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

Daniel Uteau[1]*, Silke Hafner[2], Sebastian Kouso Pagenkemper[3], Stephan Peth[1], Guido L. B. Wiesenberg[4], Yakov Kuzyakov[2,5,6], and **Rainer Horn[3]**

[1] Department of Soil Science, University of Kassel, Nordbahnhofstraße 1a, 37213, Witzenhausen, Germany

[2] Department of Soil Science of Temperate Ecosystems, Georg-August-University of Göttingen, Büsgenweg 2, 37077, Göttingen, Germany

[3] Institute of Plant Nutrition and Soil Science, Christian-Albrecht-University of Kiel, Hermann-Rodewald-Str. 2, 24118, Kiel, Germany

[4] Department of Geography, University of Zurich, Winterthurerstraße 190, 8057 Zurich, Switzerland

[5] Department of Agricultural Soil Science, Georg-August-University of Göttingen, Büsgenweg 2, 37077, Göttingen, Germany

[6] Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia

Abstract

Oxygen (O_2) supply and the related redox potential (E_H) are important parameters for interactions between roots and microorganisms in the rhizosphere. Rhizosphere extension in terms of the spatial distribution of O_2 concentration and E_H is poorly documented under aerobic soil conditions. We investigated how far O_2 consumption of roots and microorganisms in the rhizosphere is replenished by O_2 diffusion as a function of water/air-filled porosity. Oxygen concentration and E_H in the rhizosphere were monitored at a mm-scale by means of electroreductive Clark-type sensors and miniaturized E_H electrodes under various matric potential ranges. Respiratory activity of roots and microorganisms was calculated from O_2 profiles and diffusion coefficients. pH profiles were determined in thin soil layers sliced near the root surface.

Gradients of O_2 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 O_2 transport in the rhizosphere. Under water-saturated conditions and near field capacity up to –200 hPa, O_2 transport was limited, causing a decline in oxygen partial pressures (pO_2) 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 O_2 increased considerably. This was confirmed by E_H around 300 mV under aerated conditions, while E_H decreased to 100 mV on the root surface under near water-saturated conditions. Gradients of pO_2 and pH from the root surface indicated an extent of the rhizosphere effect of 10–20 mm. In contrast, E_H gradients were observed from 0 to 2 mm from the root surface. We conclude that the rhizosphere extent differs for various parameters (pH, Eh, pO_2) and is strongly dependent on soil moisture.

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

1 Introduction

One of the most important factors influencing aerobic activity in soil is the availability of molecular oxygen (O_2). Spatial distribution of O_2 in soil depends strongly on the investigated scale. At the pedon scale, higher O_2 partial pressure (pO_2) are found in the topsoil and gradually decrease with depth (*Gliński* and *Stępniewski*, 1985; *Stępniewski* and *Stępniewska*, 2009) due to larger diffusion distance to the free atmosphere. At the aggregate scale, pO_2 decreases from the outside perimeter to the aggregate center, which can reach anoxic conditions (*Sexstone* et al., 1985; *Zausig* et al., 1993). At the rhizosphere scale, O_2 distribution from the root surface into bulk soil is driven by its consumption due to respiration processes and diffusive O_2 supply (*Gliński* and *Stępniewski*, 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, O_2 has to be sufficiently transported into the rhizosphere (*Hinsinger* et al., 2009). One of the main limitations in studying pO_2 and O_2 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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* Correspondence: Dr. D. Uteau; e-mail: uteau@uni-kassel.de

