Anoxic conditions in a Douglas fir litter layer
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6 Synthesis

6.1 Research questions

The first chapter of this thesis opens with the sentence “Anoxic conditions in soil give rise to anaerobic microbial processes, such as denitrification” and ends with the conclusion that in litter layers not much is known about the occurrence of anoxic conditions and their relation with denitrification. To improve our understanding of these themes, four research questions were posed:

A. Where do anoxic conditions occur in a Douglas fir litter layer?
B. What are the conditions of oxygen diffusivity and oxygen consumption under which anoxic conditions occur in a Douglas fir litter layer?
C. How can the anoxic volume of a litter layer be modelled in a simple manner?
D. What is the relation between anoxic conditions and denitrification in a Douglas fir litter layer?

Starting from these questions an experimental and modelling approach was chosen of which the results have been presented in chapters 2 to 5. In this final chapter I give an overview of answers to the research questions posed, that are provided by these results. Furthermore, I extrapolate the results to anoxic volumes and denitrification of forest soils in general. Finally, a discussion is held on whether forest soil anoxic volumes could be estimated from humus form classification.

**Question 1: Where do anoxic conditions occur in a Douglas fir litter layer?**

Anoxic conditions in a Douglas fir litter layer occur permanently within litter particles (chapter 2), and temporarily in water filled interparticle pores (chapter 5). Within litter particles, anoxic conditions occurred at all average water contents (2-3.9 g g\(^{-1}\) dry weight) and oxygen consumption rates (0.11-0.89 μmol kg\(^{-1}\) s\(^{-1}\)). There is also a permanently existing anoxic volume inside litter particles. The anoxic volume of the litter layer did not significantly increase at higher water contents nor with a stimulated oxygen consumption rate due to addition of glucose. The most important factor for the occurrence of low to zero oxygen concentrations appeared to be particle size due to its effect on the diffusion path. The particle size above which anoxic conditions occurred was about 250 μm diameter. The particle size distribution and total volume of size classes therefore, are most important for the variability of anoxic litter layer volume per m\(^2\). Variations in particle size distributions and cumulative amounts depend on thickness and decomposition stage of the litter layer.

Within water filled interparticle pores anoxic conditions only occurred at water contents higher than 0.6-0.8 m\(^3\) m\(^{-3}\), as was demonstrated by model simulations. The model assumes oxygen saturation in adjacent air-filled pores (chapter 5). Critical factors for the development of anoxic conditions are the oxygen consumption rate in a water filled interparticle pore and the diffusion distance to the nearest air-filled interparticle pore.
Oxygen consumption rates in water filled interparticle pores depend on temperature, carbon decomposibility and microbial activity. Easily decomposable Dissolved Organic Matter (DOM) probably is an important carbon substrate for microbes in water filled interparticle pores. Sources of labile DOM are the rhizosphere, throughfall, stemflow and leachate of L horizons. Variability in DOM from these sources therefore may be important for the variability of oxygen consumption rates and anoxic conditions in water filled interparticle pores. For instance, the amount of labile DOM in throughfall was reported to be highest in summer and early autumn (Qualles and Haines, 1991; Guggenberger and Zech, 1994). Microbial activity increases with temperature; microbial activity further depends on the location in the litter layer, with the activity in the L horizon and rhizosphere being the highest.

The oxygen diffusion distance in water filled interparticle pores depends on the local water content and pore structure. Factors determining variability of water content in the field water are throughfall pattern, stemflow, drainage rate, thickness of the litter layer, root pattern and microtopography. Pore structure depends on the fabric of a humus form horizon, which varies with o.a. decomposition stage, vegetation type and soil fauna activity.

