locations where catabolic activity is high, thus giving rise to gradients. Steep oxygen gradients are also formed in microbial mats, where daytime oxygen concentration, due to photosynthetic production, may exceed saturation concentrations by several-fold.

1.2. Role of oxygen

Due to its positive redox potential, molecular oxygen is one of the most important reactants in biogeochemical

4.3. Hydrogen counter-gradients ............................................ 703
4.4. Axial dynamics of microbial activities ................................ 704
4.5. Temporal fluctuations of the oxygen status ......................... 704
5. Conclusions and outlook .................................................. 705
References ........................................................................... 706

A. Brune et al. / FEMS Microbiology Reviews 24 (2000) 691–710

FEMSRE 705 3-11-00
animal burrows on oxygen transport is therefore included.

2. Also the aerated roots of aquatic plants in their anoxic environment lead to the formation of huge oxic–anoxic interfaces. Inside the plants, O₂ usually diffuses in an aerenchyma, a contiguous gas phase connecting leaves with roots, while in the root cells and in the pore water surrounding the roots, the dramatically decreased diffusion coefficient results in steep O₂ gradients into the rhizosphere.

3. The intestinal tracts of termites represent an example of oxic–anoxic interfaces from the animal kingdom that will also serve to illustrate the importance of size for an anoxic environment. Here, it is the diffusion of oxygen into the gut which creates radial oxygen gradients and the movement of gut contents along the gut axis which leads to additional oxygen dynamics.

The review will focus on the basic principles that influence the distribution and metabolism of bacteria at oxic–anoxic interfaces, document new developments, and outline open questions for future research. For clarity and space restrictions, no attempt was made to cover all aspects and all the literature dealing with the environments discussed. The oxic–anoxic interfaces of other environments, such as biofilms, soil aggregates or flocs of organic material in the water column (marine or lake snow), and the redox processes in rice paddies are mentioned only marginally since these subjects are covered elsewhere in this volume (e.g., see reviews by Wimpenny et al. [5] and Liesack et al. [79]).

2. Sediments

2.1. General aspects

All aquatic ecosystems have some sort of an oxic–anoxic interface. High primary production or a stagnant water body may shift this boundary upwards into the water column. However, most bodies of water contain oxygen down to the bottom, with an oxic–anoxic transition within the sediment. The depth of this interface depends largely on the input of degradable organic matter to the sediment. In deep-sea sediments and in oligotrophic freshwater lakes, this interface is found at a depth of several centimeters, whereas in coastal ecosystems and in freshwater lakes with a higher productivity, oxygen rarely penetrates more than a few millimeters into the sediment – as long as oxygen transport is controlled by molecular diffusion (Fig. 1). The high respiration rates at the sediment surface are due to the accumulation of settling particles at the sediment surface, which represents a transition from a three-dimensional space (particles suspended in water) to a nearly two-dimensional area (sediment surface). Consequently, the number of bacteria found at the sediment surface is typically three orders of magnitude higher than in the water above [15].

The microbial communities of shallow-water or tidal sediments may become independent of the sedimentation of organic matter if sunlight penetrates down to the sediment surface. The steepest oxygen gradients found are formed in photosynthetic microbial mats where the oxygen concentration may exceed 1 mmol O₂ per liter during daytime. In non-photosynthetic sediments, oxygen transfer from the bulk water into the sediment proceeds mainly via molecular diffusion across the diffusive boundary layer [16–18]. The thickness of the diffusive boundary layer – and thus the steepness of the gradient and the oxygen flux into the sediment – is influenced by the roughness of the sediment surface and by the turbulence of the overlying water [18]. The sediment roughness and also the microtopography of the surface may be modified by burrowing animals as well as by waves and current [12,19,20].

2.2. Oxygen flux into the sediment

The oxygen flux into the sediment can be calculated from the slope of the oxygen profile in the diffusive boundary layer. Fick’s first law, which describes the diffusive flux, is rather simple: the net amount of a compound diffusing through a plane is given by its motility in the medium (the diffusion coefficient) and the steepness of the gradient. The diffusion coefficient (D) of oxygen in
water is $2.0 \times 10^{-5}$ cm$^2$ s$^{-1}$ at 20°C [21]. Thus, a concentration difference of 100 μM O$_2$ over 1 mm distance would correspond to a flux of 72 nmol O$_2$ cm$^{-2}$ h$^{-1}$. In order to calculate the oxygen flux ($J$) into the sediment from a given concentration gradient ($dC/dx$), the sediment-specific diffusion coefficient ($D_s$, where $D_s < D$), and the porosity ($\phi$) of the sediment have to be considered [22,23]:

$$J = -D_s \phi \frac{dC}{dx} \tag{1}$$

Pore-water profiles are linear if neither consumption nor production occurs along the gradient [22]. Non-linear concentration changes indicate the presence of oxygen-consuming or oxygen-producing processes (as long as the diffusion coefficient does not change, which usually is the case close to the water–sediment interface). Consumption or production rates can be calculated from the second derivative of the slope [22].

2.3. Electron fluxes in the sediment

The microbial processes in the water body are typically not limited by oxygen, but rather by the supply of electron donors, whereas the anoxic layers of the sediment often harbor an excess of electron donors. Anaerobic activities – fermentation and anaerobic respiration – are responsible for the metabolism of a large part of the organic carbon. The reduced products of these reactions are then reoxidized by lithotrophic and methanotrophic, aerobic activities. Therefore, the overall budget of the microbial sediment metabolism is the oxidation of the organic matter with oxygen, although the organisms primarily responsible for the oxidation of organic matter are not aerobic bacteria.

In the upper, oxidized (i.e., having a positive redox potential) layers of anoxic sediments, nitrate, Mn(IV), and Fe(III) are important electron acceptors of anaerobic respiration [24]. Reoxidation of the reduced acceptor compounds typically occurs at or below the anoxic–oxygen interface. Since Fe(II) and Mn(II) are soluble and Fe(III) and Mn(IV) are easily precipitated, the internal redox cycles are accompanied by redox-state-dependent changes in solubility [25].

In the lower, reduced sediment layers (i.e., having a negative redox potential), sulfate and CO$_2$ are the major terminal electron acceptors, which makes sulfide and methane reoxidation important processes in the upper, oxidized layers. Jørgensen [26] has shown that in marine sediments, up to 50% of the oxygen uptake is due to the reoxidation of sulfide. Since Fe(III), Mn(IV), and nitrate can serve as electron acceptors for the anaerobic oxidation of sulfide and, in theory, also of methane, no direct contact between sulfide or methane and oxygen is necessary. The oxygen-dependent oxidation of sulfide may also proceed via an internal cycle of Mn(IV) and Mn(II) without any overlap between the oxygen and sulfide gradients [27]. This prevents a chemical oxidation of sulfide with oxygen, which would compete with the activity of aerobic, sulfur-oxidizing bacteria (see below); however, sulfide is also rapidly oxidized by Mn(IV) and Fe(III).

