Anoxic conditions in a Douglas fir litter layer
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5 N$_2$O production in Douglas fir litter as affected by anoxic conditions within litter particles or in water filled interparticle pores.

*With W. Bouten, C.Rappoldt, A.Tietema and J.M. Verstraten*

*To be submitted to Soil Science Society of America Journal*

**Abstract**

Water content is an important factor in the regulation of denitrification in soil due to its effect on the development of anoxic conditions. In forest litter layers the development of anoxic conditions and their relation with denitrification rates is not completely understood. On the one hand anoxic microsites in centers of litter fragments occur at low water contents, when denitrification rates are generally low. On the other hand, oxygen concentrations in interparticle pores remain 100% saturated at high water contents, when denitrification rates can be high. At high water contents it is not clear whether any increase in anoxic volume develops in the litter fragments, the interparticle pore space or both.

Whether diffusion limitation of oxygen occurs within litter fragments or in interparticle pores may affect potential denitrification rates as the nitrate diffusion rate within litter particles is much lower than in the soil solution in interparticle pores. Furthermore the denitrifier activity may differ in litter fragments as compared to interparticle pores. The aim of this study therefore is to estimate the relation between denitrification and anoxic conditions in litter fragments and interparticle pores.

A laboratory incubation experiment was performed in which we measured nitrous oxide production of Douglas fir litter (F2 horizon) at several water contents ranging from average to extreme high values. Measurements were done under aerobic and anaerobic conditions, and with or without glucose amendments. Nitrate concentrations were always high (at least 2 mM). To get insight in the development of anoxic conditions in water filled interparticle pores a 2D oxygen diffusion grid model was used to simulate oxygen concentrations in water and organic matter at various volumes of water filled interparticle pores. To test whether diffusion constraints of nitrate and glucose could have affected measured nitrous oxide production rates within litter fragments, model simulations were done with a 1D radial diffusion-respiration model. With this model the extent of diffusion limitation was calculated under different conditions of nitrate concentration, diffusion coefficient and nitrate reduction potential.

Model simulations with the 2D grid model showed that the occurrence of high nitrous oxide production rates in samples with extreme water contents coincided with the development of anoxic conditions in water filled interparticle pores. Measured nitrous oxide production rates started to increase exponentially after 1-2 days in glucose amended samples, during which substantial microbial growth was established. For these samples model simulations showed that the increase in oxygen consumption due to microbial growth lead to anoxic conditions in water filled pores at locations which were most far from the oxygen saturated air filled pores.
It was concluded that anoxic conditions in water filled pores were the crucial factor for the development of large nitrous oxide production rates. Diffusion limitation of nitrate and glucose were estimated to be negligible under conditions of the experiment performed in this study. The occurrence of diffusion limitation was very sensitive to the nitrate reduction potential and nitrate concentration. Therefore, diffusion limitation with denitrification in litter often cannot be neglected under field conditions if the nitrate concentration is low or the nitrate reduction potential high.
5.1 Introduction

Water content is an important factor in the regulation of denitrification in soil due to its effect on the development of anoxic conditions. In aggregated soils, anoxic conditions have been observed inside mineral aggregates (Sexstone et al., 1985; Sierra and Renault, 1996). In well-drained aggregated soils, a distinction is usually made between oxygen diffusion in interaggregate and in intra-aggregate pores (Currie, 1961). At high water contents, water is held in interaggregate pores through which limitation of oxygen diffusion can occur at given oxygen consumption rates (Currie, 1965). Due to diffusion limitation, oxygen concentrations in interaggregate pores may decrease with depth, which results in an increase of the anoxic volume inside intra-aggregate pores (Smith, 1980). Anoxic conditions in aggregated soils in dependence of water content have been simulated with diffusion-reaction models (Leffelaar, 1979; Renault and Sierra, 1994).

The importance of water content and anoxic conditions for denitrification in the field is confirmed by numerous studies reporting measured correlations between field denitrification rates and water content for a range of forest and agricultural soils (Linn and Doran, 1984; Robertson and Tiedje, 1984; Davidson and Swank, 1986; Carmol and Ineson, 1999; Shelton et al., 2000). Relationships were also found between denitrification and soil properties influencing water content, such as soil texture and drainage class (Groffman and Tiedje, 1989a,b).

Reported correlations between water content and denitrification concern mineral forest or agricultural soils and seldom specifically regard the litter layer (Musacchio et al., 1996). Insight in the relation between water content, anoxic conditions and denitrification in the litter layer would be valuable as denitrification rates in the litter layer can be equally or more important than in the mineral subsoil (Martikainen et al., 1993; Musacchio et al., 1996; Dong et al., 1998; Regina et al., 1998; Papen and Butterbach-Bahl, 1999). Relationships between water content, anoxic conditions and denitrification in a litter layer are expected to differ from those in mineral soils, as the porosity as well as the water holding capacity of a litter layer are much higher (Binstock, 1984; Schaap et al., 1998). A high porosity favours oxygen diffusion into a litter layer, while temporary high water contents due to a high water holding capacity hamper oxygen diffusion.

