Oxygen and redox potential gradients in the rhizosphere of alfalfa grown on a loamy soil

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Abstract

Oxygen (O2) supply and the related redox potential (Eh) are important parameters for interactions between roots and microorganisms in the rhizosphere. Rhizosphere extension in terms of the spatial distribution of O2 concentration and Eh is poorly documented under aerobic soil conditions. We investigated how far O2 consumption of roots and microorganisms in the rhizosphere is replenished by O2 diffusion as a function of water/air-filled porosity. Oxygen concentration and Eh in the rhizosphere were monitored at a mm-scale by means of electroreductive Clark-type sensors and miniaturized Eh electrodes under various matric potential ranges. Respiratory activity of roots and microorganisms was calculated from O2 profiles and diffusion coefficients. pH profiles were determined in thin soil layers sliced near the root surface. Gradients of O2 concentration and the extent of anoxic zones depended on the respiratory activity near the root surface. Matric potential, reflecting air-filled porosity, was found to be the most important factor affecting O2 transport in the rhizosphere. Under water-saturated conditions and near field capacity up to –200 hPa, O2 transport was limited, causing a decline in oxygen partial pressures (pO2) to values between 0 and 3 kPa at the root surface. Aerobic respiration increased by a factor of 100 when comparing the saturated with the driest status. At an air-filled porosity of 9% to 12%, diffusion of O2 increased considerably. This was confirmed by Eh around 300 mV under aerated conditions, while Eh decreased to 100 mV on the root surface under near water-saturated conditions. Gradients of pO2 and pH from the root surface indicated an extent of the rhizosphere effect of 10–20 mm. In contrast, Eh gradients were observed from 0 to 2 mm from the root surface. We conclude that the rhizosphere extent differs for various parameters (pH, Eh, pO2) and is strongly dependent on soil moisture.

Key words: soil aeration / oxygen diffusion / air-filled porosity / rhizosphere / hotspots

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1 Introduction

One of the most important factors influencing aerobic activity in soil is the availability of molecular oxygen (O2). Spatial distribution of O2 in soil depends strongly on the investigated scale. At the pedon scale, higher O2 partial pressure (pO2) are found in the topsoil and gradually decrease with depth (Glin’ski and Stepniewski, 1985; Stepniewski and Stepniewska, 2009) due to larger diffusion distance to the free atmosphere. At the aggregate scale, pO2 decreases from the outside perimeter to the aggregate center, which can reach anoxic conditions (Sextone et al., 1985; Zausig et al., 1993). At the rhizosphere scale, O2 distribution from the root surface into bulk soil is driven by its consumption due to respiration processes and diffusive O2 supply (Glin’ski and Stepniewski, 1985). According to Raynaud (2010), a major part of the soil respiratory activity takes place in the rhizosphere, because of higher microbial activity compared to the bulk soil (Nunan et al., 2003) and root respiration (Kuzyakov, 2002).

The rhizosphere, i.e., the soil surrounding roots, which is influenced by its activity (Darrah, 1993), represents only about 1% of the total soil volume, but has an enormous ecological importance (Gregory, 2006; Pausch and Kuzyakov, 2011). It represents one of the hotspots in soil, where turnover of organic matter is increased compared to bulk soil due to higher microbial activity (Jones and Hinsinger, 2008). To sustain this activity, O2 has to be sufficiently transported into the rhizosphere (Hinsinger et al., 2009). One of the main limitations in studying pO2 and O2 transport in the rhizosphere are the temporal changes in air-filled porosity, microstructure formation, and displacement of the root active zone (Flessa, 1994). Con-

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centration and transport of O_2 in soil are independently well documented, but only few studies describe the spatial distribution of O_2 in the rhizosphere considering the interaction of respiration and transport at different matric potentials (Grable 1966; Grable and Siemer, 1968; Glirski and Stepienowski, 1985).