2.4. Microbial communities at the anoxic–oxygen interface

The narrow stratification of physicochemical parameters around and below the anoxic–oxygen interface is reflected in the microbial communities inhabiting the different sediment layers [28–31]. The most impressive, visible sequence is the ‘Farbstreifensandwatt’, where cyanobacteria, algae, and anoxygenic purple and green bacteria form characteristically colored layers. Although these layers may be stable over months, the system is highly dynamic and shows diurnal light cycles and often additional tidal cycles [32,33].

Despite the dynamics, there is a distinct stratification of the microbial populations in the vicinity of the anoxic–oxygen interface, as shown by studies applying molecular biological techniques [28], although all physiological groups appear to be present at their highest abundance in the uppermost layers of a given sediment or microbial mat [34]. Even the sulfate-reducing bacteria, which were expected to be restricted to the anoxic zones, are present in the upper, oxic layer in rather high numbers [29,31]. It should be kept in mind that the typical black layer beneath a bacterial mat does not indicate the presence of sulfate-reducing bacteria – which are more or less colorless – but only conditions under which iron sulfides are stable. Combining microbiological and molecular biological techniques, Sass et al. [30] have demonstrated that sulfate-reducing bacteria isolated from the oxic layers of the sediment from the oligotrophic Lake Stechlin are better adapted to oxygen than those derived from the deeper layers.

2.5. Physiological adaptations

The best-studied examples of microbial adaptations to life at the anoxic–oxygen interface are found among the bacteria involved in the sulfur cycle. It can be safely assumed that adaptations in other, less well studied groups will be similarly differentiated.

2.5.1. Sulfide-oxidizing bacteria

The classical examples of microbial adaptations to the oxygen–sulfide interface have come from studies with the free-living sulfide-oxidizing bacteria of the genera Beggiatoa [35] and Thiovulum [36,37]. These bacteria show distinct responses to oxygen and sulfide concentrations. They accumulate exactly at the interface and by their activity maintain stable gradients at increased diffusive fluxes. Sulfide oxidation and oxygen reduction occur in a sharply defined layer, typically without any overlap of oxygen and sulfide [38]. Two strategies are found which solve this problem: many sulfide-oxidizing bacteria migrate be-
tween oxic and sulfidic layers, whereas some species have developed spatially organized structures to enhance oxygen flux [37].

Since sulfide-oxidizing bacteria have no means of storing larger amounts of oxygen or sulfide, sulfide oxidation and oxygen reduction have to occur in spatially separated locations. In the sulfideic layer, sulfide is oxidized to sulfur (which can be stored in large quantities) with an electron acceptor other than oxygen. Subsequently, the bacteria migrate to the oxic sediment layer and oxidize sulfur to sulfate with oxygen. The storage of sulfur has the advantage that it is, unlike sulfide, chemically stable in the presence of oxygen.

Recently, it has been discovered that the giant bacterium *Thioplloca*, which forms huge mats along the coasts of Chile and Peru, couples sulfide oxidation with nitrate reduction [39]. These bacteria form filamentous sheaths with a length of several centimeters. Within the sheaths, the bacteria move up and down between an upper layer, which contains nitrate but little or no oxygen, and a lower layer, where oxygen and nitrate are depleted but sulfide is present. In a central vacuole, the cells accumulate the electron acceptor nitrate to enormous concentrations (up to 0.5 mol l\(^{-1}\)); a similar accumulation of oxygen – either as gas or as solute – would not be possible.

Also *Beggioatoa* spp. [40] and *Thiomargarita namibiensis* accumulate nitrate in a vacuole. However, *T. namibiensis*, the recently discovered largest prokaryote [41], seems to follow a different strategy than the filamentous species: the temporal separation of sulfur oxidation and nitrate reduction. Instead of moving up and down between the layers containing the compounds required for its energy metabolism, it appears to ‘sit and wait’ until the environmental conditions change and supply the bacterium with its electron donor or acceptor.

The prokaryotes mentioned above all have rather large cells. However, even small cells may ‘travel’ over relatively large distances, sometimes with the help of eukaryotes. Motile sulfide-oxidizing bacteria have been observed to form up to 500 μm long sulfur filaments [42]. Sulfide-oxidizing bacteria colonize the surface of a colonial *Zoothamnium*, a marine ciliate [43]. The colonies of this ciliate expand and contract, thus exposing the bacteria alternately to oxygen above and to sulfide within the boundary layer overlying a highly sulfidic substratum. Similarly, the ectosymbiotic sulfide-oxidizing bacteria covering the body of *Stilbonematinae* (marine free-living nematodes) are transported by their hosts across the oxic–anoxic interface and become alternately exposed to sulfide and oxygen [44]. More recently it has been shown that some of these ectosymbionts also use nitrate as an electron acceptor [45]. For the same reasons as outlined above, such strategies works only if the ectosymbionts can store (temporarily) either the electron donor or the acceptor.

The amazing life modes of endosymbiotic sulfur bacteria within tube worms or clams at hydrothermal vents have been described by Cavanaugh [46]. For more information on sulfur-based microbial interactions, see the review by Overmann and van Gemerden [47], this volume.

### 2.5.2. Sulfate-reducing bacteria

For many years, sulfate-reducing bacteria were regarded as strict anaerobes; however, it is now clear that they also have a set of differentiated reactions to oxygen. First, many sulfate-reducing bacteria form aggregates, resulting in a higher tolerance to oxygen exposure [48–51]. Second, at least *Desulfovibrio* species migrate in response to the oxygen concentrations in their environment. In microbial mats of Solar Lake, the numbers counted in most-probable-number (MPN) series of the uppermost layer were much lower during the day when the layer was oxic than at night under anoxic conditions [49]. Third, many, especially the fast-growing species, respire with oxygen [52]. Oxygen is preferentially used when present together with other possible electron acceptors [53]. However, although it has been demonstrated that aerobic respiration is coupled to proton translocation [54] and ATP conservation [52], aerobic growth in pure culture is poor or absent [29,55,56]. *Desulfovibrio* species may divide once [52], but prolonged exposure to oxygen halts growth and usually results in the formation of elongated cells [29]. Obviously, there are different oxygen-reducing systems present in different *Desulfovibrio* species [57]. The bacteria respire with oxygen until oxygen is depleted and then start to grow anaerobically [29,52]. The high respiration rates – exceeding those of most aerobic bacteria [58] – seem to have merely a protective function.

In oxygen gradients, bacteria form bands at the outer edge of the oxic zone (Fig. 2). This reaction includes an aerophobic reaction to high oxygen concentrations. However, for unknown reasons, the bacteria do not move to the anoxic zone where they would be able to grow, but instead accumulate at the oxic–anoxic interface inside the oxic zone. While studying the motility pattern of sulfate-reducing bacteria in oxygen gradients, Eschemann et al. [59] observed that the cells move in circles, as if they are trapped within the oxic milieu.