The relation between water content and anoxic conditions in a Douglas fir litter layer was investigated at the range of average water contents in chapter 2. We distinguished between intra- and interparticle pores. Anoxic microsites were found inside litter fragments at average water contents (0.2-0.4 m$^3$/m$^3$), when the water is fully absorbed in the intraparticle pores of the litter fragments. Within this range the anoxic volume did not increase with water content. Furthermore, the oxygen concentration in air-filled interparticle pores was oxygen saturated at all times. At water contents of field capacity and higher, the relationship between water content and anoxic conditions is unknown. At these high water contents, an increase in
anoxic volume is expected as water is now partly held in interparticle pores, which constrains oxygen diffusion. Not only the extent of increase is uncertain, but it is also unclear where the increase in anoxic volume occurs: within the intraparticle pores or in the water filled interparticle pores themselves.

A distinction between anoxic conditions within intra- and interparticle pores is important for two reasons. First, the diffusion coefficient of nitrate in the water filled interparticle pores is much higher than in the intraparticle pores of the litter. Due to the low nitrate diffusion coefficient in litter, denitrification may be limited by nitrate diffusion. Second, denitrification within litter fragments may be limited by a low denitrifier activity. Ponge (1991a) for instance showed that bacterial activity within litter fragments was only important in slightly decomposed needles, while in a more advanced stage of degradation fungal activity was dominant. This agrees with a much higher denitrification rate found in slightly decomposed (L horizon) Douglas fir needles than in intensively decomposed needles (F horizon) (Laverman, 2000).

This study treats the significance of a distinction between anoxic conditions within fragments and in water filled interparticle pores for dynamics in the denitrification process. Our specific aims are 1. to estimate the relation between denitrification and oxygen diffusion limitation in interparticle pores of Douglas fir litter and 2. to determine under what conditions denitrification within litter fragments is limited by nitrate diffusion.

These research objectives were addressed by a combination of a laboratory incubation experiment and model exercises using diffusion-reaction models specifically developed for either inter- or intraparticle pores. In the experiment it was assumed that temporal dynamics in denitrification rates are represented by temporal dynamics in nitrous oxide production rates.

5.2 Methods

Laboratory incubation experiment

Litter was sampled in July 1997 in a 36-year-old Douglas fir 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. We sampled from the 1.5-5 cm deep fermentation layer (F2) beneath a L + F (0-1.5 cm). We removed twigs, the larger roots and cones. The remaining litter largely consisted of highly fragmentated Douglas fir needles. The litter was stored at 20 °C and used for the experiments of the present study in October 1997. The pH_{w} of the litter is 3.7 (Koopmans, 1996).
**Table 5.1: Characteristics of the incubation experiment**

<table>
<thead>
<tr>
<th>Incubation Treatment</th>
<th>Solution addition l kg⁻¹</th>
<th>Water content l kg⁻¹</th>
<th>NO₃-N addition mol kg⁻¹</th>
<th>Glucose-C addition mol kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose + nitrate</td>
<td>1</td>
<td>3.15</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>glucose + nitrate</td>
<td>4</td>
<td>6.15</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>glucose + nitrate</td>
<td>6</td>
<td>8.15</td>
<td>6</td>
<td>2.5</td>
</tr>
<tr>
<td>Anaerobic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose + nitrate</td>
<td>1</td>
<td>3.15</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>glucose + nitrate</td>
<td>4</td>
<td>6.15</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>demi-water</td>
<td>1</td>
<td>3.15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>demi-water</td>
<td>4</td>
<td>6.15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

N₂O, CO₂ and O₂ production rates were measured in Douglas fir litter (F2: fragmented needles) at different water contents in a laboratory incubation experiment (Table 5.1). Samples of litter (3 g dry) were put in air-tight vessels of 300 cm³ and were moistened by spraying with demineralized water or a solution of nitrate and glucose (1mM/69 mM). Glucose was added to increase oxygen consumption rates and to stimulate denitrification due to an improved carbon availability.

N₂O- and CO₂ production and O₂ consumption rates were calculated from a concentration increase or decrease over time in the headspace. Gas concentrations were measured using Gas Chromatography, with an ECD for N₂O and a TCD for CO₂ and O₂. Production and consumption rates were determined both aerobically (ambient air) and anaerobically (N₂ atmosphere) (Table 5.1). Incubations were performed in duplicate.