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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; *Gliński* and *Stępniewski*, 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 (*Brzezińska* 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 (*Ponnamperuma*, 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; *Sposito*, 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 (*Flessa* 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 *Hafner* 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
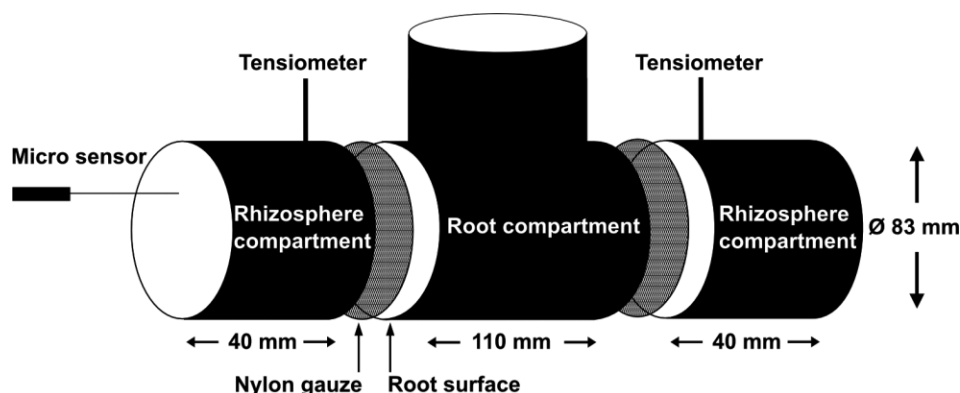
Figure 1: Setup of the experimental pots to study oxygen partial pressure and redox potential gradients in the rhizosphere of alfalfa. The three-compartment pots consisted of a central root compartment and two side compartments representing the rhizosphere of alfalfa. Nylon gauze prevented roots to penetrate into the rhizosphere compartments. The root surface of alfalfa is represented by the nylon gauze, after complete coverage by alfalfa roots.

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 O$_2$ and E$_H$ gradients were determined.

2.2 O$_2$ and E$_H$ gradients and monitoring

An O$_2$ Clark-type microsensor (Unisense A/S, Aarhus, Denmark), with 100 μm diameter tip protected by a chirurgical needle, was used to determine the O$_2$ concentration. A two-point calibration was made to convert the mV output of the O$_2$ sensors to the O$_2$ concentration. A linear interpolation was used between O$_2$ saturated water (pO$_2$ = 20.95 kPa) and a yeast-agar solution (pO$_2$ = 0 kPa). For the E$_H$ 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 E$_H$ 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 asses gradients of O$_2$ and E$_H$ 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, O$_2$ 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 pO$_2$ profile (insertion of the microsensor) was measured.

Second, gradients of O$_2$ and E$_H$ 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 O$_2$ concentration and E$_H$ 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 O$_2$ 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 pO$_2$ and E$_H$ 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 pO$_2$ and E$_H$ 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 CaCl$_2$-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 minitensiometers. 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, m^3 m^{-3}) at each matric potential. Air-filled porosity (θ$_a$, m^3 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 O$_2$ diffusivity and uptake

The O$_2$ relative diffusion coefficient (D$_s$/D$_o$) was calculated for the four matric potential ranges using the empirical Eq. 1 by (*Moldrup* et al., 1997):

$$\frac{D_s}{D_o} = 0.66 \times \theta_a \left(\frac{\theta_a}{\Theta}\right)^{\frac{12-m}{3}}, \tag{1}$$

where D_s and D_o are the diffusion coefficients (m^2 s^{-1}) of O$_2$ in soil and in free air, θ_a (m^3 m^{-3}) is the air-filled porosity at a

Table 1: Texture analysis for topsoil and subsoil and hydraulic parameters estimated by pedotransfer functions.

	Depth / cm	Sand	Silt / %	Clay	Class[a]	θr^{a} / $m^3\ m^{-3}$	θs^{a} / $m^3\ m^{-3}$	α^{a}	n^{a}
Topsoil (Ap)	0–30	16	67	17	SiL	0.071	0.460	0.0045	1.712
Subsoil (Bt)	45–75	12	59	29	SiCL	0.087	0.494	0.0070	1.565

[a]*USDA* (2011) texture classification. θr, θs, α, and n are the *van Genuchten* (1980) water retention parameters.

given matric potential, Θ ($m^3\ m^{-3}$) 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, *Gliński* and *Stępniewski* (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:

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

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
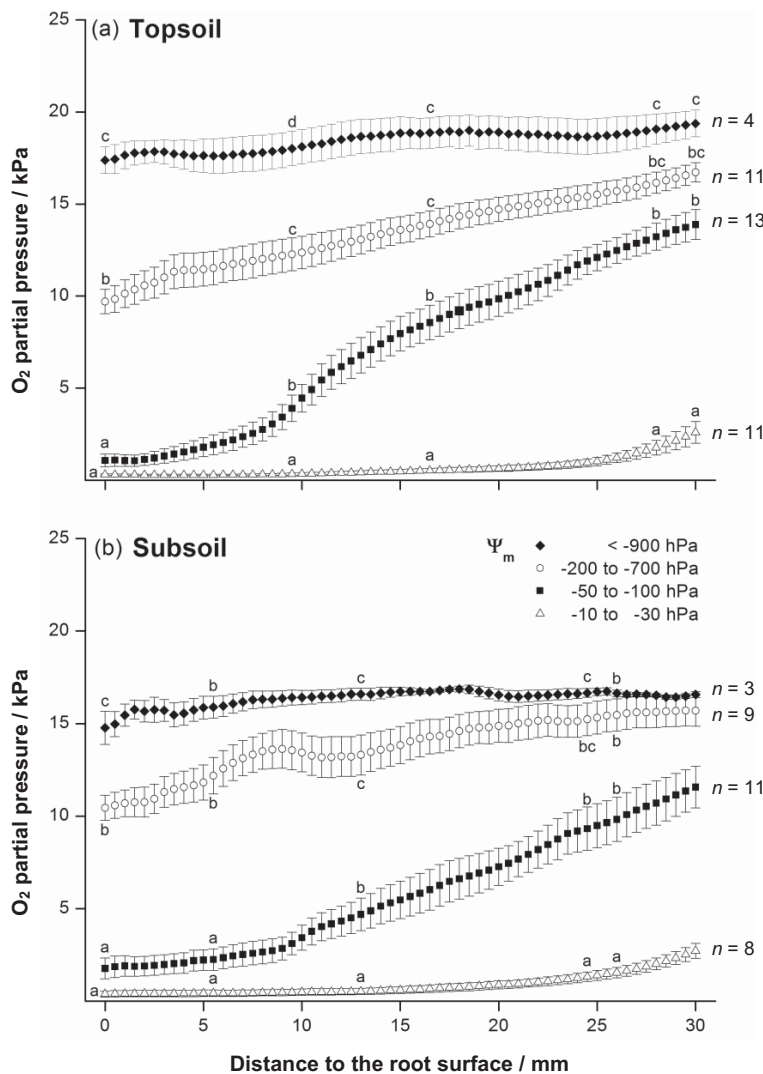
Figure 2: Oxygen partial pressure gradients from the bulk soil to the surface of alfalfa roots as a function of matric potential (Ψ_m) in top- (a) and subsoil (b). Error bars represent standard error of the mean. Different letters represent statistical differences between curves at same distance, after ANOVA test followed by Tukey HSD at $p < 0.05$.

gas diffusion coefficient of soil at a given matric potential ($m^2\ s^{-1}$). Solving for q, as the concentration C and C_0 is known, we obtain:

$$q = \frac{2D(C_0 - C)}{2Lx - x^2},\qquad(3)$$

Consumption of 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 O_2 across the profile (40 mm).

3 Results

3.1 Gradients of O_2 from bulk soil to the root surface at different matric potentials

Generally, pO_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 pO_2 was very low between 0 to 30 mm distance from the root surface. Under field capacity conditions (–50 to –100 hPa) pO_2 decreased strongly from bulk soil (13.9 kPa in top- and 11.6 kPa in subsoil) up to 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 O_2.

The low pO_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 pO_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 pO_2 levels were observed compared to drier conditions. Consequently, with respect to pO_2, we could define the rhizosphere perimeter between 10 and 25 mm from the root surface.

