Anoxic conditions in a Douglas fir litter layer
van der Lee, G.E.M.

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Abstract

The occurrence of anaerobic processes in well-drained forest soils is well-known. Moreover, the presence of anoxic microsites in litter layers has been demonstrated (chapter 2). The contribution of anaerobic and aerobic processes to total carbon decomposition is difficult to determine, since anaerobic microbial products may be reprocessed in oxic zones surrounding anoxic sites. An important controlling factor for aerobic and anaerobic microbial processes in litter layers are the low oxygen concentrations in litter particles. Knowledge of the process conditions, such as diffusion coefficient and oxygen consumption rates of litter particles, would be a first step towards understanding the dynamics of aerobic and anaerobic decomposition. Therefore, the diffusion coefficient and oxygen consumption dynamics in litter particles were quantified by comparing measured and simulated oxygen concentrations. An experiment was performed in which oxygen concentrations in litter particles were measured after a sudden change in oxygen concentration in the atmosphere around the particles. The experiment was designed to obtain information on oxygen consumption dynamics, as the diffusion coefficient is expected to be constant, but oxygen consumption rates are not. Oxygen consumption dynamics were also simulated with a 2D diffusion-reaction model, using different combinations of diffusion coefficients and oxygen consumptions. Comparison of measured and simulated concentration changes revealed that oxygen consumption in litter can be considered as a first order reaction. The diffusion coefficient of litter particles was estimated with the bulk oxygen consumption rate and the relationship between particle size and minimum oxygen concentration for the litter particles (chapter 2). The estimated diffusion coefficient was $2.5 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$, which is much lower than values reported for mineral aggregates. The low value is attributed to the polymeric nature of organic matter structure.
3.1 Introduction

Anaerobic processes in soils are important as they contribute to the production of greenhouse gases such as N\textsubscript{2}O and CH\textsubscript{4}. In forest soils, the occurrence of anaerobic processes has often been demonstrated (Goodroad and Keeney, 1984b; Groffman and Tiedje, 1989a,b; Groffman and Tiedje, 1989b; Heinrich and Haselwandter, 1997; Rashid and Schaeffer, 1987; Yavitt et al., 1995). Furthermore, the litter layer harbours a large number of anaerobic bacteria (10\textsuperscript{7} to 10\textsuperscript{8} g\textsuperscript{-1 dry matter}) (Küsel et al., 1999). Anaerobic fungi were also reported to occur in forest soil (Wainwright et al., 1994). Recently, anoxic microsites were observed in coniferous litter (chapter 2).

Although local anoxic conditions and the presence of anaerobic organisms indicate the occurrence of anaerobic processes, it is not known to what extent anaerobic litter decomposition contributes to total carbon decomposition. Anaerobic decomposition not only depends on the anoxic organic matter volume but also on the local microbial activity in these anoxic zones. Furthermore, it is not possible to use anaerobic microbial products, such as CH\textsubscript{4} or acetate to quantify the activity of anaerobic processes with, as these microbial products may be converted before they reach the (soil) atmosphere, respectively the (soil) solution (Küsel and Drake, 1999; Yavitt et al., 1995). Conversion of anaerobic products may indeed occur in litter, in which oxic zones surround anoxic centers (chapter 2).

Knowledge of the spatial distribution of microbial activity within litter is mainly qualitative and has been obtained from microscopical studies on thin sections (Ponge, 1991a,b; Dijkstra, 1996). Ponge (1991a) observed fungal colonization of the mesophyll tissues of Scots pine litter in the first decomposition stage and activity of bacteria and micro/mesofauna in later decomposition stages. Needle interiors were preferentially ingested, as the outer parts of needles (epidermis) contain more resistant lignified tissues.

More quantitative information on the conditions leading to steep oxygen concentration gradients would give insight in some important issues in soil microbial ecology. Process conditions determining litter oxygen concentration gradients are the diffusion coefficient and oxygen consumption rate of litter. The aim of the present study is to assess the diffusion coefficient in litter and the kinetics of oxygen consumption. This will be done by comparing oxygen concentrations measured with micro-electrodes and concentrations simulated with a 2D oxygen diffusion-consumption model based on thin sections. Part of the measurements have also been presented in chapter 2: (1) the stationary oxygen concentration profiles measured in a litter column and (2) the negative correlation between particle size and the minimum oxygen concentration in a particle, derived from these profiles.
3.2 Materials and methods

Stationary oxygen profiles in litter are determined by the diffusion coefficient (D) and oxygen consumption rate (Q). If both parameters are unknown, it is not possible to calculate values of D and Q from measured profiles only, since D and Q can have the same effect on oxygen concentrations. To provide additional information we performed an experiment in which we applied a stepwise increase in oxygen concentration in a litter sample, during which we measured concentration changes within litter fragments (Figure 3.1).

