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Microgradients of Microbial Oxygen Consumption in a Barley Rhizosphere Model System

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A microelectrode technique was used to map the radial distribution of oxygen concentrations and oxygen consumption rates around single roots of 7-day-old barley seedlings. The seedlings were grown in gel-stabilized medium containing a nutrient solution, a soil extract, and an inert polymer. Oxygen consumption by microbial respiration in the rhizosphere (<5 mm from the root) and in bulk medium (>30 mm from the root) was determined by using Fick's laws of diffusion and an analytical approach with curve fitting to measured microprofiles of oxygen concentration. A marked increase of microbial respiration was observed in the inner 0- to 3-mm-thick, concentric zone around the root (rhizosphere). The volume-specific oxygen consumption rate (specific activity) was thus 30 to 60 times higher in the innermost 0 to 0.01 mm (rhizoplane) than in the bulk medium. The oxygen consumption rate in the root tissue was in turn 10 to 30 times higher than that in the rhizoplane. Both microbial respiration and oxygen uptake by the root varied between different roots. This was probably due to a between-root variation of the exudation rate for easily degradable carbon compounds supporting the microbial oxygen consumption.

The rhizosphere is a concentric zone of soil around the root cylinder. Although it is only a few millimeters thick, root-derived organic matter such as exudates, mucilages, and dead epidermal cells, etc., supports high microbial activity in the zone (4, 14). Between 14 and 40% of the organic C produced in young barley or wheat plants may thus be lost again as root exudates and subsequently mineralized by microbial respiration in the rhizosphere (14, 21, 36). In addition to stimulating the activity of soil microorganisms, the root also affects the chemical microenvironment in the rhizosphere by consuming oxygen and inorganic nutrients such as nitrate and ammonium (15).

Many attempts have been made to develop theoretical models of the microscale distribution of organic and inorganic compounds (5–7, 9, 26) and microorganisms (24, 38) in the rhizosphere. Likewise, numerous experimental systems have been built to study transport and nutrient cycling in whole root systems (10, 15, 18, 20, 22, 23). However, it has always been a major problem to study the chemical microgradients around single roots, since the techniques have affected the samples or the subsamples have been too large to represent well-defined distances from the root.

In the last decade, nondestructive microsensor techniques have found numerous applications in microbial ecology (29). The advantage of these techniques is that they provide a high resolution of concentration profiles and allow simultaneous determination of several oxidants for microbial respiration, e.g., O_2, NO_3^-, and N_2O. We used a microsensor technique to study oxygen distribution around barley roots grown in a gel-stabilized soil suspension. We determined the microdistribution of microbial oxygen consumption rates in the rhizosphere and in root tissue. The method thus enabled us to detect an elevated microbial respiration in the innermost 0- to 3-mm layer adjacent to the root (equivalent to the rhizosphere). The calculations of respiration rates were based on Fick's laws of diffusion and an approach of curve fitting to measured oxygen microprofiles. A comparable approach was recently developed to calculate oxygen consumption rates in planar biofilm and sediment systems (25).

MATERIALS AND METHODS

Growth media. Sterile medium was composed of 75 g of Pluronic F127 polymer (BASF) in 250 ml of the following nutrient solution: 2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, 0.5 mM Na_2HPO_4, 0.5 μM $MnSO_4$, 1.5 μM H_3BO_3, 0.1 μM $CuSO_4$, 0.02 μM $(NH_4)_6Mo_7O_{24}$, and 0.2 μM $ZnSO_4$ (19). The Pluronic polymer, which is a low-melting-temperature polyol, is a copolymer of polypropylene oxide and ethylene oxide. The polymer liquifies when cooled to ~4°C or below and solidifies at ~15°C or above when used in a concentration of 30% (wt/wt). The compound is biologically inert and not degradable by the microorganisms. Bottles (500 ml) with nutrient medium and polymer were autoclaved and placed overnight in a refrigerator (4°C). The solution was kept in the refrigerator until used.

Nonsterile medium was made from 250 ml of nutrient solution supplemented with 6 ml of soil suspension. The latter was made of 20 g of soil in 200 ml of water mixed for 5 min and allowed to stand so that sand particles would settle. The polymer was then added, and the bottles were placed in a refrigerator until used.