The situation is different (and even more complex) when aerobic bacteria are also present. Sulfate reducers have been shown to grow continuously in coculture with aerobes [34,60,61]. Furthermore, in natural oxic environments – different from pure-culture studies – dissimilatory sulfate reduction has been detected several times (for review see [57]), a phenomenon which must be due to positive interactions with aerobic bacteria that are not yet understood in detail.

The metabolic versatility of the sulfate reducers makes it necessary to study isolates and to determine the environmental parameters if one wants to understand their metabolism in their natural locations. A sulfate-reducing bacterium like *Desulfovibrio desulfuricans* CSN is able to switch from sulfate reduction to nitrate or oxygen reduc-
tion within a minute in response to changing environmental conditions [62]. Similarly, the bacterium will carry out disproportionation of thiosulfate, sulfite, or elemental sulfur if no electron donor for the reduction of these compounds is available. If oxygen or nitrate is present, the bacterium will even oxidize sulfur compounds to sulfate. It has been pointed out that sulfate-reducing bacteria are much more versatile with respect to their use of electron acceptors than their name would imply [57]. Sulfate is only one of many electron acceptors used, and – due to the necessity of ATP-consuming activation – is not the best or preferred one.

2.6. Bioturbated sediments

Sediment-inhabiting animals have far-reaching effects on the oxic–anoxic interface and on the microbiology of sediments. Bioturbation (sediment reworking) and irrigation (enhanced water exchange between sediment and overlying water) have been studied intensively in marine (e.g., [12,19,20,63–65]) and freshwater sediments (e.g., [10,66–69]).

Since species comprising the marine macrozoobenthos are generally much larger than those occurring in freshwater sediments, their tubes reach deeper into the sediment than those of freshwater species [12,19]. However, the overall effects are quite similar. Here the focus will be on the way that chironomid larvae modify the oxic–anoxic boundary. Chironomids are midges that have evolved into many different niches. Their larvae form a dominant group in the zoobenthos of freshwater sediments. A very common habit is that of mud-dwelling worm-like larvae of medium size (millimeters to a few centimeters). The tube-building larvae that construct J- or U-shaped tubes [70] will be discussed in this context. The tubes of larvae of *Chironomus plumosus* can be as long as 20 cm and reach deep into anoxic sediment layers (Fig. 1). The walls of the tubes are reinforced with secretions and in some cases with fecal pellets [71]. These chironomids exchange the water in their burrows with undulating body movements. They need oxygen for respiration and may filter particles from the flowing water for feeding. From the oxic water pumped through a tube, oxygen diffuses into the wall and the sediment. In shallow warm bodies of water, the larvae have to pump nearly continuously to replenish the oxygen in their tubes. However, an intermittent activity has been found in the colder water of the bottom zone of deeper lakes, with about one period of pumping activity per hour, followed by a resting phase or by other activities [10]. During the latter phase, the oxygen concentration drops down both in front of the tubes and in the tube walls (Fig. 3). The amplitude declines with increasing distance from the lumen of the tube until the sediment becomes permanently anoxic about 2 mm away.

Quite a similar structure has been described from a littoral marine sediment inhabited by small polychaetes that show also an intermittent pumping activity and oxygenate the sediment surrounding their tubes [63]. On a different size scale with burrows reaching down into the sediment to a depth of 1–2.5 m, *Callianassa* (a small crab) irrigates shallow-water sediments in the Mediterranean Sea [12].

The impact of chironomid larvae on methane oxidation and production in a flooded soil has been studied in detail [69]. The number of methane-oxidizing bacteria is higher in chironomid tubes than in all other soil compartments.
However, the tube wall cannot be treated as a fully oxic system. Like methane oxidation, in vitro methanogenesis in tubes is also significantly higher than in the anoxic bulk soil, but no methane is produced in the constantly oxic surface soil. Chironomid tubes are microsites with an increased microbial activity. In the more peripheral region, methane production dominates, while at the inner tube wall close to the lumen, methane oxidation prevails. In between is probably a layer that intermittently changes between oxic and anoxic conditions. The effects of these temporal changes on microbial populations and activities are largely unknown, but the overall effect on processes may be balanced because chironomids have no net effect on methane flux across the sediment surface [69]. However, the diverse ecology of benthic animals makes it difficult to form generalizations. Depending on feeding mechanisms, the impact on sulfur cycling [64] and on carbon flow [65] may be quite different.

The described effects of bioturbation and irrigation at the oxic–anoxic interface are widely accepted. Respiratory or feeding currents as well as the movement of animals inside the burrows will transport oxygenated water through the sediment, not to mention the effects on the boundary layer. Since 1977, the concept of reduced microniches within an oxidized environment has been discussed [72]. The irrigating infauna of sediments causes the opposite effect: it creates oxic microniches within an anoxic habitat.

3. Plant roots

Plant roots form a complicated three-dimensional structure within the soil that serves to fix the plant within the substratum and to acquire essential nutrients. Local nutrient depletion makes it necessary to reconstruct and to enlarge the root system permanently. At the same time,
root exudates and sloughed-off cells (= rhizodeposition) form the main source of substrates for microorganisms. The volume of soil influenced by roots is called the rhizosphere, or more specifically the ectorhizosphere [73]. While the outer boundary of the ectorhizosphere may be difficult to define, the rhizoplane (root surface) and endorhizosphere (the various cell layers of the root itself) are easily distinguishable from each other. In the following, a special situation, the rhizosphere of wetland plants, will be discussed.

Diffusion of atmospheric oxygen is hardly hindered in a dry upland soil, but it slows down with increasing water saturation and filling-up of the pore spaces. The diffusion coefficient of oxygen in water is a factor of $10^6$ smaller than in air, rendering wetland soils anoxic almost immediately after flooding. As a consequence, wetland macrophytes had to evolve different aeration mechanisms. The basic adaptation is an aerenchyma, a porous tissue connecting the root with the emergent parts of the plants (Fig. 4) [74]. Some plants have evolved pressure-driven ventilation mechanisms (for review see [75,76]), which allow very efficient gas exchange due to a directed internal mass flow, while others support their root system by molecular diffusion alone. Among the latter is rice (*Oryza sativa*) [77,78], which will be discussed as a model plant in this review. The principal organization of the plant is shown in Fig. 4; the conceptual framework of CH$_4$ and O$_2$ turnover in the rhizosphere of a rice plant is illustrated in Fig. 5.

### 3.1. Oxygen gradients

The primary function of root aeration is to support the plant tissue with oxygen for aerobic metabolism, but it also has some secondary effects. It has repeatedly been shown that oxygen is released from rice roots. However, an individual root is not homogeneous (Fig. 4C), and the oxygenation of the rhizosphere results in a pattern first described by Armstrong [80,81], based on measurements of the radial oxygen flux. At the root apex, the tissue is highly active and no oxygen is released. A few millimeters from the apex, aerenchyma formation starts, and along this zone the highest oxygen release rates have been observed. More proximally, the exodermis becomes suberized [82], which impedes oxygen release in this zone [83,84]. Where lateral roots break through, the integrity of the root surface is disturbed, and oxygen release is increased again. Flessa and Fischer [85] recorded changes in the redox pattern in the rhizosphere when a growing root passed by. These changes were consistent with the described zonation of the root and suggest an ‘oxygenation window’ for the zone behind the root tip formed for about 2–3 days.