By metabolizing soil organic matter (SOM), aerobic microorganisms transfer electrons to an end acceptor, in this case O_2. This process plays a fundamental role for the mineralization of SOM (Brzezinska et al., 1998; Jones et al., 2004; Allaire et al., 2008). If O_2 supply is low (e.g., because of high water contents blocking diffusion pathways in the pore network), microbes might switch from aerobic to anaerobic respiration. The descending order of alternative acceptors is NO_3^-, MnO_2, Fe(OH)_3, SO_4^{2-}, and CO_2 (Ponnampерuma, 1984; Fiedler et al., 2007). Under anaerobic conditions mineralization of SOM decreases, hence, nutrient availability for plants (Drew et al., 1988). The redox potential (E_H) is therefore an important indicator determining the oxidation–reduction state in the soil (Mansfeldt, 2004). Several authors have proposed critical ranges for E_H indicating lack of O_2 (e.g., Reddy et al., 2000; Spósito, 1989). Most of them agree that in soils with neutral pH a threshold can be set between 300 and 400 mV to separate oxic from anoxic conditions. A well-known classification of the oxidation/reduction status of soils is the one proposed by Zhi-Guang (1985), in which levels > 400 mV represent an oxidizing status (O_2 as predominant electron acceptor), between 400 to 200 mV are weakly reducing (O_2, NO_3^-, and MnO_2), between 200 to ~100 mV are moderately reducing (Fe(OH)_3), and < ~100 mV are strongly reducing (SO_4^{2-} and CO_2).

Hotspots of microbiological activity occurring in the rhizosphere may have great influence on E_H. Thus, E_H is highly variable in time and space. Furthermore, its variability also depends on matric potential changes resulting from precipitation events or groundwater table changes (Fiesca and Fischer, 1992; Mansfeldt, 2003; Fiedler et al., 2007; Hinsinger et al., 2009). Due to the well-known triggering effect of O_2 on microbial activity and redox processes, gradients of E_H are expected to occur from root surface into bulk soil as a function of the air-filled porosity. Previous studies focused mainly on wetland cultivations or were conducted in sterile media (e.g., agar solutions) neglecting the highly variable properties of soils such as complex mineral composition, gas permeability, poise capacity, and microbial diversity amongst others (Fischer et al., 1989).

We hypothesize that (1) increased respiratory activity at the root surface results in decreasing pO_2 from bulk soil to the root surface, (2) the difference in pO_2 depends on matric potential, as it controls O_2 supply from bulk soil to the root surface, and (3) E_H dynamics in the rhizosphere are a function of the mentioned pO_2 gradients. To test these hypotheses, pO_2 and E_H gradients from the bulk soil to the root surface of alfalfa (Medicago sativa L.) depending on matric potential were measured in a jointed pot experiment. The aim of our study was to determine (1) the required air-filled porosity to sustain aerobic conditions in the rhizosphere and (2) the extent of the rhizosphere in terms of pO_2 and E_H.

2 Material and Methods

2.1 Experimental setup

Alfalfa (Medicago sativa L.) was grown in a three-compartment pot (Fig. 1) in a similar setup as used by Hatner et al. (2014). Soil material derived from a Haplic Luvisol (IUSS Working Group WRB, 2006) taken from the experimental station Klein Altendorf of the University of Bonn (50°37’21”N, 6°59’29”E). Two pots were completely filled with homogenized topsoil (0–30 cm, silty loam) and two with subsoil (45–75 cm, silty clay loam) at a bulk density of 1.2 g cm\(^{-3}\). The central compartment contained roots, whereas the roots could not penetrate into both rhizosphere compartments (side parts) due to a nylon gauze with a mesh size of 1 μm on one side and 30 μm on the other (Kuchenbuch and Jungk, 1982). The lateral compartments were sealed with plastic caps containing holes with a grid size of 1 cm x 1 cm, at which the O_2 and E_H microsensors could laterally be inserted.

Alfalfa was planted at a density of 0.5 g seeds per pot into the root compartment. The experiment was conducted under controlled conditions: water content was daily adjusted to 80% of...
the water holding capacity and checked gravimetrically. Plants were watered from the top of the root compartment. The photoperiod was 14 h light and 10 h dark. Light intensity was 300 μmol m⁻² s⁻¹ and the room temperature was 23°C during light and 20°C during dark periods. Two months after sowing, the whole surface of the nylon gauze was covered with alfalfa roots and O₂ and E₉ gradients were determined.