Growth chamber. The growth chamber (Fig. 1) was a Plexiglas box (3.5 by 5 by 15 cm) with a bottom of nylon mesh (mesh size, 0.15 mm), which allowed air to diffuse freely into the growth medium from both above and below. To prevent the medium from sliding through the nylon mesh before the polymer was solidified, the bottom was initially covered with autoclave tape. The boxes were sterilized by wiping them carefully with 70% ethanol and then exposing them to UV light for 30 min. The medium was then poured into the box to a thickness of 1 to 2 cm. The polymer was allowed to solidify, UV-sterilized nylon mesh was placed on top of the medium. A second 1- to 2-cm-thick layer of medium was then added. To prevent the bottom layer from

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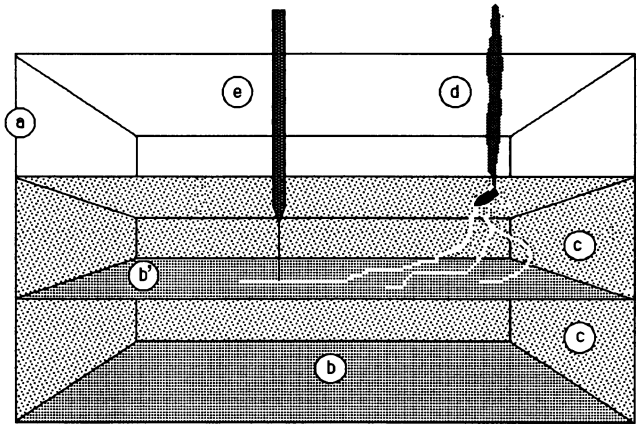

FIG. 1. Diagram of the setup used with a young barley plant in gel-stabilized soil homogenate. The growth chamber (a) was a transparent Plexiglas box (3.5 by 5 by 15 cm). The bottom was replaced by a nylon mesh (b) to provide efficient oxygen supply. Two layers of gel-stabilized soil suspension (c) were separated by another nylon mesh (b'). The roots of the barley seedling (d) grew along the latter. Oxygen concentration profiles were measured with microelectrodes (e) inserted from the top.

melting, the top layer was added at a temperature of 12 to 13°C, i.e., just before it solidified. Finally, when the top layer had solidified, the tape was removed from the bottom.

Seed inoculation and growth. Barley seeds were sterilized as follows: (i) shaking in water for 1 h; (ii) sterilizing in 2% $AgNO_3$ for 20 min; and (iii) washing once in 1% NaCl, once in sterile water, once in 1% NaCl, and finally five times in sterile water (19). After sterilization, a seed was carefully placed in each of the boxes. The boxes were covered with transparent plastic bags and placed in a chamber at 20 ± 2°C with 12-h cycles of light and dark. The boxes were tilted slightly (20° angle) to force the roots to grow along the surface of the nylon mesh. The root fragments under study with the microelectrodes were always separated by >30 mm from neighboring roots.

Oxygen measurements. Recordings of oxygen profiles were made in the medium surrounding the roots by using Clark-type oxygen microelectrodes with guard cathodes as described by Revsbech and Jørgensen (30) and Revsbech (28). The electrodes had a tip diameter of 3 to 5 μm, the 90% response time was 1 to 2 s, and the stirring sensitivity was less than 1% (i.e., oxygen consumption of the electrodes was negligible compared with the diffusive supply) (28, 32). The microelectrodes were inserted into the growth medium from above with a micromanipulator (Märzhäuser, Steindorf-Wetzler, Germany), which allowed positions of the electrode tip to be read with an accuracy of 10 μm. The position of the tip relative to the root surface could be observed with a dissection microscope. If the electrode tip was not positioned exactly perpendicular to the root surface, the electrode was pulled back and repositioned for a new measurement. If the electrode tip touched the root surface, this could be observed visually with the dissection microscope and by a slight deflection of signals on the measuring picoammeter and strip recorder.

Calculations. The experimental system was built symmetrically, so that oxygen could diffuse freely into the polymer from above and below. Diffusion was considered to be the only transport process for oxygen, and mass flow of water was ignored (see Discussion). Oxygen was considered to be consumed either by the root alone (sterile systems) or by both the root and the microorganisms in the surrounding medium (nonsterile systems).

At some distance from the root, the volume-specific rate of oxygen consumption (specific activity) in the bulk medium could be determined from Fick's second law of diffusion for a planar system by fitting small sections of parabolic curves to the curves of a measured profile. This procedure for biofilm and sediment systems has been described in detail by Nielsen et al. (25).