**Gridmodel of oxygen diffusion**

To evaluate the development of anoxic conditions in water filled interparticle pores a 2D oxygen diffusion-consumption model was developed in PCRaster GIS, comparable to the model in chapter 3 (PCRaster, 1998). This so called gridmodel simulates oxygen consumption and diffusion in a map with a distribution of organic matter particles, pores and water. This map was obtained from a thin section in which litter particles and pores could be distinguished (chapter 2, 3 and 4). The thin section was produced of litter from the same site and was previously used in chapter 2 and 3. All calculations were performed with the PCRaster package (PCRaster, 1998).
Table 5.2: Parameters used in the gridmodel

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
<th>Motivation/definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{ref}$</td>
<td>mmol m$^{-3}$ s$^{-1}$</td>
<td>0.08</td>
<td>estimated (chapter 3: $Q_{ref}$)</td>
</tr>
<tr>
<td>$Q_{ref}$</td>
<td>mmol m$^{-3}$ s$^{-1}$</td>
<td>0.6-2 (at wc 6 l kg$^{-1}$)</td>
<td>estimate based on measurements (this study)</td>
</tr>
<tr>
<td>$D$</td>
<td>m$^2$ s$^{-1}$</td>
<td>2.5*10$^{-13}$</td>
<td>estimated, chapter 3</td>
</tr>
<tr>
<td>$S_b$</td>
<td>m$^3$ m$^{-3}$</td>
<td>0.42$^a$</td>
<td>bulk porosity of organic matter</td>
</tr>
<tr>
<td>$S$</td>
<td>m$^3$ m$^{-3}$</td>
<td>0.8</td>
<td>local organic matter porosity</td>
</tr>
<tr>
<td>$\Delta x$</td>
<td>m</td>
<td>21.2*10$^{-6}$</td>
<td>scanning resolution (1200 dpi)</td>
</tr>
<tr>
<td>$\Delta t$</td>
<td>s</td>
<td>0.005</td>
<td>convergence requirements</td>
</tr>
</tbody>
</table>

$^a$estimated as total bulk porosity (0.9 (Freijer, 1994b)) minus bulk porosity of interparticle pores in thin section (0.48).

For the simulation of different water contents, the organic matter and pore space were water filled to amounts comparable to the water contents brought about in the laboratory experiment (Table 5.1). We assumed hereby that upon moistening, water is held primarily in the intra-particle pores (A-pores) of the organic matter, until saturation of intra-particle pore space ($S_b$, Table 5.2). The water content corresponding to saturation of the intra-particle pore space was estimated at 0.4 m$^3$ m$^{-3}$ ($S_b$, see Table 5.2). Above this saturated water content interparticle pores (E-pores) were filled, the smallest pores first. Filling of interparticle pores was done according to the following method. First, a water layer was added from the organic matter surfaces into interparticle pore space. Subsequently, an air layer of the same thickness as the water layer was added back again from the air-filled pores towards the organic matter surfaces. With this procedure, the water layer is erased except in pores with a radius less than the thickness of the water layer. The volume of these completely water filled pores varies with the thickness of the applied water layer. We made use of two different water layer thicknesses and obtained water filled interparticle pore spaces of 0.2 and 0.4 m$^3$ m$^{-3}$. These two values of water filled interparticle pore space corresponded with the water contents of 6 l kg$^{-1}$ and 8 l kg$^{-1}$ of the incubated litter samples.

Oxygen diffusion transport was modeled between each individual cell and its four neighbouring cells with Fick's second law, using an explicit finite difference approximation as adapted from Wang and Anderson (1982):

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

[eq. 5.1]

with:

$C_{ij}^{n}$ = concentration in cell$_i$ (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 (Table 5.2)

\( \Delta x = \Delta y = \) cell length (Table 5.2)

\( Q_{ij}^n = \) oxygen uptake in cell i,j (\( \text{mol m}^{-3} \text{s}^{-1} \)), at timestep \( n \)

\( \Delta t = \) timestep (s)

\[
Q_{ij} = Q_{\text{ref}} \ast C_{ij}/C_{\text{oxygen saturation}} \quad \text{[eq.5.2]}
\]

with:

- \( Q_{\text{ref}} = \) reference oxygen consumption rate in A-pores (\( Q_{\text{ref-A}} \)) or waterfilled E-pores (\( Q_{\text{ref-E}} \)) (\( \text{mol m}^{-3} \text{s}^{-1} \))
- \( C_{ij} = \) oxygen concentration in cell i,j (\( \text{mol m}^{-3} \))

Model parameter values are given in Table 5.2. Oxygen diffusion and consumption was only calculated for the water and organic matter fractions; for air-filled interparticle pores we assumed a constant oxygen concentration at an atmospheric level. This assumption was demonstrated to be realistic in chapter 2.