As in sediments, oxygen released into the rhizosphere is consumed by a wealth of microbial processes. The most obvious result is the formation of iron precipitates on the roots of various freshwater plants [86,87]. One may assume that microorganisms very effectively scavenge all the oxygen that is released. However, with rice microcosms grown at 25°C in the greenhouse, it has been shown repeatedly that free oxygen is present in rooted soil [84,88–90]. In contrast to this, Revsbech et al. [91] found only traces of oxygen in the rhizosphere of rice plants that were cultivated at tropical temperatures. However, given the strong temperature dependency of biological processes, this difference between the experiments may simply result from the different temperatures of the systems.

Oxygen released from the roots of wetland plants may oxygenate the rhizosphere, thereby allowing aerobic microorganisms to thrive in this particular environment, at least temporarily. Oxygen may be used to re-oxidize the reduced metabolites formed in the sulfur or iron cycle. This will result in an electron flow through different anaerobic processes as described above for sediments. The degree of oxygenation depends on different factors, such as the plant species or variety, the reducing power of the soil or sediment, and the soil temperature. Oxygen released from the root and methane produced in the anoxic bulk soil form counter-gradients in the rhizosphere, making it anoxic–anoxic boundary layer.

The diffusion between the root surface and the surrounding bulk soil can be described by a cylindrical version of Fick’s law. Net flow $F$ as a function of radius $r$ is calculated from:

$$F(r) = -2 \pi r \phi \frac{D_s}{r} \frac{\Delta C(r)}{(\ln r_2/\ln r_1)}$$

where $\phi$ is the porosity of the soil, $D_s$ is the soil diffusion coefficient, $r_2$ and $r_1$ are two points along the radius $r$, and $\Delta C(r)/(\ln r_2/\ln r_1)$ is the radial concentration gradient measured between $r_2$ and $r_1$ [92]. The principal geometry is the same as around a chironomid tube, making the gradients
steeper than those at the sediment surface, with its planar geometry. Similar models can be used to calculate the radial extension of the oxic rhizosphere [81]. Combining data for $D_1$, $O_2$ consumption rate of the sediment, and oxygen flux at the root surface, radial extensions between 0.5 and 2.9 mm have been calculated [81,84,93].

3.2. Methane oxidation and methanogenesis

In the following, the focus will be on two aerobic processes, methane oxidation and nitrification, and their related anaerobic counterparts. While the rhizosphere is at least in part oxygenated, the surrounding bulk soil is not. With rice, the bulk soil is the compartment in which methanogenesis takes place, while the oxygenated parts of the rhizosphere are thought to be the site of methane oxidation. In addition to the latter, aerobic process, an anaerobic methane oxidation in marine sediments has been described. Biogeochemical parameters show that methane is consumed in a zone deep in the sediments, where a high rate of sulfate reduction takes place simultaneously [94]. Using molecular markers, archaea were recently found to occur in sediments exactly where anaerobic methane oxidation takes place [95,96]. In contrast to the situation in marine settings, the methane turnover in rice fields has been attributed mainly to an interaction between methanogens and aerobic methane oxidizers [97]. However, there is at least one hint that anaerobic methane oxidation may also occur in rice-field soils [98].

Known methane-oxidizing bacteria are obligate aerobes and depend on methane as their sole source of carbon and energy. Methane-oxidizing bacteria form two phylogenetically distinct clusters within the $\alpha$- and the $\gamma$-Proteobacteria [99,100]. Depending on physiological and biochemical characteristics, members of the first group are also classified as type II and those of the second group as types I and X methane-oxidizing bacteria. More recently the taxa Methylocystaceae (equivalent to type II methanotrophs) and Methylococcaceae (equivalent to types I and X methanotrophs) were defined [101]. The key enzyme in methane oxidation is a monooxygenase that is found as a membrane-bound pMMO (particular methane monoxygenase) and as a soluble sMMO [75]. Both Methylocystaceae and Methylococcaceae occur in rice fields [102]. In acidic northern peatlands, a different group of methane oxidizers has been detected [103,104]. These strains are $\alpha$-Proteobacteria, phylogenetically affiliated with Beijerinckia, and assigned to the newly described genus Methylocella [105]. They possess a gene for a sMMO, but the partial sequence published to date makes a common ancestor with the phylogenetically most closely related known methane-oxidizing bacteria improbable [104].

In rice fields and in rice microcosms, aerobic methane oxidizers have been found in the anaerobic bulk soil, in the partly oxygenated rhizospheric soil, in the rhizoplane (the root surface), and in surface-sterilized roots [84,90,93,97]. Published cell numbers estimated by the MPN method are centered around $10^6$ (g dry mass soil)$^{-1}$ with deviations in both directions [90,93,106–109]. Cell numbers in the rhizosphere are $10^6$–$10^7$ (g dry mass root)$^{-1}$ [93]. From rate measurements, it has been deduced that the distribution of methane oxidizers in the rhizoplane is very patchy [93], and miniaturized MPN counts have shown large variations in numbers of methane-oxidizing bacteria ranging from <0.1 cells mm$^{-2}$ (detection limit) to >120 cells mm$^{-2}$ of root surface [110]. This patchiness has been confirmed with confocal laser scanning microscopy [111].

The finding of viable cells in surface-sterilized roots [93] provides a hint that methane-oxidizing bacteria may invade the rice plants. This has also been supported by confocal microscopy of roots from gnotobiotic rice plants that had been inoculated with pure cultures of methane-oxidizing bacteria [111]. However, the pathways and mechanisms of invasion remain unknown.

Soil-free rice roots incubated anoxically support an immediate methane production [112]. The root-associated methane production has been studied intensively [84,113,114]. In the rhizoplane, viable archaea have been localized by in situ hybridization. The root environment selects for an archaeal community that is different from that of the bulk soil [114–116]. However, while methane production is exclusively linked with archaea, not all archaea present are necessarily methanogens. Hence, the methanogenic organisms that are active in situ remain to be identified.

To summarize, in the rhizosphere and even in the rhizoplane of rice, microhabitats exist for both methane-oxidizing and methanogenic microorganisms. However, it has not been shown whether these microhabitats are spatially associated. One might speculate that methane-producing patches are point sources that favor the development of methane-oxidizing bacteria close by. One might also speculate that methane-producing archaea in the rhizosphere are especially resistant to oxygen stress. In this context, it is most interesting that in insect guts Methanobrevibacter spp. are also encountered in locations where they are potentially exposed to oxygen (see below). To the best of our knowledge, the microenvironment in the rhizosphere is characterized by counter-gradients of oxygen and methane. From studies in artificial gradient systems ([117]; P. Frenzel, unpublished data), it can be concluded that methane oxidizers live in a zone where oxygen and methane overlap. However, the conditions in a particular spot may change drastically with time. The oxic zone may be restricted to the very surface of older root segments or even disappear completely.