2.2 O₂ and E₉ gradients and monitoring
An O₂ Clark-type microsensor (Unisense A/S, Aarhus, Denmark), with 100 μm diameter tip protected by a chirurgic needle, was used to determine the O₂ concentration. A two-point calibration was made to convert the mV output of the O₂ sensors to the O₂ concentration. A linear interpolation was used between O₂ saturated water (pO₂ = 20.95 kPa) and a yeast-agar solution (pO₂ = 0 kPa). For the E₉ measurements miniaturized platinum electrodes (100 μm diameter tip, Unisense A/S, Aarhus, Denmark) were used. A two-point calibration was made with two redox buffer solutions (Mettler Toledo Intl. Inc. Urdorf, Switzerland). No pH correction was made for the E₉ measurements as the pH of the soil (pH = 6.8) was almost neutral (Bohn, 1969; Mansfeldt, 2003).

Three sets of measurements were made in the rhizosphere of the alfalfa planted pots to assess gradients of O₂ and E₉ in space and time. The three sets were conducted sequentially, whereas the replication in each set was made in parallel to ensure equal conditions.

First, O₂ gradients from the bulk soil to the root surface were measured. The sensor was pushed gently into the lateral pot (from the side) by means of a micromanipulator. Measurements were done from the bulk soil in direction to the root surface in 0.5 mm steps (10 s each step). As the total length of the lateral pot was known (40 mm), inserting the microsensor by 40 mm was necessary to get to the rooted nylon gauze. Each measurement took approx. 15 min. Measurements were made within four different matric potential ranges (–10 to –30, –50 to –100, –200 to –700 and < –900 hPa). After watering the plants, matric potential was monitored by a ceramic mini-tensiometer installed in the lateral pot at a 10 mm distance from the root surface. When reaching the desired matric potential range, a pO₂ profile (insertion of the microsensor) was measured.

Second, gradients of O₂ and E₉ were simultaneously measured by inserting microsensors with the same procedure described before. Measurements were done at near saturation (matric potential range of –10 to –30 hPa) and near field capacity (–100 to –200 hPa) to limit respiration in the pots filled with topsoil.

Third, time series measurements were carried out in the pots filled with topsoil to assess temporal variations of O₂ concentration and E₉ at the root surface. Microsensors were placed at a distance of 0–1 mm near the root surface and sealed from the outside to prevent O₂ diffusion along the sensor needle. The pots were watered until saturation after insertion of the microsensors to reduce air-filled porosity and to induce anoxic conditions. Levels of pO₂ and E₉ were monitored at 1 s resolution for 20 h. Three conditions were tested:

(1) Watering 24 h after dry conditions (< –300 hPa) to simulate a heavy precipitation event: the soil was dried by evapotranspiration until a matric potential < –300 hPa was reached. After 24 h, the soil was watered until saturation and the measurement started.

(2) Watering 1 h after dry conditions to simulate drying/wetting cycles: after saturation, the soil was dried up to matric potential < –300 hPa by evapotranspiration. After 1 h it was saturated again and the measurement started.

(3) Keeping 48 h of wet conditions to simulate longer wet periods in which soil remains saturated for more days: the saturated condition of step 2 was maintained for two days at matric potential ranges of –10 to –30 hPa. Then the soil was fully saturated and the measurement started. The stress induced to the plants did not allow further measurements of time series on comparable conditions.

2.3 pH gradients
After pO₂ and E₉ measurements were performed, the side compartments were air-dried at 20°C for 7 d. The soil was sliced parallel to the root surface at steps 2, 4, 7, 10, 15, 20, 25, 30, and 40 mm from the root surface. The soil slices (e.g., from 0–2, 2–4, 4–7 mm, etc.) were collected in individual cups and mixed with 0.01 M CaCl₂-solution for pH measurement.