Part of the added water volumes in E-pores was enclosed by organic matter cells, and was therefore cut off from a direct supply of oxygen through oxygen saturated air-filled E-pores. It was interpreted that this blocking was not representative, but merely the result of the 2D dimensionality of the map. For the calculations of oxygen concentrations in water filled E-pores we only used the set of water cells that were in contact with air-filled pores, which amounted to about 50-70\% of the added water volumes. For further calculations it was assumed that these pores were representative for all water-filled E-pores.

Oxygen concentrations in water filled E-pores were calculated until diffusion was in equilibrium with oxygen consumption of a certain model scenario. The anoxic fraction was calculated from the spatial distribution of oxygen concentration as the fraction of water filled E-pores with a concentration lower than 3\% oxygen saturation. The anoxic volume was calculated by multiplying the anoxic fraction in waterfilled E-pores with the volume of water in E-pores \( g^{-1} \).

**Cylinder model of nitrate reduction**

To evaluate under what conditions diffusion limitation of nitrate occurs in litter fragments during denitrification, we performed a sensitivity analysis with a radial diffusion-reaction model, which was developed for aggregated soils (Arah, 1990). In chapter 2 we demonstrated that the litter layer of this study can be described as an aggregated system. In this radial model, soil structure was described by an equivalent cylinder system with cylinders of 7 different radii of infinite length. This cylinder system was demonstrated to be representative for Douglas litter structure (chapter 3). Cylinder sizes were derived from particle-pore distances observed in a litter thin section of the same litter layer as used in this study (chapter 4). Cylinder
sizes and their relative weight in the representative cylinder set are given in Table 5.3.

Table 5.3: Characteristics of the representative cylinder set. Negative values result from a concave geometry of part of the organic matter surrounding pores (Rappoldt and Verhagen, 1999).

<table>
<thead>
<tr>
<th>Cylinder radius</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.021</td>
<td>-0.0092a</td>
</tr>
<tr>
<td>0.064</td>
<td>0.127</td>
</tr>
<tr>
<td>0.127</td>
<td>0.458</td>
</tr>
<tr>
<td>0.212</td>
<td>0.332</td>
</tr>
<tr>
<td>0.318</td>
<td>0.072</td>
</tr>
<tr>
<td>0.445</td>
<td>0.0117</td>
</tr>
<tr>
<td>0.594</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

In a model for aggregated soils a homogeneous external soil atmosphere is assumed; reaction kinetics only affect substrate concentrations inside aggregates. In the denitrification model, oxygen consumption and nitrate reduction are described by Michaelis-Menten kinetics:

\[
Q(r) = I(r) \frac{Q_{\text{ref}} c(r)}{K_m + C(r)}
\]

\[\text{eq. 5.3}\]

\(I(r)\) : inhibition function at radius \(r\)  
\(Q_{\text{ref}}\) : reaction potential of oxygen consumption and nitrate reduction  
\(K_m\) : Michaelis constant for reaction  
\(C(r)\) : substrate concentration at radius \(r\)

The inhibition function \(I(r)\) is only used for nitrate reduction. Inhibition of nitrate reduction is complete at an oxygen concentration of 3% oxygen saturation (value of \(I(r)\) is 0). With decreasing oxygen concentration to 0% saturation, inhibition of nitrate reduction is alleviated; \(I(r)\) linearly increases up to 1 at 0% saturation. The fraction of the equivalent cylinder set with oxygen concentrations lower than 3% was about 0.1 which agrees with measured fractions of 0.05-0.35 in litter (chapter 2: zones with concentrations 0-10% saturation).

In the sensitivity analysis of the diffusion-reaction model we calculated \(\text{NO}_3^-\) reduction rates for the equivalent cylinder set at diffusion-reaction equilibrium. The \(\text{NO}_3^-\) reduction rate for this cylinder set was calculated by summing individual rates for the different cylinder radii of the set, while correcting for the weight of each cylinder radius. We varied the nitrate diffusion coefficient, nitrate reduction potential and external nitrate concentration (Table 5.4). The nitrate diffusion coefficient in litter is unknown. For a realistic range in diffusion coefficients of nitrate in litter particles we used a range of diffusion coefficients of carbohydrates in biomembranes (Stein,
1986). Diffusional properties of organic matter resemble those of biomembranes in that both are organic polymers (Stein, 1986; Pignatello, 1989). The range in NO$_3^-$ reduction potentials is chosen so that the resulting NO$_3^-$ reduction rates for the equivalent cylinder set are representative for the experimental conditions. The resulting NO$_3^-$ reduction rates are 1, 2, or 3 times the N$_2$O production rates measured in litter samples at the start of the incubation experiment. With a NO$_3^-$ reduction rate of maximally 3 times the N$_2$O production rates, we assumed a N$_2$:N$_2$O production ratio of maximally 2. A higher ratio than 2 is not expected in soil samples with a low pH and a high NO$_3^-$ concentration such as occur in the litter samples of this study (see chapter 1).