Figure 3.1: Schematic presentation of oxygen concentration increases in pore (dashed line) and organic particles (solid line) during a stepwise increase in headspace oxygen concentration. 1 = atmospheric oxygen concentration.

The experimental procedure was as follows: a microelectrode was lowered in a litter sample and was set still at a random position in an organic matter fragment. After measurement of the equilibrium oxygen concentration, a stepwise increase in oxygen concentration was created in the sample by blowing oxygen gas through the sample headspace, resulting in a concentration of 50-70% oxygen in the sample headspace. The microelectrode measured changes in the local oxygen concentration at the electrode position inside the organic particle (Figure 3.1). Oxygen concentrations in the headspace were measured by Gas Chromatography. With these measurements a relative concentration change in oxygen concentration was calculated, defined as the ratio between the concentration change at a depth x (Δconc particle) and the concentration change at the surface (Δconc pore) (Figure 3.1). This procedure was repeated for different electrode positions.
The relative concentration change at a certain depth can provide information on oxygen consumption rate and diffusion coefficient (Goldman and Minkin, 1993), as \( D \) is constant, while \( Q \) may vary with the ambient oxygen concentration. In addition, we measured the bulk oxygen consumption rate of the litter samples by gas chromatography. (Table 3.1).

**Table 3.1:** Water content and bulk oxygen consumption rate of the litter and number of samples.

<table>
<thead>
<tr>
<th>Water content (g g(^{-1}))</th>
<th>Bulk oxygen consumption ((\mu)mol kg(^{-1}) s(^{-1}))</th>
<th>Bulk oxygen consumption (mol m(^{-3}) s(^{-1}))(^a)</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>0.11</td>
<td>(1 \times 10^{-5})</td>
<td>11</td>
</tr>
<tr>
<td>3.2</td>
<td>0.20</td>
<td>(2 \times 10^{-5})</td>
<td>5</td>
</tr>
<tr>
<td>3.9</td>
<td>0.20</td>
<td>(2 \times 10^{-5})</td>
<td>16</td>
</tr>
</tbody>
</table>

\(^a\)Calculated as: bulk oxygen consumption (\(\mu\)mol kg\(^{-1}\) s\(^{-1}\)) \(\times 10^{-6}\) \(\times\) bulk density (100 kg m\(^{-3}\)).

**Litter samples**

Douglas fir litter (F2 layer) was sampled in July 1997 in a 36 year old forest without understorey at Speuld, the Netherlands (52°13’N, 5°39’E). The forest is located on a well-drained sandy soil with a water table at a depth of 40 m throughout the year. The spatial and temporal water content dynamics of this forest are well studied (Schaap et al., 1998). We sampled from the 1.5-5 cm deep F2 layer beneath a L + F (0-1.5cm). We removed twigs, the larger roots and cones. The remaining litter largely consisted of highly fragmented Douglas fir needles. The F2 layer was selected for the relative softness of its organic matter tissues (advanced decomposition stage), which facilitated electrode insertion.

**Microelectrodes**

We used custom made oxygen electrodes with a guard cathode based on the electrodes described by Revsbech and Ward (1983) and Revsbech (1989) to measure oxygen profiles. The electrodes have an outer diameter at the tip of 7.5 \(\mu\)m and an inner diameter of 5 \(\mu\)m. They have a thick membrane (40 \(\mu\)m) to minimize oxygen use as well as for high pressure resistance. Their tips were not melted round to improve insertion into organic matter. A 0.75 V potential was applied over the electrodes. The electrodes did not show sensitivity to stirring. The 90% response time was about 8 s. Two-point calibrations were performed in \(N_2\) bubbled water (0% \(O_2\) saturation) and air bubbled water (100% \(O_2\) saturation) several times before and after the measurements.
Thin sections

Thin sections were produced from the litter columns that had been used for the micro-electrode measurements: litter columns were air dried and impregnated with unsaturated polyester resin. Thin sections were produced according to the technique described by (Jongerius and Heintzberger, 1975).

