Biophysical factors controlling root exudation and rhizosphere extension

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Maire Holz
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Amtierender Dekan: Prof.Dr. Stefan Peiffer

Prüfungsausschuss

Prof. Dr. Andrea Carminati (Gutachter)
Jun-Prof. Dr. Johanna Pausch (Gutachterin)
Prof. Dr. Egbert Matzner (Vorsitz)
Prof. Dr. Britta Planer-Friedrich

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Abstract

The rhizosphere, the region of soil near the roots, plays a crucial role in water and nutrient uptake by plants because root exudates enhance microbial activity and nutrient availability to plants. The degree to which root exudates can interact positively with plants depends on their spatiotemporal distribution around the roots. The objective of this work was therefore to understand what factors control the spatiotemporal distribution of root exudates in soil. We investigated how root traits such as root hairs and mucilage secretion and rhizosphere water related properties mediate the distribution of root exudates in soil.

The first experiment focused on the effect of soil drying on rhizosphere extension. We coupled $^{14}CO_2$ labelling and phosphor imaging of plants with neutron radiography to image ^{14}C allocation and rhizosphere water content (WC). Exudate distribution around growing roots was predicted by a numerical model. Plants grown in dry and wet soil allocated similar amounts of ^{14}C into roots but root elongation decreased by 48% in dry soil, reducing longitudinal rhizosphere extension. Rhizosphere WC was identical in dry and wet soils, presumably because of the high water retention by mucilage. The increase in rhizosphere WC enhanced root exudation in dry soil and enlarged the radial rhizosphere extension. The release of mucilage may be beneficial to plants because it maintains fast diffusion of exudates and high rhizospere extension under water limitation.

Alongside low molecular carbon compounds, roots release enzymes to mineralize nutrients. The objective of the second experiment was therefore to test how the local soil WC affects phosphatase activity in soil. Barley plants were grown in rhizoboxes and subjected to a drying cycle, while soil water content (WC) and phosphatase activity were monitored by neutron radiography and soil zymography.. Phosphatase activity and soil WC were strongly correlated in rhizosphere and bulk soil. The power-law relation between soil WC and enzyme activity confirmed our hypothesis that enzyme activity is controlled by diffusion. Phosphatase activity in the rhizosphere, having a high WC compared to the bulk soil, was significantly larger than phosphatase activity in the bulk soil. This can be explained by mucilage released into the rhizosphere which retain water upon soil drying.

The first tow studies highlight the effect of mucilage on root exudate distribution. However, so far no method is available to measure the spatial distribution of mucilage

in soil. The aim of the third experiment was therefore to test whether the C-H signal caused by fatty acids in mucilage can be measured with infrared (IR) spectroscopy to determine the spatial distribution of mucilage in soil. Measurements were carried out along transects of 1.5 mm perpendicular to root channels of plants grown in rhizoboxes. We measured distinct profiles of C-H and the amounts of mucilage we measured were comparable with results obtained in previous studies. IR spectroscopy is therefore a promising tool to measure the spatial distribution of mucilage in soil.

The aim of the fourth study was to test whether root hairs exude C to enlarge rhizosphere extension. Barley wild type (WT) and its root-hairless mutant (*brb*) were grown in rhizoboxes and labelled with $^{14}CO_2$. Root exudates were captured on filter paper and quantified by phosphor imaging. WT plants allocated more carbon (C) below ground while the hairless mutant allocated more C to shoots. Root hairs increased the radial rhizosphere extension 3-fold, from 0.5 to 1.5 mm. Total exudation was 3 times greater for WT plants compared to the hairless mutant. The increase in rhizosphere extension may enhance the positive effect of root exudates to plants.

While ^{14}C imaging of root exudates on filter paper was successfully applied in the fourth study, this approach is restricted to moist soil conditions. In the last study we therefore tested whether ^{14}C imaging on the soil-root surface can be applied to quantify root exudates. The attenuation coefficient of ^{14}C in soil was calculated and the expected ^{14}C profiles were calculated. The profiles were strongly affected by: a) the ^{14}C activity in the root, b) the root radius, c) the position of the root, d) the amount of root exudates and e) by the presence of air gaps between soil and imaging screen. Inaccurate measurements of any of these parameters would cause artefacts in the estimation of root exudates distribution in the rhizosphere using phosphor imaging.

By combining complementary imaging methods and numerical modelling we showed that root hair production and mucilage release increase rhizosphere extension and total exudation. Mucilage movement was restricted to shorter distances from the root surface (0.6 mm) compared to overall exudates (1.2 mm). Nevertheless, mucilages water retention strongly affects the diffusion of low molecular root exudates and enzymes. Root hairs increase total exudation and rhizosphere extension, which probably enhances rhizosphere interactions in larger soil volumes. Breeding for long and dense root hairs as well as increased mucilage secretion may be suitable strategies for future agriculture where nutrients are expected to become scarce.

Zusammenfassung

Die Rhizosphäre, der Boden der die Pflanzenwurzeln umgibt, spielt eine zentrale Rolle für die Nähstoff- und Waseraufnahme der Pflanzen da Wurzelexsudate, die von der Wurzel abgegeben werden die mikrobielle Aktivität und die Nährstofverfgbarkeit erhöhen. Das Ausmaß, mit dem Wurzelexsudate positiv mit der Pflanze interagieren können, hängt vor allem von der räumlichen und zeitlichen Verteilung der Wurzelexsudate in der Rhizosphäre ab. Das Ziel dieser Arbeit war es daher, zu untersuchen welche Faktoren die räumliche und zeitliche Verteilung der Wurzelexsudate im Boden bestimmen. Der Fokus lag dabei sowohl auf Wurzeleigenschaften wie der Produktion von Wurzelhaaren und der Abgabe von Mucilage als auch auf der Wasserverteilung in der Rhizosphäre.

Der Schwerpunkt des ersten Experiments lag auf der Untersuches des Effekt von Trockenstress auf die Verteilung von niedermolekularen Wurzelexsudaten im Boden. Pflanzen wurden mit $^{14}CO_2$ markiert um die ^{14}C Verteilung in Pflanzen und Boden mithilfe von Phosphor Imaging zu untersuchen. Der Wassergehalt in der Rhizosphäre wurde mithilfe von Neutronenradiographie quantifiziert. Die Abgabe und Verteilung der Exsudate wurde durch ein numerisches Modell vorhergesagt. Pflanzen im trockenen und feuchten Boden verlagerten gleich viel ^{14}C in die Wurzeln allerdings war das Wurzelwachstum für Pflanzen im trockenen Boden um 48% reduziert. Das fuührte zu einer starken Reduzierung der longitudinalen Rhizosphärenausdehnung. Der Wassergehalt in der Rhizosphäre war identisch im trockenen und feuchten Boden was durch Mucilage erklärte werden kann, die große Mengen an Wasser im Boden zurückhält. Der erhöhte Wassergehalt in der Rhizosphäre steigerte die Wurzelexsudation und die Rhizosphärenausdehnung besonders im trockenen Boden. Die Abgabe von Mucilage ist daher vorteilhaft für die Pflanze da sie die Diffusion von Wurzelexsudaten und die Rhizosphärenausdehnung besonders bei Wasserknappheit erhöht.

Neben niedermolekularen Wurzelexsudaten scheiden Wurzeln Enzyme aus um Nährstoffe zu mineralisieren. Das Ziel der zweiten Studie war es daher, zu untersuchen, wie der lokale Bodenwassergehalt die Phosphataseaktivität im Boden beeinflusst. Gerstenpflanzen wurden in Rhizoboxen angezogen und Neutronenradiographie mit Zymography kombiniert. Die Messungen wurden während eines Trockenzyklus durchgeführt. Die Phosphataseaktivität korrelierte stark mit dem Bodenwassergehalt sowohl im Rhizosphärenboden als auch im Gesamtboden. Die Tatsache, dass die Korrelation zwis-

chen Enzymaktivität und Wassergehalt durch eine Potenzfunktion beschrieben wurde, bestätigte unsere Hypothese, dass die Enzymaktivität im Boden vor allem durch Diffusion bestimmt wird. Die Phosphataseaktivität in der Rhizosphäre war im Vergleich zum Gesamtboden erhöht, da diese einen erhöhen Wassergehalt im Vergleich zum Gesamtboden aufwies. Diese Beobachtung kann durch Mucilage und EPS erklärt werden, die in die Rhizosphäre abgegeben werden und besonders under trockenen Bedingungen Wasser im Boden zurückhalten.

Die ersten beiden Studien heben die Bedeutung von Mucilage für die Verteilung der Wurzelexsudate im Boden hervor. Allerdings gibt es bisher keine Methode um die räumliche Verteilung von Mucilage im Boden zu messen. In der dritten Studie testeten wir daher ob das C-H Signal, das durch Fettsäuren verursacht wird, die in Mucilage enthalten sind, mithilfe von infrarot Spektroskopie (IR) gemessen werden kann und ob mit dieser Methode die räumliche Verteilung von Mucilage im Boden bestimmt werden kann. Die Messungen wurden entlang von 1.5 mm langen Transekten durchgeführt, die rechtwinklig zum Wurzelkanal der Pflanzen gelegt wurden. Die Ergebnisse zeigten deutliche Profile des C-H Signals, das von der Wurzel weg abfiel. Der gemessene Mucilagegehalt im Boden war vergleichbar mit den Ergebnissen voriger Studien. IR Spektroskopie stellt daher eine vielversprechende Methode für die Messung der räumlichen Verteilung von Mucilage im Boden dar.

Das Ziel der vierten Studie war es, zu testen ob Wurzelhaare organische Substanzen in den Boden abgeben und daher die Rhizosphärenausdehnung vergrößern. Gerste (WT) mit Wurzelhaaren und der vergleichbare Mutant ohne Wurzelhaare (*brb*) wurden in Rhizoboxen angezogen und mit $^{14}CO_2$ markiert. Die Wurzelexsudate wurden auf Filterpapier aufgefangen und mithilfe von Phospor Imaging quantifiziert. Pflanzen mit Wurzelhaaren verlagerten mehr Kohlenstoff (C) in den Boden, während Pflanzen ohne Wurzelhaare mehr C in den Spross verlagerten. Wurzelhaare führten zu einer 3-fach erhöhten Rhizosphärenausdehnung von 0.5 zu 1.5 mm. Die gesamte Exsudation war ebenfalls 3-fach erhöht für Pflanzen mit Wurzelhaaren verglichen mit dem Mutanten ohne Wurzelhaare. Die erhöhte Rhizosphärenausdehnung erhöht vermutlich die positiven Interaktionen zwischen Wurzelexsudaten und Pflanzen und ist daher vorteilhaft für die Pflanzen.

Während ^{14}C Imaging von Wurzelexsudaten auf Filterpapier erfolgreich in der vorigen Studie angewandt wurde, ist dieser Ansatz auf feuchte Bodenbedingungen beschränkt. Das Ziel der letzten Studie war es daher zu testen, ob ^{14}C Imaging

direkt auf der Boden-Wurzeloberfläche angewandt werden kann um Wurzelexsudate zu visualisieren und zu quantifizieren. Der Abschwächungskoeffizient von ^{14}C im Boden wurde berechnet und die zu erwartenden ^{14}C Profile berechnet. Die Profile waren stark beeinflusst von a) der ^{14}C Aktivität der Wurzel, b) dem Wurzelradius, c) der Position der Wurzel im Boden, d) der Menge der Wurzelexsudate im Boden und e) von möglichen Luftspalten zwischen Boden und Imaging Screen. Eine ungenaue Messung von einem dieser Parameter würde zu Artefakten in der Schätzung der Verteilung der Wurzelexsudate in der Rhizosphäre führen.

Durch die Kombination von verschiedenen bildgebenden Verfahren und numerischer Modellierung konnten wir zeigen, dass die Abgaben von Mucilage und die Produktion von Wurzelhaaren zu einer Erhöhung der Wurzelexsudation und der Rhizosphärenausdehnung führt. Mucilage war in einem engeneren Umkreis um die Wurzel verteilt (0.6 mm) als die gesamten Wurzelexsudate (1.2 mm). Es kann allerdings angenommen werden, dass sie aufgrund ihrer Wasserhaltefähigkeit trotzdem einen großen Einfluss auf die Verteilung von niedermolekularen Wurzelexsudaten und von Enzymen hat. Wurzelhaare führten zu einer erhöhten Wurzelexsudation und erweiterten die Ausdehnung der Rhizosphäre was vermutlich zu erhöhten Interaktionen in größeren Bodenvolumina führt. Die Züchtung auf sowohl erhöhte Mucilage Sekretion als auch auf lange und dichte Wurzelhaare könnte daher eine angemessene Strategie für die zukünftige Landwirtschaft sein, da Nährstoffe voraussichtlich knapper werden.

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In chapters marked with * I contributed as co-author, in the other chapters as first author.

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List of Abbreviations

ANOVA	Analysis of variance
brb	Barley mutant without root hairs
C	Carbon
^{14}C	Radioactive carbon isotope with atomic mass 14
d	Thickness
D	Diffusion coefficient of glucose in soil
D_0	Diffusion coefficient of glucose in water
D_{root}	Diffusion coefficient of glucose in root tissue
DRIFT	Diffuse reflectance infrared Fourier transform
EPS	Extracellular Polysaccharides
E	Exudation
FTIR	Fourir-transform infrared spectroscopy
G	Gradiation
I	Image
I_{dc}	Dark current: signal recorded by camera with no neutron beam
I_{ff}	Flat field: neutron radiograph taken without any sample
I_{norm}	Corrected image
IP	Imagin plant
IR	Infrared
k	coefficient of microbioal decomposition of root exudates
kBq	KiloBecquerel
L	Latitude
μAl	Attenuation coeffienct of neutrons by Aluminum
μH_2O	Attenuation coeffienct of neutrons by Water
$\mu soil$	Attenuation coeffienct of neutrons by Soil
MBq	MegaBecquerel
MUF-P	4-methylumbelliferyl-phosphate
n	Number of replicates
PSI	Paul Scherrer Institute
PSL	Photo stimulated luminescence
ϕ	Soil porosity

QL	Grey value of a pixel in an image
REML	Restricted maximum likelyhood
Res	Resolution
RPE	Rhizosphere priming effect
SE	Standard error
SOM	Soil organic matter
θ	Soil water content
TN	Total nitrogen
TOC	Total organic carbon
UV	Ultraviolet
WT	Barley wild type with root hairs

1 Extended Summary

1.1 Introduction

Crop production will have to be increased during the coming 40 years to meet the global food demand of the growing population (Sposito, 2013). Food security will probably be endangered by climate change, which will lead to higher temperatures, drought spells and lower potential productivity (Parry and Hawkesford, 2010; FAO, 2012; Trenberth et al., 2014). In the past, food production has mainly been increased by expanding the cultivated land area or by increasing yields through intensive agriculture. However, as most of the land is already under agricultural use and intensive cultivation inevitably leads to degradation of soil and lowers its fertility and productivity (Baligar et al., 2001) this is not an option for the future.

One strategy to increase food production is to grow crops that are adapted to low fertility environments (Lynch, 2007). In fact, availability of water and nutrients are the major constraints to world crop productivity (Parry and Hawkesford, 2010; White et al., 2013). Plant roots employ various mechanisms to increase their access to limited soil resources. Examples of such strategies include the production of root hairs, the development of an appropriate root architecture and the improvement of physical and biological soil conditions in the soil near the root, the so called rhizosphere (George et al., 2014; White et al., 2013). The rhizosphere differs in its chemical, physical and biological properties from those of the bulk soil (Hinsinger et al., 2005). These differences occur as a consequence of root growth (Aravena et al., 2014), rhizodeposition, microbial activity and the repeated wetting and drying of the soil at the root-soil interface (Gregory, 2006; Hinsinger et al., 2009). According to Jones et al. (2009) rhizodeposition is the starting point from which the rhizosphere develops and it is therefore important to improve our understanding of mechanisms and functions of rhizodeposition.

Up to one third of the carbon (C) that plants allocate to their roots is transferred to the soil as rhizodeposits (Nguyen, 2003). Plants release different compounds into the soil, for example: (1) low molecular root exudates, (2) secondary metabolites such as phytosiderophores, (3) mucilage and root cap cells (4) enzymes and (5) C allocated to symbionts (Fig. 1.1). These compounds are released by various mechanisms including secretion, diffusion and cell lysis and they play a diverse ecological role (Jones et al., 2009; Weston et al., 2012; Haichar et al., 2014).

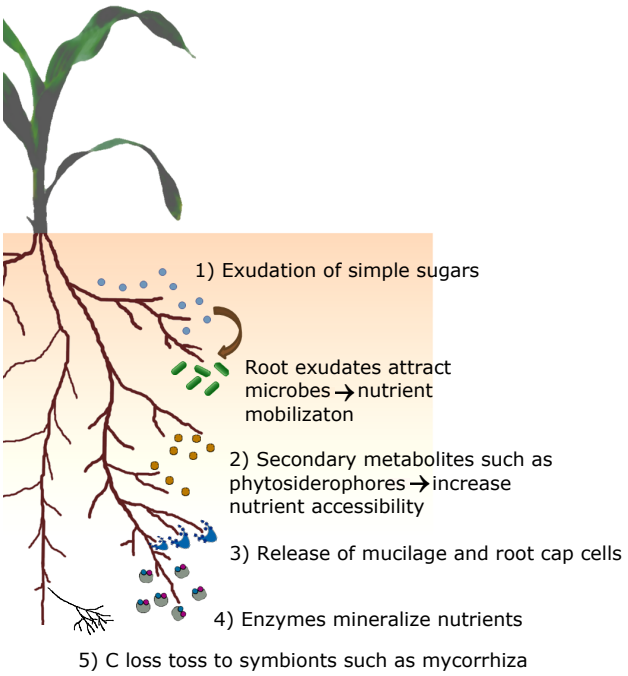

Figure 1.1: Examples of organic compounds released by the root into the rhizosphere.

Soluble root exudates in form of low molecular weight exudates play a crucial role for rhizosphere processes. On the one hand, they directly improve nutrient acquisition by mobilisation of nutrients such as phosphorus (Lynch, 2007; Marschner et al., 2011). On the other hand, they strongly affect the microbial activity and turnover of microbial biomass (Bertin et al., 2003; Helal and Sauerbeck, 1986; Kuzyakov et al., 2003), hereby indirectly influencing nutrient availability (Grayston et al., 1997) (Fig. 1.1). Several studies showed that nutrient availability increased due to a higher microbial activity in the rhizosphere compared to the bulk soil (Hamilton and Frank, 2001; Herman et al., 2006; Landi et al., 2006).

We conclude that root exudates are important agents that have positive effects on plant nutrient availability. As such, it is essential to estimate their spatial and temporal distribution in the rhizosphere because the degree to which they can positively feed back to plants, depends on the volume of soil that they occupy.

The distribution of root exudates in soil is controlled by (a) the amount of exudates released by the roots, (b) the diffusion of exudates, (c) convection, i.e. the fluxes of water to the root, (d) the soil water content, (e) decomposition of root exudates by microorganisms and (f) sorption (Jones et al., 2009; Kuzyakov et al., 2003). The water

content has a particularly strong effect on the distribution and release of root exudates as it controls its diffusion (Raynaud, 2010) and a low water content would lead to reduced C release and rhizosphere extension. Drought may additionally affect root exudation by changing the allocation of C in the plant-soil system (Reid and Mexal, 1977; Sanaullah et al., 2012), reducing microbial activity in the rhizosphere (Palta and Gregory, 1997; Zhu and Cheng, 2013) or by decreasing root elongation (Sharp et al., 2004). These processes may act in opposite directions and it is therefore still not clear whether drought will lead to increased or decreased root exudation or whether it will leave rhizodepostion unaffected (Preece and Penuelas, 2016).

Additionally, the mentioned processes may imapact differently on varying types of exudates. While low molecular weight exudates which are mainly released at the root tip (Jones et al., 2004) will certainly be strongly affected by changes in root elongation, exudates like enzymes, which are also released from older root parts (Razavi et al., 2016) may be less affected by changes in root elongation. Similarly, low molecular root exudates which are lost passively from roots (Jones et al., 2004) may be more strongly affected by diffusion (i.e. soil water content) while high molecular exudates which are actively secreted from roots (Weston et al., 2012) may be less strongly affected as plants can actively regulate their release.

Figure 1.2: Mucilage exuded from the root tips of 4 days old wheat seedlings.

Considering water dynamics in the rhizosphere, everything gets even more complex. Studies investigating the spatial distribution of root exudates have so far assumed a constant water content in rhizosphere (Darrah, 1991b,a; Raynaud, 2010). However, it has been shown that mucilage exuded by the root tip (Fig. 1.2) increases water content in the rhizosphere (Young, 1995; North and Nobel, 1997; Carminati et al., 2010; Carminati and Vetterlein, 2013). Mucilage is mainly composed of polysaccharides which are responsible for its gel-like properties (Watt et al., 1993) and its capacity to retain large volumes of water (McCully and Boyer, 1997). McCully and Boyer (1997) measured the capacity of mucilage to hold water under negative water potentials and concluded that mucilage per se has not the capacity to retain water,

Figure 1.3: Root hairs extending the root surface (left) and root hairs covered by mucilage (right).

as most if its water is drained at water potentials higher than -10 kPa. However, the remaining water is still sufficient to increase the soil water content of a few percentages, which in turn has an important role on the hydraulic conductivity of the rhizosphere (Carminati, 2012). These small changes of soil water content induced by mucilage may have a significant effect on the distribution of root exudates in the rhizosphere which strongly depends on diffusion and therefore on soil water content. It seems therefore reasonalbe to assume that the release of mucilage is a plant strategy to maintain fast diffusion of exudates and therefore an increased rhizosphere extension even under water limitation.

Plants may not only positively affect the distribution of root exudates by releasing mucilage but also by changing root morphology (Nguyen, 2003). Presumably, the production of root hairs is one strategy to increase the release of root exudates and its spatial distribution in the rhizosphere as root hairs increase the root surface from where root exudates can potentially be released (Fig. 1.3). Root hairs, releasing mucilage may further help plants to keep favourable rhizosphere conditions in dry soil. Carminati et al. (2017) showed that plants with root hairs are more efficiently taking up water from dry soil than plants without root hairs and suggested that

mucilage released from roots may play a role in this context. However, there is little direct evidence on the role of root hairs on C exudation. Although it has been shown that root hairs are covered by mucigels (Dart, 1971; Greaves and Darbyshire, 1972; Sprent, 1975) (Fig. 1.3) it is not clear whether the observed materials were released by the root hairs or just transported there simply as a result of root elongation. Moreover, there is nearly no information as to whether root hairs exude other substances apart from the observed mucigels such as low molecular exudates. Pausch et al. (2016) studied the impact of root hairs on rhizosphere priming effects (RPE). They found that the presence of root hairs increased RPE at least for young plants hinting to an increase in rhizodeposition. However, direct experimental evidence is lacking particularly on the effect of root hairs on the spatial distribution of exudates in the rhizosphere.

In conclusion, the effect of soil water dynamics on rhizodeposition and on the spatial distribution of root euxdates is still not well understood. In particular it remains unclear how plants actively, by modifying there root morphology or by releasing mucilage, control the distribution of root exudates in the rhizosphere.

1.2 Objectives and Outline

The main objective of this work was to test factors controlling the spatial distribution of root exudates in soil. We focused on the effect of drought on root exudation and how plants, for example by releasing mucilage, maintain favourable soil conditions in the rhizosphere and maintain high rhizosphere extension. The main hypotheses of the present work are summarized in Figure 1.4)

In Chapter 2 and 3 we focused on the effect of drought on root exudation and exudate distribution in the rhizosphere. In the first study (Chapter 2), we employed a numerical model to predict the release of low molecular weight exudates and its distribution in the rhizosphere depending on soil water content. In Chapter 3 we focused specifically on enzymes and how their activity is affected by soil drying and rhizosphere water retention. In these two studies, mucilage was hypothesized to retain water during soil drying (Fig. 1.4). The aim of the third study was therefore to test whether DRIFT spectroscopy can be applied to measure profiles of mucilage around roots grown in soil. While the first two chapters focused on drought, the aim of Chapter 5 was to quantify the effect of root hairs on C exudation and exudate

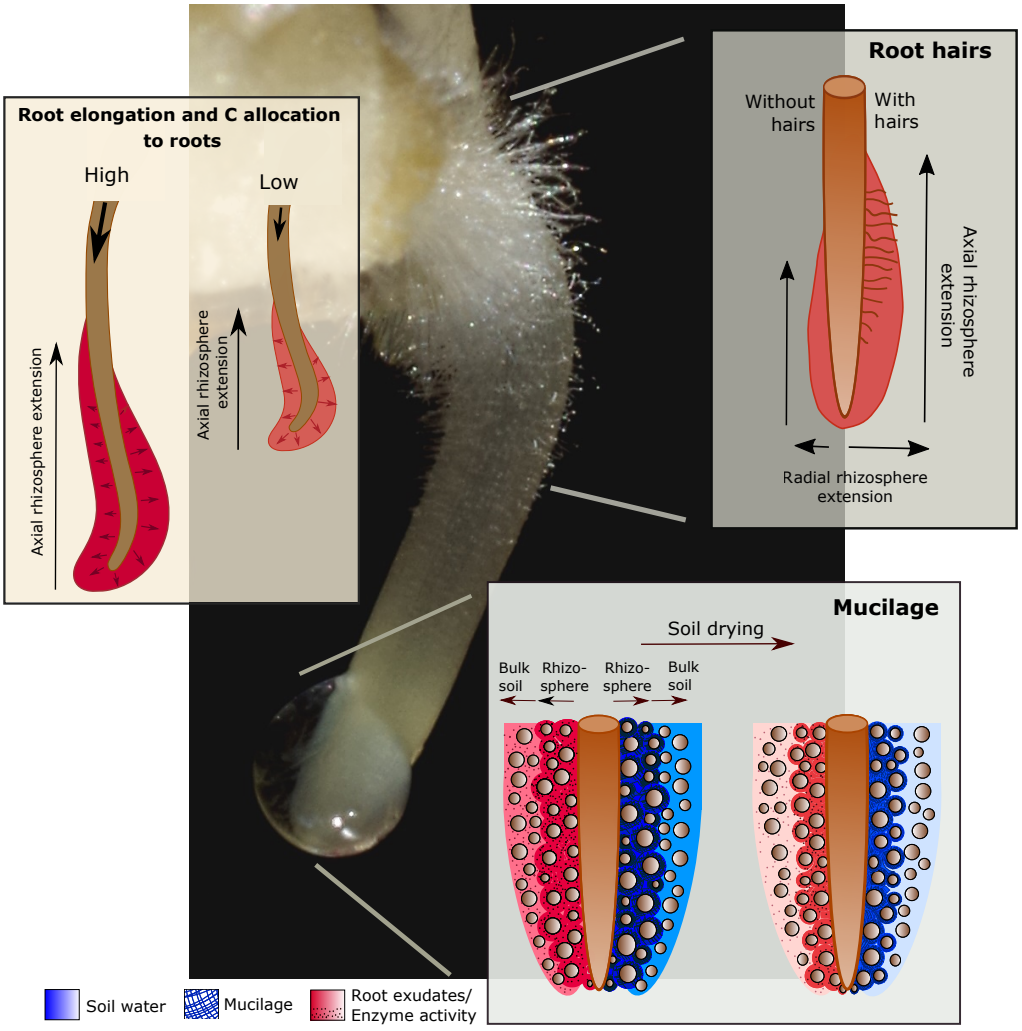

Figure 1.4: Illustration, summarizing the main hypothesis of the present work. Reduced soil water content may reduce root elongation and C allocation to root, herby reducing root exudation and radial rhizosphere extension (left). However, mucilage, may partly compensate for that effect by retaining water in the rhizosphere during soil drying. This increases diffusion of exudates and enzymes (bottom, right). Apart from soil water content and mucilage, root hairs (top, left) are likely to increase exudation of C and rhizosphere extension, herby increasing rhizosphere extension.

distribution using ^{14}C phosphor imaging of exudates collected on filter paper. In the last Chapter we finally tested whether ^{14}C phosphor imaging can be used to measure profiles of exudates around roots growing in soil which has been proven difficult previously.

With regard to the particular Chapters, the objectives of the present work were to

- test how soil water content changes the spatiotemporal distribution of root exudates in the rhizosphere and to predict root exudation by applying a numerical model (Chapter 2)
- assess whether plants maintain a high phosphatase activity in the soil close to the roots during periods of drought by retaining moisture in the rhizosphere (Chapter 3).
- test a method to measure the spatial distribution of mucilage in the rhizosphere using DRIFT spectroscopy and FTIR (Chapter 4)
- investigate how root hairs affect the C allocation in the soil-plant system, the exudation of C and its spatial distribution in the rhizosphere (Chapter 5)
- test whether phosphor imaging directly of the soil-root surface can be used to quantify C exudation and calculate profiles of root exudates around roots in soil (Chapter 6).

1.3 Material and Methods

Soil and plant material and plant growth

The soil used for the experiments described in Capters 5, 4 & 6 was a sandy soil collected close to Göttingen, Germany. TOC was 20 g kg^{-1}, TN was 1.7 g kg^{-1} and the pH amounted to 4.9. Soil particle size was distributed as follows: Clay: 8.6%, silt: 18.5%, sand: 73%. In Chapter 2 we used a mixture of fine quartz sand (70%) and silt (30%) as a soil with low organic matter content was needed. The quartz sand was collected from Duingen (Germany) and the particle size was 100-200 μm. The silt was collected from the B-horizon (2 m depth) from a field side close to Reinhausen, Göttingen (Germany) under beech forest. The particle size of the substrate mixture was distributed as follows: sand: 68.1%, silt: 25.4%, clay: 6.5%. TOC was 0.48 g kg^{-1} soil and TN was 0.019 g kg^{-1} soil. The soil pH was 4.8. In Chapter 4, a material with a relatively fine particle size and free of organic matter was needed. We therefore used a quartz material consisting of 70% quartz sand (collected

from Duingen (Germany), particle size: 0.1-0.2 mm) and 30% quartz powder (Carl Roth, particle size: <0.125 mm).