**Question 2: What are the conditions of oxygen diffusivity and oxygen consumption under which anoxic conditions occur in the litter layer?**

**Within litter:**
Steep oxygen concentration gradients in litter and anoxic sites in the larger particles (>250 μm diameter) were explained by (1) a low diffusion coefficient (2.5*10^{-13} m^2 s^{-1}) and (2) first order oxygen consumption (Q_{reference} = 0.08 mmol m^{-3} s^{-1}) (chapter 3). Diffusion coefficient and oxygen consumption rate were estimated by calibrating simulated and measured oxygen concentrations in particles.

ad (1) The low diffusion coefficient was attributed to the polymeric nature of organic matter. Low diffusion coefficients in organic matter limit the incoming flux of external oxidators. Such a situation would be favourable for microbial processes like fermentation, if local organic substrates can be used as oxidators. This would also agree with observations that the majority of anaerobic bacteria are fermenters (Gamble et al., 1977; Blössl and Conrad, 1992; Küsel et al., 1999). Small diffusion coefficients in litter may also indicate diffusion limitation of easily decomposable DOM from the external soil solution at concentrations occurring in the field. However, it is possible that with denitrification in anoxic microsites, carbon substrates are consumed that are produced by fermentation or acetogenesis within litter particles (Küsel, 1996). In this way, denitrification in anoxic microsites would not be much affected by labile DOM concentrations in the soil solution.

ad (2) First order oxygen consumption led to actual oxygen consumption rates that decrease with the distance from particle surfaces. That oxygen consumption rates
show first order kinetics, may have implications for a trophic link between aerobic and anaerobic processes in organic particles proposed by Küsel (1996). Due to low oxygen consumption rates near the interface of oxic and anoxic conditions, I do not expect intense oxidation of anaerobic organic products near this interface, but rather at or near the organic particle surfaces.

In water filled interparticle pores
Anoxic conditions in water filled interparticle pores developed at high water contents in combination with high oxygen consumption rates. In laboratory experiments with F2 litter, the critical water content appeared to be about 6 l kg⁻¹ (ca.0.6 m³ m⁻³). At this water content, anoxic condition only developed when the oxygen consumption rate was in the range of 3-3.5 μmol kg⁻¹ s⁻¹. For F2 litter, such rates are unlikely to occur under field conditions. Rates of 3-3.5 μmol kg⁻¹ s⁻¹ are associated with more easily decomposable organic matter than F2 litter.

At water contents of 8 l kg⁻¹, a significant anoxic volume occurred already at oxygen consumption rates above 0.5 μmol kg⁻¹ s⁻¹. However, such water contents are unlikely to occur in the studied litter layer. Extreme water contents may occur in litter layers containing a distinct H horizon with a low water conductivity. Furthermore, anoxic conditions in water-filled pores may be a common phenomenon in poorly drained forest soils.

How relationships between anoxic conditions, oxygen consumption rate and water content may be in other humus forms has not been studied, but seems relevant with respect to the denitrification process (see question 4). Analysis of the relation between water content and anoxic conditions could be done by studying the relationship between water content and the distribution of diffusion distances in water filled interparticle pores. At the critical water content of 0.6 m³ m⁻³ of this thesis, the maximum diffusion distance derived from thin section analysis was about 2 mm. At the water content of of 0.8 m³ m⁻³, the maximum derived diffusion distance was about 4 mm. Diffusion distances in water filled interparticle pores are treated in the next section.

Question 3: How can the anoxic volume of a litter layer be modelled in a simple manner?

Litter particle structure can be simplified to an equivalent cylinder set with only seven different cylinder radii, using the method developed by Rappoldt and Verhagen (1999) (chapter 4). With the derived cylinder set, a litter anoxic fraction can be calculated at a given oxygen consumption rate, diffusion coefficient and oxygen concentration in the interparticle pore space. However, the method only calculates the anoxic volume inside litter and not in water filled interparticle pores.