2D oxygen diffusion-consumption model

A 2D finite difference oxygen diffusion-consumption model was developed to simulate the experimental results, which were 1. the stationary oxygen concentration profiles (chapter 2), 2. the relationship between particle size and minimum oxygen concentration (chapter 2) and 3. the relative concentration change during a stepwise increase of oxygen concentration (present experiment).

Table 3.2: Diffusion coefficients and oxygen consumption rates applied in the 2D diffusion-reaction model. D and Q were either constant, decreasing or increasing with distance from the nearest pore.

<table>
<thead>
<tr>
<th>Bulk diffusion coefficient \ (m$^2$ s$^{-1}$)$^a$</th>
<th>Constant</th>
<th>Increasing</th>
<th>Decreasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen consumption rate \ (m$^3$ m$^{-3}$ s$^{-1}$)$^b$</td>
<td>Zero order</td>
<td>First order</td>
<td>Decreasing</td>
</tr>
<tr>
<td>\ (mol m$^{-3}$ s$^{-1}$)$^c$</td>
<td>1$\times$10$^{-4}$</td>
<td>4$\times$10$^{-4}$</td>
<td>1$\times$10$^{-3}$</td>
</tr>
</tbody>
</table>

$^a$ weighted average of oxygen diffusivities in air and water; $^b$ m$^3$ refers to particle volume, $^c$ see eq. 3.1.

Our 2D spatial distribution of diffusion coefficient and oxygen consumption rates was based on thin sections of litter. A thin section of a litter column was digitized and converted to a raster GIS map with cells of 21.2 by 21.2 μm (PCRaster, 1998). This cell size is sufficient to model oxygen concentration gradients with as measured oxygen concentrations changed from 100% to 0% saturation over at least 100 μm. The scanned images were classified to maps with a distinction between organic matter and pores, using a threshold grey value. The threshold value was determined as the highest grey value (256 corresponds to white) in cells which clearly corresponded to organic matter tissues in the thin section, as observed by eye. The uncertainty in this method is not quantified but is expected to be minor due to the excellent colour contrast between organic matter and pore both in the thin section and in the scanned GIS maps.

Estimates of diffusion coefficients (D) and oxygen consumption rates (Q) were assigned to all organic matter cells. Oxygen consumption was applied as either zero-order or a first-order reaction. The diffusion coefficient was either constant,
decreasing or increasing D with distance from the nearest pore. For each model run, a different set of values for D and Q was used (Table 3.2).

First order oxygen consumption was calculated by eq. 3.1.

\[
Q = \frac{[O_2]}{[O_2]_{ref}} \times Q_{ref} \quad \text{[eq. 3.1]}
\]

with:

- \(Q\) = oxygen consumption rate (mol m\(^{-3}\) s\(^{-1}\))
- \([O_2]\) = oxygen concentration (mol m\(^{-3}\))
- \(Q_{ref}\) = reference oxygen consumption rate (mol m\(^{-3}\) s\(^{-1}\))

The oxygen diffusion-consumption model was applied for the thin section grid by modeling oxygen diffusion transport between each individual cell and its four neighbouring cells. Diffusion was modeled with Fick’s 2\(^{nd}\) law (Crank, 1975):

\[
S \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} + D \frac{\partial^2 C}{\partial y^2} - Q \quad \text{[eq. 3.2]}
\]

with:

- \(S\) = organic matter porosity (intraparticle pores: 0.8 m\(^{3}\) m\(^{-3}\))
- \(C\) = concentration (mol m\(^{-3}\))
- \(D\) = diffusion coefficient (m\(^{2}\) s\(^{-1}\))
- \(Q\) = oxygen consumption rate (mol m\(^{-3}\) s\(^{-1}\))
- \(x, y\) = distance (m)
- \(t\) = time (s)

For the calculations we used an explicit finite difference approximation as adapted from Wang and Anderson (1982).