In Chapter 2 & 4 we used maize plants (*Zea mays* L.) of the seed company KWS (Variety No. 2376). In Chapter 5, 3 & 6 Barley (*Hordeum vulgare* L. cv. Pallas - wild type) and its root-hairless mutant were used (*brb*). After immersion in a 10%H_2O_2 solution for 10 minutes, the seedlings were germinated and transferred into rhizoboxes. The plants were grown in a climate chamber for approximately 4 weeks. The temperature in the climate chamber was 25 °C during day and 22 °C during night. The photoperiod was 14 hours and the photosynthetic photon flux density was 200-300 μmol m^{-2} s^{-1}.

^{14}C labelling of plants, ^{14}C allocation and phosphor imaging

^{14}C labelling and phosphor imaging was applied in Chapter 2 to identify those root regions where C is allocated to, in Chapter 5 to estimate the effect of root hairs on C exudation and in Chapter 6 to estimate the attenuation of ^{14}C in soil.

The plants were labelled in a plexiglas chamber. The label ($Na_2^{14}CO_3$ dissolved in 1M NaOH) was placed into a glass vial which was connected through plastic tubes with the chamber. By adding phosphoric acid (50%) to the label, $^{14}CO_2$ was released and pumped into the chamber where it was circulated for 2.5-5 h. 4 times during labelling 30 ml of chamber air were collected and ^{14}C in the air was measured to calculate plant ^{14}C uptake. ^{14}C imaging was conducted by placing an imaging plate on the rooted soil surfaces of the pots after labelling the plants. In Chapter 5, root exudates were additionally collected on a moist filter paper that was attached to the rhizobox for 24 h. To obtain a good signal, the screens were attached to the plant for 15 - 18 h. After this time the screens were scanned (FLA 5100 scanner, Fujifilm). The images had a spatial resolution of 50 - 100 μm.

Total dry weight as well as ^{14}C in bulk shoots, roots, root tips, rhizosphere soil and soil were measured to quantify total ^{14}C uptake and ^{14}C allocation into the plant. To collect the rhizosphere soil, the rhizoboxes were opened after ^{14}C scanning. The roots were taken out and the soil attached to the roots after gently shaking was considered as rhizosphere soil. To remove rhizosphere soil, the roots were washed with deionized water. To measure ^{14}C activity, ground shoots, roots, rhizosphere and bulk soil samples were combusted in an Oxymat OX500. The released $^{14}CO_2$ was captured in a scintillation cocktail (C400, Zinsser) and quantified using a liquid scintillation

analyzer (Tricarb, 3180, PerkinElmer). In Chapter 5 we additionally measured CO_2 efflux from soil. Immediately after labelling, the rhizoboxes where packed in a plastic bag which was closed with modelling clay at the lower part of the stem of the plants. Inside the bag a 20 ml 1 M NaOH trap was placed to trap the $^{14}CO_2$ released from soil. The ^{14}C activity in NaOH was determined using a liquid scintillation counter (Hidex, 300 SL). Total CO_2 respiration from soil was measured from a subsample (1 ml) of the NaOH trap: The carbonate in the NaOH solution was precipitated with barium chloride and the trapped CO_2-C was determined by back titration with 0.05 M HCl.

Neutron radiography

Neutron radiography was applied in Chapter 2 & 3 to monitor changes in soil water content in the bulk and rhizosphere soil based on the spatial distribution of neutrons that are transmitted through the samples. The neutron radiographs were taken in Paul Scherrer Institute (PSI), Switzerland, in the imaging stations ICON and NEUTRA.

The samples were placed in front of the imaging beam and the neutrons transmitting the sample were captured by a CCD camara and transformed into a digital image. By accounting for the attenuation coefficients of soil, water and the aluminum of the rhizoboxes, the water content in the sample can be calculted with a high spatial resolution of 100 μm. This is possible because water has a very high neutron attenuation coefficient compared to soil and aluminum.

Soil zymography

We used soil zymography in Chapter 3 to quantify phosphatase activity around the roots of plants grown in rhizoboxes. Polyamide membrane filters were soaked in phosphatase substrate (4-methylumbelliferyl-phosphate (MUF-P)) and attached to the rhizobox surface for 1h. The membranes were removed from the sample and pictures were taken at 360 nm wavelength.

For calibration, solutions with different MUF concentrations were imaged. The equation that described the correlation between MUF activity (μmol cm^{-2} h^{-1}) and the grey value from the images was used to calibrate the images and to quantify phosphatase activity (μmol cm^{-2} h^{-1}) in soil.

Image analysis

The analysis of the images was done similarly for ^{14}C images, neutron radiography and zymographs, with only small differences between the methods. The aim of the image analysis was to calculate radial or longitudinal profiles of activities/water content from the root surface. Therefore, the roots we were interested in (root tips vs. old root parts, main roots vs. lateral roots) were segmented. The segmentation was done based on the contrast between roots and soil, using either the SmartRoot plugin in ImageJ (Lobet et al., 2011) or roottracker2D (Menon et al., 2007). Possible artefacts caused by overlapping roots or roots detached from the soil were removed from the segmentation. For each image 2-10 roots were segmented depending on how many roots were clearly visible. The signal was averaged as a function of distance from the root center up to a distance of 4 cm from the root surface using the Euclidean distance mapping functions in MATLAB (The MathWorks).

DRIFT spectroscopy

Diffuse reflectance infrared spectroscopy (DRIFT) measurements in the mid-infrared range (wavelength: 2.5-25 μm, wave numbers (WN): 4000-400 cm^{-1}) was applied in Chapter 4 to measure profiles of mucilage around roots in soil. DRIFT spectra were conducted using an Agilent Cary series 600 FTIR microscope moving in the perpendicular direction from the root channel center towards the bulk soil. The spectra were recorded as 64 co-added scans at a spectral resolution of 4 cm^{-1} in steps of 100 μm from sample areas of 0.1 mm x 0.1 mm. For the rhizosphere samples, one root was chosen per plant and spectra were measured at three positions along each root (0, 1, and 3 cm behind the tip). After preprocessing of the spectra (Ellerbrock et al., 2009; Leue et al., 2010), the local peak heights of the C-H bands were normalized for the absolute peak height of the SiO_2 band at WN 1350 cm^{-1}.

Statistical approaches and modelling of root exudation

The data on C allocation in plant and soil were analysed by analyses of variances (ANOVA). To test for differences in the radial profiles of exudates in Chapter 5, a mixed effect model was used with treatment (i.e. plant type) as fixed effect and plant as random effect. To account for the differences in numbers of roots sampled per plant, the restricted maximum likelihood (REML) method was applied. In 3 we applied a

mixed effect model to account for the fact that each plant was repeatedly measured for each adjusted water content. All statistical analysis were conducted using R 3.3.1.

To model the exudation of C depending on soil water content (Chapter 2) we used the approach proposed by Kim et al. (1999) and Carminati et al. (2016). The reference system was fixed to the root tip and the diffusion-convection equation was solved numerically. The model accounted for root elongation, microbial decompostion of exudates, diffusion of exudates in soil and through the root tissue depending on soil water content. The values for rhizosphere and bulk soil water content were taken from neutron measurements. As a boundary condition we assumed zero flux at the outer boundary and a constant C concentration in the root which was calculated for plants grown in wet and in dry soil based on the ^{14}C distribution in root tips. The root segment from which exudates are released was set to 1 cm for wet plants and 0.5 cm for dry plants (Sharp et al., 2004) because we found that the root elongation rate was correlated to the length of the root zone where ^{14}C was transported to and presumable exuded from. Based on this, the amount of exudates released as well as their spatial distribution in soil was calculated.

1.4 Results and Discussion

The effect of drought on root exudation (Study 1)

The aim of this study was to investigate how the exudation of C and its spatial distribution in the rhizosphere changes with soil water content. We coupled ^{14}C imaging, neutron radiography and numerical modelling to predict root exudation.

^{14}C was allocated to root tips of main and lateral roots (Fig. 1.5). Plants grown in dry and wet soil allocated similar amounts of ^{14}C into roots but root elongation decreased by 48% in dry soil which is in line with previous findings (Sharp et al., 2004). Because the root elongation strongly correlated with the region of the root where C was allocated to and released from, the model predicted that the reduction in root elongation would result in a strong decline in root exudation for plants in dry soils. This resulted in a total exudation after the simulation time of two days that was approximately twice as high (1.78 and 1.75 μg C cm^{-3}) in wet soil compared to dry soil (0.72 and 0.9 μg C cm^{-3}). The soil water content, measured by neutron radiography differed strongly among the treatments for the bulk soil (6% vs. 20%), but was identical for the rhizosphere (31%). This may be explained by mucilage released

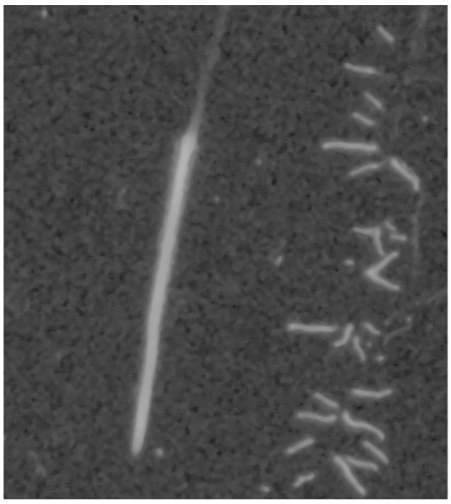

Figure 1.5: ^{14}C allocation in the root system visualized by phosphor imaging two days after plant labelling. Bright indicates high ^{14}C activity, dark indicates low ^{14}C activity.

from the root tips that can retain large amounts of water in the rhizosphere due to its gel-like structure (McCully and Boyer, 1997; Carminati and Vetterlein, 2013).

To assess the effect of the changes in rhizosphere water content, two model scenarios where tested. One scenario accounted for an increase in rhizosphere water content as observed in the experiment. As a control, another scenario was tested, assuming a constant water content towards the root. While the increase of water content towards the roots had nearly no effect for plants grown in wet soil, it increased root exudation for the dry treatment and extended its radial distribution. This can be explained by the increased diffusion of exudates in the rhizosphere due to the increased water content (Raynaud, 2010). This effect is particularly strong if the bulk soil water content is low. The changes in water content towards the root therefore partly compensated for the reduced exudation due to reduced root elongation in dry soil.

We showed that root growth and rhizosphere water content play an important role in C release by roots and in shaping the profiles of root exudates in the rhizosphere. The release of mucilage may be a plant strategy to maintain fast diffusion of exudates and high microbial activity even under water limitation.

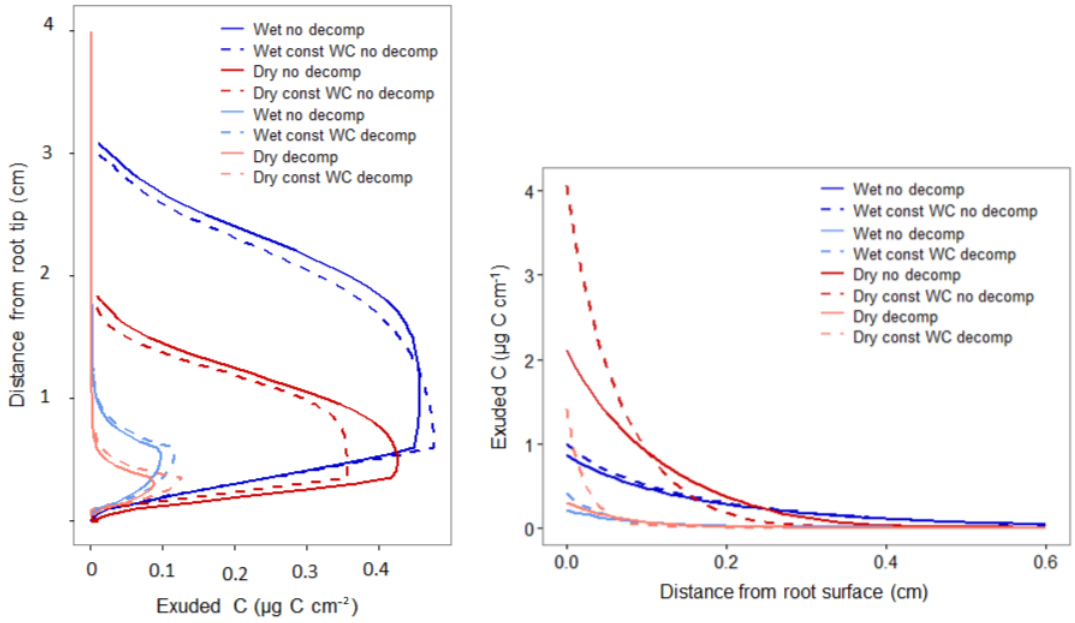

Figure 1.6: Left: Modelled axial distribution of exudates in the rhizosphere after two days of simulation. const = constant water content towards the root surface is assumed. For all other scenarios an increase in soil water content towards the root is assumed as measured with neutron radiography. decomp = microbial decomposition. Right: Modelled radial distribution of exudates in the rhizosphere after two days of simulation.

Soil drying and its effect on rhizosphere enzyme activity (Study 2)

We tested whether plants maintain a high phosphatase activity around roots during periods of drought by retaining moisture in the rhizosphere which facilitates fast diffusion of exoenzymes and thus high rates of enzymatic catalysis.

The WC in bulk soil and rhizosphere measured by neutron radiography (Fig. 1.7b, 1.8) differed significantly between the adjusted WC levels. Rhizosphere WC was approximately 2 times higher than in the bulk soil at all adjusted WCs. Particularly under dry conditions, these differences were high which resulted in an increase in the ratio between the WC in the rhizosphere and in the bulk soil of up to 2.8 with soil drying.

Similar trends as for soil WC were found for phosphatase activity (Fig. 1.7c). In bulk soil and rhizosphere, phosphatase activity decreased by 97% when the adjusted WC of the rhizoboxes dropped from 40% to 5% WC (Fig. 1.8, left). The power-law

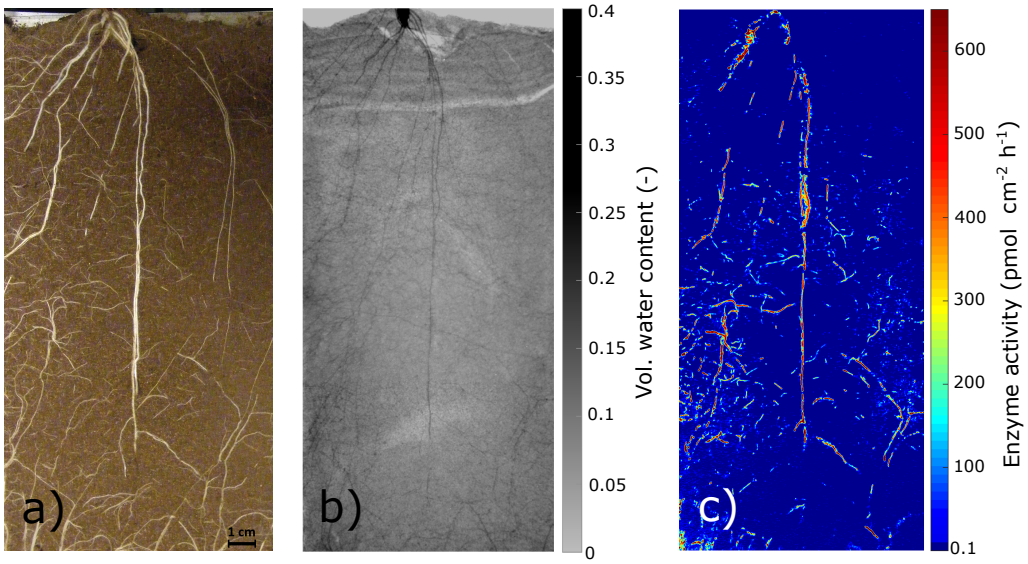

Figure 1.7: Exemplary images of a) barley roots in an open rhizobox, b) the same root system imaged by neutron radiography showing the volumetric water content and c) zymography depicting the distribution of the phosphatase activity of the same sample.

relation between soil WC and enzyme activity, which is similar to the relation between soil WC and diffusion coefficient proposed by Millington-Quirk model (Milllington and Quirk, 1961) confirms the hypothesis by Manzoni et al. (2012) that enzyme activity with soil drying is mainly controlled by diffusion. In accordance with this finding, the phosphatase activity in the rhizosphere, having a high WC compared to the bulk soil, was significantly greater than phosphatase activity in the bulk soil. With increasing soil drying, the ratio of phosphatase activity in the rhizosphere-to-phosphatase activity in the bulk soil increased. It was 9.7 at 40% adjusted WC, and reached 63.4 at 5% WC (Fig. 1.8, right).

The reason for this might be mucilage and EPS released into the rhizosphere (Carminati and Vetterlein, 2013; Young, 1995; Or et al., 2007) which retain water upon soil drying (Ahmed et al., 2014; Carminati and Vetterlein, 2013; Or et al., 2007) and therefore keep the rhizosphere moister than the bulk soil. The enhanced rhizosphere WC does not only increase the diffusion of phosphatase, but also the diffusion of inorganic phosphorus, increasing its chances to be taken up by the plant. The increase in rhizosphere WC due to mucilage and EPS has therefore a twofold positive effect

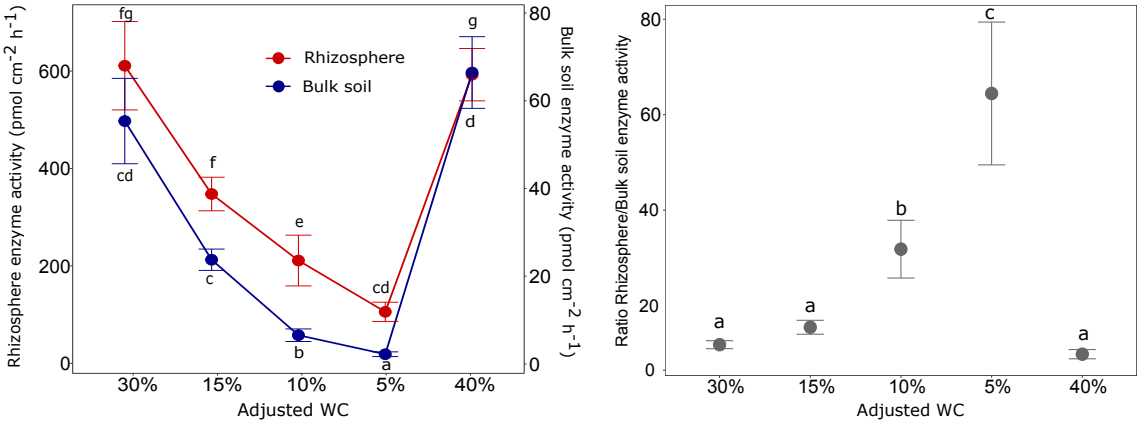

Figure 1.8: Left: Enzyme activity in the rhizosphere (red) and in bulk soil (blue) as a function of the volumetric WC to which the rhizoboxes were adjusted. Error bars indicate the standard error. Significant differences are indicated by different letters. Right: Ratio of enzyme activity of the rhizosphere to enzyme activity of the bulk soil as a function of the gravimetric WC, to which the rhizoboxes were adjusted. Error bars indicate the standard error. Significant differences are indicated by different letters.

to the plant in terms of nutrient availability in dry soil conditions, first, it increases enzyme activity, and second, it leads to a facilitated movement of phosphate towards the plant root.

Infrared spectroscopy to measure the spatial distribution of mucilage in soil (Study 3)

The aim of this study was to test a method to determine the spatial distribution of mucilage in the rhizosphere.

We used the C-H/SiO_2 ratio measured by IR spectroscopy as a proxy for mucilage content in soil, as mucilage contains fatty acids (Read et al., 2003). The calibration measurements on object slides with soil mixed with given amounts of mucilage revealed a linear relationship between mucilage content and the C-H/SiO_2 ratio. The calibration was used to convert the measured C-H/SiO_2 ratios perpendicular to the roots into mg dry mucilage per g dry soil. The measurements in the maize rhizosphere showed a distinct gradient of mucilage from the center of the root channel towards the bulk soil. The highest values of 0.02 mg g $^{-1}$ were found in the center of the root

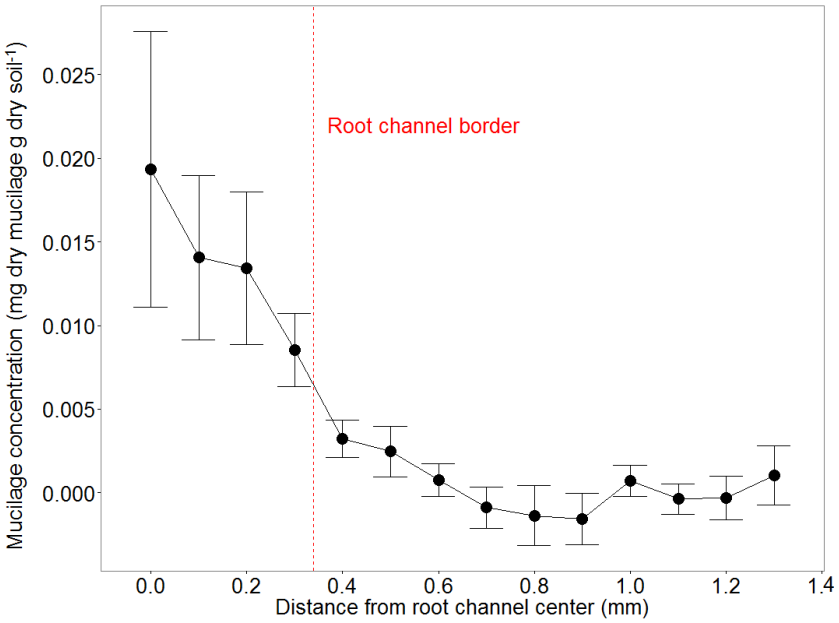

Figure 1.9: Mucilage concentration (mg dry mucilage per g dry soil) starting from the root channel center in radial direction towards the bulk soil. Mean values and standard errors of 5 replicates.

channel.

Mucilage content decreased to approximately 0 mg g $^{-1}$ at about 0.6 mm from the root surface (Fig. 1.9). This rhizosphere extension was lower compared to results obtained from ^{14}C imaging analyses (Holz et al., 2017) where root exudates diffused up to 1 mm into the bulk soil. The fact that mucilage did not move as far from the root surface as compared to overall exudates may be explained by the higher viscosity of mucilage (Read and Gregory, 1997) and reduced diffusion coefficient of mucilage compared to root exudates. Based on recalculation from the literature, we can expect a mucilage exudation rate of 0.056 mg dry mucilage per g dry soil (Chaboud, 1983). This theoretic value is 3 times higher than the values obtained by DRIFT spectroscopy; however, they still appear comparable. IR spectroscopy is therefore a promising tool to measure the spatial distribution of mucilage in soil.

Root hairs increase rhizosphere extension (Study 4)

The aim of the fourth study was to test whether root hairs lead to increased root exudation and rhizosphere extension.

The region of roots where ^{14}C was allocated correlated well with the region where

^{14}C was found on the filter paper used for the collection of root exudates (Fig. 1.10). For plants with root hairs, rhizosphere extension in radial direction reached up to 1.5 mm from the root surface, while for the mutant without hairs it reached only 0.5 mm. The increase in radial rhizosphere extension is of particular importance because it expands the volume of soil where root exudates can interact with the soil matrix and with microorganisms which may improve nuturient availibility (Hamilton and Frank, 2001; Herman et al., 2006; Landi et al., 2006).

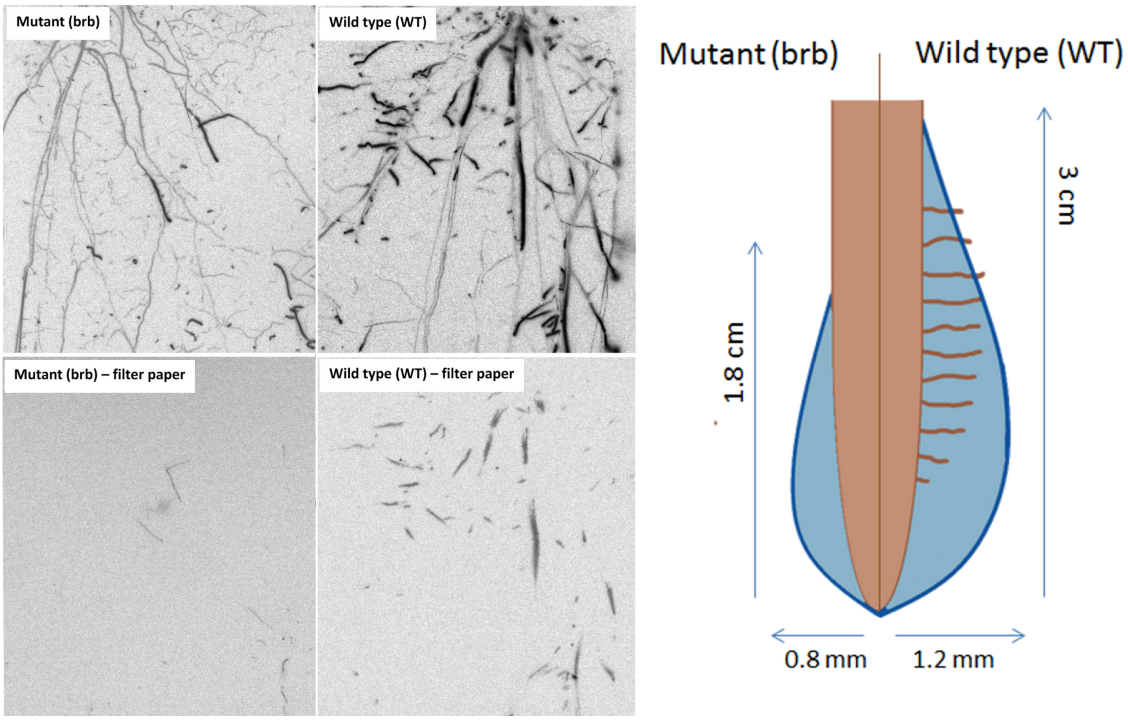

Figure 1.10: Left: ^{14}C phosphor images of the root system of barley plants (top) and of root exudates that diffused into the filter paper (bottom) which was attached to the plant during labelling. The intensity of the grey value corresponds to ^{14}C activity. The sketch on the right side indicates the changes in rhizosphere extension due to root hairs.

To separate the effect of different root elongation rates between genotypes on the axial rhizosphere extension, the ratio between axial rhizosphere extension and root elongation was calculated. The ratio was around 2 for plants with root hairs and around 1 for plants without root hairs. This indicates that the presence of root hairs extended the zone of exudation to slightly older root segments and that the root hairs themselves exude organic substances into the soil. Therefore, for plants with root

hairs, root exudation is high over a longer distance from the root tip compared to plants without root hairs. This may be considered in models of nutrient uptake which assume that nutrients are mobilized behind the zone of root exudation (Marschner et al., 2011; Kuzyakov and Xu, 2013).

Plants with root hairs allocated twice as much ^{14}C to roots and 5 times more ^{14}C to rhizosheaths compared to the mutant without hairs which corresponds with the findings from the imaging experiment. In contrast, hairless plants allocated more C to shoots (wild type: 65%; *brb*: 75%) than the wild type. ^{14}C activity in CO_2 as well as the total CO_2 respiration from soil was similar for the wild type and the hairless mutant. As plants with root hairs allocated more C belowground, while ^{14}C efflux as well as total CO_2 efflux remained unchanged, more C derived from roots was retained in soil for WT plants. The greater C allocation belowground in the presence of root hairs may therefore foster C sequestration.

Challenges and artefacts in visualization and quantification of rhizodeposition using phosphor imaging (Study 5)

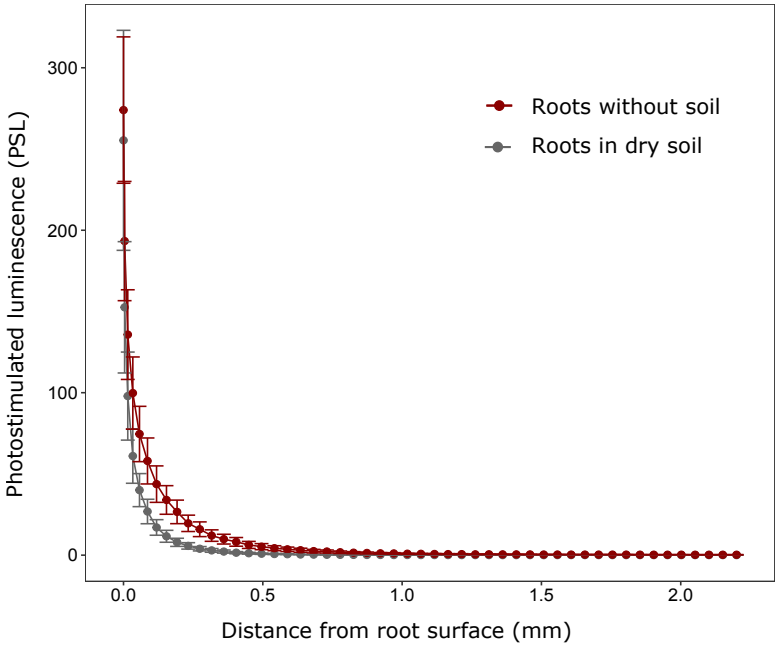

Figure 1.11: Radial profiles of ^{14}C activity of dry roots removed from soil and placed on a plane surface without soil (red line) and of the same roots placed into dry soil (grey line). Variation is given as standard error, n=4.