Near the roots, the system was considered to be radially symmetric, and diffusion fluxes of oxygen were determined from Fick's first law of diffusion applied to a cylinder:

$$J(r) = -2\pi r \times \phi \times D_s \times \frac{\delta C}{\delta r} \quad (1)$$

Where $J(r)$ is the diffusive flux at a distance r from the center of the root axis, Φ and D_s are the porosity and the diffusion coefficient, respectively (i.e., the diffusive characteristics of the medium [see below]), and $C(r)$ is the concentration of oxygen at a distance r from the root axis. Equation 1 determines the oxygen flux per unit of length of the root cylinder. To obtain the flux per unit of surface area, this number was divided by the circumference of the cylinder. The specific activity of oxygen consumption by the root cylinder could be calculated by dividing the diffusive flux at the surface of the root by the cross-sectional area of the cylinder. The diameter of the root sections studied was ~0.40 mm.

The specific activity in the zones of medium adjacent to the root was calculated from Fick's second law of diffusion applied to a cylinder:

$$\frac{\delta C}{\delta t} = \frac{1}{r} \times \frac{\delta}{\delta r}\left(r \times D_s \times \frac{\delta C}{\delta r}\right) - S \quad (2)$$

where t is the time and S is the specific activity. In a system where D_s is constant and steady state conditions ($\delta C/\delta t = 0$) are assumed (see Discussion), rearrangement and integration of equation 2 lead to the following equation for an oxygen concentration profile, which starts at the oxygen-consuming root cylinder and extends into the oxygen-consuming concentric zones of medium with microbial activity:

$$C(r) = \left[\int \frac{1}{r}\left(\int \frac{S}{D_s} \times r\, \delta r\right) \delta r\right] + (A_1 \times \ln r) + A_2 \quad (3)$$

where A_1 and A_2 are constants resulting from the integration procedure.

In practice, however, the calculations of the specific activities in the medium surrounding the root were done by fitting curves to those of measured oxygen profiles and using one of the following three sets of conditions for the radial-symmetric system.

(i) If the root is responsible for all oxygen consumption in the system, i.e., $S = 0$, equation 4 describes the oxygen profile:

$$C(r) = (A_1 \times \ln r) + A_2 \quad (4)$$

Equation 4 represents the sterile system with no microbial oxygen consumption in the medium. From this equation describing the fitted curve, a first derivative ($\delta C/\delta r$) could be obtained. This value was in turn used for the determination of oxygen flux toward the root cylinder by equation 1.