3.3. Nitrogen metabolism

Nitrogen cycling in the rhizosphere shows some parallels to methane-mediated carbon cycling. In wetland soils,
the dominating N species is ammonium. It is derived from mineralization of organic matter or, in agriculture, from urea or other ammonium-based fertilizers. Like the methane oxidizers described above, the ammonium oxidizers are strict aerobes and may be active in the oxygenated rhizosphere. They form nitrite that, in turn, may be oxidized by nitrobacteria to nitrate. Because of the location of the oxidation processes in the rhizosphere, nitrite and nitrate will either be assimilated by the root or diffuse into the surrounding anoxic bulk soil. Among the possible microbial reactions, we have chosen denitrification as an example. The potential role of aerobic denitrification [118] and of anaerobic nitrification [119] will not be discussed.

The suspected proximity of nitrification and denitrification favors a close coupling of both processes. This has been demonstrated by measuring the overall dinitrogen flux from microcosms in a helium/oxygen atmosphere [120]. The emission of dinitrogen, the end product of denitrification, ceases after an inhibitor of nitrification has been applied. This indicates a tight coupling of the two processes. Arth and Frenzel [88] used a multi-channel electrode that allowed measurement of ammonium, nitrite and nitrate within the soil at the same microsite. Using this electrode, the microenvironment in the rice rhizosphere was shown to be characterized by spatially heterogeneous substrate concentrations that changed unpredictably with time. The activities of nitrifiers and – because of the tight coupling – of denitrifiers were dependent on the presence of oxygen and ammonium. Because of the competition of ammonium oxidizers with the rice roots, ammonium becomes the limiting factor after a dense root mat has developed. Nitrification and subsequent denitrification only becomes measurable when an imbalance between nitrogen supply and nitrogen demand of the rice plant allows increased ammonium concentrations in the rhizosphere. This happens only after fertilization. Microelectrode measurements in isolated roots showed gradients of ammonium with a source in the bulk soil and a sink mainly in the rhizosphere [88]. In contrast, nitrate is produced in the same layer in which ammonium disappears and decreases both towards root surface and bulk soil. Differences in nitrification have been observed along the root [88] and agree well with the pattern of oxygen release [80,81,84].

3.4. Linking N and C cycles

Carbon and nitrogen cycles as described above are intimately interrelated (Fig. 5). Methane monooxygenases and ammonium monooxygenases are quite unspecific, and may also oxidize ammonium or methane, respectively, in addition to their preferred substrate. However, it has been shown that nitrifying bacteria are outcompeted by methanotrophs for ammonium [102,121]. Only after ammonium-based fertilization did the pore-water concentration of ammonium become high enough to support nitrification [88,120]. However, part of this nitrification activity may be due to the activity of methane-oxidizing bacteria [102,121]. In rice-planted microcosms as well as in the field, the plant itself is the top competitor for nitrogen, and fertilizer is usually taken up rapidly, making nitrifying activity a transient phenomenon [88]. In numerous other environments, it has been shown that ammonium inhibits methane oxidation. While inhibition by ammonia is mainly competitive, an additional mechanism has been proposed by King and Schnell [122,123]: in upland soils they found toxic effects due to nitrite, the end product of ammonia oxidation by methane-oxidizing bacteria [123–125]. This inhibition was more effective under methane concentrations that were higher than in situ. In contrast to these findings, methane-oxidizing bacteria in rice microcosms were not inhibited by ammonium, but instead were stimulated [102,126]. However, in rice fields, pore-water methane concentrations are much higher. Nevertheless, this finding was quite surprising, but has been since verified in additional experiments in the greenhouse and in the field (Frenzel et al., unpublished data). Obviously the ability of methane-oxidizing bacteria to fix dinitrogen [99,100] cannot compensate for the ammonium limitation in the rhizosphere.

For much too long a time, the focus of microbial ecology was on the main substrates; for methane oxidation, the focus was on the availability of methane and oxygen, with the latter assumed to be the limiting factor. This view is incomplete. Macronutrients such as N or P, as well as potential micronutrients, e.g., Cu, must also be included in the analysis. The case study described above demonstrates not only how two biogeochemical cycles are linked, but also how the availability of a nutrient may take control.

Considering these effects on the microscale of microbial ecology, the effects on the macroscale of methane emission can be summarized as follows [127]. At the plant/ecosystem level, nitrogen increases plant growth and thereby carbon supply to the rhizosphere. This will stimulate methane producers and increase methane emission from the field. At the level of the microbial community, nitrogen stimulates the growth and activity of methane-oxidizing bacteria, leading to a reduced net emission. At the biochemical level, ammonium inhibits methane oxidation because of competition at the binding site of the methane monooxygenase, hence increasing methane efflux. The balance between these processes may vary spatially and temporally depending on the molar ratio of methane and ammonium in the rhizosphere, but the overall outcome in the fertilization experiments of Bodelier et al. [102,126] was a reduction of methane emission.

The global impact of these interactions becomes evident if one takes into account the fact that rice fields are among the major sources of atmospheric methane, which is the most important greenhouse gas next to carbon dioxide. Recent estimates predict that rice production will expand by up to 70% over the next decades [128]. This will involve an intensified application of fertilizers [129]. To date it was
thought that ammonium-based fertilization would increase the methane emission due to the inhibitory effects on the MMO described above. However, understanding the microbial ecology in the rhizosphere leads one to expect that the predicted increase in application of nitrogen fertilizers will not reduce but instead promote CH$_4$ oxidation and hence decrease its emission in future rice agriculture.

4. Insect guts

The digestive tract of insects consists of three basic compartments: the foregut, midgut, and hindgut. In many herbivorous insects, the usually tubular hindgut is differentiated into one or more dilated compartments, accommodating a dense gut microbiota. Such ‘fermentation chambers’ increase the residence time of the food and allow its degradation by the microbial symbionts, analogous to the situation in the rumen, colon, or cecum of mammals. The microorganisms supplement the digestive capacities of the host, especially with their ability to hydrolyze the major structural polymers of plant cell walls (cellulose and hemicelluloses). The fermentation products, typically acetate and other short-chain fatty acids, are resorbed by the host and contribute substantially to its nutrition [130-133].

Although there is evidence that the presence of a gut microbiota is a common phenomenon among a wide range of insects and possibly other invertebrates [131,134], the only group which has been studied in detail are the wood-feeding termites (Isoptera) [130,135]. This is mainly due to the interest they received for their unusual ability to thrive on a purely lignocellulosic diet, a phenomenon which had already been attributed to their intestinal symbionts early in the last century [136].

Insect guts are surrounded by aerobic tissues aerated by the insect’s tracheal system, but the restrictions imposed by diffusive transport of oxygen across the epithelia into the gut allow anoxia to be established and maintained simply due to the removal of oxygen by the respiratory activity of the gut microbiota. Therefore, insect guts represent anoxic niches characterized by an oxic–anoxic interface with steep oxygen gradients in the microoxic periphery bordering to the host environment.