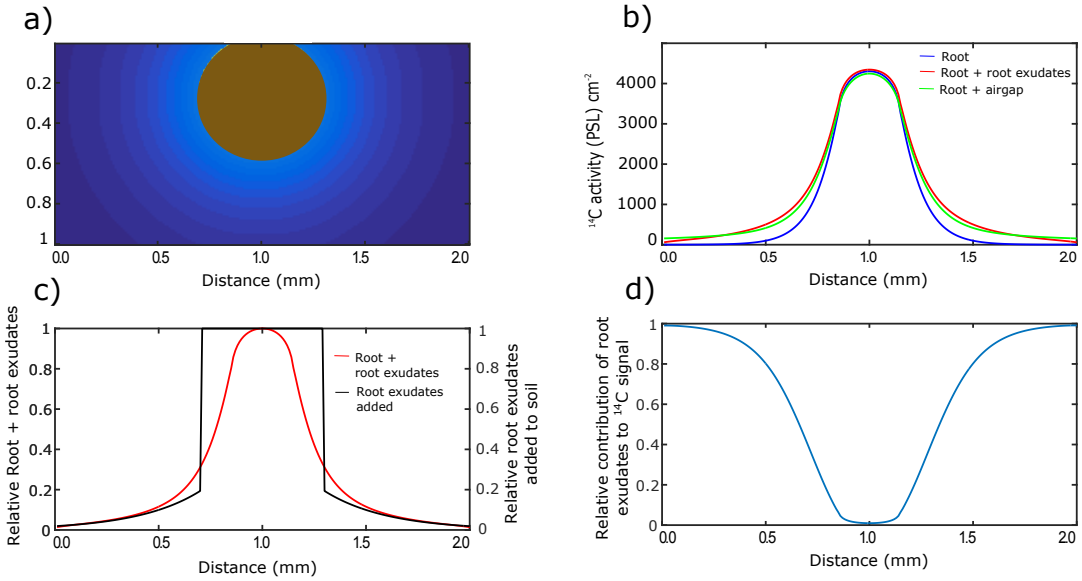

Figure 1.12: Calculated profiles of ^{14}C activity around roots. The profiles were calculated for three scenarios, first, assuming that only the root is radioactive (blue line), second, assuming that the root and root exudates in the soil around the root are radioactive (red line) and third, assuming that only the root is radioactive and that there is an airgap of 0.03 mm between the root and the imaging screen (green line) (b). c) shows the distribution of the actual root exudates in soil (black line) compared to the signal that is seen after imaging root and root exudates (red line). d) displays the relative contribution of root exudates to the ^{14}C signal measured. 1 indicates that 100 % of the signal is caused by root exudates, while 0 indicates that 100 % of the signal is caused by the activity of the root.

Because the ^{14}C filter paper approach is restricted to moist soil conditions, we tested whether phosphor imaging directly on the soil-root-surface can be used to estimate profiles of root exudates around roots in soil. Profiles of ^{14}C activity were calculated for dried roots of labelled plants that were either placed in dry soil or imaged without soil.

Profiles of ^{14}C were broader (0.5 vs. 0.25 m) and the signal intensity was higher for roots imaged without soil compared to roots placed in dry soil (Fig. 1.11). These differences reflect different attenuation of ^{14}C in soil and in air. However, the fact that even for roots placed in soil, the ^{14}C signal reached regions beyond the root surface indicates that the soil attenuated only part of the ^{14}C signal originating from roots.

To calculate the ^{14}C signal that can be assumed to originate from root exudates, we measured the attenuation of ^{14}C in soil and in water. The ^{14}C attenuation coefficient was 148 cm^{-1} for soil and 67 cm^{-1} for water which corresponds to the differences in the density of the materials which is 1 g cm^{-3} for water and 2.65 g cm^{-3} for quartz. Based on these coefficients, we calculated profiles of ^{14}C that can be expected around roots in soil. The profiles of ^{14}C were strongly affected by a) the ^{14}C activity in the root and the root radius, b) the position of the root in soil, c) the amount of root exudates in soil and d) by the presence of air gaps between sample and imaging screen. Knowing the root ^{14}C activity, its radius and position in soil, it may be possible to calculate the contribution of root exudates to the profiles of ^{14}C. However, the blurring effect of air gaps between sample and imaging screen that are difficult to quantify, makes it difficult to use ^{14}C imaging to quantify profiles of root exudates around roots in soil.

1.5 Summary, Conclusion and Outlook

The aim of the present work was to test biophysical factors, controlling the spatiotemporal distribution of root exudates in the rhizosphere. The following factors have been identified to influence root exudation and the spatial distribution of root exudates in soil: a) root elongation, b) the local soil water content, c) release of mucilage and EPS and d) the production of root hairs. It is important to consider that all these factors interact with each other and that they may not be considered independently when evaluating their effects on root exudation.

Chapter 2 and 3 highlighted the importance of soil water content for the distribution of root exudates. In Chapter 2, soil drying reduced root elongation and therefore total exudation and axial exudate distribution. In this sense, the water content had an indirect effect on root exudation. However, by reducing diffusion, soil drying directly affected root exudation and strongly reduced rhizosphere extension and total exudation. Similarly, phosphatase activity was strongly correlated with the local soil water content and declined with soil drying (Chapter 3). In both studies, we observed an increase in water content towards the roots. The increase in rhizosphere water content was attributed to mucilage, a gel-like substance released by the root tips that is able to retain large amounts of water (McCully and Boyer, 1997; Carminati and Vetterlein, 2013). By incraesing the rhizosphere water content, mucilage enhanced diffusion of exudates and enzymes. This resulted in an increase in total exudation

and radial exudate distribtution particularly under dry conditions (Chapter 2) and maintained enzyme activities even under severe soil drying (Chapter 3). By retaining water in the rhizosphere and maintaining the diffusion of exudates, both substances may therefore be beneficial to plants, particulary under dry conditions.

Despite the fact, that mucilage may be have positive effects to plants due to its water related properties, so far no method is available to estimate its distribution in the rhizosphere. In Chapter 4, we showed that the signal of C-H groups, contained in mucilage, can be measured by DRIFT spectroscopy and used as a proxy to quantify the spatial distribution of mucilage in the rhizosphere. The measured mucilage contents and the spatial extent of the mucilage-affected rhizosphere were comparable to calculated and published values. A limitation of this method, however, is that it can be applied only in soil organic matter (SOM) free material and is therefore restricted to relatively artificial soil conditions.

Apart form the factors mentioned so far, the production of root hairs also affects root exudation and rhizosphere extension. Using phosphor imaging of root exudates collected on filter paper, we showed that root hairs strongly increaese the radial and axial rhizosphere extension. The increase in axial rhizosphere extension may be considered in models of nutrient uptake where it is commonly assumed that nutrients are mobilized behind the zone of root exudation in axial direction (Marschner et al., 2011; Kuzyakov and Xu, 2013). The greater radial rhizosphere extension for plants with root hairs might be beneficial to plants because it favours plant-microbial interactions and therefore nutrient mobilization in larger soil volumes. The increase in root exudation for plants with root hairs was confirmed by the ^{14}C allocation in plant and soil. Barley with root hairs allocated more C belowground compared to plants without hairs, but this did not increase CO_2 efflux. As root C has a longer mean residence time in soil compared to shoot C (Rasse et al., 2005), it is likely that plants with root hairs foster C sequestration.

Finally, we evaluated whether phosphor imaging of soil-root surfaces can be used to visualize and to quantify root exudates around growing roots. This has proven difficult so far because of the scattering of the ^{14}C signal from the root that easily overshadows that of the root exudates. The following factors have to be considered when calculating the contribution of root exudates to the ^{14}C signal of a phosphor image taken: a) the ^{14}C activity in the root, b) the root radius, c) the position of the root, d) the amount of root exudates and e) by the presence of air gaps between soil and imaging screen. The

calculation of profiles of root exudates is highly sensitive to airgaps between sample and screen, which can hardly be quantified. It may therefore not be reliable to use such an approach in practice. Instead, the collection of root exudates on filter paper and their visualization seems to be a more trustworthy method.

By combining complementary imaging methods and numerical modelling we showed that plants engineer a suitable soil environment by the release of mucilage or by the production of root hairs. Mucilage may have positive effects on plant growth particularly in periods of drought as it retains water in the rhizosphere, herby increasing the diffusion and extension of low molecular root exudates and enzymes. Root hairs, on the other hand increase C exudation and rhizosphere extension and they may therefore enhance rhizosphere interaction in greater soil volumes which positively affects nutrient accessibility.

We tested two methods with regard to their applicability to image and quantify rhizodeposits. IR spectroscopy was successfully applied to estimate the distribution of mucilage in soil while phosphor imaging of root exudates collected on filter paper was applied to image root exudates. These methods will help to better understand factors controlling the spatial distribution of root exudates in soil. Future studies should investigate whether the root traits studied here (mucilage, root hairs, water related rhizosphere properties) do have a direct impact on nutrient accessibility. This could be done by measuring the mentioned root traits in combination with measurements of nutrient uptake or nutrient availability. Additionally, different plant varieties should be tested for their ability to increase the release of mucilage or to produce long and dense root hairs. This would enable plant breeders to develop plant varieties which are able to cope with difficult growth conditions such as water scarcity and nutrient limitation. Both are expected to occur more often in the future due to climate change.

1.6 Contributions to the included manuscripts

Study 1: Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content

Status: Published in *Plant and Soil*, 2017, doi: 10.1007/s11104-017-3522-4:

Maire Holz: 60% (experimental design, conducting the experiment, data analysis, preparation of the manuscript)

Mohsen Zarebanadkouki: 15% (conducting the experiment, data analysis)

Anders Kaestner: 5% (supervising neutron radiography measurements)

Yakov Kuzyakov: 5% (discussion of experimental design and results, comments to improve the manuscript)

Andrea Carminati: 15% (discussion of experimental design and results, data analysis, comments to improve the manuscript)

Study 2: Coping with drought: Plant roots maintain enzyme activity in drying soils by increasing water retention in the rhizosphere

Status: Submitted to *Nature Plants*, 2018

Maire Holz: 60% (experimental design, conducting the experiment, data analysis, preparation of the manuscript)

Mohsen Zarebanadkouki: 10% (conducting the experiment, data analysis)

Andrea Carminati: 10% (discussion of experimental design and results, comments to improve the manuscript)

Anders Kaestner: 5% (supervising neutron radiography measurements)

Jan Hovind: 5% (supervising neutron radiography measurements)

Marie Spohn: 10% (experimental design, conducting the experiment,discussion of experimental design and results, preparation of the manuscript)

Study 3: Spatial distribution of mucilage in the rhizosphere measured with infrared spectroscopy

Status: Submitted to *Frontiers in Environmental Sciences*, 2017

Maire Holz: 65% (experimental design, conducting the experiment, data analysis, preparation of the manuscript)

Martin Leue: 15% (conducting the experiment, discussion of experimental design and results, data analysis)

Mutez A. Ahmed: 5% (conducting the experiment)

Pascal Benard: 5% (conducting the experiment)

Horst H. Gerke: 5% (comments to improve the manuscript)

Andrea Carminati: 5% (discussion of experimental design and results, comments to improve the manuscript)

Study 4: Root hairs increase rhizosphere extension and C input to soil

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Maire Holz: 70% (experimental design, conducting the experiment, data analysis, preparation of the manuscript)

Mohsen Zarebanadkouki: 10% (data analysis)

Yakov Kuzyakov: 5% (discussion of experimental design and results, comments to improve the manuscript)

Johanna Pausch: 5% (comments to improve the manuscript)

Andrea Carminati: 10% (discussion of experimental design and results, comments to improve the manuscript)

Study 5: Quantification of root exudates around growing roots in soil using phosphor imaging

Status: In preparation

Maire Holz: 70% (experimental design, conducting the experiment, data analysis, preparation of the manuscript)

Mohsen Zarebanadkouki: 5% (data analysis)

Yakov Kuzyakov: 5% (comments to improve the manuscript)

Andrea Carminati: 20% (experimental design, discussion of experimental design and results, data analysis, comments to improve the manuscript)

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2 Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content

written by Maire Holz, Mohsen Zarebanadkouki, Anders Kaestner, Yakov Kuzyakov and Andrea Carminati

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Abstract

Aims Rhizodeposition is an important energy source for soil microorganisms. It is therefore crucial to estimate the distribution of root derived carbon (C) in soil and how it changes with soil water content.

Methods We tested how drought affects exudate distribution in the rhizosphere by coupling $^{14}CO_2$ labelling of plants and phosphor imaging to estimate C allocation in roots. Rhizosphere water content was visualized by neutron radiography. A numerical model was employed to predict the exudate release and its spatiotemporal distribution along and around growing roots.

Results Dry and wet plants allocated similar amounts of ^{14}C into roots but root elongation decreased by 48% in dry soil leading to reduced longitudinal rhizosphere extension. Rhizosphere water content was identical (31%) independent of drought, presumably because of the high water retention by mucilage. The model predicted that the increase in rhizosphere water content will enhance diffusion of exudates especially in dry soil and increase their microbial decomposition.

Conclusions Root growth and rhizosphere water content play an important role in C release by roots and in shaping the profiles of root exudates in the rhizosphere. The release of mucilage may be a plant strategy to maintain fast diffusion of exudates and high microbial activity even under water limitation.

2.1 Introduction

The rhizosphere is the small soil volume around the roots where nutrient accessibility is increased due to a higher microbial abundance and turnover compared to the bulk soil (Hamilton and Frank, 2001; Herman et al., 2006; Landi et al., 2006). Low molecular weight root exudates (hereinafter referred to as root exudates), are one important energy source for soil microorganisms (Gunina and Kuzyakov, 2015). It is therefore important to estimate the radial and longitudinal distribution of root exudates in the

rhizosphere (Toal et al., 2000; Darrah, 1991b). The distribution of C in the rhizosphere is affected by: (a) the amount of exudates released by the roots, (b) the diffusion of exudates, (c) convection, i.e. the fluxes of water to the root, (d) decomposition of root exudates by microorganisms, (e) sorption (Kuzyakov et al., 2003; Jones et al., 2009) and the presence of root hairs (Holz et al., 2017b). Although all these processes are affected by soil water content, it is still not known how drought events affect the spatial distribution of root exudates in the rhizosphere. Recently, Preece and Penuelas (2016) reviewed that rhizodeposition is increased, decreased or unaffected by drought. These contradictory results may arise because it is not yet understood how soil water content affects the complex interactions between processes and because these processes may work in opposite directions.

Diffusion has a strong influence on exudate distribution and on root exudation rate (Jones et al., 2004). It is reduced at low soil water content (Raynaud, 2010). Studies investigating the spatial distribution of root exudates have assumed a constant water content in rhizosphere (Darrah, 1991a,b; Raynaud, 2010). However, mucilage, a mixture mainly composed of polysaccharides (Chaboud, 1983) and secreted by the root tip, increases water content in the rhizosphere compared to the bulk soil (McCully and Boyer, 1997; Young, 1995; North and Nobel, 1997; Carminati and Vetterlein, 2013). By secretion of mucilage, plants may therefore augment release of low molecular weight exudates by maintaining their diffusion into the rhizosphere even at low bulk soil water contents.

Changes in root elongation (Sharp et al., 2004), which modify the length and velocity of the exuding root zone, additionally affect rhizodeposition. Preece and Penuelas (2016) showed that reduced exudation under drought can often be explained by a reduction in root growth. As low molecular weight root exudates are released from the growing tip (Jones et al., 2009; Pausch and Kuzyakov, 2011), changes in root elongation will strongly affect the distribution of root exudates. When predicting root exudation under drought, it is therefore important to consider root elongation rates. Kim et al. (1999) showed that the distribution of solutes in the rhizosphere is very different if the location of exudate release in soil is fixed or, as in real roots, it moves in the soil. The authors concluded that root exudation should be treated as a moving source term in rhizosphere models.

Drought also affects the amount of recently assimilated C, which is transported to the roots. Most studies investigating the effect of drought on the translocation

of recently assimilated C found that plants allocate relatively more C to roots when subjected to drought (Reid and Mexal, 1977; Palta and Gregory, 1997; Sanaullah et al., 2012). As root exudates are mainly lost passively (Dilkes et al., 2004), the amount of C allocated to roots will strongly affect the C loss into the soil and should therefore be considered when modelling the distribution of plant derived C in the rhizosphere.

The aim of this study was to test how soil water content changes the spatio-temporal distribution of root exudates in the rhizosphere. We considered changes in root growth, C allocation to roots and rhizosphere water content (which might be different than the average water content and which affects the diffusion coefficient). We grew maize plants in rhizoboxes and used ^{14}C imaging to identify the root parts where C was allocated and where recently assimilated C could be released into the soil. Neutron radiography was applied to measure changes in soil moisture around the roots which indirectly reflect mucilage concentration (Carminati et al., 2010). The amount of C translocated into the roots, root elongation as well as the changes in soil water content towards the roots were used as parameters for a numerical model of exudate diffusion and degradation in the rhizosphere. The application of the model enabled us to quantify the radial and longitudinal distribution of exudates in the rhizosphere for varying parameters such as root growth rate, soil water content and degradation rate.

Our hypotheses were: i) mucilage increases the water content close to the root, hereby increasing exudate diffusion in the rhizosphere. This increase will be more pronounced in dry soil. The higher water content will additionally increase microbial activity and exudate decomposition; ii) drought reduces root elongation and the length of the root exudation zone leading to reduced longitudinal rhizosphere extension and reduced total exudation. These hypotheses were tested combining the imaging results with the simulation.

2.2 Materials and Methods

Soil preparation and plant growth

Before germination, maize seeds (KWS 2376) were immersed in a 10% H_2O_2 solution for 10 minutes to avoid seed-borne diseases. Seedlings were planted after 3 days of germination. Maize plants were grown for six weeks in rhizoboxes with a size of either 12 x 30 x 1 (for neutron radiography) or 30 x 30 x 1 cm (^{14}C imaging). The

boxes were kept at an angle of approximately 55° to make sure that the roots were growing close to the lower side of the rhizoboxes. The soil was a mixture of fine quartz sand (70%) and silt (30%). The quartz sand was collected from Duingen (Germany) and the particle size was 100-200 μm. The silt was collected from the B-horizon (2 m depth) from a field side close to Reinhausen, Göttingen (Germany) under beech forest. The texture of the material was the following: sand: 68.1%, silt: 25.4%, clay: 6.5%. The total C content of the soil was 0.48 ($\pm$ 0.027) g kg^{-1} soil and the total N content was 0.019 ($\pm$ 0.0013) g kg^{-1} soil. The soil pH was 4.8 ($\pm$ 0.47). Prior to plant growth the following nutrients were added per kg of soil: NH_4NO_3-N: 0.3 g, $Ca(H_2PO_4)_2$-P: 0.06 g, K_2SO_4-K: 0.03 g, $CaSO_4$-Ca: 0.05 g, $MgSO_4$-Mg: 0.05 g, H_3BO_4-B: 2 mg, $CuSO_4$-Cu: 0.03 mg, $MnSO_4$-Mn: 1.5 mg, $(NH_4)_2MoO_4$-Mo: 0.03 mg, $ZnCl_4$-Zn: 1.2 mg, FeNaEDTA-Fe: 3.6 mg. Soil water content in the samples was checked gravimetrically each day and it was adjusted by adding water from the top with a syringe. A 1 cm thick layer of gravel was added on top of the samples to prevent evaporation and to avoid strong fluctuations in soil water content. The soil water content was kept to 1% in the first five weeks of plant growth. One week before measurements, the volumetric soil water content was brought to either 20% or 6%, which correspond to a soil matric potential of -89 hPa and -500 hPa, as measured using a pressure plate apparatus. The two treatments are referred to as wet and dry treatment. The temperature in the climate chamber was 25 °C during day and 22 °C during night, the photoperiod was 14 hours and the photosynthetic photon flux density was 200 μmol m^{-2} s^{-1}. During the growth period, photos of the root system were taken in regular intervals to monitor root elongation. For each plant a photo one day before labelling, at the day of labelling and one day after labelling was chosen. The elongation rate was calculated using the Smart Root plugin in ImageJ: Roots from each image were segmented and the length of the segment was calculated by the program. From the differences in root length at different days the elongation rate was calculated. Four replicates per treatment were used for neutron radiography measurements, and five were used for ^{14}C imaging.

Plant labeling, ^{14}C imaging and quantification of ^{14}C in plant and soil

^{14}C imaging was conducted with plants grown under same conditions as for neutron radiography. Each plant was labelled with 1 MBq $^{14}CO_2$ (specific activity of 2,205,200 MBq mmol C^{-1}). The labelling procedure was done according to Kuzyakov

et al. (2006). The plants were labelled in a plexiglas chamber (0.6 x 0.6 x 0.8 m) in which a fan was placed in order to distribute the ^{14}C in the whole chamber. The label ($NaH^{14}CO_3$ dissolved in 1M NaOH) was placed into a glass vial which was connected through plastic tubes with the chamber. By adding phosphoric acid (50%) to the label, $^{14}CO_2$ was released and pumped into the chamber where it was circulated for 2.5 h. 4 times during labelling (after 5, 30, 60 and 120 min) 30 ml of chamber air were collected from the chamber with a syringe. After injection of this air into a scintillation cocktail (C 400, Zinsser Analytics) and measurement of the ^{14}C in the liquid by a ^{14}C counter (Hidex, 300 SL), ^{14}C activity in the chamber air was back-calculated. To capture the ^{14}C that was remaining in the chamber in the end of labelling, the chamber air was pumped through a 1M NaOH solution for 30 minutes. The ^{14}C in the solution was determined with a ^{14}C counter (Hidex, 300 SL) after adding scintillation cocktail to the solution. These measurements showed that the plants had taken up approx. 80% of the added ^{14}C at the end of labelling.

^{14}C imaging was conducted by placing an imaging plate (Storage phosphor screen, BAS-IP MS 2040 E, VWR) on the rooted soil surfaces of the pots after labelling the plants. Images of each root system were taken 2, 3 and 4 days after labelling. To obtain a good signal, the screens were attached to the plant for 17 h. After this time the screens were scanned (FLA 5100 scanner, Fujifilm). The images had a spatial resolution of 100 μm. Total dry weight as well as ^{14}C in bulk shoots, roots, root tips, rhizosphere soil and soil were measured to quantify total ^{14}C uptake and ^{14}C allocation into the plant. To collect the rhizosphere soil, samples were opened after ^{14}C scanning. The roots were taken out and the soil attached to the roots after gently shaking was considered as rhizosphere soil. To remove rhizosphere soil, the roots were washed with deionized water. The rhizosphere soil was collected and freeze dried to avoid microbial decomposition of exudates. Similarly, root material was freeze dried to avoid microbial decomposition. To measure the ^{14}C content in the root tips we chose the same tips chosen for ^{14}C image analysis - i.e. the tips of crown roots which showed a high ^{14}C activity in the images (Fig. 2.1,2.2). The segmentation procedure is explained in detail in the paragraph Quantification of ^{14}C images. The roots were cut 3 cm behind the tip. Root material and rhizosphere soil was freeze dried and ground. Shoot material and bulk were dried at 40 °C and ground. To measure ^{14}C activity, ground shoots, roots, rhizosphere and bulk soil samples were combusted in an Oxymat OX500. The released $^{14}CO_2$ was captured in a scintillation cocktail (C400, Zinsser)

and quantified using a liquid scintillation analyzer (Tricarb, 3180, PerkinElmer).

Quantification of ^{14}C images

To quantify the length of the root segments, where ^{14}C was allocated to, we segmented those regions of the crown roots which showed a high ^{14}C activity (i.e. the same root tips which were later on destructively sampled for ^{14}C analysis). The roots were segmented based on the contrast between roots and soil using the program Roottracker 2D. As the program segments the whole root system, the regions with high ^{14}C which we were interested in were chosen for analysis visually thanks to the high contrast in ^{14}C content between the apical growing regions and the more proximal segments: the starting point of this region was set to the location where the ^{14}C signal started to increase compared to the average signal of the bulk root (i.e. the proximal root parts). The procedure of segmentation is exemplary shown in Figure 2.2. The length of this region with high ^{14}C was quantified using image processing toolbox in MATLAB (The MathWorks). Overlapping roots were excluded from the analysis. We assumed that the root region which showed a high ^{14}C activity was the region where ^{14}C was exuded from, as shown by Dennis et al. (2010).

Neutron radiography

Neutron radiography experiments were performed at the ICON imaging station (Kaestner et al., 2015) at Paul Scherrer Institute (PSI) in Switzerland. We used a CCD camera detector with an array of 2160 by 2560 pixels, a field of view of 11.7 cm by 13.8 cm, and a spatial resolution of 0.1 mm. We took 12 radiographs (for the big rhizoboxes) and 6 radiographs (for the small rhizoboxes) with 39% horizontal and 27% vertical marginal overlaps to scan the entire samples. Neutron radiography was used to quantitatively measure the distribution of water in the rhizosphere based on the spatial distribution of neutrons that are transmitted through the samples. As soil mixed with mucilage has a higher water holding capacity than soil without mucilage, it is possible to relate the soil water content to mucilage concentration (Kroener et al., 2014). After keeping the samples at water contents of 6% and 20% for one week, neutron radiography measurements were conducted.

A detailed description of quantification of neutron radiographs can be found in Zarebanadkouki et al. (2012). Briefly, neutron radiographs were corrected for the flat field

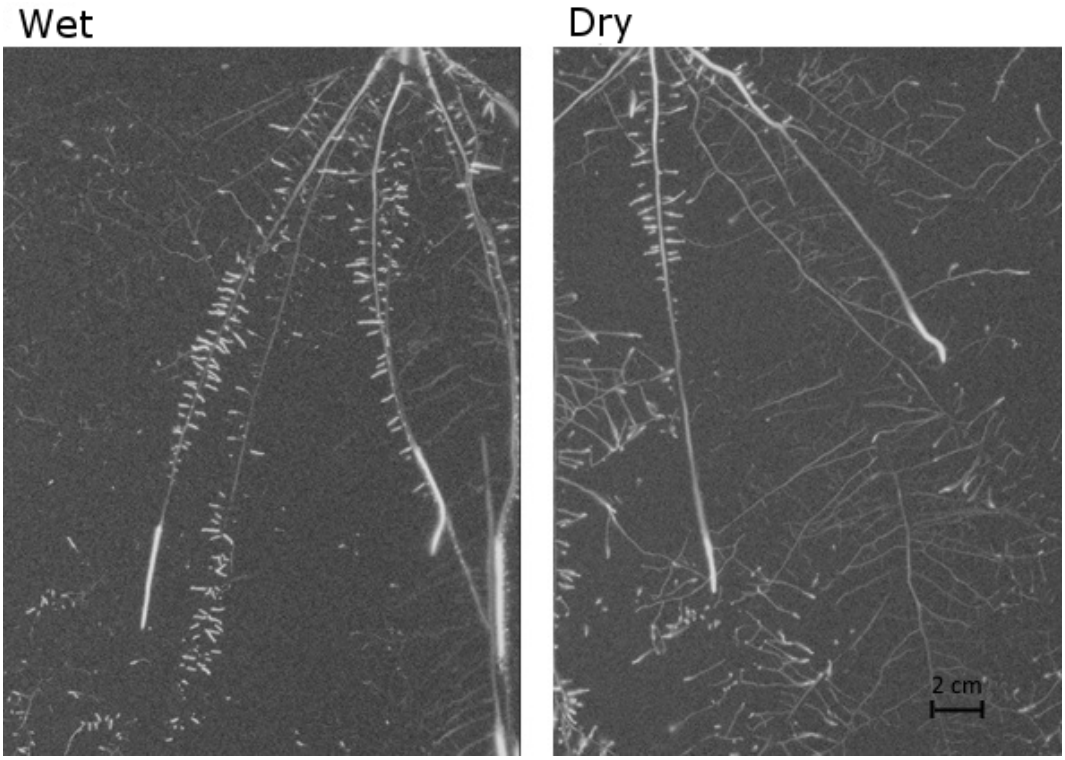

Figure 2.1: ^{14}C images of a plant grown in wet soil (left side) and a plant grown in dry soil (right side). Bright color indicates high ^{14}C activity; dark color indicates low ^{14}C activity. The sample size was 20 x 30 cm.

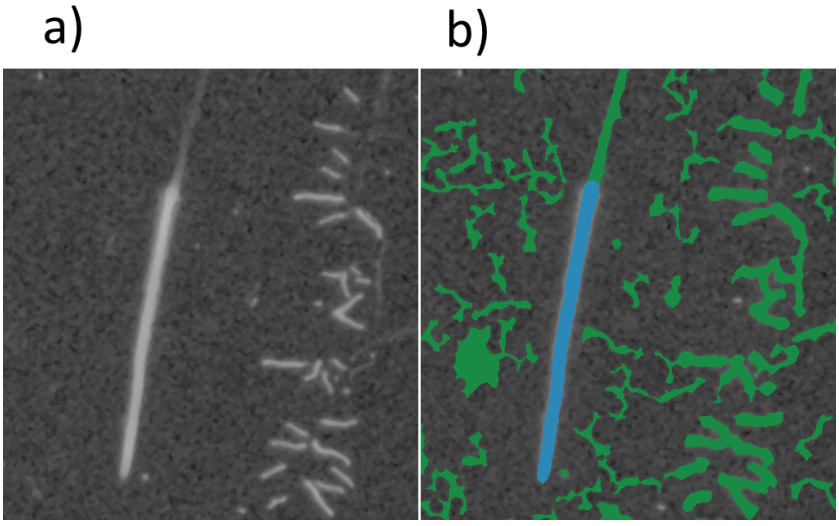

Figure 2.2: Process of image segmentation: (a) original image, (b) segmented image (blue: segmented root, green: noise), that is excluded from the analysis (e.g. crossing roots). Note that this is exemplary shown for ^{14}C imaging. However, the procedure is the same for neutron radiographs.