Changes in E_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_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_H were determined (Fig. 3). Under nearly saturated conditions, the E_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 pO_2 and E_H at the root surface

A time delay of a few hours between changes of O_2 concentration and E_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 O_2 concentrations did not have an influence on E_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_H was observed. This could be due to facultative microorganisms that might have changed to anoxic respiration after several hours of O_2 lack (Fig. 4b). Only under prolonged periods of water saturated conditions (Fig. 4c), E_H reached moderately reducing values, in which reduction of other elements as Fe and Mn started.

## 3.3 pH gradients from bulk soil to the root surface

The 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 O_2 in the rhizosphere as affected by matric potential

The pO_2 decreased linearly from bulk soil in direction to the root surface, indicating that the O_2 decrease was determined by O_2 supply and constant O_2 consumption (Fig. 2). On the contrary, an increase in slope steepness of the linear relation
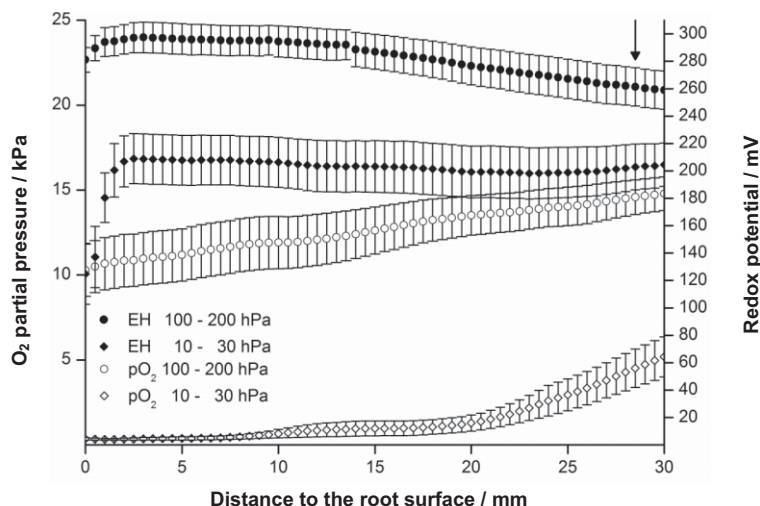
Figure 3: Oxygen partial pressure (pO_2) and associated redox potential (E_H) values in topsoil as affected by matric potential. The pO_2 is significantly different from 0 to 30 mm. Values of E_H show significant differences up to 28.5 mm (arrow). Error bars represent standard error of the mean. Statistical differences between curves at same distance were tested by ANOVA followed by Tukey HSD at $p < 0.05$, $n = 15$.
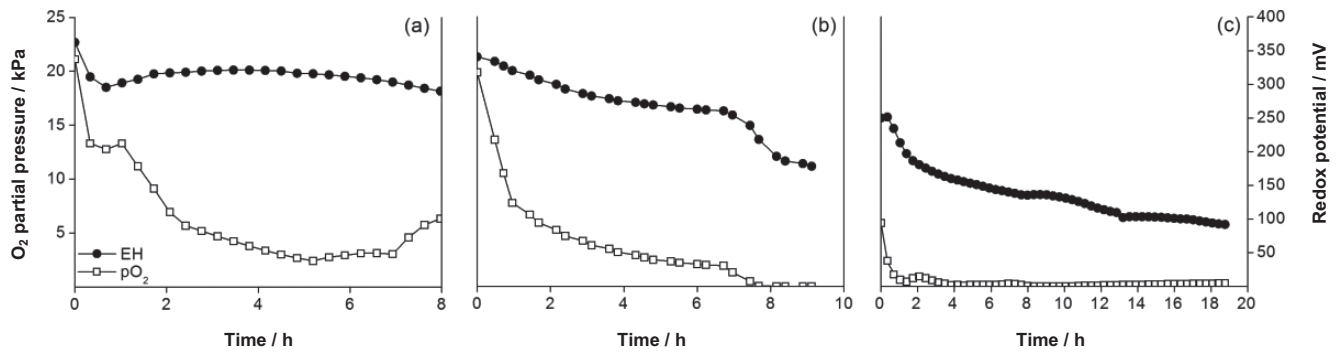
Figure 4: Dynamics of oxygen partial pressure (pO_2) and redox potential (E_H) at alfalfa root surface in topsoil. (a) saturation of the soil after 1 d of dry conditions (< -300 hPa); (b) saturation after one hour of dry conditions (< -300 hPa); (c) after 2 d of wet conditions (-10 to -30 hPa).