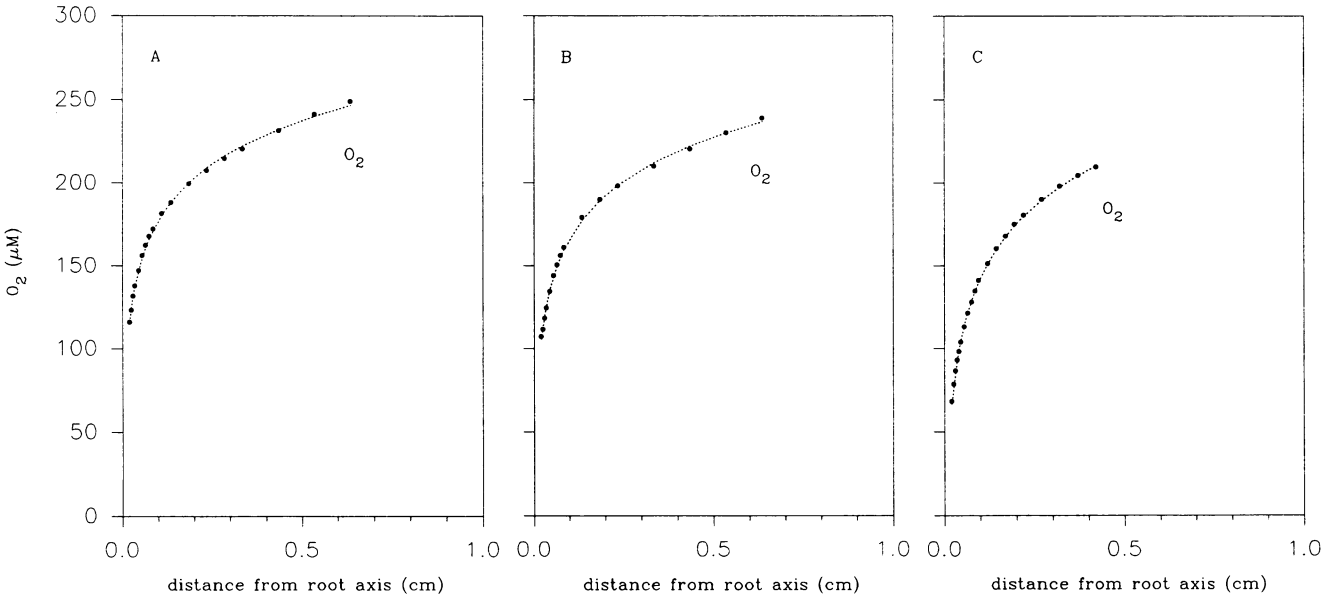

FIG. 2. Oxygen profiles from three sterile barley rhizosphere systems. Profiles were determined with microelectrodes (●), and curve fitting (– – –) with equation 4 was performed (see the text): (A) $C(r) = (37.6 \times \ln r) + 263.7$; (B) $C(r) = (38.2 \times \ln r) + 254.2$; (C) $C(r) = (47.2 \times \ln r) + 251.3$.

(ii) If the medium surrounding the root cylinder is divided into discrete concentric zones, each with constant specific activity (S_0), equation 5 describes the oxygen profile:

$$C(r) = \left(\frac{S_0}{4D_s} \times r^2\right) + A_1 \times \ln r) + A_2 \quad (5)$$

Here, the specific activity (S_0) in a particular zone can be calculated from the coefficient to r^2. With this approach the oxygen profile is subdivided into overlapping sections, each of which was subjected to curve fitting. Two adjacent zones, I and II [with curve fits $C_I(r)$ and $C_{II}(r)$], were separated at the point where $C_I(r) = C_{II}(r)$ and $\delta C_I/\delta r = \delta C_{II}/\delta r$. This implied that the oxygen flux at this point was the same whether determined by $C_I(r)$ or $C_{II}(r)$.

(iii) If the medium surrounding the root is considered as a continuous zone in which the specific activity is inversely proportional to the distance from the root axis (i.e., $S = S_0/r$), equation 6 describes the oxygen profile:

$$C(r) = \left(\frac{S_0}{D_s} \times r\right) + (A_1 \times \ln r) + A_2 \quad (6)$$

Here, the specific activity (S) at a distance r can be calculated by multiplying the coefficient to r with the diffusion coefficient and dividing by r. Thus, when this calculation method was used, it was possible to obtain a continuous distribution of the microbial oxygen respiration rates in the medium, as opposed to the discontinuous distribution obtained from equation 5.

Diffusive characteristics. Porosity (Φ) can be defined as the pore volume relative to the total volume (pores plus particles). Since the soil suspension was very dilute (~0.25% particles), we considered the porosity of the medium to be 1. The diffusion coefficient (D_s) of the gel-stabilized growth medium was determined by a method described in detail by Revsbech (29). This method determines the porosity multiplied by the diffusion coefficient, i.e., $\Phi \times D_s$, of the medium relative to the diffusion coefficient in water or 0.2 to 2% agar (the latter have diffusion characteristics indistinguishable from that found in water [29]). By this method we determined the ratio $(\Phi \times D_s)_{medium}/D_{s\ water}$ to be 0.80 ± 0.03 (standard error, $n = 4$). Using a D_s value for oxygen in water of 2.06×10^{-5} cm^2 s^{-1} at 20°C (2), we thus obtained a $(\Phi \times D_s)_{medium}$ value of 1.65×10^{-5} cm^2 s^{-1}. Having defined Φ to be 1, the value represents D_s and was used in the calculations.

RESULTS

Sterile systems. Oxygen concentration profiles were determined in the rhizosphere toward the roots of 7-day-old barley seedlings at a point ~1 cm above the root tip. Results from three different sterile systems (without microbial activity) are shown in Fig. 2. In accordance with equation 4, logarithmic curves could be fitted to these profiles, thus indicating that the oxygen transport toward the roots was purely diffusive. The fluxes of oxygen toward the roots of the three plants (A, B, and C) were 31, 32, and 39 pmol cm^{-2} of root surface s^{-1}, respectively. The specific activities were subsequently calculated to be 3.1, 3.2, and 3.9 nmol cm^{-3} of root tissue s^{-1}, respectively. The excellent fit with logarithmic curves (equation 4) also showed that there was no oxygen consumption by microbial activity outside the root cylinder.