(I_{ff}, radiograph taken without any sample) and the dark current (I_{dc}, signal recorded by the camera when there was no beam) according to:

$$I_{norm} = \frac{I - I_{dc}}{I_{ff} - I_{dc}} \tag{2.1}$$

where I_{norm} is the corrected image and I is the original image. The signal obtained in the neutron radiographs included the attenuation of the three compounds of the sample: (a) the aluminum container, (b) soil and (c) water (including the roots). The attenuation coefficient of dry soil (μ_{soil}) and aluminum (μ_{Al}) was obtained from neutron radiography of an unplanted dry sample (packed in similar way as for planted container). The neutron attenuation of water (μ_{H_2O}) was calculated from neutron radiography of a control sample with known thickness of water (0.3 cm). The calculated attenuation coefficients were $\mu_{soil} = 0.28$ cm^{-1}, $\mu_{Al} = 0.02$ cm^{-1} and $\mu_{H_2O} = 4.72$ cm^{-1}. For the pixel containing no root the contribution of H_2O to the neutron attenuation was calculated as

$$-log\Big(\frac{I_{norm}}{I_{dry}}\Big) = \mu_{H_2O} d_{H_2O} \tag{2.2}$$

where I_{dry} is the unplanted dry sample and dH_2O is the thickness of water (cm). Volumetric water content in the soil was defined as

$$\theta = \frac{d_{H_2O}}{d_{tot}} \tag{2.3}$$

where d_{tot} is the inner thickness of the container in beam direction ($d_{tot} = 1$ cm). The roots were segmented based on the contrast between roots and soil using the program Roottracker 2D. Noises (e.g. crossing roots or cracks in soil) were also removed by the segmentation. The calculation of water content in the rhizosphere was done for main roots and lateral roots separately. For the lateral roots, segments of 0.5-1 cm were chosen, for the main roots segments of 1-2 cm were chosen. Per sample 2-15 roots were segmented, depending on how many roots were clearly visible. The length and diameter of roots and of rhizosphere soil were calculated using the Euclidean distance map (Soille, 2003) (MATLAB, The MathWorks). The average of signal was calculated for each distance from the root surface up to a distance of 3 cm from root surface. To calculate the profile of water content in soil as a function of distance from the root we needed the radial profile of soil water content while the 2D radiographs give Cartesian profiles. Neutron attenuation projected in each pixel of the

2D radiographs contains information on the average water content across the sample in the beam direction. The pixels close to the root therefore are an average of the water content in the rhizosphere and in the portion of the soil in front of and behind of the rhizosphere. Assuming radial symmetric profiles of water content around the roots, the actual 3D (radial) profile of water content in the rhizosphere was calculated following the approach given in Zarebanadkouki et al. (2016)

$$\theta(x) = \frac{2}{d_{tot}} \int_{x}^{\sqrt{x^2+\left(\frac{d_{tot}}{2}\right)^2}} \theta(r) \sqrt{\frac{r^2}{r^2 - x^2}} dr \tag{2.4}$$

where d_{tot} is the inner thickness of the sample, r is the radial distance from the root center, and x is the apparent distance from the root center in the radiograph. Equation 2.4 is derived by changing the Cartesian coordinate into radial coordinate. The average signal measured at the position x on the detector is the average of the actual water content between the distances of x and $\sqrt{x^2 + \left(\frac{d_{tot}}{2}\right)^2}$ from the root surface, which are the limits of integration in Equation 2.5. The term $\sqrt{\frac{r^2}{r^2-x^2}}$ comes from the transformation into the radial coordinates. Finally, the profiles of water content from the root surface were calculated in the same way as described for ^{14}C images.

Model

We used the approach proposed by Kim et al. (1999) and Carminati et al. (2016) to predict the distribution of root exudates in soil. The reference system was fixed to the root tip and the diffusion-convection equation was solved numerically. The following equation was used:

$$\theta \frac{\delta}{\delta t} C = \frac{1}{r} \frac{\delta}{\delta r} \left(r D(\theta) \frac{\delta C}{\delta z} \right) + \left(D(\theta) \frac{\delta C}{\delta z} \right) - \left(\theta v \frac{\delta^2 C}{\delta z^2} \right) - kC \tag{2.5}$$

where t is the time, v is the root elongation (wet: 2 cm d^{-1}; dry 1 cm d^{-1}) and k is the degradation coefficient of glucose (0.01) (Gunina and Kuzyakov, 2015). We used glucose to represent root exudates as the degradation coefficient and the diffusion coefficient for this compound are available in literature. The degradation coefficient was defined in a way that it decreased with soil water content (Palta and Gregory, 1997; Zhu and Cheng, 2013). More precisely, we assumed that k decreased as the cubic root of the water content. r and z are the radial and longitudinal directions of the system. $D(\theta)$ is the coefficient of diffusion of exudates (cm^2 s^{-1}) at varying water

contents θ and it is calculated using the equation proposed by Millington and Quirk (1961):

$$D(\theta) = D_0 \frac{\theta^{\frac{10}{3}}}{\phi^2} \tag{2.6}$$

where θ is the soil water content (cm^3 cm^{-3}), D_0 is the diffusion coefficient of glucose in pure water (5x10^{-6} cm^2 s^{-1}), and ϕ is the soil porosity (0.366 %). The boundary conditions were zero flux at the outer boundary and constant C concentration in the root C_0. To calculate C_0 the ^{14}C activity per gram of root tip (wet: 193 kBq; dry: 279 kBq) was converted into ng of labelled C g^{-1} root: The specific activity of the $NaHCO_3$ tracer (2205200 MBq mmol C^{-1}) was converted into the weight of ^{14}C per ^{14}C activity which gave 3.24 x 10^{-5} ng C Bq^{-1}. By multiplying the ^{14}C activity g^{-1} root (kBq) by this number we obtained the amount of ^{14}C g^{-1} root (ng). To obtain the total amount of Carbon (^{12}C + ^{14}C) we multiplied this number by 617570 which was the ratio between ^{12}C and ^{14}C in the chamber air during labelling. Finally the value was multiplied by the root tissue density of herbaceous plants of 0.22 g cm^{-3} (Birouste et al., 2014) to obtain volumetric units (μg cm^{-3}). This resulted in a C concentration of 852.25 ($\pm$ 370.54) μg C cm^{-3} for plants grown in wet soil and 1222.78 ($\pm$ 123.51) μg C cm^{-3} for plants grown in dry soil. As these differences were not statistically different the average of both (1037.52 μg C cm^{-3}) was used for the C concentration (C_0) in the root. The exudation rate per root surface was given by:

$$E(z) = D_{root}\left[C_{root} - C(r = r_{root}, z)\right] \tag{2.7}$$

where E (μg C cm^{-2} s^{-1}) changes along the root, D_{root} = 1.15 x 10^{-4} cm h^{-1} is the diffusion coefficient of the root, taken from (Farrar et al., 2003), and C ($R = r_{root}, z$) is the concentration in the soil at the root surface at a given distance z from the root tip. The root segment from which exudates are releases was set to 1 cm for wet plants and 0.5 cm for dry plants (Sharp et al., 2004) because we found that the root elongation rate was correlated to the length of the root zone where ^{14}C was transported to and presumable exuded from. This zone was half as long as for plants in dry soil compared to plants in wet soil. The spatial discretization was 0.1 cm in longitudinal direction and 0.01 cm in radial direction.

Table 2.1: Shoot, root and rhizosphere (rhizo) dry weight and total ^{14}C as well as ^{14}C translocated from the shoot (^{14}C per gram shoot (kBq)) into the root (^{14}C per gram root (kBq)) and from the root into the rhizosphere (^{14}C per gram rhizo (rhizosphere soil) (kBq)). Variation is given as standard error. The number of replicates was 5 per treatment. The p value is presented as follows: ns: $p \geq 0.05$, *: $p < 0.05$.

	Wet plants	Dry plants	p-value
Dry weight shoot (g)	2.97 (0.23)	2.29 (0.22)	ns
Dry weight root (g)	1.34 (0.12)	0.95 (0.03)	*
Dry weight rhizo(g)	19.00 (3.83)	7.60 (0.38)	*
Total ^{14}C in shoot (kBq)	340.8 (60.0)	209.2 (12.3)	*
Total ^{14}C in root (kBq)	69.07 (13.34)	34.44 (8.23)	*
Total ^{14}C in rhizo (kBq)	4.61 (1.45)	2.87 (1.28)	ns
^{14}C per gram shoot (kBq)	113.3 (9.88)	96.3 (12.35)	ns
^{14}C per gram root (kBq)	51.32 (5.81)	36.47 (8.11)	ns
^{14}C per gram rhizo (kBq)	0.25 (0.054)	0.37 (0.13)	ns
^{14}C per gram root tip (kBq)	193.67 (28.11)	279.79 (83.89)	ns
^{14}C translocated from shoot to root (%)	46.21 (5.81)	15.93 (2.70)	*
^{14}C translocated from root to rhizo (%)	6.77 (1.72)	8.11 (2.50)	ns

Statistics

The significances of differences in plant biomass and ^{14}C allocation between the treatments (dry/wet soil) were tested using R 3.3.1. A one way ANOVA followed by a post hoc test (Tukey-Test) was used. The level of significance was $\alpha = 0.05$. To test for significances in root elongation between treatments a mixed effect model ($\alpha = 0.05$) with treatment as fixed effect and plant as random effect was applied. To account for the differences in numbers of roots sampled per plant, the restricted maximum likelihood (REML) method was applied.

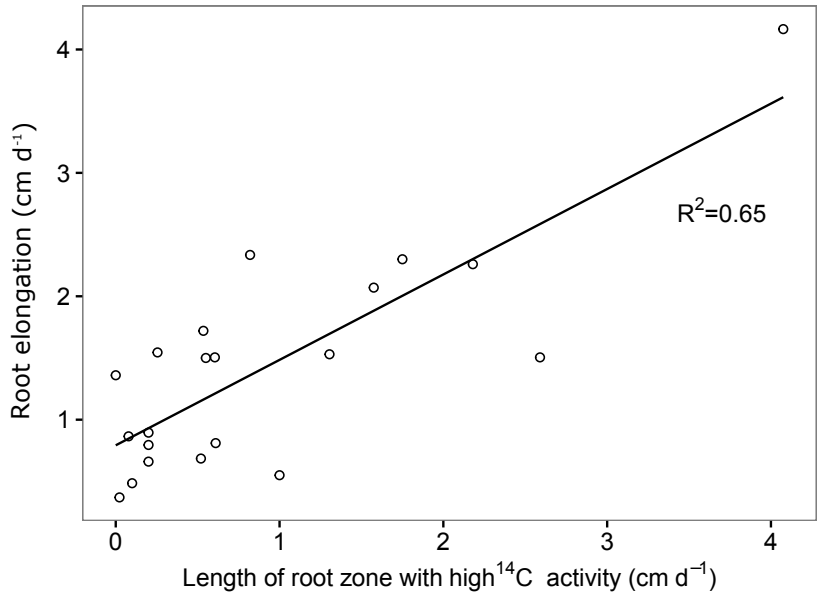

Figure 2.3: Correlation between the root elongation rate (cm d^{-1}) and the length of the root zone which showed a high ^{14}C activity in the phosphor images (cm d^{-1}). The correlation between the two variables was significant based on a level of $p < 0.05$.

2.3 Results

Plant biomass and ^{14}C allocation in plants

Shoot biomass was similar for plants grown in wet and in dry soil. Root biomass and dry weight of rhizosphere soil were higher for plants grown in wet soil (Table 2.1) compared to plants grown in dry soil. Wet plants showed a higher ^{14}C activity in shoots and roots, while the ^{14}C activity in rhizosphere was independent on soil moisture. Similarly, ^{14}C activity per gram of root and rhizosphere soil as well as in the root tips was similar for both moisture levels. While plants grown in wet soil transported higher amounts of assimilates (^{14}C) from the shoot to the root compared to the dry treatment, ^{14}C transported from root to rhizosphere was similar for both soil water contents (Table 2.1).

^{14}C imaging and root growth

Root elongation of main roots was twice as high in wet compared to dry soil (2 cm d^{-1} vs. 1 cm d^{-1}) (Table 2.2). For lateral roots elongation rates were similar for plants grown in dry and in wet soil. ^{14}C was mostly allocated to the root tips of either

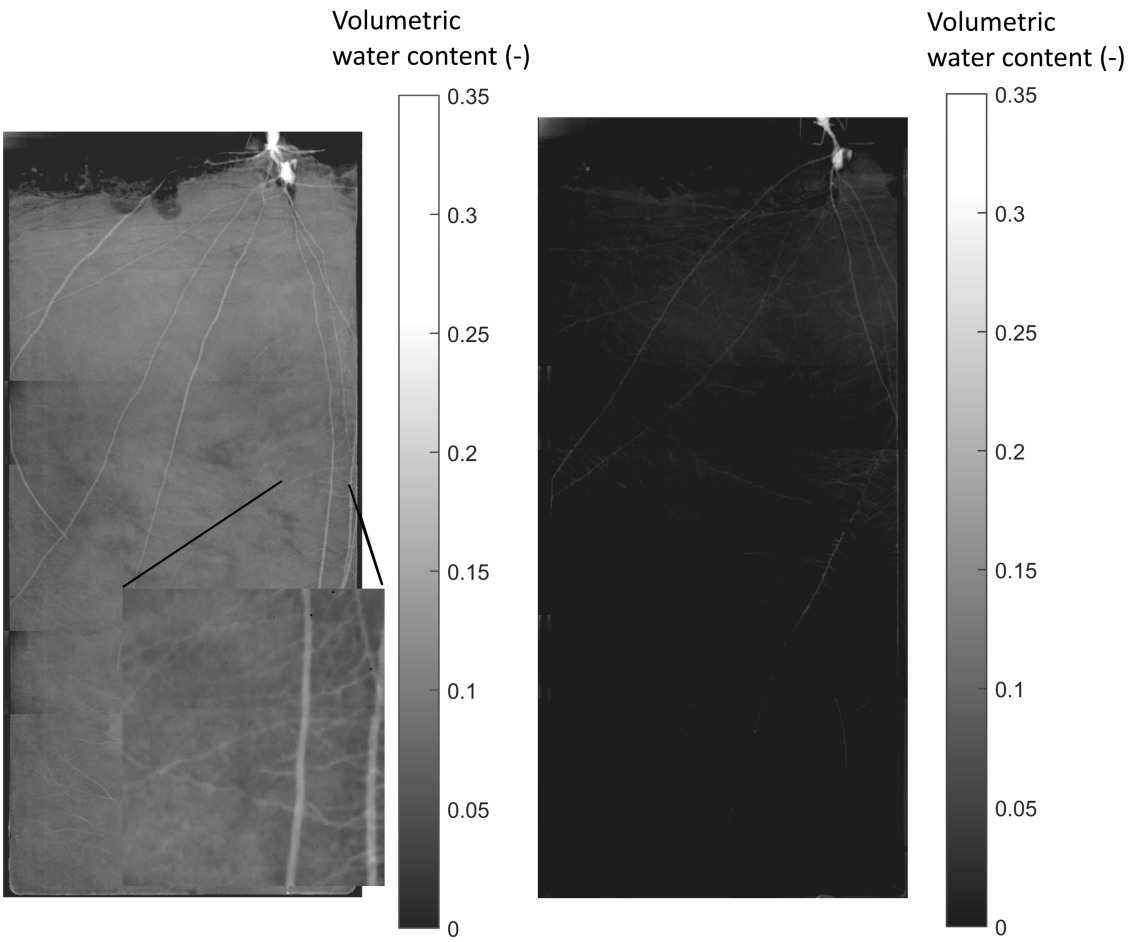

Figure 2.4: Water content (WC) for the 6 week old maize plants measured by neutron radiography. Left side: moist soil and closeup (20% vol. WC), right side: dry soil (6% vol. WC). The inner size of the sample was 15 x 30 cm.

Table 2.2: Root elongation rate for main and lateral roots of plants grown under dry and wet conditions. Variation is given as standard error. The number of replicates was 5 per treatment. The p value is presented as follows: ns: $p \geq 0.05$; *: $p < 0.05$.

	Main roots			Lateral roots		
	Wet	Dry	p-value	Wet	Dry	p-value
Root elongation	2.06	1.08	*	0.23	0.16	ns
(cm d^{-1})	(0.57)	(0.23)		(0.02)	(0.04)	

main roots or lateral roots (Fig. 2.1). Nearly no ^{14}C activity was found around old root segments. The length of the segments of the main roots with high ^{14}C activity correlated well with the root elongation rates ($R^2 = 0.65$) (Fig. 2.3.)

Neutron radiography

Neutron radiography showed that the water content close to the root surface was similar for both treatments although the samples had different bulk soil water content (Fig. 2.4). The water content in the rhizosphere was always higher than in the bulk soil (Fig. 2.5). It increased 7 times for the main roots and 6 times for lateral roots in dry soil at the root surface compared to the bulk soil. In wet soil, the water content in the rhizosphere was 1.5 times higher compared to bulk soil. At the root surface, water content ranged around 30%. The water content reached the bulk soil value at a distance of 0.3-0.4 from the root surface (Fig. 2.5).

Model

Total exudation after the simulation time of two days was approximately twice as high in wet soil compared to dry soil (Table 2.3, Fig. 2.6). For wet soil it ranged around 1.78 and 1.75 μg C cm^{-3}, for dry soil between 0.72 and 0.9 μg C cm^{-3}. The amount of total exuded C did not change even if microbial decomposition of exudates was included in the model. Only in the scenario with a constantly low water content of 6%, the microbial decomposition resulted in a slightly higher total C exudation (no decomposition: 0.72 μg C cm^{-3}; with decomposition: 0.77 μg C cm^{-3}). Unlike total exudation, microbial decomposition reduced the amount of exudates that were found

Table 2.3: Modelled results on total exudation and the amount of exudates which remained in soil after the simulation time of two days. The Wet and Dry treatments assume a water content which increases towards the roots (as seen in neutron radiographs). For the constant water content we assumed that the water content does not change in the rhizosphere and is constantly 15% (wet) and 6% (dry).

		Exudates remaining in soil after 2 days (μg C)	Tot. exudation after 2 days (μg C)
Wet	No decomposition	1.78	1.78
	Decomposition	0.12	1.77
Wet const. WC	No decomposition	1.77	1.75
	Decomposition	0.12	1.77
Dry	No decomposition	0.88	0.89
	Decomposition	0.05	0.90
Dry const. WC	No decomposition	0.72	0.72
	Decomposition	0.07	0.77

in soil after two days of simulation of approximately 10 times for wet soil and of 15 times for dry soil. These changes were independent of changes in rhizosphere water content for wet soil. Instead, in dry soil an increased rhizosphere water content resulted in a lower amount of exudates remaining after two days (0.72 μg C cm^{-3}) compared to the scenario with constant rhizosphere water content (0.88 μg C cm^{-3}). The decrease in root elongation (and in the exuding root segment) caused a strong reduction in longitudinal rhizosphere extension (Fig. 2.7) the predicted longitudinal extension of rhizosphere in wet soil was around 6 cm, in dry soil it was ca. 4 cm. Microbial decomposition further decreased the extension to around 2 cm in wet soil and ca. 1 cm in dry soil. (Fig. 2.6, 2.7). Assuming increased soil water content towards the root enhanced the longitudinal rhizosphere extension in dry soil if microbial decomposition was not considered. The radial extension of rhizosphere was highest in wet soil if microbial decomposition was not considered. Decomposition strongly reduced the extension to 0.25 cm for wet soil and 0.1 cm for dry soil. Radial profiles were steeper in dry soil compared to wet soil if constant water content was assumed (Fig. 2.7).

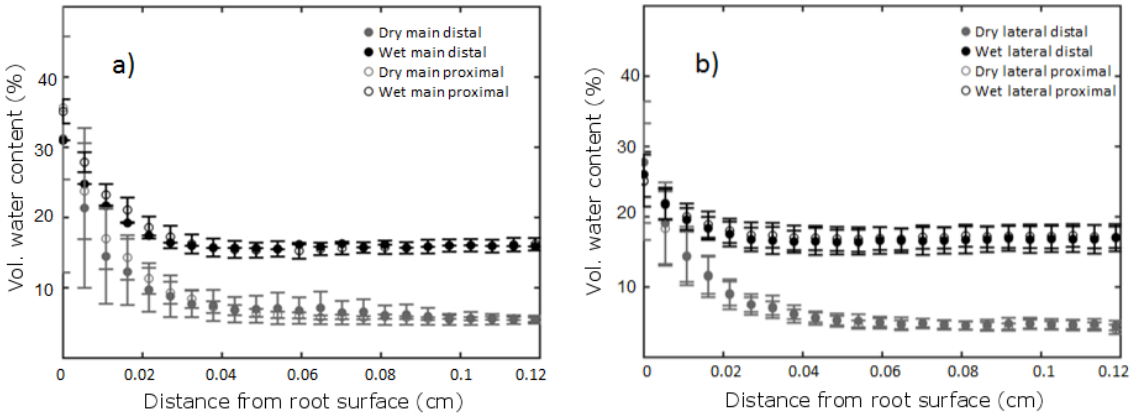

Figure 2.5: a) Water content in the rhizosphere of the proximal parts (i.e. the old parts) and the distal part (i.e. root tips) of a) the main roots and b) of the lateral roots. Variation is given as standard error, n = 5.

However, the diffusion of exudates into the soil was higher (Fig. 2.7) if the increase of water content towards the root was considered: this resulted in an increase in radial rhizosphere extension in dry soil. In the wet soil however, the change in rhizosphere water content had minor effects on the radial distribution of exudates.

2.4 Discussion

Drought is expected to increase the translocation of recently assimilated C to roots (Sanaullah et al., 2012; Palta and Gregory, 1997; Reid and Mexal, 1977). Similarly to Canarini and Dijkstra (2015), we found that the amount of C allocated in the roots was equal for plants grown in wet and in dry soil when normalized for the root biomass. In contrast, the total ^{14}C input into roots was reduced under dry conditions. The reduced photosynthesis and the reduction of biomass probably decreased the total C input into the soil (Canarini and Dijkstra, 2015). It should be considered that drought in our study lasted for only one week and therefore the effects on C allocation were less strong compared to studies with longer drought periods (Fuchslueger et al., 2013). ^{14}C activity was highest around the tips of main and lateral roots (Fig. 2.2). The region of high ^{14}C activity on the images corresponds well with the root zone where ^{14}C is exuded (Dennis et al., 2010). We therefore conclude that exudation took place at the root tips, which showed a high ^{14}C activity (Haichar et al., 2014; Personeni et al., 2007). High exudation at root tips can be explained by their high

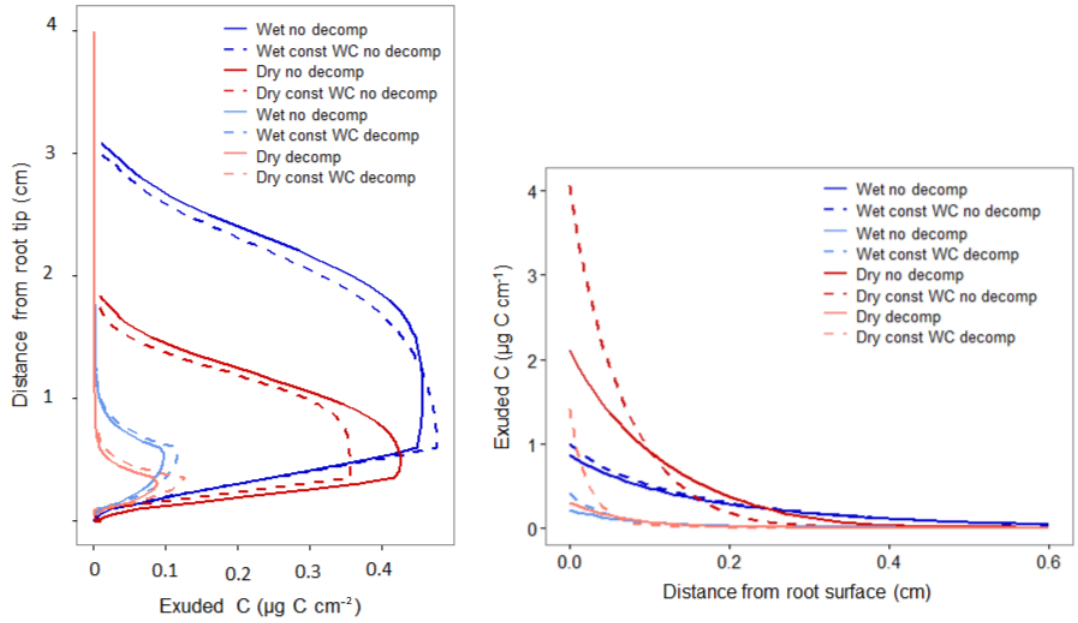

Figure 2.6: Left: Modeled axial distribution of exudates in the rhizosphere after two days of simulation. const = constant water content towards the root surface is assumed. For all other scenarios an increase in soil water content towards the root is assumed as measured with neutron radiography. decomp = microbial decomposition. Right: Modeled radial distribution of exudates in the rhizosphere after two days of simulation.

sugar concentration compared to older root zones, as well as by their high diffusional permeability compared to older root parts (Nguyen, 2003; Jones et al., 2009).

The modeled exudation rates were comparable with rates measured in other studies (Toal et al., 2000; Nguyen, 2003) but at the lower range. The reason might be that the model did not consider root hairs or mycorrhization, which increase C release. Root hairs increase the spatial extend of root exudates in soil (Holz et al., 2017b) however, for simplicity they were not considered in the model. The radial rhizosphere extension of 1.5-6 mm was comparable with values reported in other studies (Hafner et al., 2014; Schweinsberg-Mickan et al., 2012; Kuzyakov et al., 2003; Toal et al., 2000). Total root exudation was higher for the wet soil compared to the dry soil because: a) exudate diffusion increased with water content, and b) the increased length of exudation zone for plants in moist soil caused a higher exudation compared to plants in dry soil. Decomposition decreased the amount of exudates that remained in soil

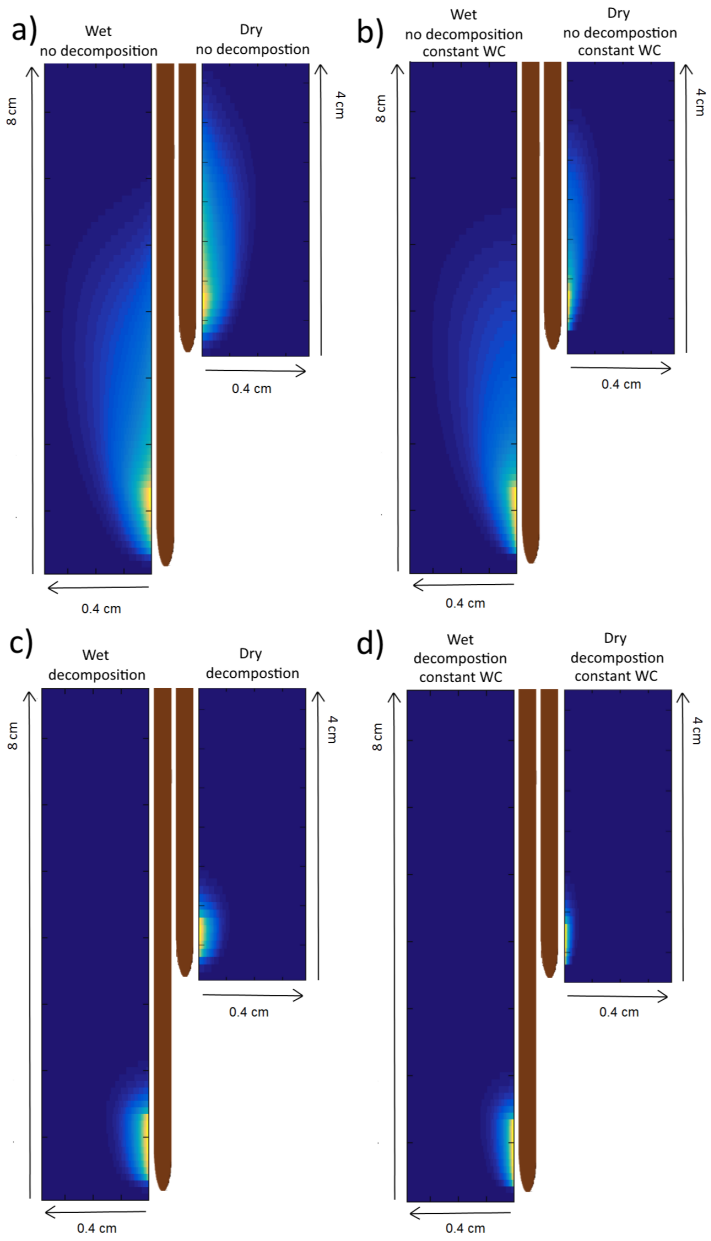

Figure 2.7: Simulated distribution of exudates two days after release from root tip into the soil for all modelled scenarios: a, b) no microbial decomposition, c, d) constant rhizosphere water content, i.e. the water content does not increase towards the root surface. Yellow indicates high concentration and blue indicates null concentration. Note that the differences in the lengths of the roots for wet and dry soil are caused by differences in root elongation rate.

after two days but did not change the total exudation. This was unexpected because microbial decomposition may increase root exudation due to the removal of exudates that increases the concentration gradient (Nguyen, 2003; Meharg and Killham, 1995). However, the exudation in our model depended solely on the concentration gradient between C inside the root and C in the first node of soil at the tip where exudates were released. The differences in concentration gradients in this zone were relatively small between scenarios with and without microbial decomposition. Therefore higher gradients (i.e. higher decomposition rates) or increased root permeability would have been needed for decomposition to increase total exudation.