\[
C_{ij}^{n+1} = C_{ij}^n + \left[ \left( \frac{C_{i+1,j}^n + C_{i-1,j}^n - 2C_{ij}^n}{\Delta x^2} \right) + \left( \frac{C_{i,j+1}^n + C_{i,j-1}^n - 2C_{ij}^n}{\Delta y^2} \right) \right] \times \left( \frac{D \Delta t}{S} \right) - \frac{Q_{ij}^n \Delta t}{S} \quad \text{[eq. 3.3]}
\]

with:

- \(C_{ij}^n\) = concentration in cell \(i,j\) (mol m\(^{-3}\)) at timestep \(n\)
- \(C_{i+1,j}^n, C_{i-1,j}^n, C_{i,j+1}^n, C_{i,j-1}^n\) = concentration in upper, lower, left or right neighbouring cell
- \(D\) = diffusion coefficient (m\(^{2}\) s\(^{-1}\))
- \(S\) = local organic matter porosity (0.8 m\(^{3}\) m\(^{-3}\))
- \(\Delta x=\Delta y=cell\ length\ (21.2*10^{-6} \text{ m following from the scanning resolution of 1200 dpi})\)
- \(Q_{ij}^n\) = oxygen uptake in cell \(i,j\) (mol m\(^{-3}\) s\(^{-1}\), at timestep \(n\)
- \(\Delta t\) = timestep (s)
Simulation of experimental results

For the simulation of oxygen profiles in a litter column under normal conditions, an atmospheric oxygen concentration was applied in interparticle pores. With this boundary condition, the model was run until the oxygen concentration in organic matter was in equilibrium. From the resulting map with equilibrium concentration, vertical profiles of oxygen concentration were sampled at random positions. These profiles were compared with measured profiles. For the simulation of a stepwise increase in oxygen concentration, the model was first run for atmospheric oxygen concentration in pores and subsequently for an oxygen concentration of 80% oxygen. Relative concentration changes in oxygen concentration were calculated for cells in organic matter similar to calculations in Figure 3.1.

Particle sizes were calculated from the simulated oxygen vertical profiles by summing adjacent numbers of grid cells in a profile with a concentration lower than 100%. These particle sizes were related to the minimum simulated oxygen concentration in those particles. This relationship had been calculated in a similar way for the measured oxygen concentration profiles (chapter 2).

In the model simulations we did not take account of the differences in water content as had been established in the experiments (Table 3.1). Differences in water content are not relevant for the objects of this study as the value of D within the organic matter does not depend on the water content within organic matter (see chapter 2).

Estimation of diffusion coefficient and oxygen consumption rate

The diffusion coefficient of litter particles is estimated with the bulk oxygen consumption rate and the relationship between particle size and minimum oxygen concentration for the litter particles (chapter 2). Calculations are performed according to the following procedure: first, from the comparison of measured relative concentration changes and those simulated at different scenario’s of D and Q, the most realistic scenario is derived (Table 3.2). Then oxygen consumption rates are applied that agree with measured bulk oxygen consumption rates. Using this Q, a value of D is finally estimated by optimising D based on the best fit between the simulated and measured minimum oxygen concentrations.
3.3 Results

*Simulated oxygen profiles*

With the oxygen diffusion-consumption model we simulated maps with equilibrium oxygen concentrations in the organic matter cells for all scenario's of diffusion coefficient and oxygen consumption rate (Table 3.2). An example of such an equilibrium map is presented in Figure 3.2. From maps with calculated equilibrium oxygen concentrations, vertical sections in oxygen concentration were plotted versus depth (Figure 3.3a). The general pattern of simulated profiles resembled that of a measured oxygen profile (Figure 3.3b). However, with different scenario's of D and Q (Table 3.2), we could produce strongly resembling concentration patterns. Therefore, no information on the validity of the diffusion coefficient and oxygen consumption rate could be derived from simulated profiles only.