near to the root surface indicated increasing O_2 consumption due to higher biological activity (Fig. 6). Below a matric potential of –50 hPa, O_2 consumption was relatively evenly distributed, with a small increase near the root surface. Between –50 to –100 hPa, 100 times less O_2 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 O_2 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 O_2 to the rhizosphere and O_2 consumption decreased close to zero (Fig.6).

4 Discussion

4.1 Distribution of O_2 in the rhizosphere

It is well known that the distribution of O_2 depends on a variety of factors. Here we showed that, as hypothesized, the distribution of O_2 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 O_2 consumption near to the root is mainly controlled by matric potential (Fig. 2). Biologically-mediated processes of O_2 consumption, such as root and microbial respiration, also played a funda-

mental role. To which extent O_2 can be transported in the rhizosphere is 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 O_2 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 O_2 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 O_2 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-
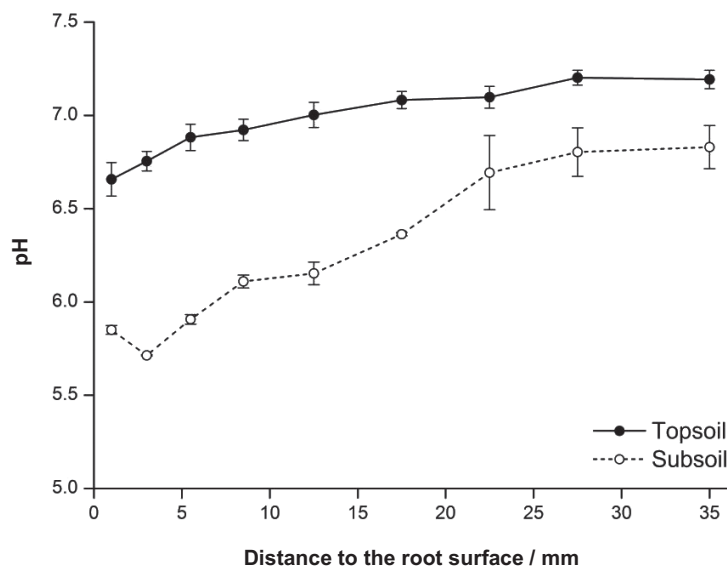
Figure 5: pH gradient from the bulk soil to the root surface for top and subsoil. Error bars represent standard error of the mean ($n = 4$).
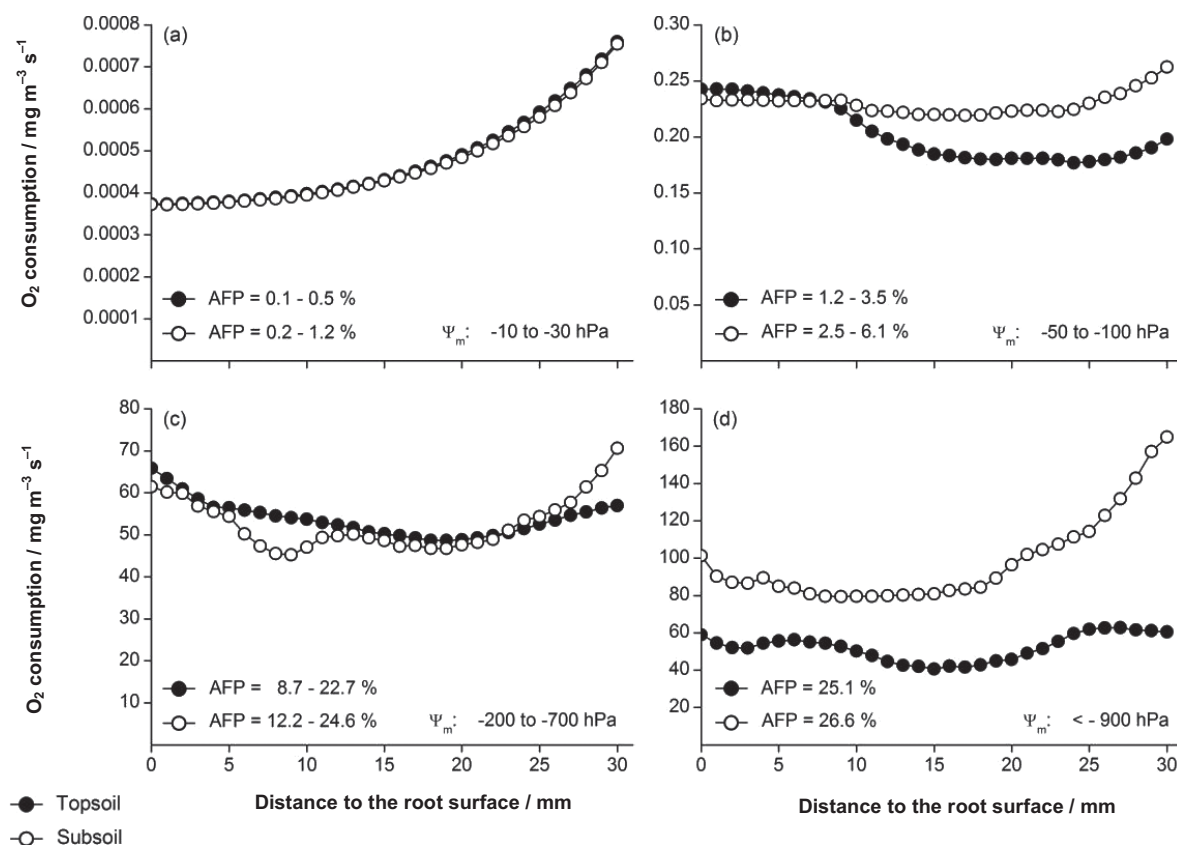
Figure 6: Calculated O_2 consumption (mg O_2 m^{-3} soil s^{-1}) depending on matric potential (Ψ_m) ranges in top- (black dots) and subsoil (white dots). AFP = Air-filled porosity.