To further verify the mathematical modelling and the curve-fitting approach, curves described by equation 6 were fitted to the three oxygen profiles in Fig. 2. Small differences of the measured profiles and the fitted logarithmic curves were demonstrated (data not shown); however, the range of specific activities calculated in the innermost zone closest to the root surface was less than 2.8 pmol cm^{-3} s^{-1} and thus negligible compared with that of the nonsterile systems (see below). Sterility of the bulk medium was further confirmed by the absence of bacterial colony formation when samples of the bulk medium were spread on nutrient agar plates.

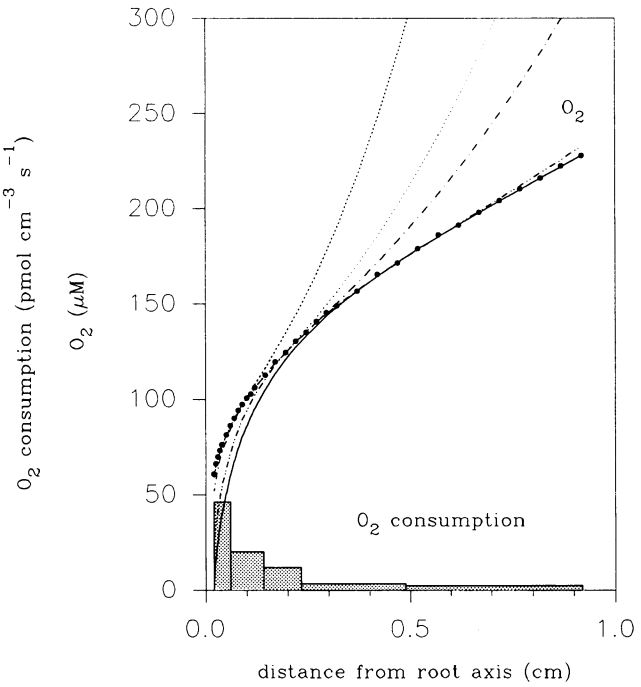

FIG. 3. Oxygen profile from a nonsterile barley rhizosphere system determined with a microelectrode (●). Curve fitting in overlapping sections of the profile with the discontinuous approach outlined in equation 5 was performed (see the text): (– – –) $C_I(r)$ = (709.2 × r^2) + (20.9 × ln$r$) + 142.4; (. . .) $C_{II}(r)$ = (308.8 × r^2) + (23.7 × ln$r$) + 151.9; (— · — ·) $C_{III}(r)$ = (183.4 × r^2) + (28.7 × ln$r$) + 164.6; (— ·· — ··) $C_{IV}(r)$ = (51.7 × r^2) + (42.9 × ln$r$) + 192.8; (——) $C_V(r)$ = (35.5 × r^2) + (50.6 × ln$r$) + 202.4. Each of the equations gave an excellent fit for a specific section of the profile. Two adjacent sections, e.g., I and II, were separated at the point where $C_I(r) = C_{II}(r)$ and $\delta C_I/\delta r = \delta C_{II}/\delta r$. Oxygen consumption rates (▥) were determined from the curve fits (see the text).

Finally, the oxygen profiles in the bulk medium (>30 mm from the roots) showed a constant concentration corresponding to air saturation (data not shown), again indicating an absence of oxygen consumption.

All results from the sterile systems confirm that the rhizosphere model system was indeed radially symmetric around the root and that diffusion was the only transport process for oxygen (see Discussion). Both observations are important for interpretation of the subsequent experiments with nonsterile systems.

Nonsterile systems. Oxygen profiles were measured toward roots of 7-day-old seedlings in three nonsterile systems. Again, the measurements were done ~1 cm above the root tips (Fig. 3 and 4).

In two of the nonsterile systems (B and C), the microbial oxygen consumption in the bulk medium was calculated from profiles measured more than 30 mm away from the roots (data not shown). The profiles could in each case be fitted with a parabolic curve, as shown for planar systems (25), indicating constant specific activities of 1.4 pmol cm^{-3} s^{-1} in experiment B and 3.8 pmol cm^{-3} s^{-1} in experiment C in the bulk medium outside the rhizosphere. The bulk specific activity was not determined in experiment A; however, assuming that the constant specific activity is reached ~0.9 cm away from the root axis, as judged from such distances in experiment B and C, the value in experiment A would be 3.2 pmol cm^{-3} s^{-1}.