The length of the zone with active exudation was reduced by 50% for plants grown in dry soil compared to plants under optimal moisture. This caused a strong reduction in the longitudinal rhizosphere extension for plants in dry soil. The decrease in the length of the exuding root zone and the shortening of longitudinal rhizosphere extension was one of the strongest effects that the drought treatment had. However, the increase in water content close to the root, which was more pronounced in dry soil, partly compensated this effect: the increase in exudate diffusion in the vicinity of the roots increased a) the total exudation and b) the radial extension in the rhizosphere. Most likely, the increase in water content close to the root was caused by the large water retention capacity of mucilage. Mucilage is a mixture of polysaccharides and some lipids exuded by the root tip and it increases the soil water holding capacity (Carminati and Vetterlein, 2013; Carminati et al., 2010; North and Nobel, 1997; Young, 1995). Little is known about the effect of soil drying on mucilage release. Iijima et al. (2000) found that mechanical impedance increases the number of sloughed root cap cells and the amount of mucilage. Additionally, reduced root elongation rate in dry soil will lead to a higher mucilage concentration per rhizosphere soil volume (assuming that mucilage release per time is not decreased in dry soil). We therefore expect that a higher mucilage concentration in the rhizosphere of the dry soil led to a strong increase in local soil moisture resulting in comparable water contents under dry and wet conditions.

Although we expected that increasing water content towards the root would increase the spread of exudates, the opposite was true if microbial decomposition was included into the model: microbial activity increases with soil water content that are comparable with the wet treatment used in our study (Zhu and Cheng, 2013; Palta and Gregory, 1997). This led to faster decomposition of root exudates in wet soil.

The fast decomposition of exudates might be beneficial for plants because increased microbial abundance and turnover will lead to increased nutrient release from microorganisms (Kuzyakov and Xu, 2013; Herman et al., 2006; Hamilton and Frank, 2001) and from microbial decomposition of soil organic matter (Chen et al., 2013).

2.5 Conclusions

The distribution of root exudates in the rhizosphere depends on various processes including root elongation rate and exudation rates which are both affected by the local water content in the rhizosphere. Soil water content increased towards the root particularly in dry conditions, most likely due to an increased secretion of mucilage that holds high amounts of water in the rhizosphere. The increased water content had the following effects: a) it enhanced the total exudation especially for plants grown in dry soil, b) it extended the rhizosphere volume in the radial direction and c) it accelerated microbial decomposition of exudates in the vicinity of roots.

Drought directly affected exudation by reducing the root growth and the zone, where exudates are released, and caused a strong decrease in total exudation in dry soil. However, this negative effect of drought on exudation was partly compensated by the increase of water content towards the root. We conclude that roots enhance the diffusion of exudates into the soil by releasing mucilage which increases the local water content in the rhizosphere. Hereby, they stimulate interactions with microorganisms and contribute to a higher nutrient accessibility.

2.6 Acknowledgement

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3 Coping with drought: Plant roots maintain enzyme activity in drying soils by increasing water retention in the rhizosphere

written by Maire Holz, Mohsen Zarebanadkouki, Andrea Carminati, Jan Hovind, Anders Kaestner and Marie Spohn

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Abstract

Severe soil drying negatively impacts nutrient uptake and enzyme activity in the rhizosphere and it is not clear how plant roots adjust to it. Here we propose that water retention in the rhizosphere is functional to maintain high enzyme activity around roots under dry conditions. We show that plants maintain a high phosphatase activity around roots during periods of drought by retaining moisture in the rhizosphere, which facilitates fast diffusion of exoenzymes and thus high rates of enzymatic catalysis. Barley plants were grown in rhizoboxes and subjected to a drying cycle, while soil water content (WC) and phosphatase activity were monitored by neutron radiography and soil zymography. The rhizosphere WC increased with soil drying relative to the WC of the bulk soil probably due to plant-derived mucilage. Enzyme activity increased in the rhizosphere compared to the bulk soil from a ratio of 10 at 40% WC to a ratio of 63 at 5% WC. Enzyme activity and local soil WC were strongly correlated (rhizosphere: $R^2 = 0.53$, bulk: $R^2 = 0.63$), indicating that diffusion controls soil enzyme activity. Our study demonstrates that the activity of exoenzymes in soil strongly depends on the local soil WC and that by retaining water in the rhizosphere plants maintain an increased enzyme activity around roots exposed to drought. This is beneficial for plant nutrition because the high WC of the rhizosphere facilitates a high rate of phosphatase activity and phosphorus (P) diffusion, and thus plant P acquisition.

3.1 Introduction

Although drought events will likely occur more frequently in the future (FAO, 2012; Trenberth et al., 2014), little is known about the effect of soil drying on plant nutrient acquisition and about plant strategies to adjust to soil drying and limited nutrient

availability. A key place where still unknown adaptation strategies take place is the rhizosphere, the interface between soil and roots (Gregory, 2006; Neumann et al., 2009). Studying the rhizosphere is difficult using traditional methods. However, novel imaging techiques allow to explore the effect of soil drying on processes of nutrient acquisition in the rhizosphere in situ (Oburger and Schmidt, 2016).

Plants release organic compounds such as exoenzymes into the soil. Some of them, namely phosphatases, are of great importance for plant nutrition because they catalyze the hydrolysis of organic phosphorus (P), which results in the formation of inorganic P that can be taken up by plants. Phosphatases are produced and released into the soil by soil microorganisms and by plant roots (Nannipieri et al. 2011; Rejsek et al. 2012, and references therein). Plants increase phosphatase activity in the rhizosphere directly by the release of phosphatases (George et al., 2002; Tarafdar and Claassen, 1988) and indirectly (Rejsek et al., 2012) by stimulating microbial activity in the rhizosphere (Landi et al., 2006) and therefore phosphatase production (Spohn and Kuzyakov, 2013). In short, the rhizosphere is a hot spot of enzyme activity (Richardson et al., 2009).

Exoenzyme movement in soil is mainly controlled by diffusion and consequently by soil WC. The higher the soil WC, the higher the diffusion rate, and thus the higher the chance that an enzyme and a substrate molecule meet, and that the substrate is enzymatically transformed (Allison et al., 2011; Koch, 1990). In drying soils, water films become thinner and increasingly discontinuous, the water-filled cross-section for the substrates to diffuse decrease and becomes more tortuous, and thus diffusion of solutes and enzyme activity is strongly decreased (Burns et al., 2013; Manzoni et al., 2012). In a meta-analysis Manzoni et al. (2012) revealed that diffusion rather than biological processes impede microbial and enzyme activity in dry conditions. However, direct experimental evidence of such effects in the rhizosphere is lacking, which is in part because until recently there has been no method to determine soil enzyme activity in situ. Only a recently developed imaging technique allows for determining soil enzyme activity at different soil WCs (Spohn et al., 2013).

For the rhizosphere to act as a hot spot of enzyme activity, it has to maintain high soil moisture content to sustain diffusion of enzymes. An increased soil WC in the rhizosphere compared to the bulk soil has been repeatedly reported (Carminati et al., 2010; Holz et al., 2017a; North and Nobel, 1997; Young, 1995) and was explained by the release of mucilage by plant root tips (Ahmed et al., 2014; Carminati et al.,

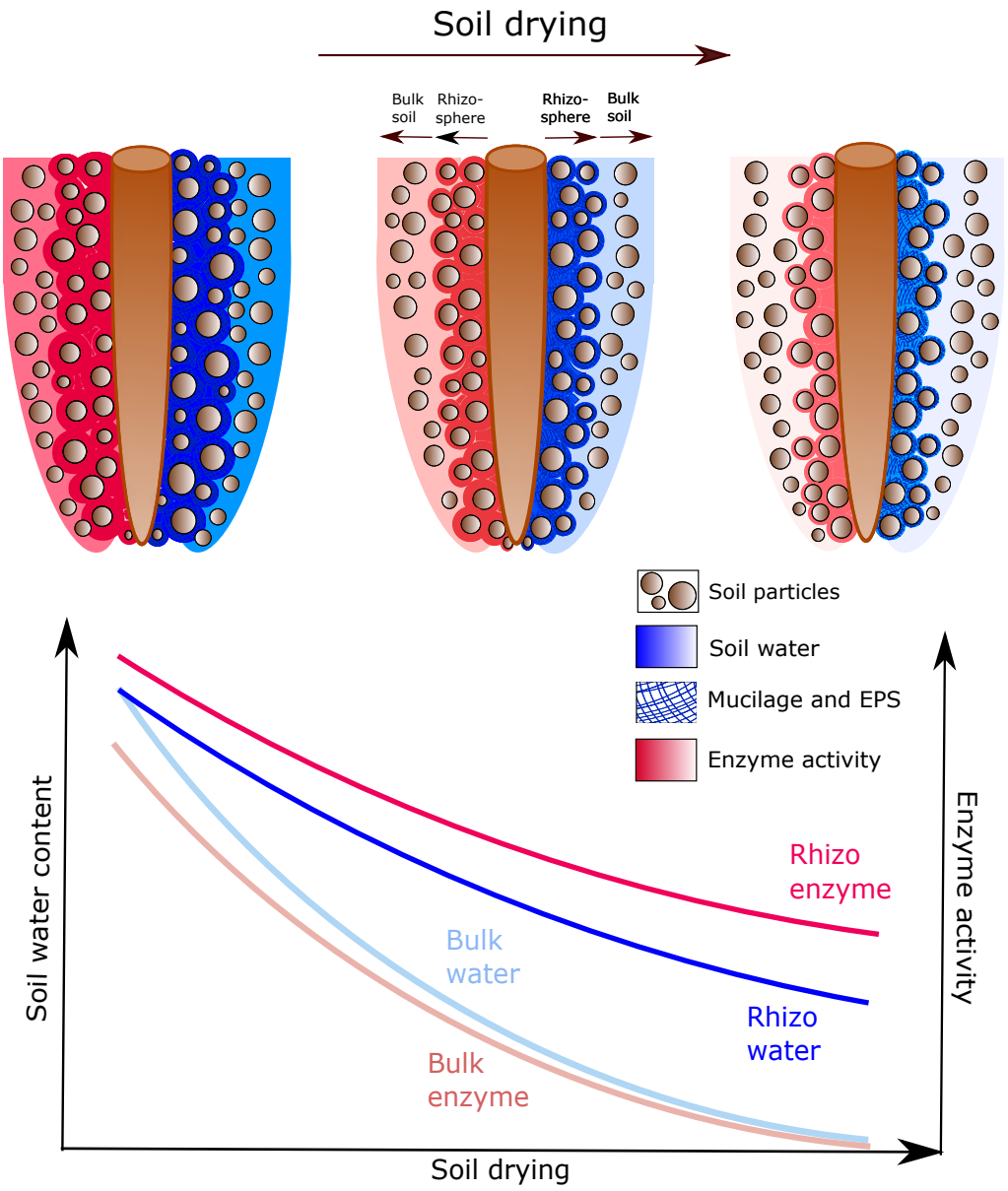

Figure 3.1: Conceptual model describing the change in enzyme activity and water content in bulk soil and rhizosphere with soil drying. The bulk soil dries more strongly than the rhizosphere, in which water is retained by mucilage and EPS. Thus, enzyme activity, which strongly depends on diffusion, decreases less with soil drying in the rhizosphere than in the bulk soil.

2010; McCully and Boyer, 1997) and of extracellular polymeric substances (EPS) by soil microorganisms (Or et al., 2007). Both substances are able to store an amount of water up to 1000 times of their own dry weight due to their gel-like structures (McCully and Boyer, 1997; Roberson and Firestone, 1992). When the soil dries, these substances retain water and thus keep the rhizosphere moist, while the bulk soil dries out more quickly.

The aim of this study was to test whether plants maintain a high phosphatase activity in the soil close to the roots during periods of drought by retaining moisture in the rhizosphere that allows for fast diffusion of exoenzymes and substrate, and thus high rates of enzymatic catalysis (Fig. 3.1). This would be beneficial for plant nutrition because it allows plants to maintain a high rate of P mineralization in the rhizosphere even during periods of drought. To test this concept, we grew barley plants in rhizoboxes and subjected them to a drying cycle. During the drying cycle phosphatase activity was monitored by soil zymography, which allows for measuring soil enzyme activity under in situ WCs (Spohn and Kuzyakov, 2013), and soil WC in the soil-root system was visualized over time using neutron radiography (Zarebanadkouki et al., 2012).

We hypothesized that: (i) barley maintains a high WC in the rhizosphere compared to the bulk soil during periods of drought due to the effect of mucilage and EPS that keep the rhizosphere moist; and (ii) phosphatase activity in the rhizosphere changes less with soil water content (WC) than phosphatase activity in the bulk soil due to mucilage and EPS that retain water in the rhizosphere, and thus facilitate fast diffusion.

3.2 Material and Methods

Experimental design

Barley plants were grown in rhizoboxes that allowed us to determine the distribution of water and exoenzyme activity in soil. Barely seeds were immersed in a 10% H_2O_2 solution for 10 min to avoid seed-borne diseases before germination. Seedlings were planted 3 days after germination. The barley plants were grown in rhizoboxes with an inner size of 10x20x0.6 cm for 27 days (Figure 3.2a). The boxes were inclined by 55 °C during the entire cultivation, to make the roots grow along the bottom wall of the boxes. The soil was a sandy soil collected from an A horizon of a cropland close

to Göttingen, Germany. TOC was 2.0%, TN was 0.17% and the pH amounted to 4.9. Soil particle size was distributed as follows: Clay: 8.6%, silt: 18.5%, sand: 73% (Holz et al., 2017b). The plants were kept in a climate chamber and during the total growth period, the volumetric soil WC was maintained at 23-25%. The temperature was 25°C during the day and 22°C during the night, the photoperiod was 14 hours and the photosynthetic photon flux density was 300 μmol m^{-2} s^{-1}. After transferring the plants for neutron radiography and zymography measurements to Paul Scherrer Institute (Switzerland), they were adjusted to different WCs. In order not to stress the plants due to many repeated measurements, the plants were divided into two groups. Each group comprised 12 plants, 6 wild type plants with root hairs (WT) and 6 plants without root hairs (brb) (n = 6). The reason for using genotypes with and without hairs was to estimate the effect of root hairs on both local water content and enzyme activity as well as potential artefacts originating from the root hairs being interpreted as regions with higher water content or enzyme activity. Group one was adjusted to 5% WC, and after the neutron and zymography measurement were completed, it was rewetted to 40% WC. Group two was adjusted to 30% WC, and after the measurements to 15% and finally to 10%. At each WC, enzyme activity and WC were determined. The adjustments of the WCs were done as fast as possible, and the WC of the rhizoboxes was changed from 30 to 10% within 10 hours. Neutron radiography and zymography measurements were performed directly after each other with not more than 1.5 hours of difference.

Neutron radiography

The distribution of water was determined by neutron radiography, which quantifies the spatial distribution of neutrons that are transmitted through the samples (Figure 3.2b). Neutron radiography measurements were performed at the NEUTRA imaging station at the Paul Scherrer Institute (PSI) in Switzerland. We used a CCD camera detector with an array of 2160 x 2560 pixels, a field of view of 15.3 x 18.1 cm, and a spatial resolution of 0.07 mm. We took two radiographs per rhizobox with 39% vertical overlap to scan the entire sample.

A detailed description of quantification of neutron radiographs can be found in Zarebanadkouki et al. (2012). Briefly, neutron radiographs were corrected for the flat field (I_{ff}, radiograph taken without any sample) and the dark current (I_{dc}, signal recorded by the camera when there was no beam) according to:

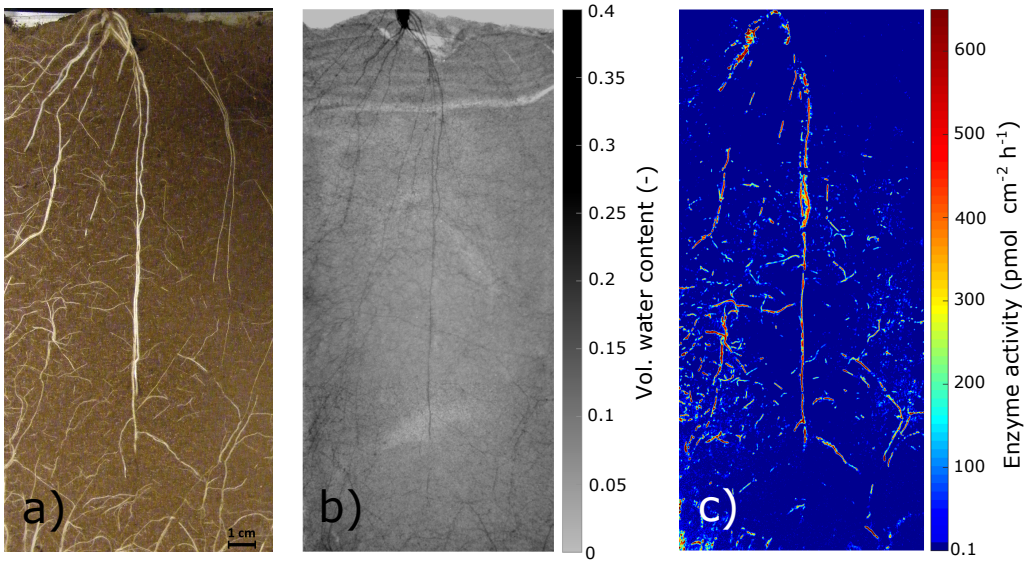

Figure 3.2: Exemplary images of a) barley roots in an open rhizobox, b) the same root system imaged by neutron radiography showing the volumetric water content and c) zymography depicting the distribution of the phosphatase activity of the same sample.

$$I_{norm} = \frac{I - I_{dc}}{I_{ff} - I_{dc}} \tag{3.1}$$

where I_{norm} is the corrected image and I is the original image. The signal obtained in the neutron radiographs included the attenuation of the three compounds of the sample: (a) the aluminum container, (b) soil and (c) water (including the roots). The attenuation coefficient of dry soil (μ_{soil}) and aluminum (μ_{Al}) was obtained from neutron radiography of an unplanted dry sample (packed in similar way as for planted container). The neutron attenuation of water (μ_{H_2O}) was calculated from neutron radiography of a control sample with known thickness of water (0.3 cm). The calculated attenuation coefficients were $\mu_{soil} = 0.28$ cm^{-1}, $\mu_{Al} = 0.02$ cm^{-1} and $\mu_{H_2O} = 4.72$ cm^{-1}. For the pixel containing no root the contribution of H_2O to the neutron attenuation was calculated as

$$-log\left(\frac{I_{norm}}{I_{dry}}\right) = \mu_{H_2O} d_{H_2O} \tag{3.2}$$

where I_{dry} is the unplanted dry sample and d_{H_2O} is the thickness of water (cm). Volumetric WC in the soil was defined as

$$\theta = \frac{d_{H_2O}}{d_{tot}} \tag{3.3}$$

where d_{tot} is the inner thickness of the container in the beam direction ($d_{tot} = 0.6$ cm). To calculate the profile of WC in soil as a function of distance from the root, the radial profile of soil WC was needed while the 2D radiographs give Cartesian profiles. Neutron attenuation projected in each pixel of the 2D radiographs contains information on the average WC across the sample in the beam direction. The pixels close to the root are therefore an average of the WC in the rhizosphere of the soil in front of and behind of the rhizosphere. Assuming radial symmetric profiles of WC around the roots, the actual 3D (radial) profile of WC in the rhizosphere was calculated following the approach given in (Zarebanadkouki et al., 2016):

$$\theta(x) = \frac{2}{d_{tot}} \int_x^{\sqrt{x^2+\left(\frac{d_{tot}}{2}\right)^2}} \theta(r) \sqrt{\frac{r^2}{r^2 - x^2}} dr \tag{3.4}$$

where d_{tot} is the inner thickness of the sample, r is the radial distance from the root center, and x is the apparent distance from the root center in the radiograph. Equation 3.4 is derived by changing the Cartesian coordinates into radial coordinates. The average signal measured at the position x on the detector is the average of the actual WC between the distances of x and $\sqrt{x^2 + \left(\frac{d_{tot}}{2}\right)^2}$ from the root surface, which are the limits of integration in Equation 3.5. The term $\sqrt{\frac{r^2}{r^2-x^2}}$ comes from the transformation into the radial coordinates.

The roots were segmented based on the contrast between roots and soil using the program Roottracker 2D (Menon et al., 2007). In cases where the contrast between roots and soil was too low for the program to detect roots, they were segmented manually. The root diameter of segmented roots was calculated using the Smart Root plugin (Lobet et al., 2011) in ImageJ (https://fiji.sc/). Noises (e.g. crossing roots or cracks in the soil) were also removed by segmentation. Per image, 2-8 roots were segmented, depending on how many roots were clearly visible. The length and diameter of roots and of rhizosphere were calculated using the Euclidean distance map (Soille, 2003) (MATLAB, The MathWorks). The average signal was calculated for each distance from the root surface up to a distance of 3 cm from the root surface. The first 0.5 mm of the radial measurements were averaged as rhizosphere WC, while the measurements in 0.55-30 mm distance from the root surface were averaged as bulk soil WC.

Zymography

The distribution of phosphatase activity was determined by soil zymography. The measurements were conducted according to Spohn and Kuzyakov (2013) but without an agarose gel as in Razavi et al. (2016). Briefly, polyamide membrane filters (Tao Yuan, China) with a pore size of 0.45 μm were cut into 10 x 20 cm pieces. Each membrane filter was placed in a solution of 5 ml of water containing 2.2 mg 4-methylumbelliferyl-phosphate (MUF-P), which is a substrate for phosphatases that turns fluorescent after hydrolyzation. The membrane was soaked with the solution and then quickly dried for 3 min at room temperature before attaching it to the soil in the rhizoboxes for 60 min. Subsequently, the membrane was removed from the soil surface and remaining soil was carefully removed with a small brush. The membranes were placed on an epi-UV-desk (Desaga) in the dark, and pictures were taken at 360 nm wavelength (Figure 3.2c) with a digital camera (Nikon D3100). Additionally to the actual membrane, a small piece of membrane (2x6 cm) without substrate was also imaged to control for the background signal.

For calibration, solutions with 0, 19, 37, 62, 87 and 125 μM 4-methylumbelliferone (MUF) were prepared. Pieces of membranes with a size of 4 x 4 cm were cut and coated with the solutions and imaged in the same way as the zymographs. From the amount of solution taken up per cm of membrane and the MUF concentration of the solution, the concentration of MUF per area was calculated. For the calculation of the phosphatase activity, the amount of MUF per area was divided by the incubation time. The correlation between MUF activity (μmol cm^{-2} h^{-1}) and the grey value from the images was described be the following equation:

$$y = 94.5x^{2.069} \tag{3.5}$$

where y is the phosphatase activity (pmol cm^{-2} h^{-1}) and x is the grey value of the image. The equation obtained from the calibration (Eq. 3.5) was applied to all images to convert the grey value to phosphatase activity. The signal of the control membrane was subtracted from the image to remove the background signal. Based on the contrast between roots and soil, root segments of 3 - 5 cm were segmented using the SmartRoot plugin in ImageJ. Possible artefacts caused by overlapping roots or roots detached from the soil were removed from the segmentation. 2 - 8 roots per image were segmented depending on how many roots were clearly visible. The signal

was averaged as a function of distance from the root center up to a distance of 4 cm from the root surface using the Euclidean distance mapping functions in MATLAB (The MathWorks). The first 0.5 mm of the radial measurements were averaged as phosphatase activity in rhizosphere, while the measurements in 0.55 - 50 mm were averaged as phosphatase activity in bulk soil.

Statistical analysis

The significances of differences between plant types (WT, brb) were tested by fitting a linear model in R (version 3.3.1.) The model included plant type (WT, brb), WC (5%, 10%, 15%, 30%, 40%) and soil region (rhizosphere, bulk soil) as fixed factors and plant number (i.e. replicate 1 - 6) as random factor to account for the fact that measurements on different WCs and soil regions were conducted on the same plants (i.e. repeated measurements). The model revealed that the plant genotype did not have a significant influence on phosphatase activity. The factor plant genotype was therefore removed from the model, which increased the power of the model by increasing the degrees of freedom. The final model included WC and soil region as fixed factors and plant number as random factor and the number of replicates was 12. To achieve a normal distribution of the residuals of the model, zymography data were log transformed while WC data (from neutron measurements) were square root transformed. Following the model, a post hoc test (Tukey HSD) was conducted to test for significant differences between factor levels. The level of significance was $p < 0.05$. To test for significances in the correlation between WC and phosphatase activity in rhizosphere and bulk soil, a Pearson correlation test was conducted in R. The test revealed that both correlations were significant. The level of significance was $p < 0.05$.

3.3 Results

The WC in bulk soil and rhizosphere measured by neutron radiography differed significantly between all adjusted WC levels (Fig. 3.3, left) with only one exception. The WC in the rhizosphere was approximately 2 times higher than in the bulk soil at all adjusted WCs (Fig. 3.3, right). The differences between rhizosphere and bulk soil WC were particularly high under dry conditions. The WC in the bulk soil decreased 7.6 times as the adjusted WC of the rhizoboxes decreased from 40% to 5%, while it decreased only 3.8 times in the rhizosphere. This resulted in a change in the ratio of

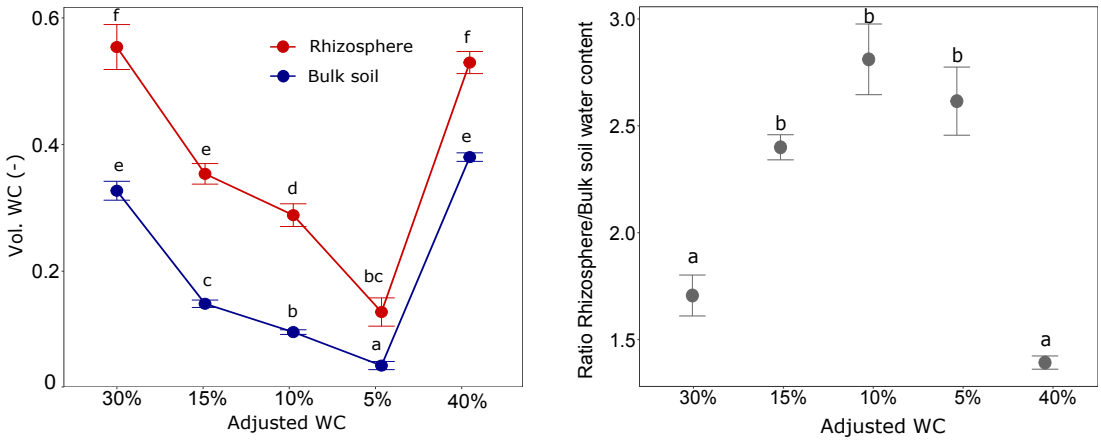

Figure 3.3: Left: Volumetric water content (vol. WC) of the rhizosphere (red) and of the bulk soil (blue) measured with neutron radiography as a function of the volumetric water content (vol. WC), to which the rhizoboxes were adjusted. Error bars indicate the standard error, n = 12. Significant differences ($p < 0.05$) are indicated by different letters. Right: Ratio between volumetric water content (vol. WC) of the rhizosphere to (vol. WC) of the bulk soil measured by neutron radiography as a function of the gravimetric WC, to which the rhizoboxes were adjusted. Error bars indicate the standard error, n = 12. Significant differences ($p < 0.05$) are indicated by different letters.

the WC in the rhizosphere and in the bulk soil. While the rhizosphere-to-bulk soil WC ratio was 1.4 at 40% adjusted WC of the rhizoboxes, it increased to 2.8 at an adjusted WC of 10%.

Similar trends as for soil WC were found for phosphatase activity. In bulk soil and rhizosphere, phosphatase activity decreased significantly when the adjusted WC of the rhizoboxes dropped from 40% to 5% WC (Fig. 3.4, left). Phosphatase activity in the rhizosphere was significantly larger than phosphatase activity in the bulk soil at all WCs. While the ratio of phosphatase activity in the rhizosphere-to-phosphatase activity in the bulk soil was 9.7 at 40% adjusted WC, it increased to 63.4 at 5% WC (Fig. 3.4, right). Phosphatase activity strongly increased both in the rhizosphere and in the bulk soil when the WC of the rhizoboxes was adjusted from 5 to 40% (Fig. 3.4), confirming a strong, reversible effect of WC on phosphatase activity.