In model calculations of chapter 5 it was shown that anoxic conditions could develop in water filled interparticle pores at extreme water contents and stimulated oxygen
consumption rates. Comparison between model results and measurements of nitrous oxide production revealed that the anoxic volume in water filled interparticle pores is an important factor for the development of high nitrous oxide production rates. In chapter 5 simulation models were applied on the complex pore structure of the thin section. Although these simulations were successful, they required too much computer time to make it a suitable method for general application. Therefore, it is desirable to have a simple method for simulation of anoxic volumes in water filled interparticle pores, in addition to that for litter particles (chapter 4).

Simulated oxygen concentrations in water filled interparticle pores indicate that the simplification method of Rappoldt and Verhagen (1999) applied to litter structure, may also be applied to water filled interparticle pores (chapter 5). This is because the calculated oxygen concentrations in water filled interparticle pores in the fabric of the thin section showed a clear relation with the distance to an air-filled pore (Figure 6.1). In Figure 6.1 a normalized distance distribution is shown from each point in a water filled interparticle pore to the nearest air-filled pore. The distribution is calculated as described in chapter 4. Such a distance distribution varies with water content and therefore should be calculated for each water content. With these distance distributions, equivalent cylinder sets for “structures” of water filled interparticle pores can be derived in a similar way as was done for the organic matter fabric. With the derived cylinder sets, relations can be derived between the anoxic volume in water filled interparticle pores and the characteristic oxygen penetration depth similar to those for the organic matter fabric (chapter 4).

To make the simplification method applicable to water-filled pores, some future developments are necessary. For instance, the general applicability of 2-dimensional thin sections should be tested (see chapter 5). Furthermore, it must be possible to distinguish between oxygen diffusion in water filled interparticle pores and in the surrounding litter. For this, it should be known what the oxygen consumption rates are in water filled interparticle pores and what they are in litter.

**Question 4: What is the relation between anoxic conditions and denitrification in the litter layer?**

The permanent anoxic volume inside litter particles leads to low nitrous oxide production rates in the F2 litter layer (< 0.05 mmol kg⁻¹ s⁻¹) (chapter 5). In contrast, the temporary anoxic volume within water filled interparticle pores gives rise to high nitrous oxide production rates (0.5-2.5 mmol kg⁻¹ s⁻¹) at field nitrate concentrations (1-2 mM) (chapter 5).
Figure 6.1: Normalized distribution of distances from a location in a water filled interparticle pore to the nearest air filled pore. Within a distance class the distribution of oxygen concentration levels is drawn in different grey values. The distance distribution corresponds with a water content of 0.8 m³ m⁻³. The oxygen concentration levels are calculated with a potential oxygen consumption rate in water filled interparticle pores of 0.3 *10⁻³ mol m⁻³ s⁻¹, which resulted in a bulk oxygen consumption rate of 0.8 μmol kg⁻¹ dry s⁻¹ (0.8*10⁻⁴ mol m⁻³ s⁻¹).

The low nitrous oxide production rates within litter particles were attributed to a limited denitrifier activity inside litter particles. Possibly, microbial activity inside the litter was dominated by fungi. The activity of denitrifiers depends on colonization of needle interiors by denitrifying bacteria. The degree of colonization of needles by bacteria is related to the successive stages of decomposition (see chapter 1); which are reflected in a stratified pattern of ectorganic horizons in the litter layer. Variability of denitrifier activity in organic particles is probably an important factor for the variability of denitrification rates within anoxic microsites in litter.

Within litter anoxic microsites, it was found that nitrous oxide production was not limited by nitrate, since model simulations showed that nitrate concentrations in anoxic microsites were generally not much lower than those outside the litter particles (1.5 mM). Only in the largest size classes of the equivalent cylinder set did limitation of nitrate diffusion occur, but these size classes comprised only a minor part of the total. The absence of diffusion limitation was explained by a high external nitrate concentration, a low denitrification potential and a high degree of fragmentation of the litter, through which particles in the smallest size class dominated.
Nitrous oxide production was high when anoxic conditions developed in water filled interparticle pores at extreme water contents and oxygen consumption rates (chapter 5). Production rates were about one third of the rate observed under complete anoxic conditions. This potentially high nitrous oxide production was attributed to a considerable activity of facultative anaerobes at the litter surfaces and in water filled interparticle pores. The high nitrous oxide production coincided with considerable microbial growth due to the earlier added glucose.