![Oxygen Concentration Map](image)

**Oxygen concentration**

(% saturation)

<table>
<thead>
<tr>
<th>Value</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.56</td>
<td></td>
</tr>
<tr>
<td>82.79</td>
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<td>71.83</td>
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<td>23.99</td>
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<tr>
<td>12.22</td>
<td></td>
</tr>
<tr>
<td>8.4612</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.2: Map with equilibrium oxygen concentrations for a scenario of and a constant oxygen diffusion coefficient ($0.4*10^{-11}$ m$^2$ s$^{-1}$) and first order oxygen consumption kinetics (with constant $Q_{ref}$ of $1.2*10^{-3}$ mol m$^{-3}$ s$^{-1}$). Real size of image: 1.2*1.7 cm.
Figure 3.3: Fig. 3.3a (top): Simulated oxygen profile with the 2D diffusion-reaction model. Vertical transect of the map with equilibrium oxygen concentrations of Figure 3.2, taken at a random position. Model scenario of a constant oxygen diffusion coefficient (\(0.4 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}\)) and first order oxygen consumption kinetics (with constant \(Q_{\text{ref}}\) of \(1.2 \times 10^{-3} \text{ mol m}^{-3} \text{ s}^{-1}\)). Fig. 3.3b (bottom): Example of a typical oxygen profile. Water content = 3.2 g g\(^{-1}\), bulk oxygen consumption rate = \(0.69 \mu\text{mol kg}^{-1} \text{s}^{-1} = 6.9 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}\) (sample was sprayed with 69 mM glucose solution).
Simulated relative concentration changes

Different scenarios of D and Q led to different results in calculated relative concentration changes during a stepwise increase in headspace oxygen concentration (Figure 3.4). This difference was especially found between scenarios of zero- and first-order oxygen consumption. With zero-order oxygen consumption, the relative concentration change showed a positive but highly scattered correlation with initial oxygen concentration in a particle (Figure 3.4). This scatter was always observed, no matter whether the diffusion coefficient or oxygen consumption rate was constant, increasing or decreasing with distance to the nearest pore. A scatter in concentration change means that different relative concentration changes occur at locations with the same initial oxygen concentration. Differences in relative concentration are caused by different increases in oxygen fluxes during the stepwise increase. Differences in oxygen fluxes appeared to be caused by differences in particle size and structure (results not shown).

![Graph showing relative concentration change vs. initial oxygen concentration](image)

**Figure 3.4:** Relation between relative concentration change and initial oxygen concentration simulated with the 2D oxygen diffusion-consumption model. Symbols (▲): zero-order oxygen consumption, constant oxygen diffusion coefficient \((0.4\times10^{-11}\ \text{m}^2\ \text{s}^{-1})\) and consumption rate \((4\times10^{-4}\ \text{mol}\ \text{m}^{-3}\ \text{s}^{-1})\); (□): first order oxygen consumption (constant \(Q_{\text{ref}} 1.2\times10^{-3}\ \text{mol}\ \text{m}^{-3}\ \text{s}^{-1}\)) and constant oxygen diffusion coefficient \((0.4\times10^{-11}\ \text{m}^2\ \text{s}^{-1})\); (■): first order oxygen consumption, increasing diffusion coefficient \((0.4\times10^{-12}\ \text{in outer layer of organic matter fabric, 0.4}\times10^{-11}\ \text{m}^2\ \text{s}^{-1}\ \text{within outer layer of organic matter fabric})\).

With first-order oxygen consumption, the relative concentration change showed a positive relation with initial oxygen concentration in a particle, without any scatter at all. At constant Q and D, the relationship was linear over the whole range of initial oxygen concentrations, while at increasing D and/or Q, the relationship showed a
curved shape in the lower range of initial oxygen concentrations (Figure 3.4). With first-order oxygen consumption, differences in increases in oxygen flux lead to congruent increases in consumption, and can so result in the same relative concentration change. In this way effects of particle size and structure are excluded.

The measured relative concentration change appeared to be linearly related to the initial oxygen concentration, with very little scatter (Figure 3.5). This linear relationship agrees with a scenario of first-order oxygen consumption. Since the measured relationship between relative concentration change and initial oxygen concentration was linear over the whole range of initial oxygen concentrations, it further agreed with a scenario of a constant D. A realistic oxygen diffusion-consumption scenario of constant D and first-order Q results in a strongly decreasing actual oxygen consumption rate with oxygen concentration and therefore with distance from the particle surfaces (Figure 3.6).

![Graph](image)

**Figure 3.5:** Measured relative concentration change in organic particles during a stepwise increase in oxygen concentration around the particles, in dependence of the initial oxygen concentrations before the stepwise increase. Samples at different water contents gave similar results, therefore samples at different water contents are indicated by the same symbol.