We found a significant relationship between phosphatase activity and WC both

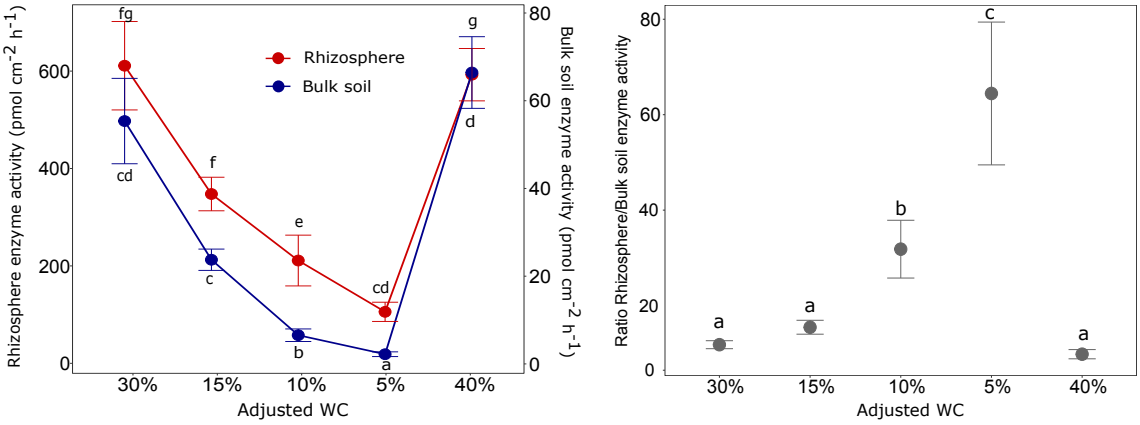

Figure 3.4: Left: Enzyme activity in the rhizosphere (red) and in bulk soil (blue) as a function of the volumetric WC to which the rhizoboxes were adjusted. Error bars indicate the standard error, n = 12. Significant differences ($p < 0.05$) are indicated by different letters. Please note that enzyme activity in rhizosphere and bulk soil refer to different y-axes. Right: Ratio of enzyme activity of the rhizosphere to enzyme activity of the bulk soil as a function of the gravimetric WC, to which the rhizoboxes were adjusted. Error bars indicate the standard error, n = 12. Significant differences ($p < 0.05$) are indicated by different letters.

for rhizosphere and bulk soil that could be approximated by quadratic equations (Fig. 3.5). While for the rhizosphere, the factor (1936.4) was higher than for the bulk soil (308.3), the coefficient of determination was slightly higher for the bulk soil ($R^2 = 0.63$) compared to rhizosphere ($R^2 = 0.55$).

3.4 Discussion

Here we showed that barley maintains a high enzyme activity close to the root during periods of drought by retaining water in the rhizosphere that allows for fast diffusion of exoenzymes (Fig. 3.1). This is beneficial for plant nutrition because the high WC of the rhizosphere facilitates a high rate of phosphatase activity, and thus plant P acquisition. Moreover, we showed for the first time based on a novel in situ imaging method that diffusion of enzymes is highly dependent on the local soil WC.

Previous studies also reported reduced enzyme activity with decreasing soil WC (Sanaullah et al., 2011; Sardans and Peñuelas, 2005; Steinweg et al., 2012). However, in these studies, enzyme activity was determined in soil solution, which until recently was

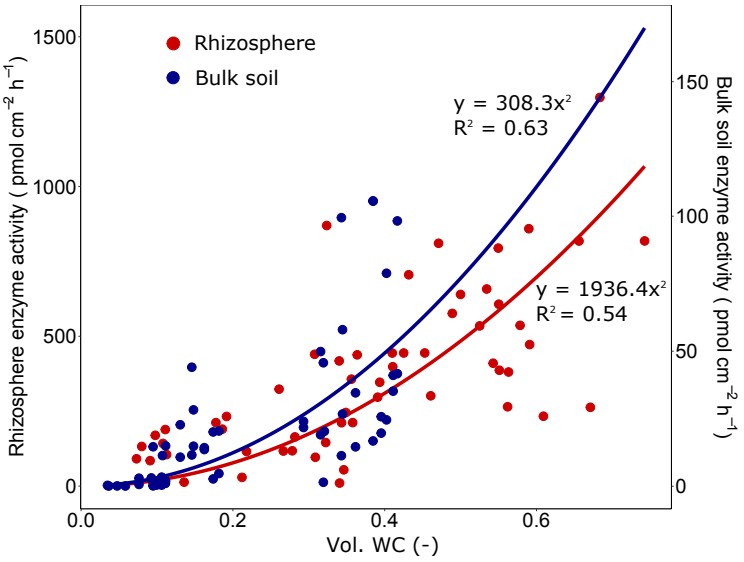

Figure 3.5: Correlation between vol. water content and enzyme activity in rhizosphere (red) and bulk soil (blue). The two correlations were significant based on the Pearson correlation test with p = 2.363 x 10^{-07} for the bulk soil and p = 5.84x10^{-10} for the rhizosphere. Please note that enzyme activity in rhizosphere and bulk soil refer to different y-axes.

the only way to determine soil enzyme activity (Burns et al., 2013). Thus, the previous studies reflect changes in the enzyme concentration resulting most likely from changes in the enzyme release rates of the organisms in response to drought. In our study, moisture levels were adjusted rapidly to avoid biological adaptation such as a change in enzyme release from plants or microorganisms. The power-law relation between soil WC and enzyme activity (Fig. 3.5), which is similar to the relation between soil WC and diffusion coefficient proposed by Millington-Quirk model (Milllington and Quirk, 1961) confirms our hypothesis that enzyme activity with soil drying is mainly controlled by diffusion. Particularly the fact that immediately after rewetting of the samples, enzyme activity rose to the original activity under moist conditions (Fig. 3.4, left), highlights the importance of diffusion for enzyme activity. Therefore, previous studies that determined enzyme activity in soil solution strongly overestimated enzyme activity because they oversaw the effect that soil WC has on enzyme activity through modified diffusion rates. Our study demonstrates that a decreased soil WC from nearly saturated to 5% can decrease enzyme activity by 97% exclusively due to the effect of soil WC on the diffusion rate of the enzymes. Thus, our study confirms the hypothesis

stated by Manzoni et al. (2012) that diffusion rather than biological processes controls enzyme activity in dry soils.

In our study, the driest conditions corresponded to a soil water potential below -3.9 MPa at which plant roots and microorganisms experience strong stress (Manzoni et al., 2012). However, in the rhizosphere this stress was alleviated since the rhizosphere had a higher WC than the bulk soil and this difference increased with increasing soil drying. Increased rhizosphere WCs have been observed in previous studies (Carminati et al., 2010; Holz et al., 2017a; Young, 1995). The reason for this is mucilage released from the root tips (Carminati and Vetterlein, 2013; Young, 1995) and possibly also EPS released from microorganisms (Or et al., 2007) whose activity and abundance is increased in the rhizosphere compared to the bulk soil (Dennis et al., 2010; Jones and Hinsinger, 2008). Both substances, mucilage and EPS retain water upon soil drying (Ahmed et al., 2014; Carminati and Vetterlein, 2013; Or et al., 2007) and therefore keep the rhizosphere moister than to the bulk soil. Moreover, it is possible that release of water from the root tips might have further increased the water content and water potential in the rhizosphere. The difference between rhizosphere and bulk soil WC increased with soil drying (Fig. 3.3, right). This can be explained by the fact that with soil drying, the concentration of mucilage and EPS increases in the liquid solution.

The enhanced rhizosphere WC does not only increase the diffusion of phosphatase, but also the diffusion of inorganic phosphorus, increasing its chances to be taken up by the plant. The increase in rhizosphere WC due to mucilage and EPS has therefore a twofold positive effect to the plant in terms of nutrient availability in dry soil conditions, first, it increases enzyme activity, and second, it leads to a facilitated movement of phosphate towards the plant root.

In conclusion, our study demonstrates that the activity of exoenzymes in the rhizosphere strongly depends on soil WC and that plants that retain water in the rhizosphere during periods of drought maintain an increased enzyme activity in the rhizosphere. This is beneficial for plant nutrition because the high WC of the rhizosphere facilitates a high rate of phosphatase activity, and thus plant P acquisition. Thus, in the light of an increasing frequency of droughts, our findings suggests that plant species that release high amounts of mucilage will be able to maintain high rates of nutrient mineralization and acquisition in the rhizosphere. This has direct implications for the selection of agricultural plants because it suggests that varieties that

release large amounts of mucilage will be able to better cope with drought.

3.5 Acknowledgements

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4 Spatial distribution of mucilage in the rhizosphere measured with infrared spectroscopy

written by Maire Holz, Martin Leue, Mutez A. Ahmed, Pascal Benard, Horst H. Gerke and Andrea Carminati

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Abstract

Mucilage is receiving increasing attention because of its putative effects on plant growth, but so far no method is available to measure its spatial distribution in the rhizosphere. We tested whether the C-H signal related to mucilage fatty acids is detectable by infrared spectroscopy and if this method can be used to determine the spatial distribution of mucilage in the rhizosphere. Maize plants were grown in rhizoboxes filled with soil free of organic matter. Infrared measurements were carried out along transects perpendicular to the root channels. The profiles of the C-H proportions showed a decrease of C-H with increasing distance: 0.8 mm apart from the root the C-H signals achieved a level near zero. The measured concentrations of mucilage were comparable with results obtained in previous studies, which encourages the use of infrared spectroscopy to quantitatively image mucilage in the rhizosphere.

4.1 Introduction

Mucilage is a gel-like substance released from the root-cap cells of plants (Oades, 1978). It has been claimed to provide several benefits for plant growth, such as the lubrication during root penetration (Iijima et al., 2004) or the increase in water uptake due to its high water-holding capacity (Ahmed et al., 2014; Carminati and Vetterlein, 2013). The extent of such benefits depends on the spatial distribution of mucilage around roots. To date, there is no experimental method to non-invasively and quantitatively image mucilage in soils and our knowledge of mucilage spatial distribution remains largely speculative.

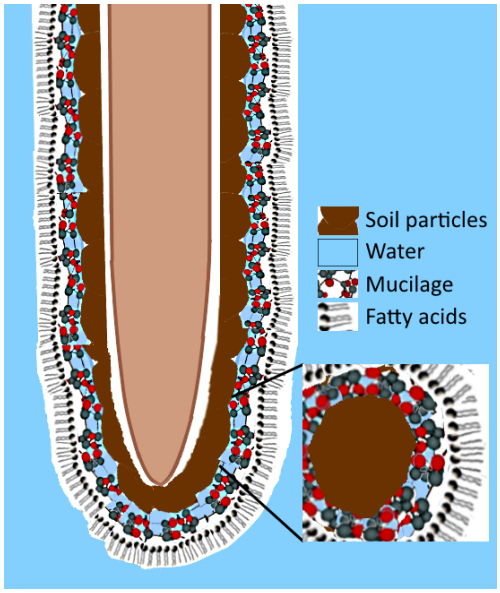

Figure 4.1: Conceptual model describing the hydrophobicity around root after soil drying caused by fatty acids contained in mucilage.

Among the many effects of mucilage on soil properties, one spectacular effect is the rhizosphere water repellency (Ahmed et al., 2016; Carminati and Vetterlein, 2013; Hallett, 2003; Moradi et al., 2012; Whalley et al., 2004). Mucilage has been claimed to be responsible for the observed hydrophobicity (Ahmed et al., 2015; Benard et al., 2017) as it contains phospholipids (Read et al., 2003), which are hydrophobic due to their nonpolar fatty acid chains (C-H groups) (Figure 4.1). Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy can be used to map functional groups such as C-H groups at intact soil surfaces at the mm-scale (Leue et al., 2010, 2015). In combination with microscopic techniques, DRIFT spectroscopy can be used to determine organic functional groups also at the μm-scale. The objective of this study was to test whether DRIFT spectroscopy and Fourier-transform infrared spectroscopy (FTIR) microscopy allow rapid identification of the distribution of mucilage in the rhizosphere.

In order to achieve this goal we mapped the spatial distribution of mucilage across maize root channels grown in rhizoboxes by evaluating the signals from the C-H groups.

4.2 Material and Methods

Quartz material consisting of 70% quartz sand (collected from Duingen (Germany), particle size: 0.1-0.2 mm) and 30% quartz powder (Carl Roth, particle size: $<$ 0.125 mm) was used as a growth medium free of soil organic matter. For the calibration, maize root mucilage was extracted as described in Zickenrott et al. (2016).

Dried quartz material was mixed with different amounts of mucilage at concentrations of 0.0, 0.025, 0.05, 0.1, 0.25, 0.5, and 1 mg/g (gram of dry mucilage per gram of dry soil). The mixtures were applied on object slides in four replicates and were air-dried. For the mapping of the spatial distribution of mucilage across root channels, maize (*Zea mays L.*) plants (KWS 2376) were grown in rhizoboxes with an inner size of 10 x 20 x 1.5 cm filled with the same quartz substrate as used for the calibration. Before germination, maize seeds were immersed in a 10% H_2O_2 solution for 10 minutes to avoid seed-borne diseases. Prior to plant growth the following nutrients were added per kg of soil: N: 0.3 g, P: 0.06 g, K: 0.03 g, Ca: 0.05 g, Mg: 0.05 g, B: 2 mg, Cu: 0.03 mg, Mn: 1.5 mg, Mo: 0.03 mg, Zn: 1.2 mg, Fe: 3.6 mg. During plant growth, the water content was adjusted to 20% and the rhizoboxes were kept at an angle of approximately 55 C ° to make sure that the roots were growing close to the lower side of the rhizoboxes. The temperature in the climate chamber was 25 °C during day and 22 °C during night, the photoperiod was 14 hours and the photosynthetic photon flux density was 300 μmol m^{-2} s^{-1}. After four weeks of growth, plants were cut, the soil samples were dried at 35 °C and the roots were removed from the soil manually (Fig. 4.2).

Diffuse reflectance infrared spectroscopy (DRIFT) measurements in the mid-infrared range (wavelength: 2.5 - 25 μm, wave numbers (WN): 4000 - 400 cm^{-1}) was applied to the calibration samples at the object slides using a BioRad FTS 135 spectrometer (Cambridge, Massachusetts, USA). Each DRIFT spectrum was recorded by 16 co-added scans with a spectral resolution of 4 cm^{-1} against a gold background (Leue et al., 2015). At the intact root channels and surrounding substrate, DRIFT spectra were conducted using an Agilent Cary series 600 FTIR microscope (Agilent Cary series 600, Agilent Technologies, Inc., Santa Clara, CA, USA) moving in the perpendicular direction from the root channel center towards the bulk soil. The spectra were recorded as 64 co-added scans at a spectral resolution of 4 cm^{-1} in steps of 100 μm from sample areas of 0.1 x 0.1 mm. For the rhizosphere samples, one root was chosen

Figure 4.2: Rhizobox with soil and plants before (left side) and after the roots were excavated from the soil (right side). Measurements were conducted after removing the roots at three position along the root (0, 1 and 3 cm behind the tip) as indicated by the red arrows.

per plant and spectra were measured at three positions along each root (0, 1, and 3 cm behind the tip). After preprocessing of the spectra (Ellerbrock et al., 2009; Leue et al., 2010), the local peak heights of the C-H bands were normalized for the absolute peak height of the SiO_2 band at WN 1350 cm^{-1}. The relationship between mucilage content and C-H signal intensities obtained from the calibration samples was used to quantify the mucilage content in the rhizosphere of the maize roots. After calibration, the background signal (i.e., the average signal in the bulk soil where no mucilage was expected) was subtracted from each measured value.

4.3 Results and Discussion

The calibration measurements on the object slides revealed a linear relationship between mucilage concentration and the C-H/SiO_2 ratio (Figure 4.3). The measurements in the maize rhizosphere showed a distinct gradient of mucilage from the center of the root channel towards the bulk soil. The highest values of 0.02 mg g^{-1} were found in the center of the root channel. Mucilage concentration decreased to approximately 0 mg g^{-1} with increasing distance from the root channel (Figure 4.4). This pilot experiment showed that infrared spectroscopy can be applied to detect profiles of mucilage

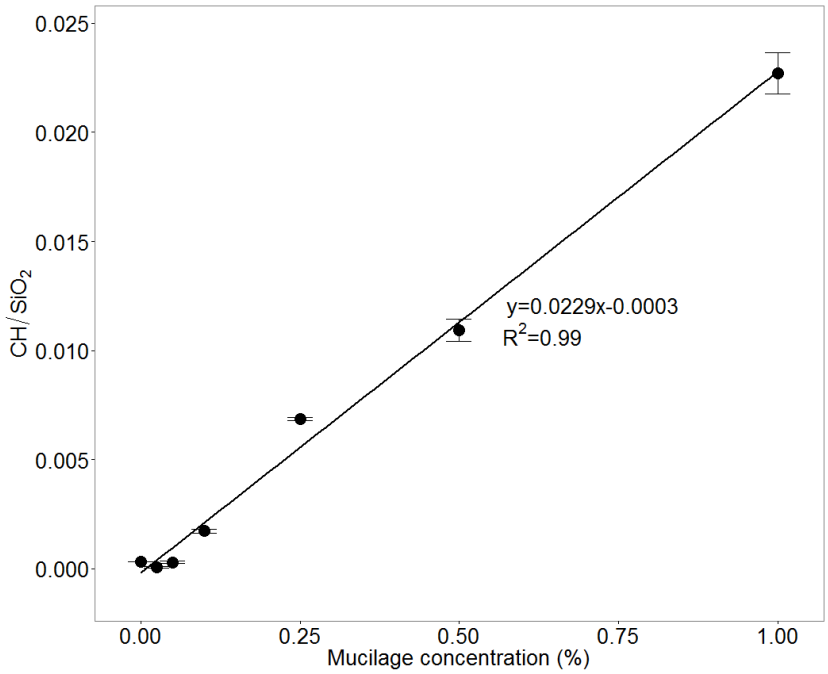

Figure 4.3: Ratio between CH and SiO_2 signal for soil mixed with different maize-mucilage concentrations ranging from 0 to 1 mg dry mucilage per g dry soil.

around roots grown in soil. The profiles of mucilage content were relatively steep: at 0.6 mm distance from the center of the root channel, mucilage content decreased below the limit of detection. The extent of the rhizosphere affected by mucilage (0.6 mm) was smaller compared to results obtained from ^{14}C imaging analyses. Holz et al. (2017) showed that overall root exudates diffused up to 1 mm into the bulk soil. The fact that mucilage did not move as far from the root surface as compared to overall exudates may be explained by the higher viscosity of mucilage compared to root exudates (Read and Gregory, 1997) and reduced diffusion coefficient of mucilage compared to root exudates.

We found contents (i.e., average values for channel) of up to 0.02 mg dry mucilage per g dry soil inside the root channel. Based on the literature, we can expect a mucilage exudation rate of 15 μg d^{-1} per root tip (Chaboud, 1983). Assuming a maximum diffusion length of 1 mm into the soil, a mean root radius of 0.5 mm, a soil bulk density of 1.43 g cm^{-3} as for our samples and a root elongation of 3 cm d^{-1} we expect a mucilage concentration in the rhizosphere of 0.056 mg dry mucilage per g dry soil. This theoretic value is 3 times higher than the values obtained by DRIFT spectroscopy; however, they still appear comparable.

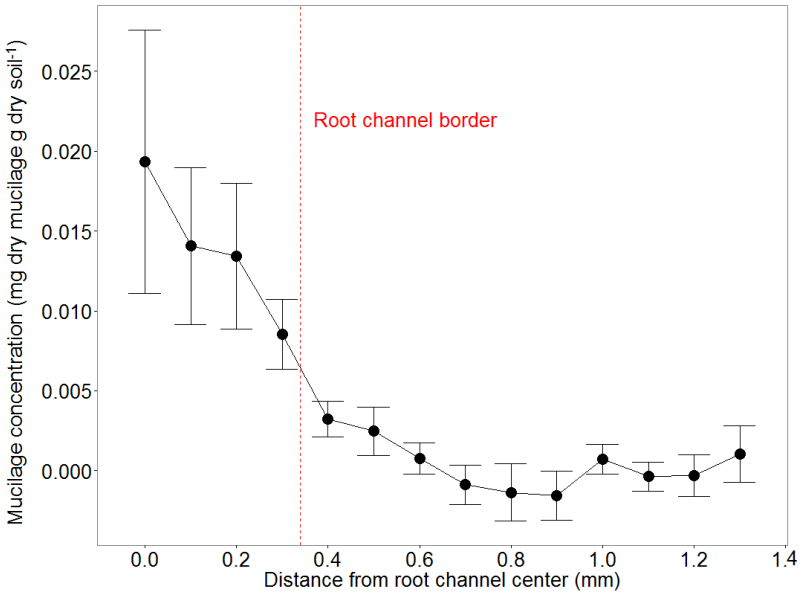

Figure 4.4: Mucilage concentration (mg dry mucilage per g dry soil) starting from the root channel center in radial direction towards the bulk soil. Mean values and standard errors of 5 replicates.

One opportunity for increasing the precision of the DRIFT measurements could be the use of the same DRIFT spectrometer (here: FTIR microscope) for both, the calibration and intact sample measurements. The use of a finer substrate to optimize the application of the DRIFT technique could improve the spectral analysis of the mucilage and the identification resolution.

Along the axial direction of the root channels, the mucilage concentration inside the channels as well as the gradient perpendicular to the channels remained constant (data not shown). Probably the measured root channel segments at distances of 0, 1, and 3 cm behind the tip were still too young to show differences in mucilage concentration and distribution. Probably longer time intervals (i.e. older root segments) would be necessary to detect changes in mucilage quantity or quality due to decomposition. Future studies with older plant roots should test if the distribution or quantity of mucilage changes along the root channels (in the axial direction).

4.4 Conclusions

The results suggest that DRIFT spectroscopy and FTIR microscopy are promising techniques to quantify the spatial distribution of mucilage in the rhizosphere. The

measured mucilage contents and the spatial extent of the mucilage-affected rhizosphere were comparable to calculated and published values. Future studies could investigate the effect of factors such as: root hairs, root age, root type, plant species, soil texture and soil water content on the spatial distribution of mucilage in the rhizosphere.

4.5 Acknowledgements

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5 Root hairs increase rhizosphere extension and carbon input to soil

written by Maire Holz, Mohsen Zarebanadkouki, Yakov Kuzyakov, Johanna Pausch and Andrea Carminati

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Abstract

Background and Aims Although it is commonly accepted that root exudation enhances plant-microbial interactions in the rhizosphere, experimental data on the spatial distribution of exudates are scarce. Our hypothesis was that root hairs exude organic substances to enlarge the rhizosphere farther from the root surface.

Methods Barley (*Hordeum vulgare* L. cv. Pallas - wild type) and its root-hairless mutant (*brb*) were grown in rhizoboxes and labelled with ^{14}C. A filter paper was placed on the soil surface to capture, image and quantify root exudates.

Key Results Plants with root hairs allocated more carbon (C) to roots (wild type: 13%; *brb*: 8% of assimilated ^{14}C) and to rhizosheaths (wild type: 1.2%; *brb*: 0.2%), while hairless plants allocated more C to shoots (wild type: 65%; *brb*: 75%). Root hairs increased the radial rhizosphere extension 3-fold, from 0.5 to 1.5 mm. Total exudation on filter paper was 3 times greater for wild type plants compared to the hairless mutant.

Conclusion Root hairs increase exudation and spatial rhizosphere extension, which probably enhance rhizosphere interactions and nutrient cycling in larger soil volumes. Root hairs may therefore be beneficial to plants under nutrient limiting conditions. The greater C allocation belowground in the presence of root hairs may additionally foster C sequestration.

5.1 Introduction

Future agriculture will be limited by drought in many parts of the world due to climate change (FAO, 2012; Parry and Hawkesford, 2010; Parry et al., 2005). It is therefore crucial to improve our understanding of processes that have the potential to increase the ability of plants to extract water and nutrients from the soil. Root traits, such as length and density of root hairs, have been proposed to increase plant productivity especially when soil resources are limited (Bardgett et al., 2014; Jungk, 2001; Pausch et al., 2016).

Root hairs are tubular extensions of epidermal cells (Peterson and Farquhar, 1996), which emerge right behind the zone of root elongation (Jungk, 2001). For cereals, approximately 20-90 root hairs emerge per mm of root length (Gahoonia and Nielsen, 1997) and they grow 0.2-1.0 mm into the soil (Schweiger et al., 1995; Haling et al., 2010; Brown et al., 2012). Root hairs contribute between 70-90% of the total root surface area and there is evidence that they improve nutrient acquisition (Bates and Lynch, 1996; Gilroy and Jones, 2000; Brown et al., 2012). However (Jungk, 2001) calculated that the increase in root surface due to root hair length alone could not explain increased nutrient influx into the root. The author proposed that other processes such as exudation of organic substances by root hairs might additionally increase nutrient availability.

Little is known about the role of hairs in root exudation. Root exudates were defined as materials released from the roots such as simple sugars, amino acids or polysaccharides but did not include dying root cells or root hairs (Leinweber et al., 2008). Although it has been shown that root hairs are covered by mucigels (Dart, 1971; Greaves and Darbyshire, 1972; Sprent, 1975) it is not clear whether the observed materials were released by the root hairs or just transported there simply as a result of root elongation. Moreover, there is nearly no information as to whether root hairs exude other substances apart from the observed mucigels such as low molecular exudates. Pausch et al. (2016) studied the impact of root hairs on rhizosphere priming effects (RPE). They found that the presence of root hairs increased RPE at least for young plants hinting to an increase in rhizodeposition. However, the contribution of root hairs to the total root exudation and to the spatial distribution of exudates in the rhizosphere remains unclear.

The aim of this study was to test how root hairs affect the carbon allocation in the

soil-plant system and the root exudation. We grew barley (*Hordeum vulgare L. cv. Pallas* - wild type WT) and its root-hairless mutant (*brb*) for four weeks and labelled them with $^{14}CO_2$ to trace the carbon allocation in plant and soil. ^{14}C imaging was used to quantify the effect of root hairs on exudation and rhizosphere extension. We hypothesized that: i) the presence of root hairs increases the amount of ^{14}C allocated to the roots (relative to the total assimilated ^{14}C) because carbon is needed for root hair production and maintenance; ii) root exudation is greater in the wild type because of the presence of root hairs; and iii) direct exudation from root hairs increases the radial and axial (longitudinal) rhizosphere extension.

5.2 Material and Methods

Sample preparation and plant growth

Before germination, barley seeds (wild type: WT, bald root barley: *brb*) were immersed in a 10% H_2O_2 solution for 10 min to avoid seed-borne diseases. Seedlings were planted after 3 days of germination.

The growth of plants was staggered over time for all experiments: per sowing time, 3 - 4 plants were grown. This was done because the exemption limit for radioactive substances allows the handling of only limited amounts of ^{14}C at a time in a laboratory. Further, the chamber for labelling could not fit more than a maximum of 5 plants. The sowing dates were between April and June 2016. The two treatments (WT, *brb*) were randomly distributed within and between the sowing times. The barley plants were grown for four weeks in rhizoboxes with a size of 12 x 20 x 3.5 cm for 30-31 days (Fig. 5.1). The soil used was a sandy soil (A-horizon) collected from a field site close to Reinhausen (Göttingen, Germany). C_{tot} was 2.0% and N_{tot} was 0.17% and pH was 4.9. The soil texture was distributed as follows: Clay: 8.6%, silt: 18.5%, sand: 73%. The volumetric soil water content was kept at 23 - 25% vol. water content during plant growth. The temperature in the climate chamber was 25 °C during the day and 22 °C during the night, the photoperiod was 14 hours and the photosynthetic photon flux density was 200 μmol m−2 s−1. During the growth period, photographs of the root system were taken at regular intervals to monitor root elongation.

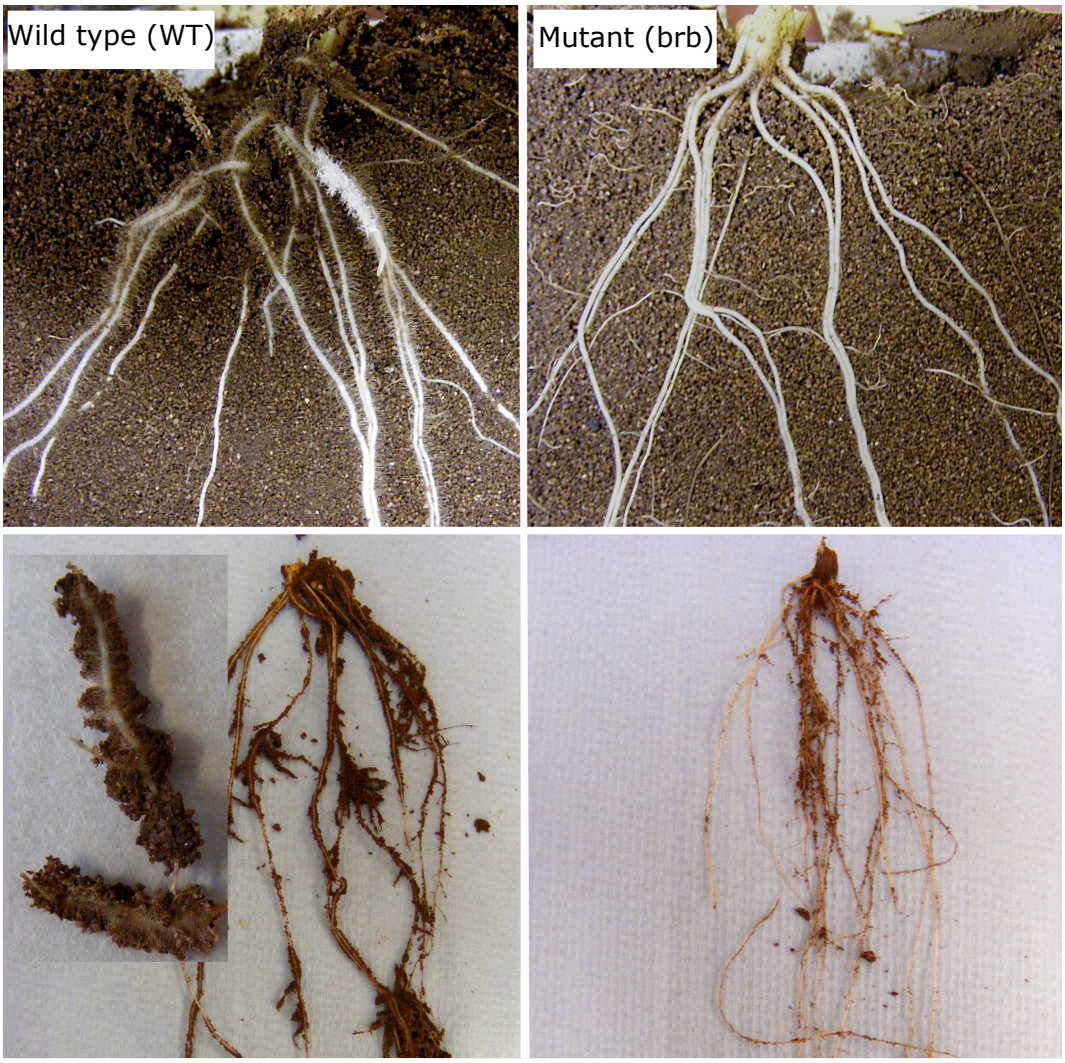

Figure 5.1: Top: Close up of the root system of barley plants after four weeks of growth. Left side: wild type with root hairs (WT); Right side: mutant without root hairs (*brb*). Bottom: rhizosheaths around barley roots after taking them out of the soil and gently shaking them. Left side: wild type with root hairs (WT); Right side: mutant without root hairs (*brb*).