The concentration profiles measured toward the roots in the nonsterile systems could not be modelled by the simple equation 4 (data not shown) as in the sterile systems, since oxygen was now consumed by the microorganisms in the medium as well as by the roots. To map the distribution of microbial oxygen consumption closer to the roots, we tried the discontinuous approach of modelling as determined by equation 5, assuming discrete, concentric activity zones. Curve fitting was performed in overlapping sections of the profile, and adjacent sections were separated as outlined

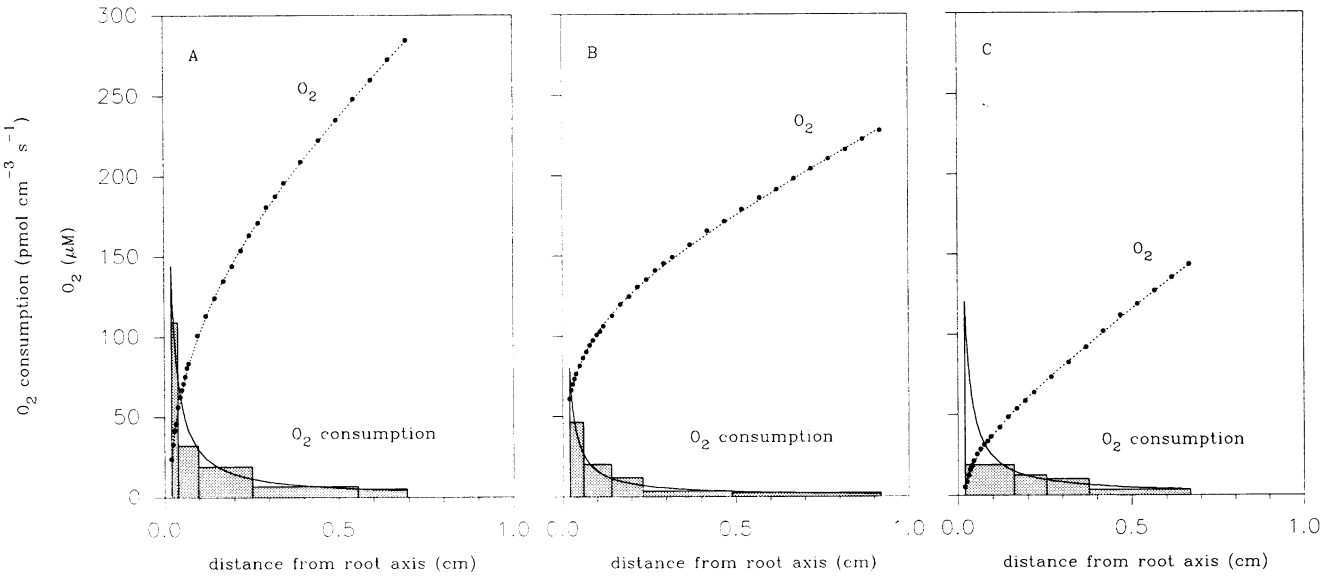

FIG. 4. Oxygen profiles from three nonsterile barley rhizosphere systems, including system B from Fig. 3, showing different oxygen consumption rates. Profiles were determined by microelectrodes (●), and curve fitting (– – –) with the continuous modelling approach outlined in equation 6 was performed (see the text): (A) $C(r)$ = (175.1 × r) + (40.0 × ln$r$) + 177.0; (B) $C(r)$ = (97.5 × r) + (21.4 × ln$r$) + 141.2; (C) $C(r)$ = (145.7 × r) + (12.0 × ln$r$) + 50.5. Oxygen consumption rates (specific activities) were estimated from equation 6 for the continuous modelling approach (——) and equation 5 (see the text and Fig. 3) for the discontinuous modelling approach (▥).

earlier. Figure 3 shows the curve fits and estimated specific activities in discrete zones around the root determined by this approach. The specific activity decreases from 46.3 pmol cm^{-3} s^{-1} in a 0- to 0.4-mm-thick zone at the root surface to 2.3 pmol cm^{-3} s^{-1} at a distance of ~5 mm from the root surface. The latter value thus approaches the background level of 1.4 pmol cm^{-3} s^{-1} measured in the bulk medium.