2.4 Estimation of soil air-filled porosity
Air-filled porosity was estimated from the water retention curve and matric potential measurements read from the mini-tensiometers. The texture of top and subsoil was analyzed by the method of wet sieving and precipitation (USDA, 2011). Sand, silt, and clay contents (Table 1) were used to fit the pedotransfer functions with the ROSETTA program (Schaap et al., 2001), which were used to calculate the van Genuchten (1980) soil water retention curve parameters (with restriction m = 1 – n⁻¹). The model number 3 was used, which considers textural percentages and bulk density as estimators. Although the uncertainty of the used pedotransfer functions may be elevated in some cases, our study was conducted with homogenized (unstructured) soil, thus, the estimated parameters are greatly related to texture and bulk density. The calculation of the water retention curve parameters allowed the estimation of the volumetric water content (θ, m³ m⁻³) at each matric potential. Air-filled porosity (θₐ, m³ m⁻³) was then calculated by the difference between total porosity and the corresponding water contents for each matric potential range.

2.5 Calculation of O₂ diffusivity and uptake
The O₂ relative diffusion coefficient (Dₒ/Dₛ) was calculated for the four matric potential ranges using the empirical Eq. 1 by Moldrup et al., 1997:

$$\frac{D_o}{D_s} = 0.66 \times \theta_a \left( \frac{\theta_a}{\theta_s} \right)^{\frac{12 \cdot m}{Q}}$$  \hspace{1cm} (1)

where Dₒ and Dₛ are the diffusion coefficients (m² s⁻¹) of O₂ in soil and in free air, θₐ (m³ m⁻³) is the air-filled porosity at a
given matric potential, \( \Theta \) (m\(^3\) m\(^{-3}\)) is the total soil porosity and "m" is an empirical parameter set equal to 6 for homogenized repacked soil (Moldrup et al., 1997).

Respiratory activity for each matric potential range was calculated by numerical modeling using a monolayer profile and constant diffusion coefficient. Assuming a constant O\(_2\) consumption rate \( q \) at a distance \( x \) from the free atmosphere with a known diffusion coefficient in soil \( D \), Glin´ski and Stepniewski (1985) calculated the O\(_2\) concentration \( C \) by combining Fick’s first law with the accumulated O\(_2\) uptake (that is assumed to be steady state) using Eq. 2:

\[
C = C_0 - \frac{q(2Lx - x^2)}{2D},
\]

where \( C \) is the O\(_2\) concentration (g m\(^{-3}\)) at a distance \( x \) (m) from a total layer of length \( L \) (m), \( C_0 \) (g m\(^{-3}\)) is the O\(_2\) concentration at the upper boundary condition (free atmosphere), \( q \) is the O\(_2\) consumption rate per unit soil (g m\(^{-3}\) s\(^{-1}\)) and \( D \) the
gas diffusion coefficient of soil at a given matric potential (m² s⁻¹). Solving for \( q \), as the concentration \( C \) and \( C_0 \) is known, we obtain:

\[
q = \frac{2D(C_0 - C)}{2Lx - x^2}.
\]  

(3)

Consumption of \( \text{O}_2 \) was compared between topsoil and subsoil pots to assess microbial activity in relation to the distance to the root surface and the transport of \( \text{O}_2 \) across the profile (40 mm).

3 Results

3.1 Gradients of \( \text{O}_2 \) from bulk soil to the root surface at different matric potentials

Generally, \( p\text{O}_2 \) increased with decreasing matric potential; however, differences between top- and subsoil were not observed (Fig. 2). Under nearly saturated conditions (−10 to −30 hPa) the \( p\text{O}_2 \) was very low between 0 to 30 mm distance from the root surface. Under field capacity conditions (−50 to −100 hPa) \( p\text{O}_2 \) decreased strongly from bulk soil (13.9 kPa in top- and 11.6 kPa in subsoil) up to \( \text{O}_2 \) depletion (1.1 kPa and 1.8 kPa, respectively) at the root surface. As the soil became dryer than field capacity (matric potential < −200 hPa), the air-filled porosity was high enough (> 9% for top- and 12% for subsoil) to supply the root surface with \( \text{O}_2 \).