$^{14}CO_2$ pulse labelling and CO_2 measurement

After four weeks of plant growth each plant was labelled with 0.5 MBq $^{14}CO_2$ (specific activity of 59.6 mCi mmol C^{-1}) for 4.5 hours. Labelling was always conducted at noon for plants of all sowing times. The labelling technique has been described in detail elsewhere (Kuzyakov et al., 2006). At the end of labelling, approximately 70% of the added ^{14}C had been taken up by the plants. This was tested by collecting 30 ml of chamber air with a syringe 4 times during labelling (after 5, 30, 60, 120 min) and injecting it into a scintillation cocktail (C 400, Zinsser Analytics).^{14}C activity was quantified by a liquid scintillation counter (Hidex, 300 SL). The activity of ^{14}C in the 30 ml of chamber air that had been taken out of the chamber was back calculated to the volume of the whole chamber. Doing this for all 4 measuring times, we calculated the amount of ^{14}C in the chamber over time which is inversely proportional to the uptake of ^{14}C.

For the measurement of ^{14}C allocation in the soil-plant systems (Exp. 1) we used five replicates of each barley type (WT and *brb*) and four replicates for the measurement of ^{14}C respiration over time (Exp. 2). For ^{14}C imaging of roots and exudates three replicates were used (Exp. 3). Immediately after labelling, the rhizoboxes used for the first and second experiment where packed in a plastic bag which was closed with modelling clay at the lower part of the stem of the plants. Inside the bag a 20 ml 1 M NaOH trap was placed to trap the $^{14}CO_2$ released from soil.

For the first experiment the trap was left inside the bag for 24 hours. After that time, the trap was removed and a scintillation cocktail (Eco Plus) was added to NaOH with the ratio of 4:1. ^{14}C activity in NaOH was determined using a liquid scintillation counter (Hidex, 300 SL). Shoots were cut and dried at 40 °C. The roots were taken out of the soil and the soil attached to the roots after being gently shaken (rhizosheath) was collected. Roots were dried at 40 °C. Rhizosheath and bulk rhizosphere soil (the soil not adhering to the roots) were freeze dried to avoid microbial degradation of labile carbon compounds. The terminology bulk rhizosphere soil was chosen as the root density in the rhizoboxes was high so that we assume that the whole soil volume was affected by the activity of the roots. However, it is important to bear in mind that the so defined bulk rhizosphere also contains some small roots and root hairs because it is technically impossible to remove all the roots from the soil after harvesting the plants. Care must therefore be taken when interpreting the results on ^{14}C in bulk

rhizosphere soil as a considerable amount might be caused by ^{14}C in root debris. The biomass of the dried plant samples was determined gravimetrically. To measure ^{14}C activity, ground shoots, roots, rhizosheath and bulk rhizospere soil samples were combusted in an Oxymat OX500. The released $^{14}CO_2$ was captured in a scintillation cocktail (C400, Zinsser) and quantified using a liquid scintillation analyzer (Tricarb, 3180, PerkinElmer). Total CO_2 respiration from soil was measured from a subsample (1 ml) of the NaOH trap: The carbonate in the NaOH solution was precipitated by barium chloride and the trapped CO_2-C was determined by back titration with 0.05 M HCl.

In the second experiment (Exp. 2), $^{14}CO_2$ production in soil was measured over 17 days. The NaOH traps were exchanged after 1, 2, 4, 9 and 17 days and ^{14}C in the traps was measured as described above. After 17 days, plant biomass and ^{14}C in plant and soil material was determined as described above.

For the third experiment (Exp. 3), rhizoboxes were opened immediately before labelling and a moist filter paper (Whatman, 1001 - 917, 11 μm) was attached to the root surface in order to capture root exudates (Dennis et al., 2010). Note that, although we defined root exudates in this study as materials released from the roots such as simple sugars, amino acids or polysaccharides, we cannot exclude that sloughed off root cells or the contents of damaged root hairs are also contained in the material captured in the filter paper. As the mechanical stress at the rhizobox surface is rather low, we assume that those compounds make up only a small portion of ^{14}C captured at the filter paper and that the ^{14}C in the filter paper is mainly due to root exudates. The presence of root hairs on the filter paper was checked with a microscope. The filter paper was covered by a thin plastic film to avoid drying of the filter paper. Finally, a thin layer of foam material was placed between the plastic film and the cover of the rhizobox to achieve a good contact between soil surface and filter paper. 18 hours after labelling, the filter paper was carefully removed from the soil surface and dried in an oven (40 °C) to avoid the decomposition of root exudates captured in the filter paper. ^{14}C imaging was conducted by placing an imaging plate (Storage phosphor screen, BAS-IP MS 2040 E, VWR) both on the filter paper and on the rooted soil surfaces of each rhizobox. A thin plastic film was placed between the sample (or filter paper) and the imaging screen to protect the imaging screen against the moist soil. The screens were attached to the roots (or filter paper) for 15 h. After this time the screens were scanned (FLA 5100 scanner, Fujifilm) with a spatial resolution of 50 μm.

Quantification of root elongation and image analysis

During the growth period, photographs of the root system were taken at regular intervals to monitor root elongation. For each plant photographs taken one day before labelling, on the day of labelling and one day after labelling were analyzed. The elongation rate was calculated using the Smart Root plugin (Lobet et al., 2011) in ImageJ (https://fiji.sc/): Roots from each image were segmented and the length of the segment was calculated. Root elongation rate was calculated based on the changes in root length over time.

For quantification of ^{14}C images, the images were converted from a log into a linear system by applying the following equation:

$$PSL = \left(\frac{Res}{100}\right)^2 * \frac{4000}{S} * 10^{L*\left(\frac{QL}{G}-\frac{1}{2}\right)} \tag{5.1}$$

where *PSL* (Photostimulated Luminescence) is the quantified value of the image in linear scale, *Res* is the resolution of the image in μm ($Res = 50$ μm), S is the sensitivity ($S = 5000$), L is the latitude ($L = 5$) and G is the gradation ($G = 65535$). After conversion of the images, the background noise was removed: The part of the image where the screen was not in contact with the sample was selected and subtracted from the part of the image where the root system was visible. Based on the contrast between roots and soil/filter paper the root tips that showed a high ^{14}C signal in the images were segmented using the SmartRoot plugin in ImageJ. In this way only the roots that were at the sample surface were selected. Possible artifacts caused by overlapping roots or roots detached from the soil were removed from the segmentation. 2 - 5 roots per sample were segmented depending on how many roots were clearly visible. The signal was averaged as a function of distance from the root center up to a distance of 4 cm from the root surface using the Euclidean distance mapping functions in MATLAB (The MathWorks). To quantify total exudation, the *PSL* values around each root tip were summed up and a mean of total exudation per tip and treatment was calculated.

Statistical analysis

The significances of differences between treatments (WT, *brb*) were tested using R 3.3.1. After testing for normal distribution and homogeneity of variances, a one-way ANOVA was conducted followed by a post hot test (Tukey-Test). The level of

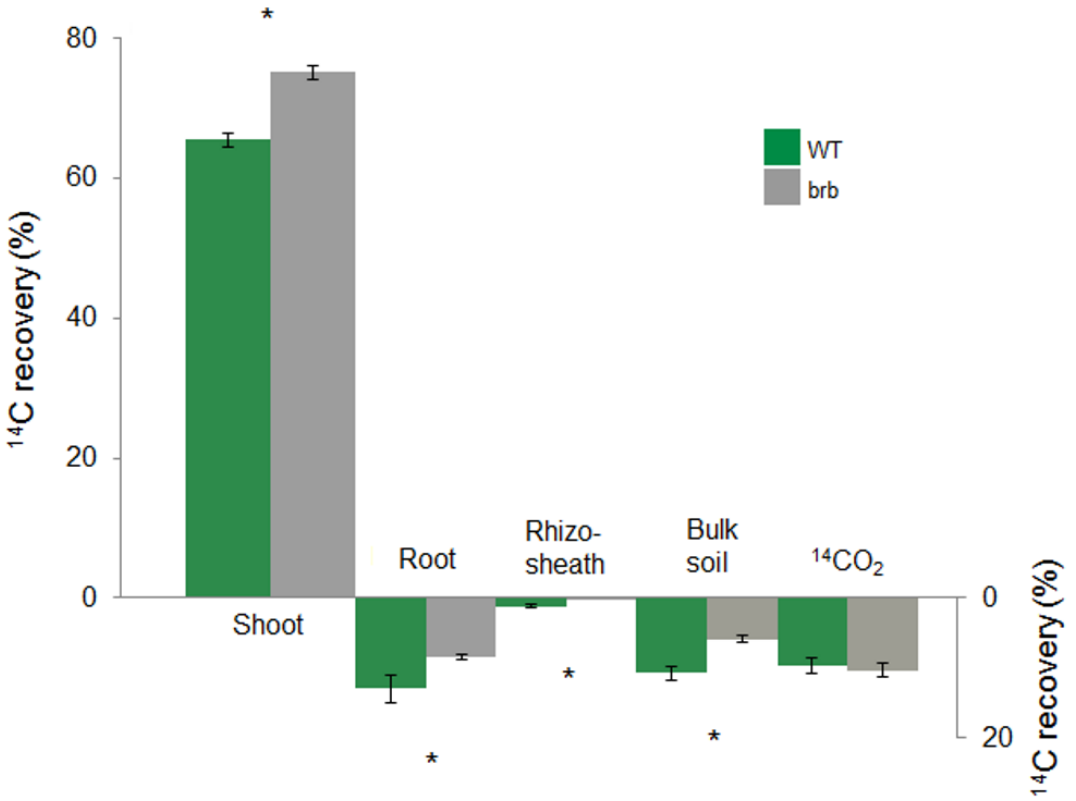

Figure 5.2: ^{14}C recovered in shoots, roots, rhizosphere, bulk soil and CO_2 of the barley wild type (WT) and the mutant without root hairs (*brb*) one day after labelling. Variation is given as standard error. The number of replicates was 5. The p value is presented as *: $p < 0.05$.

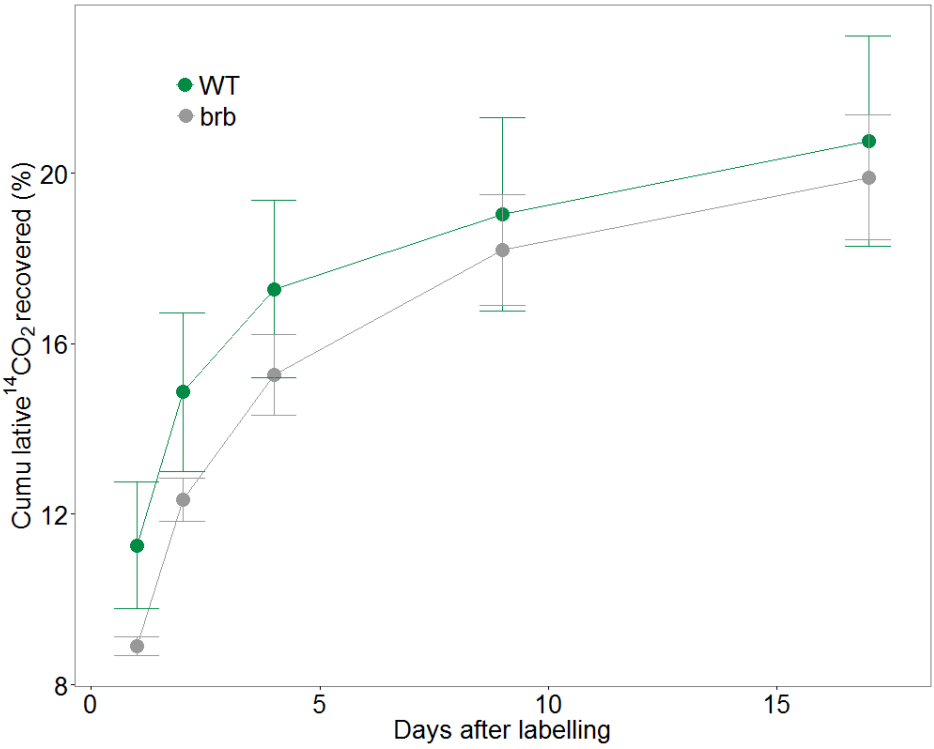

Figure 5.3: ^{14}C recovery (% ± SE) in CO_2 from soil and root respiration over a period of 17 days. Differences between treatments were not significant ($p < 0.05$).

significance was $\alpha = 0.05$. To test for significances in the radial rhizosphere extension, total exudation and the ratio between axial rhizosphere extension and root elongation between treatments a mixed effect model ($\alpha = 0.05$) with treatment as fixed effect and plant as random effect was applied. To account for the differences in numbers of roots sampled per plant, the restricted maximum likelihood (REML) method was applied.

5.3 Results

Plant Biomass, ^{14}C recovery and total CO_2 efflux

Shoot biomass measured in the first experiment was similar for plants with and without root hairs. Root biomass was 3 times greater in plants with root hairs compared to the hairless mutants and the rhizosheath was 10 times greater in WT compared to the hairless mutants. The specific ^{14}C activity of shoots was similar in both plants, while it was 22% greater in the roots and 80% greater rhizosheath of *brb* plants compared to WT plants (Table 5.1). Similarly, total ^{14}C in shoots did not differ between the

Table 5.1: Shoot, root and rhizosheath dry weight and specific and total ^{14}C activity for the barley wild type (WT) and the mutant without root hairs (*brb*) one day after labelling. Variation is given as standard error. n = 5. The p value is presented as follows: ns: $p \geq 0.1$, (*): $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

	WT	*brb*	p-value
Dry weight shoot (g)	0.37 (0.07)	0.31 (0.06)	ns
Dry weight root (g)	0.13 (0.03)	0.05 (0.01)	*
Dry weight rhizo(g)	5.75 (0.83)	0.49 (0.09)	*
^{14}C in shoot (kBq g^{-1})	492 (32.3)	494 (39.2)	ns
^{14}C in root (kBq g^{-1})	273 (29.9)	333 (29.2)	*
^{14}C in rhizosheath (kBq g^{-1})	0.55 (0.1)	0.99 (0.16)	***
^{14}C in bulk rhizosphere (kBq g^{-1})	0.03 (0.007)	0.01 (0.002)	*
Total ^{14}C in shoot (kBq)	173 (27.1)	143 (16.9)	ns
Total ^{14}C in root (kBq)	37.5 (9.64)	16.2 (2.38)	(*)
Total ^{14}C in rhizosheath (kBq)	3.43 (1.00)	0.45 (0.06)	*
Total ^{14}C in bulk rhizosphere (kBq)	3.68 (0.46)	2.90 (0.39)	ns
^{14}C in CO_2	41.1 (9.74)	33.8 (5.45)	ns

genotypes, while total ^{14}C in root and rhizosheath soil was greater in WT compared to *brb*. ^{14}C activity in CO_2 as well as the total CO_2 respiration from soil was similar for the wild type and the hairless mutant. ^{14}C recovery was calculated as percentage of total ^{14}C measured in all pools one day after labelling. On average, 70% of the ^{14}C was recovered in shoots, 10% in roots, 1% in rhizosheath, 9% in bulk rhizosphere soil and 10% in CO_2. Plants without root hairs allocated relatively more ^{14}C to shoots compared to plants with root hairs. In contrast, WT allocated twice as much ^{14}C into roots compared to *brb* and allocated 5 times more ^{14}C into rhizosheath (Fig. 5.2). For both plant genotypes, similar amounts of ^{14}C were found in the respired $^{14}CO_2$. The cumulative ^{14}C respiration over the sampling period 17 days after labelling was measured in Exp. 2 and was similar for WT and *brb* (Fig. 5.3). After 17 days, 21% of $^{14}CO_2$ was recovered for plants with root hairs and 19% for plants without root hairs.

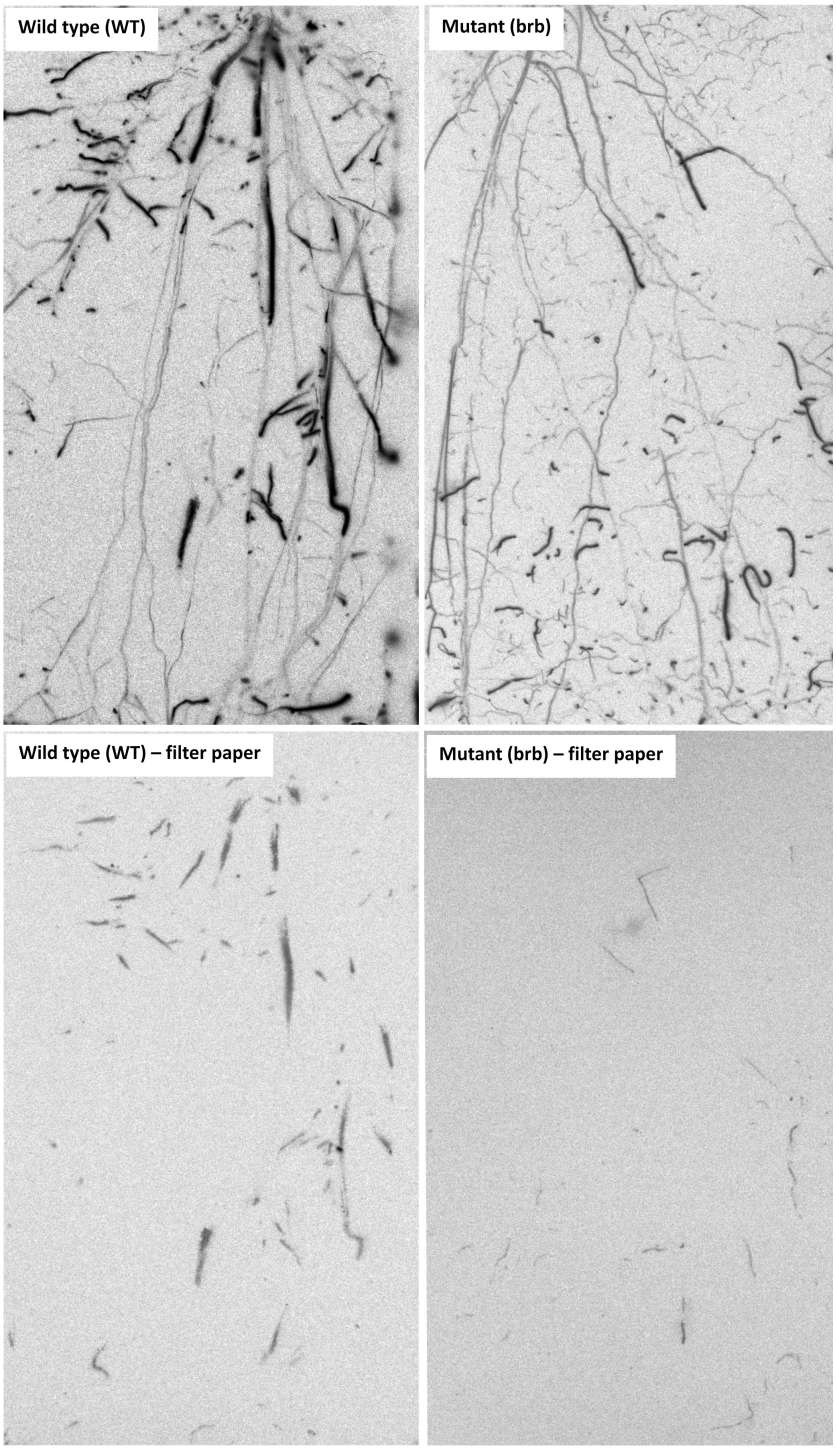

Figure 5.4: ^{14}C phosphor images of the root system of the barley plants (top) and of the root exudates that diffused into the filter paper (bottom) which was attached to the plant during labelling. The intensity of dark colour corresponds to ^{14}C activity.

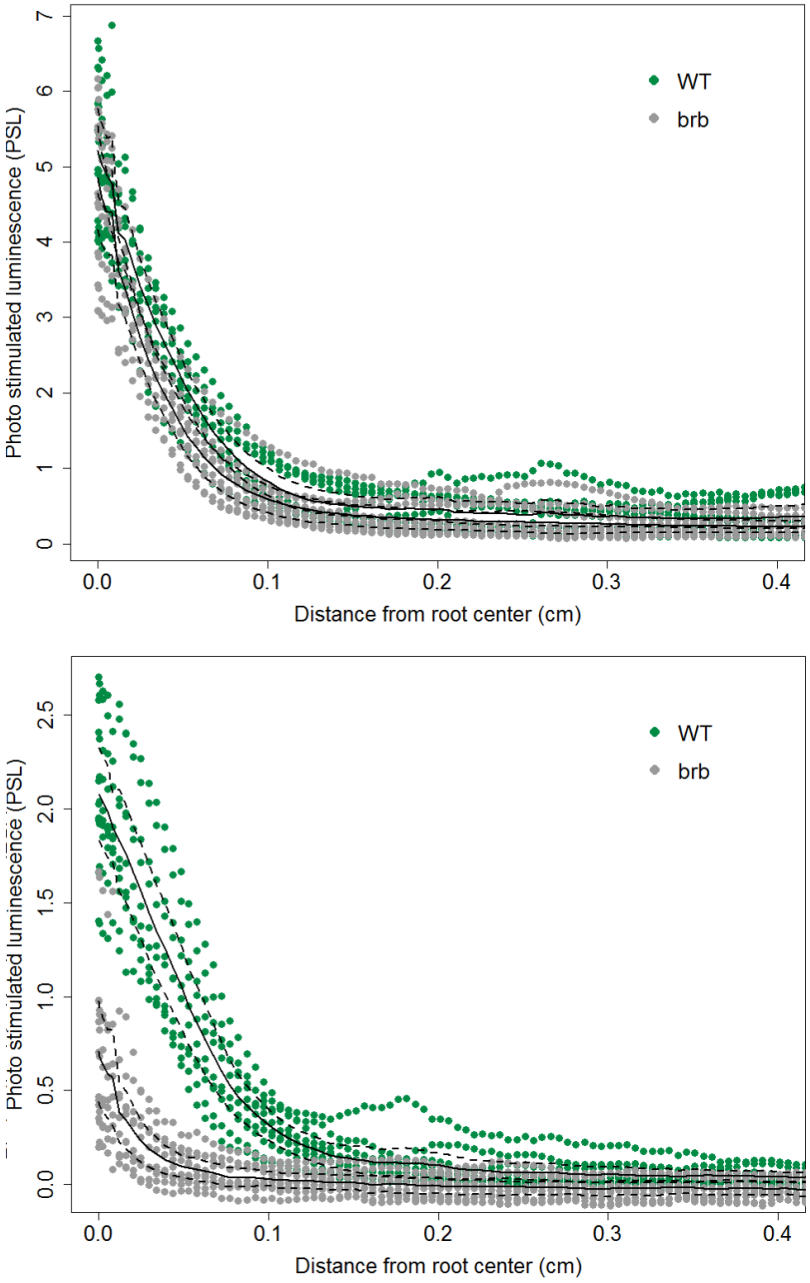

Figure 5.5: Radial profiles around the roots in the soil (top) and of ^{14}C activity (i.e. root exudates) on filter paper (bottom). The data sets were fitted using a linear model (solid lines). Confidence intervals are shown as dashed lines. Differences between the profiles were significant for the profiles on filter paper (bottom) but not for those of the roots in soil (top). Two to five roots were analyzed from each of 3 replicate plants.

^{14}C imaging and rhizodeposition

Root hairs were clearly visible along all the roots of WT plants grown in rhizoboxes (Fig. 5.1, top). Root hairs reached a length of up to ca. 1 mm and were in general longer around older root parts (visual, qualitative impression). Root hairs favored rhizosheath formation while nearly no soil was attached to the roots for the mutant without root hairs after their removal from soil (Fig. 5.1 bottom). ^{14}C was allocated to growing root tips of main and lateral roots (Fig. 5.4, top). The region of roots where ^{14}C was allocated correlated well with the region where ^{14}C was found on the filter paper (Fig. 5.4, bottom). The radial profiles of ^{14}C activity around roots imaged directly on the soil samples were similar for plants with and without root hairs (Fig. 5.5, top). The activity decreased to zero at a distance of 1 mm from the root center. The ^{14}C activity on filter paper (i.e. root exudates) at the location of the root center was approximately 3 times lower compared to the ^{14}C activity of roots in soil for WT plants and was approximately 10 times lower for the hairless mutant (Fig. 5.5, bottom). This is because only a small portion of the ^{14}C taken up by roots is exuded as exudates. Plants with root hairs showed broader profiles of exudates compared to plants without root hairs. The ^{14}C activity at the location of the root center was on average around 2 *PSL* for WT plants and 0.5 *PSL* for plants without root hairs. For plants with root hairs ^{14}C activity decreased to zero at a distance of 1.5 mm from the root center, while for the mutant without hairs it decreased to zero at a distance of 0.5 mm from the root center (Fig. 5.5, bottom).

To separate the effect of different root elongation rates between genotypes on the axial rhizosphere extension, the ratio between axial rhizosphere extension and root elongation was calculated. This ratio was around 2 for plants with root hairs and around 1 for plants without root hairs (Fig. 5.6, left). This means that the presence of root hairs extended the zone of exudation to slightly older root segments. Root hairs caused a 3-fold increase in total exudation on filter paper compared to plants without root hairs (Fig. 5.6, right).

5.4 Discussion

Root exudates can increase nutrient availability in soil. On the one hand, they directly improve nutrient acquisition by mobilisation of nutrients such as phosphorus, iron and micronutrients (Dakora and Phillips, 2002; Hinsinger, 2001; Lynch, 2007; Marschner

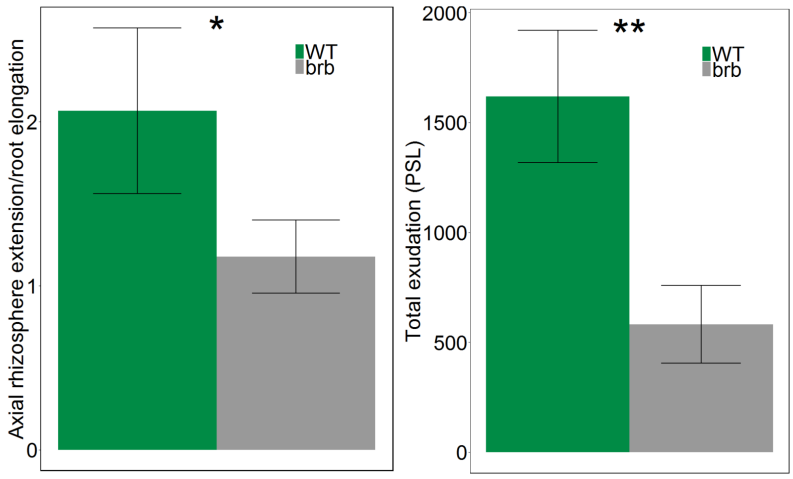

Figure 5.6: Left: Ratio between axial rhizosphere extension and root elongation for the barley wild type (WT) and the mutant without root hairs (*brb*). Variation is given as standard error (n = 3). The p value is presented as follows: *: p < 0.05. Right: Total exudation of barley plants with root hairs (WT) and without root hairs (*brb*) calculated based on the ^{14}C activity on the filter paper, which was attached to the roots and soil in the rhizobox during labelling. Variation is given as standard error (n = 3). The p value is presented as follows: **: $p < 0.01$.

et al., 2011), particularly in nutrient-poor soils. On the other hand, they strongly affect soil microbial activity and turnover of microbial biomass (Bertin et al., 2003; Gunina and Kuzyakov, 2015). Several studies have shown that nutrient availability increases due to higher microbial activity in the rhizosphere compared to the bulk soil(Hamilton and Frank, 2001; Herman et al., 2006; Landi et al., 2006). It is likely that increased rhizodeposition by plants with root hairs increases nutrient availability under nutrient limiting conditions. Apart from the increase in total exudation for WT plants, the increase in radial rhizosphere extension is of particular importance because it expands the volume of soil where root exudates can interact with the soil matrix and with microorganisms.

Root hairs also increased the axial extension of the rhizosphere as indicated in Figure 5.7. Commonly, root exudation is highest at the root tip and immediately behind the tip (Jones et al., 2009). Conceptual models of plant-microbe interactions along the root axis assume that root exudation and microbial activity are high in immediate vicinity of the root tip where nutrients are immobilized by microorganisms

(Kuzyakov and Xu, 2013). Behind the root tip, in the root hair zone, exudation is assumed to be low and microbes start to starve so that nutrients are mobilized and can be taken up by the plants (Marschner et al., 2011; Kuzyakov and Xu, 2013). We found that plants with root hairs increase axial rhizosphere extension compared to hairless plants. This indicates that the root hairs themselves exude organic substances. We conclude that root exudation is high over a longer distance from the root tip compared to what is commonly expected. For models of nutrient uptake this would imply that nutrients are mobilized farther behind the root tip than so far assumed.