A diffusion limitation of nitrate is indicated by a decrease in nitrate concentration and a subsequent decrease in total NO$_3^-$ reduction rate. This decrease follows from eq. 5.3, in which reaction rates depend on substrate concentrations according to Michaelis Menten kinetics.

**Table 5.4: Parameters used in the cylinder model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
<th>Source/motivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$_m$ O$_2$</td>
<td>mmol m$^{-3}$</td>
<td>200</td>
<td>prescribed to fit results of chapter 3</td>
</tr>
<tr>
<td>K$_m$ NO$_3^-$</td>
<td>mmol m$^{-3}$</td>
<td>1</td>
<td>set low to address only effects of diffusion</td>
</tr>
<tr>
<td>Q$_{ref}$ O$_2$</td>
<td>mmol m$^{-3}$ s$^{-1}$</td>
<td>0.08</td>
<td>same as in gridmodel (Table 5.2)</td>
</tr>
<tr>
<td>Q$_{ref}$ NO$_3^-$</td>
<td>mmol m$^{-3}$ s$^{-1}$</td>
<td>0.000152, 0.000456, 0.000912</td>
<td>estimated based on experimental results</td>
</tr>
<tr>
<td>D$_{eff}$ O$_2$</td>
<td>m$^2$ s$^{-1}$</td>
<td>2.5*10$^{-13}$</td>
<td>bulk diffusion coefficient, same as in gridmodel (Table 5.2)</td>
</tr>
<tr>
<td>D$_{eff}$ NO$_3^-$</td>
<td>m$^2$ s$^{-1}$</td>
<td>2.5<em>10$^{-13}$-1</em>10$^{-14}$</td>
<td>bulk diffusion coefficient; estimates based on diffusion coefficients of carbohydrates in biomembranes (Stein, 1986)</td>
</tr>
</tbody>
</table>

external [NO$_3^-$] | mmol m$^{-3}$ | 100, 500, 1000, 1500 | prescribed to fit results of chapter 3                                          |
5.3 Results

Measured N₂O production was low at all applied additions of glucose/nitrate solution during the first 24 hours of aerobic incubation (Figure 5.1). Thereafter N₂O production rates increased for samples with water contents of 6 and 8 l kg⁻¹ (Figure 5.1) where the greatest increase was measured for the highest water content (8 l kg⁻¹). This lag phase in N₂O production of 24 hours was not found for samples under anaerobic incubation (Figure 5.2). Under anaerobic incubation N₂O production started immediately at a rate about as high as 250 times the initial rates measured under aerobic incubation (Figure 5.2). Furthermore, no differences were measured under anaerobic incubation that could be attributed to water content (Figure 5.2). The only difference found was due to amendment of glucose and nitrate, which caused a slightly higher N₂O production rate than additions of demineralized water.

![Figure 5.1: N₂O production rates under aerobic conditions at different water contents, established by amendments of 1, 4 and 6 l kg⁻¹ of a glucose-nitrate solution (1mM/69 mM). Dashed line, (Δ): water content 8 l kg⁻¹; dotted line, (○): water content 6 l kg⁻¹; solid line (□): water content 3 l kg⁻¹. Open symbols: samples A; closed symbols: samples B. Symbols represent averages over the period between the moment at a symbol and that at the preceding symbol.](image)

The CO₂ production and O₂ consumption rates showed the same lag phase as N₂O production rates under aerobic incubation, but differed in their response to water content after 24 hours (Figure 5.3). While N₂O production rates at the highest water content increased much faster than at water content 6 l kg⁻¹, CO₂ production and O₂ consumption rates showed similar increases for both water contents.
Figure 5.2: N$_2$O production under anaerobic conditions at different water contents, established by either demineralized water or a glucose-nitrate solution (1 mM/69 mM). Symbols (Δ) water content 3 l kg$^{-1}$ with glucose and nitrate; (○) water content 6 l kg$^{-1}$ with glucose and nitrate; (□) water content 3 l kg$^{-1}$ without glucose and nitrate (X) water content 6 l kg$^{-1}$ without glucose and nitrate. Open symbols: samples A; closed symbols: samples B.

Anoxic volumes in water filled E-pores simulated with 2D grid model

The distribution of air-filled interparticle pores, water-filled interparticle pores, organic matter and unselected water-filled interparticle pores is presented in Figure 5.4 for water content 6 and 8 l kg$^{-1}$. At a water content of 6 l kg$^{-1}$, air-filled interparticle pores are regularly distributed over the grid, with small distances in between. At a water content of 8 l kg$^{-1}$, the number of air-filled pores has strongly decreased and distances between air-filled pores are larger than at a water content of 6 l kg$^{-1}$. As a consequence, the volume of water filled interparticle pores that is dependent on a certain air-filled pore for its oxygen supply is larger at a water content of 8 l kg$^{-1}$ than of 6 l kg$^{-1}$. Likewise, distances over which oxygen must diffuse from an air filled interparticle pore to certain points in the water filled interparticle pore space are also larger at a water content of 8 l kg$^{-1}$ than of 6 l kg$^{-1}$.