Figure 4 shows a comparison of specific activities determined by equation 5 in three different root systems, including system B from Fig. 3. A finer sectioning, resulting in better resolution, of the innermost zone is observed in system A, whereas the best possible sectioning in C is coarser than that in B. This illustrates a major limitation of the discontinuous approach; some profiles cannot easily be sectioned into narrow, discrete zones, and the result is a relatively coarse profile of the specific activity. Furthermore, single off measurement in the oxygen profile may strongly influence the curve fitting.

Therefore, a curve fit of the oxygen profiles in the three systems with the continuous approach of modelling (equation 6) is included in Fig. 4. This gave a smoother picture of the estimated specific activities but was in good accordance with the activities determined by the discontinuous approach with equation 5 (Fig. 4). Thus, in systems A and B the overall activities in the rhizosphere (total areas under the curves) differ by only 5 to 6% with the two calculation models. In system C, however, the difference is 27%, presumably because the curve fitting in the innermost zone was poor by the discontinuous approach. With the continuous model, the maximum specific activity in the innermost zone closest to the root surface (rhizoplane) ranged from 80.4 to 144.5 pmol cm^{-3} s^{-1} in the three experiments. These rates were 30 to 60 times higher than the activities in the bulk medium. Finally, the specific activity within the root cylinder could be determined from the oxygen flux at the root surface. The rates were 3.59, 1.93, and 1.23 nmol cm^{-3} s^{-1} in systems A, B, and C, respectively, and thus 10 to 25 times higher than those in the rhizoplane.

DISCUSSION

Root respiration has been studied in several plant species by following the oxygen consumption in closed water systems containing complete root systems (37). It was shown that oxygen consumption rates were of the same order of magnitude (~1 μg cm^{-2} h^{-1} or ~9 pmol cm^{-2} s^{-1}) for many different species (unfortunately, barley was not among the investigated species). However, oxygen uptake by the roots declined with the age of the root system. By comparison, our approach of using a microsensor technique and calculating the oxygen consumption rates from diffusion-reaction models (equation 3 and derivatives thereof) gave slightly higher rates (~3.5 μg cm^{-2} h^{-1} or ~30 pmol cm^{-2} s^{-1}) in the barley roots, independent of whether the oxygen uptake rates were calculated from the profiles in sterile or nonsterile medium. The deviation from literature values may reflect the fact that we only examined the youngest and most active parts of the roots.

Since our estimated rates of oxygen consumption by young and active root tissue (30 pmol cm^{-2} s^{-1}) were, not surprisingly, slightly higher than the rates for whole root systems (9 pmol cm^{-2} s^{-1}), the microsensor approach seemed to be quantitative. This was obviously promising for determination of the microbial oxygen consumption in the rhizoplane, the rhizosphere, and bulk medium. For this purpose, the diffusion-reaction models described by equation 5 (discontinuous model) and equation 6 (continuous model) were therefore developed. Both the continuous and the discontinuous approaches of modelling seemed valuable, although each had obvious advantages and disadvantages. (i) Problems associated with subsectioning the measured profiles in the discontinuous model are avoided with the continuous approach. In the continuous model, however, the specific activity is considered inversely proportional to the distance from the root axis; this excludes detection of spatial heterogeneity, which may be detected by the discontinuous model. (ii) The specific activity at the root surface (rhizoplane) can be estimated more precisely with the continuous approach than with the discontinuous approach. (iii) The models required that the diffusion coefficient D_s was constant throughout the medium, that the systems were in steady state (i.e., $\delta C/\delta t = 0$) and that the oxygen transport in sterile and nonsterile systems could be adequately described by molecular diffusion. The assumption of a constant D_s value seems reasonable, since the medium was a homogenized soil suspension stabilized with a polymer. Steady state in the 1-week-old systems was confirmed by several measurements at the same point (data not shown), showing that the concentration profile did not change over a time period of more than 1 h (usually the time needed for determining a profile was 10 to 15 min). The assumption of pure molecular diffusion was supported by an estimate of the potential error introduced by oxygen transport from mass flow of water. Darrah (6) and Newman and Watson (24) determined a water velocity of 0.009 to 0.017 cm day^{-1} at the root surface of actively growing plants. With this range of water velocity and an oxygen concentration of 290 nmol cm^{-3} (μM) in air-saturated water at 20°C, the mass flow rate of oxygen toward a root should be 2.5 to 5.0 nmol cm^{-2} day^{-1} or 0.03 to 0.06 pmol cm^{-2} s^{-1}. This is 3 orders of magnitude lower than the transport rate by molecular diffusion. We therefore concluded that the oxygen transport in our experiments was adequately described by molecular diffusion only.