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 E_H in dependence of matric potential

We hypothesized that E_H dynamics are a function of root-influenced O_2 gradients. Hence, if the O_2 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 E_H 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 E_H in dryland conditions and in natural soil was introduced by *Fischer* et al. (1989). They determined the root tip as the active part on E_H 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, E_H 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 E_H within the first millimeters close to the root surface, which is in agreement with the findings of *Fischer* et al. (1989). According to the Nernst equation, low E_H 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 E_H is negligible. Since the low E_H values of 130 to 200 mV were measured together with pO_2, we state an O_2 lack (pO_2 < 1 kPa) as the primary effect on the rhizosphere E_H profile defining reducing conditions.

Saturation after a dry period impacts directly the pO_2 but does have little immediate influence on E_H. 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 O_2 concentration and changes in E_H 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), E_H 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 E_H following limited O_2 availability is reduced to a few hours. This process could represent intermittent

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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_H, because Fe^{2+} and Mn^{2+} availability increases (*Ponnamperuma*, 1984; *Flessa* and *Fischer*, 1992; *Stępniewski* and *Stępniewska*, 2009), most authors agree that a decrease in pO_2, and consequently in E_H, 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 (*Stępniewski* and *Stępniewska*, 2009), or reduced root growth (*Ponnamperuma*, 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 understanding on root- and rhizosphere-related processes, which are especially relevant for nutrient uptake and C storage in the subsoil (*Rumpel* and *Kögel-Knabner*, 2011; *Kautz* et al., 2013)

5 Conclusions

This study dealt with O_2 availability and transport in alfalfa rhizosphere. This study confirmed our three hypotheses, as O_2 and E_H 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_H was observed only up to about 2 mm. Matric potential played a predominant role in the O_2 transport, with a limiting threshold of –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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