*Estimation of diffusion coefficient with minimum concentrations in particles*

From the linear relationship between relative concentration change and initial oxygen concentration, we derived that Q is first-order and D is constant. However, we cannot estimate values of D, since different combinations of D and Q all result in the same
straight line. Therefore, we used the measurements of the bulk oxygen consumption rate and the relation between particle size and minimum oxygen concentration to calibrate values of D and Q (see Methods). Simulated and measured relations between particle size and minimum oxygen concentrations are presented in Figure 3.7. The D within litter is thus estimated at $2.5 \times 10^{-13}$ m s$^{-2}$ and $Q_{ref}$ at $8 \times 10^{-5}$ mol m$^{-3}$ s$^{-1}$. The combination of D and $Q_{ref}$ resulted in a bulk oxygen consumption rate of $1.1 \times 10^{-5}$ mol m$^{-3}$ s$^{-1}$, which is comparable to the measured rate in field-moist litter (Table 3.1).

![Figure 3.6: Vertical profile of actual (○) and reference (□) oxygen consumption rate with depth. Particles and pores are indicated by arrows.](image)

**3.4 Discussion**

The general trend between particle size and minimum oxygen concentration is reproduced with the model. Nevertheless, some differences between the simulated and measured relationships occur. First, the simulated relation shows less scatter than the measured one. The simulated relationship is based on a constant diffusion coefficient and first order oxygen consumption with a constant $Q_{ref}$. This means that estimated values of D and $Q_{ref}$ are independent of the distance to an air-filled pore. The scatter in the measured relationship probably evolves from variations in D and $Q_{ref}$ of litter particles that are also independent of distance to an air-filled pore. A certain heterogeneity in D and $Q_{ref}$ of litter particles is indeed expected as the litter in the thin section shows different sorts or litter particles: needle fragments with and without an epidermal layer, and excrements. These particles will individually differ in microbial activity and decomposition stage, resulting in corresponding differences in D and $Q_{ref}$. 

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Second, simulated minimum oxygen concentrations are more often zero at particles larger than 0.25 mm than measured ones. Measured minimum oxygen concentrations may be higher due to nearby inter- or intraparticle pores that were unobserved in the thin section. This can be an effect of the 2-dimensionality of the thin section. Furthermore, small intraparticle pores in connection with interparticle pore space may sometimes not appear as pores in the thin section due to the scanning resolution.

![Graph](image)

**Figure 3.7:** Measured and simulated minimum oxygen concentration in particles as a function of particle size (in vertical direction). Symbols (□) measured; (▲) simulated, best fit with measurements at $D = 2.5 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$ (constant), $Q_{\text{ref}} = 8 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$ (first order, constant). The estimated $Q_{\text{ref}}$ led to a bulk oxygen consumption rate of $1.1 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$.

The estimated value of $D$ should be seen as an order of magnitude, due to uncertainties in for instance the applied value of $Q$. The actual consumption rate within organic matter may be lower than the bulk consumption rate, as the measured bulk consumption also includes consumption at the surface of organic matter. Oxygen consumption at the surface of organic matter in the order of the measured rate does not result in lowered oxygen concentrations within organic matter (results not shown). This is due to the high diffusion coefficients in water and air compared to organic matter. An uncertainty in $Q$ leads to a proportionally equal uncertainty in $D$ as the estimated value of $D$ linearly depends on the prescribed value of $Q$. 

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**Low diffusion coefficient in litter**

The estimated diffusion coefficient of oxygen in litter of about $2.5 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$ is more than ten times lower than the lowest values in the literature observed for mineral aggregates ($1.4 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ (Sierra et al., 1995) to $3 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ (Zausig and Horn, 1992)). For mineral aggregates, much higher diffusion coefficients ranging $0.4 \times 10^{-9}$ m$^2$/s to $2 \times 10^{-8}$ m$^2$/s$^{-1}$ have also been reported (Greenwood and Goodman, 1967; Rappoldt, 1995; Sexstone et al., 1985; Zausig et al., 1990).