4.1. Radial oxygen gradients

In contrast to the situation in sediments or biofilms, where a one-dimensional model may sometimes suffice to describe oxygen fluxes at the oxic–anoxic interface (Eq. 1), the tubular and spheroid gut compartments of insects are always three-dimensional systems with a radial symmetry [132]. In that respect, the situation resembles that of the roots of aquatic plants immersed in anoxic sediment or soil (see above), although the direction of oxygen diffusion is inverted. In a tubular or spherical system, however, the direction of oxygen flux is important, since at a constant total-area-integrated flux, there is an inverse-proportional relationship between the steepness of the radial gradients and the radial distance [137]. This means that even in the absence of any O$_2$ consumption along the gradient, the steepness of the radial O$_2$ gradient towards a hypothetical sink at the gut center would increase, whereas the steepness of the radial O$_2$ gradient from a root cylinder towards a hypothetical sink in the rhizosphere would decrease with the distance from the root (Eq. 2).

Due to the inverse-proportional relationship between surface area and volume of any spherical body, any surface-related processes will increase in importance with decreasing size of the system. The intestinal volume of the...
dilated hindgut paunch of a termite (\( < 1 \mu l \)) is roughly \( 10^8 \) times smaller than that of the rumen of a cow, and it has been estimated that the influx of oxygen per unit volume across the gut epithelium will be about 500 times larger (assuming a spherical geometry) in a termite gut than in the bovine rumen [132].

This situation has two fundamental consequences for any small gut. The first is that despite the efficient removal of oxygen within a fraction of a millimeter below the gut epithelium, a substantial part of the gut volume is rendered (micro)oxic (Fig. 6A,B). Since the diameter of the anoxic portion of the gut lumen decreases with the total gut diameter, there must be a lower size limit below which systems of tubular or spherical geometry cannot be anoxic at any given O\(_2\) removal rate, as also pointed out by Ploug et al. [137] for marine aggregates. In termite guts, the tubular midgut region and the narrow constrictions connecting the individual hindgut dilatations are generally oxic throughout their lumen [138–140], although the case of the first proctodeal segment (P1) of *Nasutitermes lujae*, which shows a considerable intestinal alkalinity (pH 10.5), illustrates that even a tubular section with a diameter of \(< 250 \mu m\) may possess an anoxic status [139]. Such exceptions may be due to high respiratory rates of the epithelial cells, possibly related to the maintenance of the steep pH gradients across these epithelia, or to a diffusion barrier formed by specific cuticular structures. However, it is also possible that chemical rather than biological processes may be responsible for an exceptionally high O\(_2\) consumption rate, as indicated by the results of a recent study [141] of the extremely alkaline P1 segment of soil-feeding termites (pH \( \approx 12 \); see Fig. 7A).

As a second consequence, the influx of O\(_2\) into the gut has to be balanced by a continuous removal of oxygen by the intestinal microbiota located in the gut periphery. Irrespective of the actual geometry, Fick’s first law of diffusion (Eqs. 1 and 2) actually demands that the influx rate of oxygen into the gut will actually increase with the efficiency at which oxygen is removed. In small guts, one therefore has to expect a significant impact of oxygen influx on the composition and the metabolic properties of the gut microbiota and on the carbon and electron flow within the gut microbial community.

### 4.2. Impact of oxygen influx

Several cultivation-based studies of the hindgut microbiota of the termite *Reticulitermes flavipes* did indeed demonstrate a dominance of aerotolerant lactic acid bacteria and other, facultatively anaerobic and even strictly aerobic bacteria among the carbohydrate-utilizing isolates [143,144]. Strictly anaerobic isolates were rare, which contrasts strongly with the situation in the bovine rumen or the human colon [145,146]. To date, coccoid lactic acid bacteria have been found in the intestinal tracts of all termite species investigated, comprising three of the five families of lower termites (Masto-, Kalo-, and Rhinotermitidae) and several species of higher termites (Termitidae), including the globally most important soil-feeding taxa [144,147] and references therein.

Although the viable count of coccoid lactic acid bacteria represents only about 3% of the total microbial cell count in the *R. flavipes* hindgut, this is not unreasonable in view of the large morphological diversity among the hindgut microbiota of this termite. Genetic fingerprinting of the hindgut microbial community has established that one of the lactic acid bacteria isolates in fact represents a dominating sequence type among the *R. flavipes* gut microbiota [147]. Nevertheless, it has to be kept in mind that the majority of the microorganisms in termite guts, as in any other environment, have so far escaped cultivation, and that nothing is known about the physiological properties of the uncultivated organisms or their relation to oxygen [135].

The lactic acid bacteria colonizing the hindguts of *R. flavipes* (wood-feeding) and *Thoracotermes macrothorax* (soil-feeding) show a high genetic diversity; representative
strains were phylogenetically classified by 16S rRNA gene sequence analysis as lactococci and enterococci, probably representing new species [147]. All Lactococcus and Enterococcus strains isolated from *R. flavipes* and *T. macrothorax* possess NADH oxidase, pyruvate oxidase, and superoxide dismutase, which are the key enzymes conferring on lactic acid bacteria the ability to reduce oxygen and a tolerance to its toxic metabolites [144,147]. In the presence of oxygen, the isolates shift their fermentation balance completely from lactate to acetate. Oxygen reduction rates of cell suspensions were quite high (69 nmol min⁻¹ (mg dry mass)⁻¹ for *Enterococcus* strain RfL6) [147], but are still surpassed by the respiratory activity of sulfate-reducing Desulfovibrio species (see above), which represent another population of potentially oxygen-reducing anaerobes among the termite gut microbiota [148,149].

In principle, fermenting bacteria may gain a considerable increase in their energy budget by shunting electrons from the oxidative branch of fermentative pathways to an external electron acceptor [150]. Also Desulfovibrio spp. can couple O₂ reduction with electron transport phosphorylation [52]. Nevertheless, it remains to be established whether the O₂-reducing potential of lactic acid bacteria and sulfate-reducing bacteria observed in pure culture is of any relevance for the electron flow in the termite gut. A key to answering this question will be the localization of the respective populations with respect to the oxygen gradient.

The influx of oxygen via the gut epithelium and its reduction in the hindgut periphery has a significant impact on carbon and electron flow within the hindgut microbial community of *R. flavipes*. Using microinjection of radioisotopes into intact termite guts, it has been demonstrated that the absence of oxygen in the incubation atmosphere decreases the turnover rates of lactate and shifts the hindgut metabolism of glucose and lactate towards more reduced products [151]. Lactate has been identified as an important intermediate in the hindgut metabolism of this termite, whereas the low turnover rates of injected glucose indicate that free glucose is not an important intermediate under in situ conditions [151]. The in situ fluxes of carbon through the intestinal lactate pool represents one-third of the total carbon flux in the animal. Consequently, the low concentrations of lactate in the hindgut fluid are due to a rapid turnover of lactate rather than to the absence of lactate production, as had been suggested on the basis of earlier results [144]. Although the rapid turnover of the lactate pool would consolidate the presence of lactic acid bacteria and the low lactate concentrations in the hindgut fluid, the immediate precursor of lactate and the organisms responsible for its formation remain unknown.