6.2 General discussion

*Extrapolation of the occurrence of anoxic conditions and denitrification to forest soils in general.*

For the laboratory experiments of this study only organic material of the F2 horizon was used of a Douglas fir forest soil. This material consisted of highly fragmented needles and excrements. The F horizon is only one horizon of the humus form, which can consist of a L, F, H and Ah horizon, according to the classification system of Green et al. (1993). These horizons are distinguished based on morphological properties, such as degree of fragmentation, amount of fine substances (humus), aggregation etc. Due to their differences in structure, humus form horizons are also expected to differ in the development of anoxic conditions and subsequent denitrification. The potential for anoxic conditions and denitrification of the different humus form horizons L, H and Ah is discussed below. Treated separately are non-horizon components of the litter layer, namely woody tissues (branches and fir cones), dead roots and the rhizosphere. Furthermore, distinctions in anoxic conditions and denitrification between needle litter and leaf litter layers are treated.

*L horizon (intact needles)*

The L horizon is more favourable than the F2 horizon for the development of anoxic conditions in litter particles, since particle sizes are larger and oxygen consumption rates higher. The development of anoxic conditions in water filled interparticle pores is improbable in the L horizon due to the low water holding capacity of this layer.

Regarding denitrification, there are several studies giving evidence that the denitrifier activity in the L horizon is probably higher than in the F horizon. First, Ponge (1991a) observed that bacterial numbers were most abundant in relatively young needles in the first stage of microfaunal attack. Furthermore, Küsel (1996) found the anaerobic carbon decomposition potential to be highest in the L horizon. Finally, Laverman (2000) recently demonstrated high denitrification rates in L horizon material under aerobic incubation that were only 8-15 times lower than rates measured under anaerobic incubation. In contrast she found denitrification rates below detection limit in F material under aerobic incubation. At the high denitrification rates measured by Laverman in intact needles, denitrification was probably limited by nitrate diffusion according to model calculations performed in chapter 5.
**H- and Ah horizon**
The organic matter in the H and Ah horizons mainly consists of humus. Humus particles are extremely small and moreover exhibit low oxygen consumption rates due to the poor substrate quality. Therefore, anoxic conditions within these humus particles is improbable when the oxygen concentration outside these particles is atmospheric. More important perhaps, are anoxic conditions in water filled interparticle pores as pore structure is more dense than in the F horizon and water holding capacity is high. Furthermore, at temporarily high water contents bulk horizon diffusivity of oxygen may sometimes not be sufficient to maintain oxygen concentrations in air-filled pores at atmospheric levels. Schaap et al. (1998) demonstrated that water contents higher than at field capacity frequently occurred in the Ah horizon. At low oxygen concentrations in pore space, anoxic conditions could also occur within organic matter particles. Whether anoxic conditions in water-filled pores and organic matter particles develop, highly depends on oxygen consumption rates. Temporary fluxes of easily decomposable DOM from upper horizons or from throughfall may be an important factor in this respect. It is hypothesized, that under anoxic conditions in water filled interparticle pores, denitrification rates are probably high, similar to those measured in the F2 layer in chapter 5. Indeed, considerable denitrification rates have been frequently reported for forest soils from which the litter layer was removed (Dong et al., 1998; Papen and Butterbach-Bahl, 1999).

In the above discussion, I did not take account of aggregation effects in the H or Ah horizon. Aggregation may lead to anoxic conditions and subsequent denitrification in organo-mineral aggregates. Aggregates of this nature do not occur in an undisturbed F3 horizon. The relative importance of anoxic conditions in water filled interparticle pores or inside organomineral aggregates is unknown and has to be subject of future studies.