The low \( p\text{O}_2 \) for the conditions −10 to −30 and −50 to −70 hPa did not differ significantly up to 9.5 (in topsoil) and 13.0 mm (in subsoil) distance to the root surface, respectively (Fig. 2). Under drier conditions (−200 to < −900 hPa) the \( p\text{O}_2 \) did not reach levels lower than 10 kPa, but still decreased near to the root surface. At 28 mm for topsoil and 24.5 mm for subsoil, under field capacity similar \( p\text{O}_2 \) levels were observed compared to drier conditions. Consequently, with respect to \( p\text{O}_2 \), we could define the rhizosphere perimeter between 10 and 25 mm from the root surface.

Changes in \( E_{\text{H}} \) within the rhizosphere showed a strong interaction with the water saturation defined as matric potential.

Under nearly saturated conditions (−10 to −30 hPa), lower \( E_{\text{H}} \) values were measured close to the root surface (2 mm) compared to the bulk soil, while under drier conditions (−100 to −200 hPa) only slight differences in \( E_{\text{H}} \) were determined (Fig. 3). Under nearly saturated conditions, the \( E_{\text{H}} \) values changed from weakly to moderately reducing at the root surface according to Zhi-Guang (1985), while under drier conditions the levels were classified as weakly reducing.

3.2 Dynamics of \( p\text{O}_2 \) and \( E_{\text{H}} \) at the root surface

A time delay of a few hours between changes of \( \text{O}_2 \) concentration and \( E_{\text{H}} \) occurred after saturation of the compartments filled with topsoil (Fig. 4). Starting from matric potential < −300 hPa (Fig. 4a), a short period of 7 h of low \( \text{O}_2 \) concentrations did not have an influence on \( E_{\text{H}} \). According to the classification of Zhi-Guang (1985), during the complete measurement a weakly reducing status was observed (Fig. 4a). After short drying (−300 hPa for 1 h) and rewetting (−10 hPa), a change in \( E_{\text{H}} \) was observed. This could be due to facultative microorganisms that might have changed to anoxic respiration after several hours of \( \text{O}_2 \) lack (Fig. 4b). Only under prolonged periods of water saturated conditions (Fig. 4c), \( E_{\text{H}} \) reached moderately reducing values, in which reduction of other elements as Fe and Mn started.

3.3 \( \text{pH} \) gradients from bulk soil to the root surface

The \( \text{pH} \) of the studied bulk soil was neutral with values of 6.5 to 7.2. A gradient could be observed near to the root surface where more acidic conditions (down to 5.7) were found. The root-induced acidification was stronger in the pots filled with subsoil compared to those filled with topsoil (Fig. 5).

3.4 Consumption of \( \text{O}_2 \) in the rhizosphere as affected by matric potential

The \( p\text{O}_2 \) decreased linearly from bulk soil in direction to the root surface, indicating that the \( \text{O}_2 \) decrease was determined by \( \text{O}_2 \) supply and constant \( \text{O}_2 \) consumption (Fig. 2). On the contrary, an increase in slope steepness of the linear relation...
near to the root surface indicated increasing O₂ consumption due to higher biological activity (Fig. 6). Below a matric potential of –50 hPa, O₂ consumption was relatively evenly distributed, with a small increase near the root surface. Between –50 to –100 hPa, 100 times less O₂ was consumed in comparison to drier conditions. The air-filled porosity was found to be low (2% to 4% for topsoil and subsoil, respectively) causing low diffusion of O₂ to the root surface. Under nearly saturated conditions (matric potential of –10 to –30 hPa) the air-filled porosity was too low to deliver enough O₂ to the rhizosphere and O₂ consumption decreased close to zero (Fig. 6).

4 Discussion

4.1 Distribution of O₂ in the rhizosphere

It is well known that the distribution of O₂ depends on a variety of factors. Here we showed that, as hypothesized, the distribution of O₂ from bulk soil to the root surface is driven by rhizosphere respiration. At the same time, our second hypothesis was confirmed, as the ability to balance O₂ consumption near to the root is mainly controlled by matric potential (Fig. 2). Biologically-mediated processes of O₂ consumption, such as root and microbial respiration, also played a fundamental role. To which extent O₂ can be transported in the rhizosphere depends on air-filled porosity. Thus, the matric potential is the driving parameter, because it controls the presence of water-blocked pores. A direct relation can be established between air-filled porosity and the Fick’s gas diffusion coefficient. Many authors suggest an exponential increase of the diffusion with higher air-filled porosity (Buckingham, 1904; Millington and Quirk, 1961; Ball, 1981) while others suggest a linear relation (Penman, 1940; Anderson et al., 2000) or combined relations (Deepagoda et al., 2011).