### 4.3. Hydrogen counter-gradients

In the hindgut of *R. flavipes*, the oxygen gradients into the gut are opposed by steep counter-gradients of hydrogen (Fig. 6B). Hydrogen accumulates to substantial partial pressures in the gut lumen, but only small amounts escape from the gut since it is consumed on its way to the epithelium [138]. While hydrogen formation in lower termites is attributed to the fermentative activity of the anaerobic flagellates [130,152], the sources of H₂ among the largely prokaryotic gut microbiota of higher termites are not clear. Methanogenesis and homoacetogenesis, which seem to occur simultaneously in the hindguts of all termites, albeit at different rates [153], are considered important hydrogen sink reactions in all termite hindguts.

In view of the microoxic conditions encountered in the hindgut periphery, the gut would also appear to be the ideal habitat for aerobic H₂-oxidizing ‘Knallgas’ bacteria. The presence of such bacteria in *R. flavipes* hindguts has been confirmed [144], but hydrogen microsensor measurements under defined gas headspace have shown that at least part of the H₂-oxidizing activity in the gut periphery can be attributed to anaerobic microorganisms (Fig. 6B). This observation is astonishing in view of the microoxic conditions in this locality, but it agrees well with the findings that the hindgut cuticle is densely colonized by methanogenic archaea of the genus *Methanobrevibacter* [154,155] and that hindgut methanogenesis is strongly stimulated by exogenous H₂ [138]. The biochemical basis for the apparent oxygen tolerance of the isolates [154] and the significance of the considerable rates of H₂-dependent oxygen reduction observed in dense cell suspensions of *Methanobrevibacter curtulicaris* (A. Tholen and A. Brune, submitted for publication) remain to be established.

In contrast to the methanogenic activity, the in situ rates of reductive acetogenesis are virtually unaffected by the incubation atmosphere [151]. The lack of stimulation by externally supplied H₂ and the insensitivity of the activity to the presence of oxygen in the gut periphery indicates that the homoacetogenic population(s) are located within the hindgut lumen, where reductive acetogenesis appear to be saturated for H₂ due to the high H₂ partial pressure [151]. Only in starved termites, whose hindguts exhibit strongly decreased luminal H₂ partial pressures [138], has a significantly decreased rate of reductive acetogenesis been observed [151]. These observations add strong support to the hypothesis that the coexistence of methanogenic and homoacetogenic microorganisms within the hindgut of *R. flavipes* are due to a spatial separation of the respective populations rather than to a direct competition for hydrogen [138,132].

In other termites, the methanogenic populations are not restricted the gut wall but also occur in the lumen, mostly associated with the intestinal protozoa. The specific location of the methanogenic and other H₂-consuming populations may be responsible for the differences in CH₄ and H₂ emission rates observed between and within different termite species [140,153,156]. Little is known about the identity of the homoacetogens, but since the first sprochetal isolates from *Zootermopsis angusticollis* have been iden-
tified as homoacetogens [157], it is not unlikely that the closely related spirochetal clones from the hindguts of R. flavigus, Coptotermes formosanus, and Zootermopsis angusticollis share the same type of metabolism [158]. This is supported by the fact that many spirochetes are attached to the potentially H2-producing intestinal protozoa [133].

In theory, radial concentration gradients should build up for any fermentation product accumulating in the gut lumen, resulting in radial fluxes towards the gut epithelium. Although the hindgut of R. flavigus contains significant numbers of microorganisms capable of aerobic oxidation of most metabolites found in the hindgut fluid [144], little is known about the importance of aerobic oxidation processes in the gut periphery. Initial results, obtained by microinjection of radiolabeled metabolites into R. flavigus hindguts, have shown that acetate oxidation in the hindgut may account for 4% of the total carbon flux through this termite [151].

4.4. Axial dynamics of microbial activities

While the steep radial gradients of metabolites in the hindgut paunch of R. flavigus indicate heterogeneity in the radial distribution of the microbial activities responsible for their formation, the dynamics of H2 and O2 profiles along the gut axis also indicate that each gut region is colonized by a different microbiota. The axial differentiation of the hindgut is most pronounced among the higher termites (Termitidae), which may possess up to five separate compartments (Fig. 7A). Microelectrode studies of the soil-feeding Cabitermes and related species of the subfamily Termitinae have revealed a considerable axial dynamics not only in oxygen status and intestinal pH [132,140,159], but also in other parameters such as H2 partial pressure [140] and the redox state of both mineral and organic gut contents (A. Kappler and A. Brune, submitted for publication). Together with the radial gradients which superimpose the axial profiles in each of the compartments [140], the hindgut microenvironment provides a patchwork of microhabitats for a diverse microbiota.

In the gut of soil-feeding termites, all major compartments are also anoxic at the center (Fig. 7B), although O2 consumption in the extremely alkaline P1 region (pH > 12) appears to be due to a chemical reaction rather than to biological activity [141], a phenomenon which may be of importance for the transformation and mineralization of humic substances during gut passage [160,161]. Hydrogen accumulates only in the anterior, highly alkaline hindgut, rendering the mixed segment (ms) and the third proctodeal segment (P3) significant sources of H2, whereas posterior to the P3 segment, H2 concentrations are below the detection limit (< 100 Pa) [140]. The posterior hindgut (P3a and P4b compartments) harbors significant populations of methanogens and homoacetogens [142] and also the bulk of the methanogenic and homoacetogenic activities (Fig. 7C). Since hydrogen emission rates of isolated hindguts surpass the rates found with living termites, it has been postulated that a cross-epithelial H2 transfer from the anterior (ms–P3) to the posterior gut regions (P4a–P5) would not only fuel methanogenesis in these compartments, but might also create microniches favorable for homoacetogenesis [140,142].

In contrast to wood-feeding termites, where CO2 is the only important electron acceptor in the anoxic gut regions, the inorganic soil components, especially the large pool of iron (hydr)oxides, represents a substantial source of potential electron acceptors in soil-feeding termites. There is strong evidence that humic-acid-mediated microbial reduction of ferric iron represents an important intestinal process (A. Kappler and A. Brune, unpublished results). In view of the large oxygen fluxes into the gut, reoxidation of ferrous iron by chemical or microbial processes is feasible, but remains to be investigated.

Apart from the observations mentioned above, little is known about the spatial organization of the termite gut microbiota. Using fluorescent in situ hybridization with group-specific rRNA-targeted probes, Berchtold et al. [162] have demonstrated a principal difference in the density and radial distribution among the major phyla of the microbiota in the two major hindgut regions of Mastotermines darwiniensis. The thick gut wall of the posterior region is colonized preferentially by Gram-positive cocci and rod-shaped and filamentous bacteria of the Cytophaga-Flexibacter-Bacteroides phylum, whereas α-Proteobacteria occur mainly free in the lumen. The anterior, thin-walled region of the paunch is not densely colonized, and although this does not significantly affect the oxygen status of the lumen, the microbiota is morphologically and phylogenetically different from that in the posterior region [162].