Figure 5.5 presents the simulated anoxic volume in water filled interparticle pores at water contents of 6 and 8 l kg$^{-1}$, in dependence of oxygen consumption rate. Furthermore, measured N$_2$O production rates in dependence of oxygen consumption rate are also shown for these water contents. At a water content of 8 l kg$^{-1}$, anoxic conditions develop already at low oxygen consumption rates (Figure 5.5). The anoxic volume in water filled interparticle pores further increases with oxygen consumption rate. The increase is irregular, which is probably related to the small number of
selected water-filled interparticle pores and the limited representativity of the thin section segment (chapter 4). At the water content of 8 l kg\(^{-1}\), calculated anoxic volumes were correlated with measured N\(_2\)O production (Figure 5.5). In contrast, at a water content of 6 l kg\(^{-1}\) anoxic conditions only develop at extreme oxygen consumption rates. At an oxygen consumption rate of 3 \(\mu\)mol kg\(^{-1}\) s\(^{-1}\), corresponding with \(3\times10^{-4}\) mol m\(^{-3}\) s\(^{-1}\), the anoxic volume is still insignificant.

Figure 5.3: CO\(_2\) production (positive) and O\(_2\) consumption (negative) at different water contents, effected by amendments of 1, 4 and 6 l kg\(^{-1}\) of a glucose-nitrate solution (1mM/69 mM). Dashed line, (\(\Delta\)): water content 8 l kg\(^{-1}\); dotted line, (\(\circ\)): water content 6 l kg\(^{-1}\); solid line (\(\bullet\)): water content 3 l kg\(^{-1}\). Open symbols: samples A; closed symbols: samples B.

**Nitrate diffusion and reduction within particles**

Figure 5.6 shows the total nitrate reduction rate of the cylinder set at different nitrate reduction potentials, initial nitrate concentrations and diffusion coefficients. Diffusion limitation is indicated by a decrease in total nitrate reduction rates in respect to potential reduction rates, caused by decreased equilibrium nitrate concentrations in the anoxic zones of the cylinders. Diffusion limitation was negligible under conditions representative for the laboratory experiment, as is demonstrated by small differences between total nitrate reduction rates and potential reduction rates of the equivalent cylinder set (Figure 5.6). In the incubation experiment the nitrate concentration was at least 1.5 mM. At this concentration, diffusion limitation in the sensitivity analysis is most severe at the highest nitrate reduction potential of 0.79 nmol kg\(^{-1}\) s\(^{-1}\) and the lowest diffusion
coefficient of $1 \times 10^{-14} \text{ m}^2 \text{s}^{-1}$. Under these circumstances, total denitrification rate is still more than 90% of the potential rate in absence of diffusion constraints. Figure 5.6 shows that diffusion limitation can be important at a nitrate concentration of 0.1 mM, when total nitrate reduction rate is decreased with 35 to 75%.

**Figure 5.4:** On the left: distribution of organic matter, air-filled E-pores and water-filled pores (selected and unselected) at water content 6 l kg$^{-1}$ (upper graph) and 8 l kg$^{-1}$ (lower graph). On the right: simulated oxygen concentrations in selected water filled E-pores at water content 6 l kg$^{-1}$ (upper graph) and 8 l kg$^{-1}$ (lower graph). At water content 6 l kg$^{-1}$, oxygen consumption rate is 3.2 μmol kg$^{-1}$ s$^{-1}$, at water content 8 l kg$^{-1}$, oxygen consumption rate is 0.8 μmol kg$^{-1}$ s$^{-1}$. 

Right: Oxygen concentration
- 100 (%saturation)
- 75
- 50
- 25
- 0

Left:
- organic matter
- air-filled pore
- water-filled pore

Water content 6 l kg$^{-1}$

Water content 8 l kg$^{-1}$
Figure 5.5: On the left axis measured N\textsubscript{2}O production rates in dependance of O\textsubscript{2} consumption rates (symbols). Symbols: (Δ) N\textsubscript{2}O production at water content 8 l kg\textsuperscript{-1}; (○) idem at a water content of 6 l kg\textsuperscript{-1}. Open symbols: samples A; closed symbols: samples B. Dashed line: anoxic volume at water content 8 l kg\textsuperscript{-1}; solid line: idem at 6 l kg\textsuperscript{-1}. On the right axis simulated anoxic volumes in water filled E-pores in dependance of O\textsubscript{2} consumption rate (lines).