**Branches and fir cones**
Branches and fir cones may comprise a considerable part of total litter fall. In general, woody material is more resistant to decomposition than needle or leaf litter due to its relative large content of lignocellulose and small amount of nitrogen (Dickinson and Plugh, 1974). Therefore, decomposition of wood has been found to be dominated by fungi, although some wood decomposing bacteria have been observed (Dickinson and Plugh, 1974).

The oxygen consumption rates of fresh fir cones and branches from our study site were of the same order as those of the LF horizon (data not shown). Therefore, anoxic sites can also be expected in cones and branches, especially since their size is much larger than that of needles. However, if denitrifiers are present in those woody materials, denitrification is probably limited by nitrate diffusion due to the large size of branches and cones. It is hypothesized therefore, that denitrification in branches and cones probably is negligible.
Dead roots/Rhizosphere

Dead roots (fine) can be a source of relatively easily decomposable substrate in H or Ah horizons, although dead roots are more resistant than plant or tree litter (Dickinson and Plugh, 1974). Anoxic sites in decomposing roots are not improbable, due to activity of fungi and bacteria in interiors of dead roots. The importance of denitrification is unknown in decaying root interiors.

Microbial activity in the rhizosphere is much higher than in the surrounding bulk soil due to labile organic root exudates (Norton et al., 1990); differences easily amount to one order. The contribution of bacteria to microbial activity is also higher than in the surrounding soil, as well as potential denitrification rates (Dickinson and Plugh, 1974; Scott Smith and Tiedje, 1979). When the rhizosphere is part of a water filled interparticle pore and anoxic conditions develop due to high oxygen consumption rates, denitrification rates may become of considerable importance. Denitrification rates in the rhizosphere depend on nitrate concentration, as denitrifiers have to compete for nitrate with tree roots.

From the above discussion of the potential for anoxic conditions and denitrification, I hypothesize that denitrification may be most important inside litter particles in the L horizon and in water filled interparticle pores in H and Ah horizon or rhizospheres. However, extensive additional research is required, due to the complexity of humus forms. Important factors of interest are litter type, nutrient status and activity of soil fauna.

Leaf litter layers of deciduous trees

Litter layers of deciduous forests differ from litter layers of coniferous forests regarding the shape of the leaves/needles as well as in the structure of the litter layer. These differences have influence on the development of anoxic conditions and therefore denitrification, which is explained below. The shape of leaves resembles that of sheets, while that of needles is more like cylinders. For oxygen diffusion through organic matter towards oxygen consuming microbial communities in the leaf interior, the leaf thickness is important. This thickness in general seems smaller than the diameter of coniferous needles and may vary between tree species. The limited thickness of leaves decreases the oxygen diffusion distance towards the center of the leaf, and therefore leaves may be less favourable for the development of anoxic conditions than coniferous needles. However, in slowly decomposing needle litter, anoxic microsites were already observed in particles with a diameter of 250 μm. Therefore, anoxic microsites may be expected in even smaller particles at much faster decomposition rate and consequently oxygen consumption rate as occur in relatively young leaf litter (L horizon).

Leaf litter layers differ in structure from needle litter layers because leaves in the F horizon tend to be arranged along horizontal planes. Such F horizons form a so-called matted structure, which can be compact or non-compact (Green et al., 1993). The pores between these decomposing leaves must also be arranged on horizontal
planes, and may have a limited height in compact matted F horizons. Under wet conditions, these horizontally positioned pores will be water filled. In such thin water layers between broad leaves the oxygen diffusion distance through this water layer to the nearest air-filled pore may amount up to centimeters. Therefore, due to microbial oxygen consumption within the leaf or on the leaf surface, anoxic microsites may easily develop. The occurrence of anoxic conditions in these horizontal pores was also suggested by Küsel (1996). The high diffusion distances in water filled pores between leaves differ from those in coniferous litter layers. In a needle litter layer, diffusion distances of only 2 to 4 mm developed at extreme water contents. These diffusion distances led to anoxic microsites in water filled interparticle pores at sufficiently high oxygen consumption rates (chapter 5).