Low water content will favor O₂ transport but will be a limiting factor for root water and nutrient uptake and for microbial respiration. Balogh et al. (2011) found higher respiration rates under water contents around 30–40% in a structured silty-clay-loam, which represented field capacity conditions (around –100 to –200 hPa). Under these conditions our homogenized soil was too saturated (Fig. 2) to transport enough O₂ to the rhizosphere. A balance between air-filled porosity and water content should be optimal for plant growth. Our results showed a clear distinction beyond the threshold of –200 hPa matric potential, at which air-filled porosity reached 9% and 12% for top- and subsoil, respectively. Above this critical values enough O₂ could be transported to the rhizosphere and soil respiration increased exponentially (Figs. 2 and 6). This agrees with the general rule of 10% air-capacity estab-
lished by Wesseling and van Wijk (1957) and confirmed by others (e.g., Grable, 1966; Robinson, 1964). Textural differences between topsoil and subsoil were not sufficient to influence air-filled porosity, making the calculated respiration similar for both materials, which might be due to the fact that we established the same bulk density for both soil materials.

### 4.2 Dynamics of E₈ in dependence of matric potential

We hypothesized that E₈ dynamics are a function of root-influenced O₂ gradients. Hence, if the O₂ supply is not sufficient because of a high water saturation, anoxic conditions occur, resulting in reduced (or almost none) microbial respiration (Hinsinger et al., 2006). In spite of its obviousness, most studies on E₈ in dryland cultivations do not report of root effects on its spatial variation (Richter et al., 2007). One of the few works describing rhizosphere gradients of E₈ in dryland conditions and in natural soil was introduced by Fischer et al. (1989). They determined the root tip as the active part on E₈ variation and could observe its influence up to 3 mm from the root surface. A limitation of this study was the constant water content, fixed at –60 hPa throughout the experiment, which resulted in variations inside the aerobic respiration range (400 to 800 mV). Other studies report effects of water table fluctuations, temperature, and SOM. Mansfeldt (2003; 2004) has found annual fluctuations between –160 and 560 mV in a Typic Endoaquoll marsh induced by water table fluctuations. These ranges are typical for long periods of water saturation followed by dryer conditions during summertime. In our study, E₈ values were also driven by matric potential (Fig. 3), showing weakly reducing conditions with a matric potential < –200 hPa and moderate reducing conditions when nearly saturated. We also found a critical spatial dependence of E₈ within the first millimeters close to the root surface, which is in agreement with the findings of Fischer et al. (1989). According to the Nerst equation, low E₈ in the rhizosphere could be an effect of pH changes: 59 mV per pH unit (Fiedler et al., 2007). We could observe a significant acidification of 0.5 to 1 pH unit near the root surface (Fig. 5). However, the effect of up to 1 pH unit on E₈ is negligible. Since the low E₈ values of 130 to 200 mV were measured together with pO₂, we state an O₂ lack (pO₂ < 1 kPa) as the primary effect on the rhizosphere E₈ profile defining reducing conditions.