A major achievement in the present work was the detailed recording of both the rate and the spatial distribution of microbial oxygen consumption in the rhizosphere of barley seedlings. The oxygen consumption by microorganisms in the narrow 0- to 3-mm inner zone corresponding to the rhizosphere was much higher than that in the surrounding bulk medium. The rhizosphere activity could also be clearly distinguished from that of the root tissue. As mentioned previously, as much as 40% of the organic C synthesized by barley plants may be lost immediately by root exudation (rhizodeposition) (14, 21, 36). Our observations of high microbial activity in the rhizosphere may be even more profound in natural soil systems; according to Trofymow et al. (35), rhizodeposition can thus be 2 to 4 times higher in natural soil than in laboratory systems with roots in liquid medium.

In these experiments we studied root sections with a well-defined cylindrical geometry, i.e., root sections without root hairs or side roots. This allowed us to better distinguish between the respiratory activity of the root and that of the microorganisms. However, it was impossible to distinguish between the respiratory activity of the plant cells (root tissue) and that of a possible microbial population in the root (endorhizosphere). The latter could therefore explain the differences observed for root respiration in sterile and nonsterile systems; the rates were thus slightly higher and more variable in individual roots in the nonsterile systems. We are presently trying to map the oxygen consumption in root

segments with root hairs and sites of high exudation like the root tip (35) and branching points of the root. Although the conical shape of the root tip (outermost 2 to 3 mm at the root apex) has so far made it impossible to quantify oxygen consumption rates, the measured profiles have indicated that the activity in this region is higher than that further back on the root (data not shown). This could be due to high oxygen consumption in the root meristem as well as a high microbial activity in the surrounding medium caused by intensive exudation in the apex region (35). High microbial respiration at the root tip could further be enhanced by degradation of the mucus, which is continously sloughed off as the root penetrates new medium.

The stagnant gel system puts a diffusional constraint on the supply of oxygen to the root and the microorganisms in the rhizosphere. However, the microbial oxygen consumption should not be affected by exhaustion of oxygen until the concentration level approaches the K_m value, which is reported to be as low as 1 to 10 μM (31). Our study nevertheless demonstrates that the rhizosphere can easily develop a very low oxygen partial pressure (Fig. 3C), eventually leading to complete anoxia. This makes the gel-stabilized model system of the rhizosphere very interesting in relation to studies of nitrogen transformations, e.g., microbial nitrification and denitrification.

One of our future aims is to apply the microsensor technique to nonsterile rhizosphere systems and study the interactions between root activity and bacterial nitrification and denitrification in oxygen microgradients. Although ion-selective microelectrodes have been used to study fluxes of ammonium and nitrate at the surfaces of barley roots in water culture (12), the microsensors may allow a number of processes that are controlled by oxygen and nitrate availability to be studied under near-in situ conditions. (i) Coupling between nitrification and denitrification at oxic-anoxic interphases could be studied.

(ii) Competition between denitrifying bacteria and plant roots for available nitrate could also be studied. This competition is also affected by oxygen concentrations, however, because root uptake of nitrate is increasingly inhibited at lower oxygen concentrations (3) and denitrification is typically inhibited at higher oxygen concentrations (3, 34).

(iii) Microsensors can also be used to study differential inhibition of nitrifying bacteria by lower oxygen concentrations, since ammonium oxidizers may be more tolerant than nitrite oxidizers to lower oxygen concentrations (8, 11). This may possibly explain why nitrite accumulates in a low-oxygen rhizosphere (1). At low oxygen levels, nitrous oxide may also accumulate from release by the ammonium oxidizers (13, 16, 27, 33).

(iv) The system could be used to study differential inhibition of reaction steps in denitrification, since nitrous oxide reductase is the most oxygen sensitive of the denitrification enzymes (17, 34). Thus, in the presence of small amounts of oxygen, nitrous oxide rather than dinitrogen may be the predominant product of denitrification.

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