5.4 Discussion

Relation between denitrification and anoxic condition in water filled interparticle pores

An increase in CO\textsubscript{2} production after addition of glucose indicates the occurrence of microbial growth. This was confirmed by the microscopical observation of bacterial cell division after one day. As CO\textsubscript{2} production rates were comparable for water contents of 6 and 8 l kg\textsuperscript{-1}, microbial activity developed similarly at both water contents. However, the increase in N\textsubscript{2}O production widely differed at the two highest water contents. Calculations with the gridmodel showed that a different increase in N\textsubscript{2}O production can be explained by a different increase in anoxic volume at the refered water contents. A high water content alone is not sufficient to give rise to anoxic conditions in water filled E-pores. Significant anoxic volumes only develop at stimulated oxygen consumption rates, as was the case after microbial growth. The limited anoxic volume in E-pores at the initial oxygen consumption rate of the experiment explains the low N\textsubscript{2}O production during the first 24 hours of incubation.
At a water content of 6 l kg⁻¹ the anoxic volume in water-filled E-pores was negligible. This is explained by the regular distribution of air-filled interparticle pores, which was sufficient to supply water-filled E-pores with oxygen. An insignificant anoxic volume in E-pores explains the low N₂O production of sample A at this water content (Figure 5.1). However, the N₂O production of sample B was about twenty times higher, suggesting that anoxic conditions had developed in the water-filled E-pores of this sample. This may have been caused by a local sparse distribution of air-filled E-pores, resulting from a local distribution of mainly small pores, of from an inhomogeneous water distribution. Apparently, the water content of 6 l kg⁻¹ is a critical content for the studied litter samples, above which the number of air-filled E-pores further decreases and water-filled E-pores with anoxic conditions develop. A high variability of the number of water-filled E-pores with anoxic conditions leads to a corresponding high variability in N₂O production rates at water contents around 6 l kg⁻¹.

The correlation between anoxic volume in water-filled E-pores and the measured N₂O production at the water content of 8 l kg⁻¹, indicates that a certain potential N₂O production rate exists per m³ anoxic volume of water-filled E-pores. Such a potential N₂O production per anoxic volume in E-pores would be a useful measure as it may enable the prediction of N₂O production rates for other water contents. Furthermore, such a potential N₂O production rate could be measured for different litter layers.

Figure 5.6: Total NO₃⁻ reduction rate of the equivalent cylinder set (y-axis) at three different nitrate reduction potentials (NO₃⁻RP) and four initial nitrate concentrations. Values are indicated in the figure.
However, the calculated anoxic volume in E-pores of the 2D thin section grid deviates from the volume of the real 3D sample and the extent of this deviation is yet unknown. The calculation of anoxic conditions in a 3D sample at the resolution used in this study has been limited by current computer capacity. Measurement of the water distribution at the resolution used in this study has also been limited, but then by the capacity of measurement devices (Hainsworth and Aylmore, 1983; Hopmans et al., 1992; Peyton et al., 1994). Limitations of computer capacity and measurement devices are expected to be alleviated. For future studies, calculations of anoxic conditions in 3D samples therefore seem a useful approach to further explore the relation between denitrification and anoxic conditions in water-filled E-pores.

While anoxic conditions in water filled E-pores appeared to be a critical factor for the rate of N₂O production in laboratory incubations, the question rises whether anoxic conditions also determine denitrification rates under field conditions. The importance of anoxic conditions in water-filled E-pores depends on the range of water contents and oxygen consumption rates occurring in the field. As for water contents, Schaap et al. (1998) measured maximum water contents corresponding to about 6 l kg⁻¹. At this water content, anoxic conditions only develop at an oxygen consumption rate of about 3 μmol kg⁻¹s⁻¹; but the resulting anoxic volume was still small (Figure 5.5). In the litter layer of this study oxygen consumption rates of this order only occur in the surface layer of slightly decomposed needles, which have a lower water holding capacity (CO₂ prod. 2-2.5 μmol kg⁻¹s⁻¹, laboratory incubation 20°C, Wessel, 1997). Therefore, anoxic conditions in water filled pores in the litter layer are expected to be exceptional in the Douglas fir forest at Speuld.

Maximum water contents largely depend on the drainage rate of the litter layer and furthermore also on the thickness and structure of the litter layer. For instance, the drainage rate will be lower in litter layers with a distinct H horizon. Higher water contents than reported by Schaap et al. (1998) will also occur in litter layers on poorly drained mineral soils. As the litter layer of this study was relatively thin and lacked a humus layer, water contents of 8 l kg⁻¹ as brought about in the experiment are exceptional in Speuld. At water contents of 8 l kg⁻¹, anoxic conditions develop at less extreme oxygen consumption rates (Figure 5.5). Relevant oxygen consumption rates for those situations are 2 μmol kg⁻¹s⁻¹ and higher, which commonly occur in litter of the F1 horizon (Yavitt et al., 1995; Küsel, 1996). In measured bulk oxygen consumption rates, no distinction can be made between oxygen consumption within intraparticle or interparticle water filled pores. However, high oxygen consumption rates within intraparticle pores should also lead to anoxic conditions in water filled pores, which was indeed demonstrated in earlier model calculations (results not shown).
Nitrate diffusion constraints in litter particles?