Saturation after a dry period impacts directly the pO₂ but does have little immediate influence on E₈. This is in accordance to the findings of Ewing et al. (1991) and Reddy and Patrick (1975), who reported a shift between a decrease in O₂ concentration and changes in E₈ that can be as long as 2 d in soil samples. In our case of a rapid change between wet and dry conditions followed by re-wetting (Fig. 4b), E₈ seems to respond faster than the cited shifts. This is because our study deals with a more densely colonized environment, in which changes are much faster than in bulk soil experiments because of the higher microbial activity. Thus, the delay in response time of E₈ following limited O₂ availability is reduced to a few hours. This process could represent intermittent
showers in the field, which have a great impact on denitrification, as N loss is reported to be greater under wetting/drying cycles (Patrick and Gotoh, 1974). Although some authors see a benefit in low $E_w$, because $Fe^{2+}$ and $Mn^{2+}$ availability increases (Ponnampерума, 1984; Flessa and Fischer, 1992; Ste˛pniewski and Ste˛pniewska, 2009), most authors agree that a decrease in $pO_2$, and consequently in $E_w$, leads to a decrease in the nutrient uptake ability of most plants (Drew et al., 1988; Fiedler et al., 2007) together with other negative effects like lower mineralization of SOM, Fe and S phytotoxicity (Pezeshki et al., 1988), loss of mineral N (Ste˛pniewski and Ste˛pniewska, 2009), or reduced root growth (Ponnampерума, 1984; Fiedler et al., 2007) among others. In our study, limiting conditions for aerobic respiration were found only directly at the root surface and under nearly saturated conditions, which in the field could represent longer humid periods.

4.3 pH gradients from the bulk soil to the root surface

The greatest differences between topsoil and subsoil were found in the rhizosphere effect on pH (Fig. 5). Rhizosphere changes in pH are mostly driven by respiration activity and the $CO_2$ produced, especially in the region of the root tip (Flessa and Fischer, 1992). The $CO_2$ concentration in the rhizosphere is known to be about 10 to 100 times higher than that of the atmosphere (Pausch and Kuzyakov, 2012). In addition to water, it forms $H_2CO_3$ which as an acid decreases the pH. Rhizosphere pH levels can be up to 1–2 units below bulk soil pH (Hinsinger et al., 2009), which is confirmed by our results. As respiration rates were similarly distributed for the topsoil and the subsoil, similar pH gradients with respect to the root surface are expected in both materials. Nevertheless, in the subsoil a greater root induced acidification was observed, about one pH unit versus 0.5 pH units in the topsoil (Fig. 5). This can be explained on the one hand by the higher C content in the pots with topsoil (0.99% versus 0.52% in the subsoil pots, data not shown). SOM is known to play a key role in the pH buffering capacity, increasing it considerably in the pedon but also in the mm-scale, which could be observed in the rhizosphere of our experiment. On the other hand, the availability of soluble minerals is lower in deeper soil horizons, forcing plants to release more exudates and to larger distances to assess the nutrients (e.g., Gocke et al., 2014; Kautz et al., 2013). pH gradients were observed up to 20 mm from the root surface, which is a larger distance than reported by various authors based on short-term experiments of about 0.2 to 10 mm (Fischer et al., 1989; Kuzyakov et al., 2003; Sauer et al., 2006; Hinsinger et al., 2009). Our experiment (based on a root-mat technique) gives information of the average effect of many roots, possibly resulting in an overestimation of processes in contrast to single root measurements. This also explains the high respiration activity up to 20 mm as stated before. However, in the long-term, i.e., as a consequence of the whole lifetime of a root, even larger distances of rhizosphere effects of 5 cm or even more were described (Gocke et al., 2014). This clearly shows the high variability of rhizosphere effects at different distances depending on the experimental set-up and a certain need to investigate rhizosphere effects in the long-term to overcome effects of single experiments. There is also a need to improve our understand-

5 Conclusions

This study dealt with $O_2$ availability and transport in alfalfa rhizosphere. This study confirmed our three hypotheses, as $O_2$ and $Eh$ dynamics were clearly root driven. We found an extent of the rhizosphere for $O_2$ concentration up to 20 mm, while the root influence over $E_w$ was observed only up to about 2 mm. Matric potential played a predominant role in the $O_2$ transport, with a limiting threshold of $\approx 200$ hPa below which $O_2$ supply was not limited. About 9–12% air-filled porosity was found to be sufficient to transport $O_2$ for rhizosphere aerobic respiration activity. Under more saturated conditions, the $O_2$ consumption rates decreased about 100 times and moderately reducing conditions were found. Although these results were produced under controlled conditions with homogenized soil, the determined thresholds allow a better assessment of optimal air-filled porosity in natural environments.

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