This low oxygen diffusion coefficient in litter may be understood when we consider that the structure of organic matter is comparable to that of a cross-linked polymer (Pignatello, 1989). Diffusion of particles in a polymer network deviates from diffusion in fluids, and consequently also in soil solution of mineral aggregates, because polymer molecules cannot flow around the diffusing particle (Stein, 1986). In a polymer, particles diffuse through its free volume, which is composed of many transient holes of different sizes. For a suitable diffusion path, each adjacent hole must be larger than the volume of the diffusing particle (Stein, 1986). This largely determines the diffusion coefficients of a substrate through polymers. The estimated value of $2.5 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$ falls in the range of those of most organic polymers ($1 \times 10^{-10}$ to $1 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$) (Yasuda and Stannett, 1975).

A low oxygen diffusion coefficient following from the polymeric structure of organic matter also has consequences for diffusion of substrates within litter particles in general. For instance, apart from diffusion limitation of oxygen, limitation by diffusion of other oxidators may occur. Therefore, denitrification within litter particles could be limited by nitrate diffusion. Diffusion limitation of oxidators from the soil solution may favour micro-organisms within litter that make use of local organic oxidators, which can occur in fermentation processes. Furthermore, diffusion limitation of substrates may enhance the coupling of microbial processes, where one microbial population can consume microbial products of adjacent other populations.

**Implications of first order oxygen consumption**

A first order oxygen consumption scenario as observed in the present study has also been found in several studies on mineral soil aggregates (Sierra and Renault, 1995, 1996). First order uptake of substrates in soil has been explained by diffusion limitation (Myrold and Tiedje, 1985b). However, Sierra and Renault (1995) observed first-order oxygen uptake in the absence of diffusion limitation but measured inhibition of oxygen uptake by CO$_2$. Consequently, the explanation of first-order oxygen consumption may be a complex phenomenon and combined responses of different microbial populations might be involved.

Estimated actual oxygen consumption rates strongly decreased with distance from the particle surfaces. (Figure 3.6). Microscopical observations on litter thin sections
clearly show a preferential decomposition of needle interiors by (1) soil flora and (2) soil fauna, with the outer epidermal layer remaining to the last (Ponge, 1991a). This has been attributed to a better substrate quality of the mesophyll tissues than of the epidermal layer.

ad (1) Low oxygen consumption rates in needle interiors suggest the occurrence of microbial decomposition processes in which no or little oxygen is consumed. Of course, in the anoxic sites, anaerobic decomposition will occur, but sites with zero oxygen concentrations form only a small part of the total organic matter volume, while zones with low oxygen concentrations on the other hand form a relative large part. At such low concentrations, carbon decomposition may occur by anaerobic decomposition processes which are active in the presence of oxygen. For instance, fermentation has been demonstrated to occur in the presence of oxygen and in some cases even to be stimulated by low oxygen concentrations (Gottschal, 1986). Gottschal (1986) further referred to a study in which fermentation products became more oxic as oxygen partial pressure increased, resulting in an increased yield of acetate. This agrees with observations of acetate being the most important fermentation product in litter (Küsel and Drake, 1999).

ad (2) The activity of micro- and mesofauna is expressed by a fragmentation of litter due to tunnelling or communiton of whole litter particles (Ponge, 1991b; Dijkstra, 1996). This fragmentation has been reported to be important for organic matter decomposition by increasing the organic matter surface exposed to microbial attack (Dickinson & Plugh, 1974). The first order oxygen uptake derived from the present study, implies that fragmentation may improve oxygen supply inside the particles, by which aerobic microbial carbon decomposition is increased.

3.5 Conclusions

Stationary oxygen profiles can be simulated with different scenarios of diffusion coefficients and oxygen consumption rates. Therefore, unique values of D and Q cannot be derived from the comparison of measured and simulated profiles only. A suitable method to determine a realistic scenario of D and Q is the measurement and simulation of relative concentration changes following a stepwise increase in oxygen concentration around litter particles. Values of D and Q are estimated from the relation between measured minimum oxygen concentration and particle size and the bulk oxygen consumption rate of the litter. A realistic scenario of D and Q is a constant D of $2.5 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$ and first order oxygen consumption with a constant $Q_{ref}$ of 0.08 mmol m$^{-3}$ s$^{-1}$. The low value of D is attributed to the polymeric structure of organic matter. First order oxygen consumption rates and a low value of D result in strongly decreasing actual oxygen consumption with distance from the nearest air-filled interparticle pore.