Aerobic N$_2$O production rates measured in this study were not limited by nitrate diffusion (Figure 5.6) or glucose diffusion (results not shown). At the same time however, aerobic N$_2$O production rates were about 250 times lower than under anaerobic conditions, although the anoxic fraction was estimated to be about 0.05-0.35 (chapter 2). Therefore, low aerobic N$_2$O production rates suggest a lower denitrifier activity within litter particles than at the surfaces. This is plausible since Ponge (1991a) stated that the presence of a dominant bacterial activity within litter depended on the decomposition stage, with a dominance of bacteria in slightly decomposed material and a dominance of fungi in a more advanced stage. Assuming this situation to be representative, decomposition in the litter particle centers (F2) of this study would be expected to be dominated by fungi. A denitrifier activity which decreases with decomposition stage agrees with a declining potential denitrification rate with litter age and decomposition stage (Pang and Cho, 1984; Heinrich and Haselwandter, 1991).

Diffusion limitation only occurred in the sensitivity analysis at a combination of low nitrate concentrations and high nitrate reduction potentials (Figure 5.6). Important for this result was the fact that the particle size distribution of the litter was dominated by small particles, which alleviate diffusion constraints. Low nitrate concentrations did not occur in our experiment, in which concentrations were at least 1.5 mM after amendments. Results of the sensitivity analysis showed that diffusion constraints were negligible at concentrations as low as 1 mM. Such concentrations are of the same order as concentrations just under the litter layer reported by Koopmans (1996) for the forest of this study. Concentrations of this order are common for nitrogen saturated litter layers (Van Breemen and Verstraten, 1991; Tietema et al., 1992a). The dominance of small particles in the particle size distribution of the litter resulted from a high degree of fragmentation. Larger particle sizes enhance diffusion constraints: in the two largest cylinder classes, diffusion limitation did frequently occur in the sensitivity analysis. However, due to their small relative weights in the representative cylinder set, this did hardly affect total denitrification.

Concluding from the above discussion, we expect the absence of nitrate diffusion limitation to be representative for highly fragmented needle litter layers and also for H horizons of forests with a high atmospheric N-input. In unfragmented litter layers diffusion limitation is expected to occur more frequently as particle size distribution of unfragmented litter is dominated by larger particles and the denitrifier activity is higher. Assuming the particle size distribution to be dominated by the two largest cylinder classes of the cylinder set of fragmented litter, denitrification rates can be decreased by 55 to 70% at the highest nitrate reduction potential applied in this study (Table 5.5).
Table 5.5. Nitrate reduction rates (nmol kg\(^{-1}\) s\(^{-1}\)) in the two largest cylinder classes of the equivalent cylinder set at the NO\(_3^\) reduction potentials of the sensitivity analysis presented in Table 5.4. External NO\(_3^\) concentration is 1 mM.

<table>
<thead>
<tr>
<th>Cylinder radius mm</th>
<th>Diffusion coefficient</th>
<th>NO(_3^) reduction potential 0.79 nmol kg(^{-1}) s(^{-1})</th>
<th>NO(_3^) reduction potential 2.4 nmol kg(^{-1}) s(^{-1})</th>
<th>NO(_3^) reduction potential 4.7 nmol kg(^{-1}) s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>2.5*10(^{-13})</td>
<td>0.064</td>
<td>0.19</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>2.5*10(^{-14})</td>
<td>0.064</td>
<td>0.19</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>1.25*10(^{-14})</td>
<td>0.064</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>0.59</td>
<td>2.5*10(^{-13})</td>
<td>0.086</td>
<td>0.26</td>
<td>0.51</td>
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<tr>
<td></td>
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<td>0.086</td>
<td>0.20</td>
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<tr>
<td></td>
<td>1.25*10(^{-14})</td>
<td>0.081</td>
<td>0.13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

5.5 Conclusions

Anoxic conditions in water filled interparticle pores are an important factor for denitrification in the litter layer of this study. N\(_2\)O production rates in anoxic volumes of water filled E-pores were high, which was associated with a high activity of denitrifiers. On the other hand, N\(_2\)O production rates in anoxic sites within intraparticle pores were low, which was attributed to a limited denitrifier activity.

Diffusion limitation of nitrate inside litter particles was negligible, due to a low NO\(_3^\) reduction potential, a high external nitrate concentration and the predominantly small size of the particles. Diffusion limitation did occur in the largest particles of the litter layer, which indicates that diffusion limitation could be important in unfragmented litter. Furthermore, diffusion limitation occurs when the external nitrate concentration is below 0.1 mM.