A layered structure of leaf litter layers may affect bulk oxygen diffusion rates, when the horizontally layered pores between leaves are discontinuous. Freijer (1994a) measured bulk oxygen diffusivity of a needle litter layer and a leaf litter layer. He indeed observed a lower diffusivity in leaf litter than in needle litter, even when the bulk porosity of the leaf litter layer was higher than that of the needle litter layer. This difference in bulk diffusion may especially become important at high water contents (0.5-0.6 m$^3$ m$^{-3}$). Evidence that a leaf litter layer can act as a diffusion barrier was found by Dong et al. (1998), who measured an increased contribution to surface nitrous oxide fluxes from the mineral soil when the litter layer had been removed. When bulk oxygen diffusivity is not sufficient for the oxygen demand in the litter layer, a vertical concentration gradient develops, which enhances the formation of anoxic microsites in litter particles in deeper horizons.

In conclusion, the above described differences between needle type litter and leaf type litter indicate that oxygen dynamics between the two litter types may differ significantly. We hypothesize that anoxic conditions within leaves occur to a lesser extent than in needles and anoxic conditions in water filled pores between leaves to a larger extent. For investigation of differences between deciduous and coniferous litter layers, experiments on oxygen dynamics in deciduous litter layers are necessary.

**Extrapolation of humus form classification to estimates of humus form anoxic volumes**

Humus form development is intimately related to soil type, climate and vegetation type. The humus form classification system of Green et al. (1993) has been applied on a wide range of forest ecosystems in British Columbia, North America and Europe (Green et al., 1993; Sevink, 1997; Sevink et al., 1998). Depending on climate, forest type and soil type, typical humus forms were classified.

In the above section I argued that humus form horizons differ in their potential for anoxic conditions and denitrification. Furthermore, the contribution of woody
tissues and roots to humus form horizons is expected to be important as well as the litter type, being either needle litter or leaf litter. It is conceivable that morphological properties of humus form horizons correspond to a “typical” organic matter and pore structure. Such a general relation between morphological properties and horizon structure would mean that horizon structure can be represented by an equivalent cylinder system with the method of Rappoldt and Verhagen (1999). By combining such representative cylinder sets for each humus form horizon, representative cylinder systems of pores and organic matter could be derived for the whole humus form.

The possible relation between humus form and typical humus form structure, enables extrapolation of regional distribution of humus forms to estimates on litter layer anoxic volumes in different climatic zones, on mineral substrates etc. For quantification of actual anoxic volumes based on humus form structure, diffusion coefficients and oxygen consumption rates must be known. With respect to this, diffusion coefficients are expected to vary with decomposition stage and vegetation type, as both factors influence the “crystallinity” of organic matter polymers. In a similar way, oxygen consumption rates also depend on decomposition stage and vegetation type as these determine the decomposibility of the organic matter. Finally, it has to be investigated what the relation is between anoxic volumes and denitrification rates. Important in this regard is the distinction between anoxic volumes in water filled interparticle pores and those in organic matter tissues (chapter 5).

In the literature, various models have been developed for the prediction of greenhouse gases in soil (Vinten et al., 1996; Li et al., 2000; Riley and Matson, 2000). A humus form cylinder system is easily implemented in diffusion/reaction modules for the estimation of soil anoxic volumes. This would mean an important progress in application of these models on forest ecosystems, by taking account of the specific structural properties of litter layers. However, applicability of humus form structure in models or estimates on soil anoxic volumes remains speculative and should first be tested before any definite conclusions can be drawn.

I end this synthesis with the general conclusion that anoxic conditions are a common phenomenon in litter layers. In consequence of this conclusion, studies on anaerobic microbial processes in forest soils should pay specific attention to the litter layer. Furthermore, the general occurrence of anoxic conditions leaves us to the question what the role is of anaerobic microbial processes in litter decomposition.