In general, there is a large phenotypic and (phylo)genetic diversity among the hindgut microbiota of any termite investigated (for a review, see [135]), but morphological and molecular studies have revealed little or no information on the physiological properties of the respective types. Conversely, cultivation-based approaches often lack information on the numerical significance of the respective populations. It has been pointed out that even if both abundance and physiological activities have been determined, as in the case of aerobic bacteria capable of degrading aromatic compounds when oxygen is present [148], the ecological significance of a specific population and their metabolic potential remains uncertain until their exact location (e.g., relative to the oxygen gradient) has been established [135].

4.5. Temporal fluctuations of the oxygen status

Ebert and Brune [138] have demonstrated that the diffusive influx of oxygen and its removal by the respiratory activity of the hindgut microbiota represents a fine-tuned equilibrium, which can be easily disturbed by increasing
the diffusive influx of oxygen, e.g., by incubating the insects under a headspace with increased oxygen partial pressure. When the influx of oxygen surpasses the reductive capacities of the gut microbiota, the gut becomes completely oxic, and luminal production of hydrogen ceases completely. Since such conditions can also be brought about by starvation [138], it is likely that the nutrient status of a termite (controlling the supply of reductant and thereby also the respiratory capacity of the gut microbiota) will change the penetration depth of oxygen and thus influence the position of the oxic–anoxic interface.

Intestinal H₂ production in soil-feeding Termitinae is severely affected by the starvation situation with which the termites are inevitably faced in the laboratory. H₂ partial pressures in the anterior hindgut of Cubitermes spp. decrease progressively with time during the first weeks after collection [140]. However, high H₂ partial pressures in the P3 of Cubitermes orthognathus, which range from 0.1 to 1 kPa in starved individuals, can be restored by feeding topsoil from the collection site to small batches of termites. Within 24 h, the H₂ partial pressure in the P3 rises to maximum values (1.5–9 kPa), which are even higher than those measured 2 weeks after collection [140].

5. Conclusions and outlook

In any environment, oxygen is not only responsible for the direct mineralization of organic matter, but also for the reoxidation of the reduced electron acceptors of anaerobic respiration processes. The relative position of the oxic–anoxic interface therefore enforces a characteristic layering of microbial processes which directly or indirectly depend on oxygen, and the basic principle controlling the flow of electrons from organic matter to oxygen is molecular diffusion.

At the macroscale, the oxic–anoxic interface in sediments appears as a more or less planar boundary. However, at the microscale of bacteria, the geometry of sediment surface reveals a fractal dimension that makes a simple one-dimensional model difficult to apply. In addition, the diffusion limitation is often overcome by physically or biologically driven mixing or advection, or by the locomotory activity of animals or specifically adapted microorganisms.

In contrast to the situation in sediments, the three-dimensional characteristics of the oxic–anoxic interface are quite obvious in animal burrows, around plant roots, and in the intestinal tracts of animals. The case of the small guts illustrates that in non-planar systems, the relative importance of transport processes at the oxic–anoxic interface are strongly scale-dependent.

In most environments, the position of the oxic–anoxic interface is subject to temporal fluctuations. In the case of sediments, the deposition of fresh organic matter, periodic flooding, temperature-mediated changes in microbial activity, or mixing events caused by bioturbation or wave movement will affect the relative position of the oxic–anoxic interface, but also all dependent processes, which will induce a time series of successive environmental changes in a given locality. Also during growth of a plant root, the oxygen status of the root zone will change with changes in the supply of oxygen or root secretions during different stages of root development. In the case of insect guts, the feeding activity of the host will determine the availability of endogenous reductant, which in turn will influence the depth of oxygen penetration into the intestinal tract. In small guts, anoxic conditions may be merely a transient feature for a certain period after feeding.

In a stratified environment, microbial communities are typically layered. Nevertheless, fluctuations in the relative position of the oxic–anoxic boundary may necessitate specific adaptations enabling a microorganism to colonize microniches characterized by a fluctuating oxygen status. Many oxygen-tolerant anaerobes are well adapted to survive oxygen stress. A most intriguing phenomenon, however, is the recently discovered ability of bacteria generally considered to be ‘strict anaerobes’ to maintain a functional energy metabolism in the presence of – or even based on – molecular oxygen.

Pure cultures are important to define the metabolic potential and to identify specific adaptations to changing environmental conditions of a given microorganism, although the mere presence of a given metabolic potential does not indicate whether that activity is actually expressed in situ. In order to assign functions to microbial populations, it is necessary to determine their in situ activities and to characterize their microhabitats at high resolution [135]. For example, both lactic acid bacteria and sulfate-reducing bacteria in the termite gut have the potential to reduce O₂, yet it is essential to know their location relative to the steep O₂ gradient to be able to predict whether they will actually do so in situ.

Gradient cultivation may help to discover novel metabolic groups or increase the percentage of successfully cultivated microorganisms of a specific guild. However, the gradient situation gives rise to microenvironmental conditions which are difficult to mimic by cultivation. Many techniques necessary to study microbial populations and their activities in situ, such as microelectrodes, in situ hybridization, and single-cell isolation, are already available [163], although they are still relatively new and not broadly used (see also Fröhlich and König [164] and Spring et al. [165], this volume).

Molecular methods based on phylogenetic markers such as the 16S rRNA gene sequence do not require cultivation and are therefore widely used to characterize microbial communities (reviewed by Ludwig and Amann, this volume [166]). Cryosectioning techniques allow the characterization of the topology of microbial communities within a structured environment. However, the 16S rRNA gene se-
quence rarely allows inferences to be drawn regarding the physiological properties of a given phylotype. Only genes whose products are directly involved in a specific metabolic pathway can be used as molecular markers for assigning a potential function to a phylotype and, provided that the marker is sufficiently conserved, may even allow its assignment to a specific guild. Since the mere presence of a gene does not necessarily reflect that it is actively expressed, the ecological relevance of such studies will increase if the expression of a gene is studied at the mRNA level [167].

Intriguing possibilities for linking community structure and function, however, arise from the combination of molecular techniques and methods allowing inferences on the activity of individual microbial cells or spatially organized populations. It has been demonstrated that in situ activities may be assigned to individual cells in a microbial community by the combination of fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes and microautoradiography [168]. Even the classical activity-based staining of cells with tetrazolium salts acquires a new dimension when the cells carrying the formazan are separated from the inactive populations by density gradient centrifugation and analyzed by molecular cloning or fingerprinting [169]. Also the recently demonstrated coupling of activity-dependent labeling of molecular biomarkers using radioactive or stable isotopes with molecular biological methods has provided cultivation-independent means for a functional identification [102,170].

Acknowledgements

We wish to thank the two anonymous reviewers of this article for their constructive criticism, and Karen A. Brune for editing the manuscript.

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