We used ^{14}C imaging to estimate rhizosphere extension and total exudation on filter paper. Based on the distribution of ^{14}C on filter paper, we found that root exudation was approximately 3 times greater for the wild type compared to the hairless mutant. These differences are smaller than the 8 times difference in the total carbon found in the rhizosheaths (Table 5.1). This great difference can be explained by the 10 times larger rhizosheath mass for WT plants which confirms former experiments with the same genotypes (Haling et al., 2010). Indeed, a good part of organics might move farther away from the root surface and still contribute to rhizosphere processes, which is likely to happen for plants without root hairs. ^{14}C allocation in the rhizosheath should therefore not be confounded with ^{14}C allocation in the rhizosphere. A more extensive discussion on the differences between rhizosheath and rhizosphere terminology is given in (York et al., 2016).

^{14}C imaging allows estimating the spatial distribution of C in the soil-plant system. However, care must be taken when interpreting the images in terms of root exudation. When calculating the profiles of ^{14}C as a function of distance from the root center for images taken from the soil-root surface (i.e. intact samples with roots in the soil) we did not find differences between the treatments (Fig. 5.5, top). In contrast, significant differences between treatments where found when analyzing the spatial distribution of ^{14}C on filter paper. Possibly, the ^{14}C signal originating from the roots in the intact samples is so strong that it overshadows the signal from root exudates. This is particularly problematic if the soil-root surface is not perfectly flat (which normally is the case). In this case small gaps are present between the soil-root surface and the imaging screen and the β electrons from ^{14}C decay from the roots travels in any direction through air where it is barely attenuated. Consequently, the β signal hits the screen at a position farther away from the root, where it actually does not originate from. Using filter paper we avoided those two sources of errors because: a) the signal

of roots is excluded from the image; b) the surface of the paper is perfectly flat and air gaps between the surface and the screen can be excluded.

So far, rhizodeposition from root hairs has been observed for specific compounds such as mucigels (Dart, 1971; Greaves and Darbyshire, 1972; Sprent, 1975), acid compounds (Yan et al., 2004) and sorgoleone (Czarnota et al., 2003; Dayan et al., 2009). Sorgoleone secretion is specific for Sorghum plants and was seldom observed at root hairs of other graminaceous plants (Weston et al., 2012). There is less information on the effect of root hairs on total rhizodeposition. Pausch et al. (2016) quantified the effect of roots hairs on rhizosphere priming effect (RPE). Priming was increased for plants with root hairs at tillering stage compared to plants without root hairs which showed negative RPE. Because plants had similar root biomass the authors concluded that the presence of root hairs may explain differences in RPE. Possibly priming was increased in WT plants because the extension of the rhizosphere and because the extension of the rhizosphere by root hairs accelerated SOM decomposition (Pausch et al., 2016). We found that barley plants with root hairs exuded significantly more C than hairless mutants, which suggests that exudates are not only released from the tips of main and lateral roots but also from root hairs. The increase in RPE found by Pausch et al. (2016) may therefore not only be explained by a shift in microbial utilization of exudates but also by an increase in total exudation for plants with root hairs. The images showing increased root exudation for WT plants fitted well with the data on ^{14}C recovery: plants with root hairs allocated more carbon to roots and rhizosheath soil while plants without root hairs allocated relatively more C to shoots. For both plant types $^{14}CO_2$ efflux from soil was similar although root exudation was increased for WT plants. It follows that more C derived from roots was retained in soil for WT plants compared to plants without root hairs. There are two possible explanations for this observation: 1) roots hairs decrease the local soil water content in the rhizosphere, as shown by (Segal et al., 2008), which might result in a slower decomposition of ^{14}C labeled compounds in the rhizosphere (Sanaullah et al., 2012), thus reducing $^{14}CO_2$ efflux from soil; .2) alternatively, or additionally, the metabolic quotient (qCO_2) of microorganisms in the rhizosphere of WT plants decreased, i.e. microorganisms used C more efficiently for the build-up of biomass and respired relatively less C compared to the *brb* plants. As plants with root hairs allocated more C belowground, while ^{14}C efflux as well as total CO_2 efflux remained unchanged, more C derived from roots remained in soil for WT plants. Considering that the mean residence time in soils of

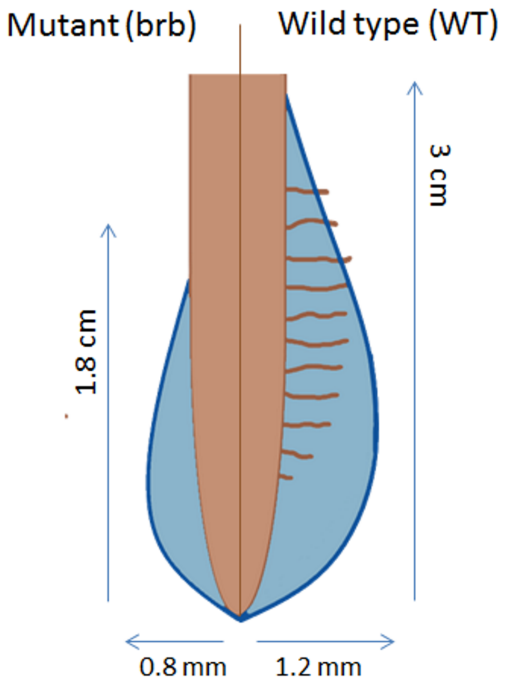

Figure 5.7: Distribution of root exudates in radial and axial direction for plants with root hairs (right) and the mutant without root hairs (left)

root-derived C is 2.4 times greater than that of shoot-derived C (Rasse et al., 2005) root hairs may play a significant role in soil C sequestration.

5.5 Conclusions

Plants with root hairs exuded significantly more carbon into the soil compared to plants without hairs and extended the rhizosphere in radial and axial directions (Fig. 5.7). The higher exudation and the increased rhizosphere extension might be an advantage for plants with root hairs because both favor plant-microbial interactions and therefore nutrient mobilization in the rhizosphere. Barley with root hairs allocated more C belowground compared to plants without hairs, but this did not increase CO_2 efflux. As root carbon has a longer mean residence time in soil compared to shoot carbon, it is likely that plants with root hairs foster C sequestration. Breeding for long root hairs and a high root hair density may be a suitable strategy for future agriculture, where nutrients are expected to become scarce and where C sequestration is a major issue due to climate change and resource depletion.

5.6 Acknowledgements

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6 Challenges and artefacts in visualisation and quantification of rhizodeposition using phosphor imaging

written by Maire Holz, Mohsen Zarebanadkouki, Yakov Kuzyakov and Andrea Carminati

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Abstract

Root exudates are an important energy source for soil microorganisms but quantifying their spatial distribution in soil is challenging. We tested whether phosphor imaging of labelled C can be used to accurately estimate profiles of root exudates in the rhizosphere. First, the attenuation coefficient of ^{14}C in soil and in water was measured. Secondly, barley plants were labelled with ^{14}C and a set of experiments were conducted to determine the origin of the ^{14}C signal detected with phosphor imaging. Selected roots of the labelled plants were imaged in soil, in air and after being replaced in a dry soil. Profiles of ^{14}C were broader for roots in air compared to those for the same roots replaced in a dry soil. However, even after replacing the roots in dry soil, the ^{14}C signal reached regions beyond the root surface, indicating that the ^{14}C signal was only partly attenuated by the soil. This was confirmed by the measured ^{14}C attenuation coefficient which was 148 cm^{-1} for soil and 67 cm^{-1} for water. Based on these coefficients we calculated profiles of ^{14}C that can be expected in the rhizosphere. The profiles of ^{14}C are strongly affected by: a) the ^{14}C activity in the root, b) the root radius, c) the position of the root in soil, d) the amount of root exudates in soil and e) by the presence of air gaps (or regions with high porosity) between soil and imaging screen. Inaccurate measurements of any of these parameters would cause artefacts in the estimation of root exudates distribution in the rhizosphere using phosphor imaging.

6.1 Introduction

Phosphor imaging is used in many scientific fields to quantify the distribution of radioactive tracers in samples (Amemiya and Miyahara, 1988). It has been applied in geology to detect radioactive elements in rocks (Cole et al., 2002), in molecular biology to image DNA adducts (Ottow et al., 2005; Reichert et al., 1992; Story et al., 1994) or in quantitative whole-body autoradiography to determine the distribution

of pharmaceutical substances in rodents (Richards, 2006; Solon and Lee, 2001). To obtain images, a phosphor imaging screen that is coated with BaFBr:Eu2+ crystals is placed on the sample (Amemiya and Miyahara, 1988). Once, electrons from β-decay hit the screen, their energy is stored in the BaFBr:Eu2+ crystals and they are released upon photo stimulation with light with a wavelength of 350 - 450 nm (Leblans et al., 2011). The spatial resolution of the obtained image is controlled by the imaging process itself rather than by the characteristics of the storage phosphor reader or by the imaging plates (Johnston et al., 1990). This is for two reasons; first, in case of small gaps between the sample and the imaging screen the recorded signal will be diffused in all directions (Fig. 6.1, top). Secondly, depending on the energy of the radioactive substance, the radiation will go through the sample in any direction, at least if part of the radioactive source is below the surface of the sample (Fig. 6.1, bottom). The distances that the radiation can go through the sample depends on its energy and will blur the image (Holz et al., 2017b; Johnston et al., 1990) (Fig. 6.1).

Recently, phosphor imaging has been applied in soil science to estimate ^{14}C-carbon allocation in plants and soil. While the imaging of C allocation into the plant roots works well (Pausch and Kuzyakov, 2011), it has proven more difficult to map C exudation from roots and the spatial distribution of the exuded C in rhizosphere soil (Holz et al., 2017b). Because so far, there is no method available to determine the spatial distribution of root exudates in soil in situ, it is tempting to apply ^{14}C imaging to image root exudates in the rhizosphere. Holz et al. (2017b) used ^{14}C imaging to Barley plant with and without root hairs after labelling the shoots with ^{14}C-CO_2. The radial profiles of ^{14}C from the root surface towards the bulk soil did not differ when images where taken from the rhizobox surface. However, when a filter paper was placed on the rhizobox surface and the captured root exudates where images, the radial profiles increased dramatically for plants with root hairs compared to plants without root hairs. This indicates that the images taken from the rhizobox surface do not reflect the real ^{14}C distribution in the rhizosphere and can therefore not easily be used to estimate profiles of root exudates around roots. This is probably due to the above mentioned reasons, which would lead to an overshadowing of the root exudates in the rhizosphere by the ^{14}C signal that is originally coming from the ^{14}C activity in the root itself. However, this assumption has not been proved experimentally.

The aim of this study was to test whether phosphor imaging can be used to calculate profiles of root exudates around growing roots. The study was divided in two

experiments. The aim of the first experiment was to test whether what is seen as ^{14}C signal around roots can be considered to be root exudates. To test this, barley plants where labelled with ^{14}C-CO_2. Root tips, showing a high ^{14}C activity were removed from soil one day after labelling and images were taken from the rhizobox soil, presumably containing the root exudates. After this, the removed root tips were dried and placed into dry soil and imaged to estimate the contribution of ^{14}C to the profiles, originating actually from the root itself and not from root exudates. The aim of the second experiment was to calculate an attenuation coefficient of ^{14}C in soil and water. Defined amounts of ^{14}C were given to different soil depths to calculate the attenuation of the ^{14}C signal. The advantage of such a coefficient would be that the proportion of the signal of the ^{14}C originating from the root instead of from root exudates could be calculated and the ^{14}C imaging could be used to actually quantify the distribution of root exudates in the rhizosphere.

6.2 Material and Methods

Sample preparation and plant growth

For the first experiment four barley plants (Hordeum vulgare L. cv. Pallas) were grown in rhizoboxes. The seeds were immersed in a 10% H_2O_2 solution for 10 min before germination to avoid seed-borne diseases. The barley plants were grown in rhizoboxes with a size of 12 x 20 x 3.5 cm for 30 - 31 days. The soil used was a sandy soil (A-horizon) collected from a field site close to Reinhausen (Göttingen, Germany). Ctot was 2.0% and Ntot was 0.17% and pH was 4.9. The soil texture was distributed as follows: Clay: 8.6%, silt: 18.5%, sand: 73%. The volumetric soil water content was kept at 23 - 25% vol. water content during plant growth. The temperature in the climate chamber was 25 °C during the day and 22 °C during the night, the photoperiod was 14 hours and the photosynthetic photon flux density was 200 μmol m^2 s^1.

$^{14}CO_2$ pulse labelling and ^{14}C imaging of plants

The procedure of ^{14}C labelling is described in detail in (Holz et al., 2017b). After four weeks of plant growth each plant was labelled with 0.5 MBq $^{14}CO_2$ (specific activity of 59.6 mCi mmolC^{-1}) for 4.5 hours. Labelling was always conducted at noon for plants of all sowing times. At the end of labelling, approximately 70% of the added ^{14}C had been taken up by the plants. This was tested by collecting 30 ml of chamber

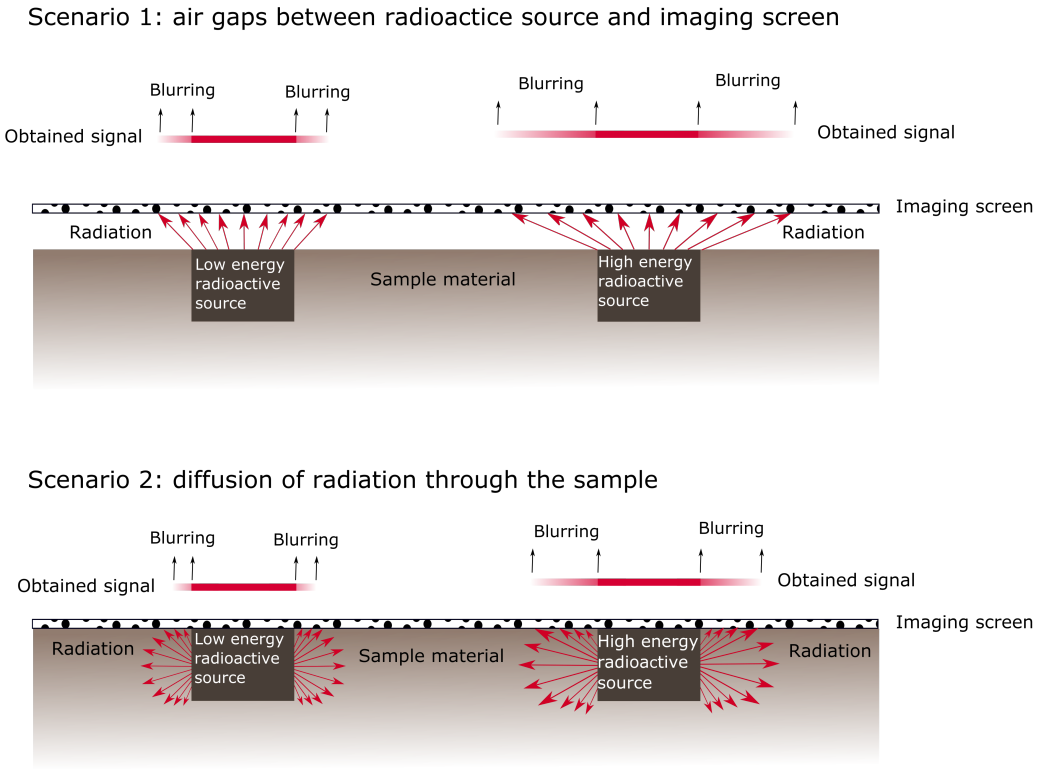

Figure 6.1: Conceptual model of artefacts that may occur during phosphor imaging and that may blur the image and reduce the spatial resolution. Scenario 1 shows the diffusion of radiation trough air, in case of small gaps between the sample and the imaging screen. Scenario 2 describes the travelling of radiation trough the sample from deeper sample layers which again leads to a blurring of the image.

air with a syringe 4 times during labelling (after 5, 30, 60, 120 min) and injecting it into a scintillation cocktail (C 400, Zinsser Analytics).^{14}C activity was quantified by a liquid scintillation counter (Hidex, 300 SL). The activity of ^{14}C in the 30 ml of chamber air that had been taken out of the chamber was back calculated to the volume of the whole chamber. Doing this for all 4 measuring times, we calculated the amount of ^{14}C in the chamber over time which is inversely proportional to the uptake of ^{14}C.

After labelling, one phosphor image was taken per rhizobox to detect root parts with high ^{14}C activity. This was done by placing an imaging plate (Storage phosphor screen, BAS-IP MS 2040 E, VWR) on the samples. A thin plastic film was placed between the sample and the imaging screen to protect the imaging screen against

the moist soil. The screens were attached to the samples for 15 h. After this time the screens were scanned (FLA 5100 scanner, Fujifilm) with a spatial resolution of 50 m. Those root parts, showing a high ^{14}C activity (i.e. the growing root tips) were carefully removed from the soil and dried in a drying oven at 40 °C for 48 h. After removal of the root tips, the rhizoboxes were imaged again as described above to test, whether the signal originating from root exudates would be detectable in the soil regions where the root tips had been growing (Fig. 6.2a,b). The dried roots where imaged twice. First, they were placed on a plexiglas plate without any soil to estimate the effect of the air gap between the sample and the imaging screen on the blurring of the ^{14}C signal around the roots (Fig. 6.2c). After this, the roots were placed on a rhizobox surface filled with dry soil. The roots were carefully pressed in the soil in order to obtain a totally plane surface. This was done to test whether there would be a ^{14}C signal detectable outside the root radius which would indicate that the β-radiation from ^{14}C decay was going through the soil (Fig. 6.2d).

Image analysis

The quantification of ^{14}C images was done as described in Holz et al. (2017b). First, the images were converted from a log into a linear system by applying the following equation:

$$PSL = \left(\frac{Res}{100}\right)^2 * \frac{4000}{S} * 10^{L*\left(\frac{QL}{G}-\frac{1}{2}\right)} \tag{6.1}$$

where *PSL* (Photostimulated Luminescence) is the quantified value of the image in linear scale, *Res* is the resolution of the image in μm ($Res = 50$ μm), S is the sensitivity ($S = 5000$), L is the latitude (L= 5) and G is the gradation ($G = 65535$). After conversion of the images, the background noise was removed: The part of the image where the screen was not in contact with the sample was selected and subtracted from the part of the image where the root system was visible. Based on the contrast between roots and soil, the root tips that showed a high ^{14}C signal in the images were segmented using the SmartRoot plugin in ImageJ. Possible artefacts caused by overlapping roots or roots detached from the soil were removed from the segmentation. 2 - 5 roots per sample were segmented depending on how many roots were clearly visible. The signal was averaged as a function of distance from the root center up to a distance of 4 cm from the root surface using the Euclidean distance mapping functions

in MATLAB (The MathWorks). To quantify total exudation, the PSL values around each root tip were summed up and a mean of total exudation per tip and treatment was calculated.

Calculation of the ^{14}C attenuation coefficient and of ^{14}C profiles

To measure the ^{14}C attenuation in soil and in water, we used 2.5 cm deep PVC plates into which rectangular holes with a size of 8 x 12 cm were placed. The holes had the following depths: 0.5, 1, 2, 4, and 8 mm. 1 x 1 cm sized pieces of filter paper were soaked in 2 ml solution containing 1 MBq of ^{14}C-glucose, The pieces of filter paper were dried for one hour at 60 °C and 4 pieces of filter paper were placed into each hole of the PVC plates, covered with a thin plastic film and then covered with dry soil. After placing an imaging plate on the soil surfaces for 18 hours, phosphor imaging was conducted as described above. To measure the attenuation of ^{14}C in water, the same pieces of filter paper were covered with water instead of soil and the imaging was done similarly as for soil. To calculate the attenuation coefficients, the signal of ^{14}C coming from the filter papers was related to the respective soil thickness (Fig. 6.5). The data were fitted to a falling exponential function according to the Beer-Lambert-Law:

$$I = Exp^{-\mu * x} \tag{6.2}$$

where $I=$ is the ^{14}C signal obtained from the image, μ is the attenuation coefficient of ^{14}C and x is the soil thickness. Knowing I and x, it was possible to calculate the attenuation coefficient of ^{14}C for soil and for water. Based on the attenuation coefficient in soil, we calculated the ^{14}C signal that would be captured by the imaging screen. We considered a soil region with a size of 200 pixel in x direction and 100 pixel in y direction having a pixel size of 0.01. The root had a diameter of 0.6 mm and it was placed in the center of the soil region. It was assumed to be either slightly below, right at the soil surface or slightly above the soil surface, creating a small air gap between the sample and the imaging screen (Fig. 6.6, left). The activity of the root was assumed to be 1000 PSL and the soil in the first pixel at the root surface had a ^{14}C activity that corresponds to 5 - 30% of the ^{14}C activity of the root. Root exudates were assumed to decreased exponentially from the root surface (Fig. 6.8c). We assumed a volumetric soil water content of 15% and a bulk soil density of 1.5 g cm^{-3} which yielded an effective attenuation of ^{14}C in soil of 95.4 cm^{-1}. The signal

of ^{14}C was calculated for one position of the root in radial direction from the root surface into the bulk soil. In this sense, we assumed that the ^{14}C was homogeneously distributed in the root in axial direction. For each pixel in y direction, the ^{14}C signal was calculated considering the ^{14}C activity in the root, in the root exudates and the attenuation of the ^{14}C signal travelling through the soil. For the air gap between the sample and the screen in that cases where the root was placed slightly above the soil surface, an attenuation coefficient of 0 was assumed.

Statistical analysis

Statistical analyses were done using R 3.3.1. To test for significances in the radial rhizosphere extension (Fig. 6.3,6.4) between treatments, a fixed effect model ($\alpha = 0.05$) with treatment as fixed effect was applied.

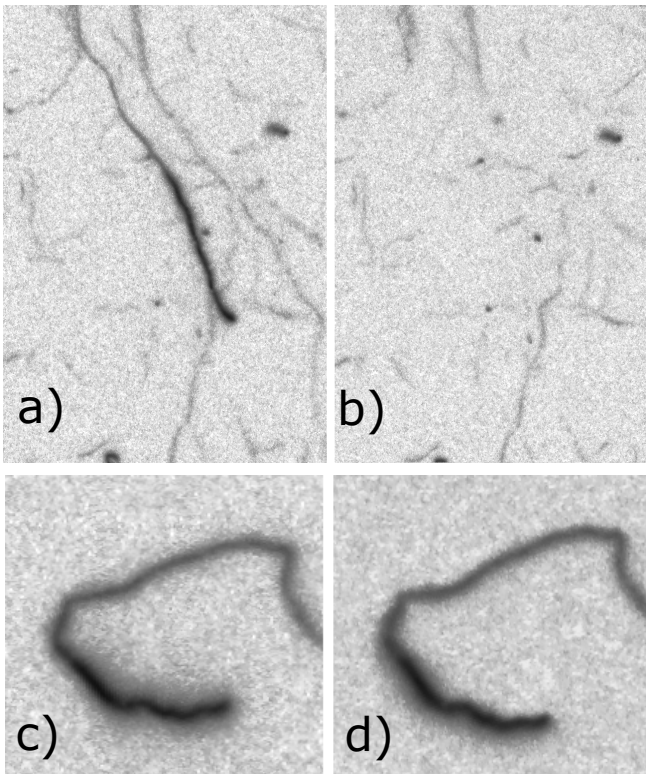

Figure 6.2: Exemplary ^{14}C images of the barley roots. a) image of a rhizobox with roots and b) after removing the root tip with high ^{14}C activity. c) shows an imaged root placed on plexiglas without soil and d) shows the same root after placing it in dry soil. Dark indicates high ^{14}C activity, bright indicates low ^{14}C activity.

6.3 Results and Discussion

High ^{14}C activity was found around the root tips of young, growing roots (Fig. 6.2). This is in line with previous studies which showed that root tips are important hotspots in soil (Kuzyakov and Blagodatskaya, 2015) and that recently assimilated C within the root system is mainly allocated to root tips and released from there (Dennis et al., 2010; Holz et al., 2017a; Jones et al., 2004; Pausch and Kuzyakov, 2011).

The radial profiles of ^{14}C activity around roots grown in rhizoboxes extended up to 0.5 mm from the root surface into the bulk soil. At the root surface they reached values of around 40 PSL and the activity decreased to approximately 0 after 1 mm (Fig. 6.3). In contrast, no profiles of ^{14}C were found after removing the roots and the signal scattered around 0, even at the positions where the root tip had formerly been seen (Fig. 6.3). One reason might be that root exudates are easily available to microbes and that microbial activity in the rhizosphere is high compared to the bulk soil (Gunina and Kuzyakov, 2015; Landi et al., 2006). Therefore, a high proportion of root exudates might have been decomposed already at the time of ^{14}C imaging. Furthermore, it is possible that during the removal of the root, a small portion of rhizosphere soil, and with it some root exudates, were accidentally removed. However, even though it might be possible that microbial decomposition of exudates or partly removal of rhizosphere soil reduced root exudates, we would still have expected to see some signal of ^{14}C in the soil region where the roots had been. The fact that no ^{14}C was seen around roots after removing them from soil although broad ^{14}C profiles are seen around roots in soil raises the question whether profiles of ^{14}C around roots in soil can be interpreted as root exudates. Although it has been suggested that ^{14}C imaging can be used to detect root exudates in soil (Pausch and Kuzyakov, 2011), it is not clear whether the imaged ^{14}C around roots is actually the signal originating from root exudates or rather the blurred signal originating from the root (Dennis et al., 2010; Holz et al., 2017b).

The profiles of ^{14}C for dried roots placed into dry soil were steeper and showed a smaller extension (0.25 vs. 0.5 mm) than the profiles of dried roots imaged without soil which reflect different attenuation of ^{14}C in soil and in air. The fact that even for roots in soil, the ^{14}C signal reached regions beyond the root radius, confirms our assumption that ^{14}C originating from the plant root is not totally attenuated in soil but is scattered over short distances in all direction, blurring the ^{14}C image (Fig. 6.1,

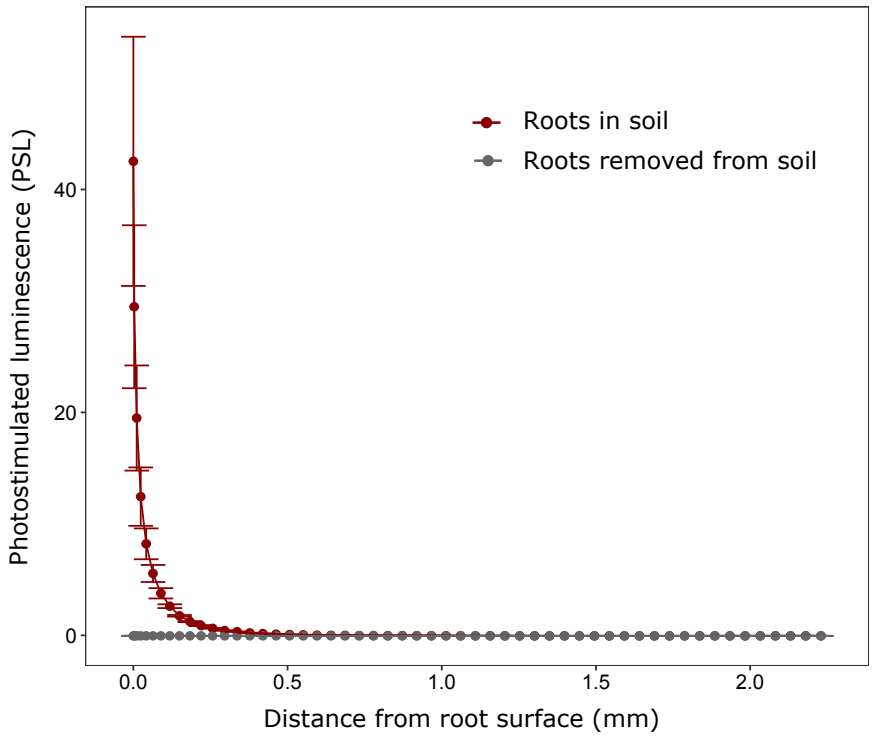

Figure 6.3: Radial profiles of roots in rhizoboxes (red line) and of the ^{14}C signal after removal of the root tips (grey line). Variation is given as standard error, n=4. The treatments differed significantly ($\alpha = 0.05$).

bottom). Additionally, air gaps between the sample surface and the imaging screen would further extend the profiles of ^{14}C measured. This may happen if the root is placed slightly above the soil surface or if there is some loose soil around the root in the rhizobox. These findings indicate that the profiles of ^{14}C around roots visualised by phosphor imaging may not simply be used to calculate profiles of exudates around roots because a) air gaps may blur the signal (Fig. 6.1, top) and b) the ^{14}C signal detected by the screen at a given position may not come from that position but from a deeper soil layer and eventually from the root while it might be interpreted as root exudates (Fig. 6.1, bottom). We therefore aimed to quantify the effect of both effects on profiles of ^{14}C around roots by calculating the attenuation of ^{14}C in soil. The ^{14}C activity with thickness of soil and water followed a strong exponential decay (Fig. 6.5) which is in accordance with previous findings on attenuation of β-radiation in different materials (Özmutlu and Ahmet Cengiz, 1990). While for soil, the signal decreased to 0 with a soil thickness of around 0.25 mm, water attenuated less ^{14}C and the signal reached 0 with a water thickness of around 0.5 mm. The

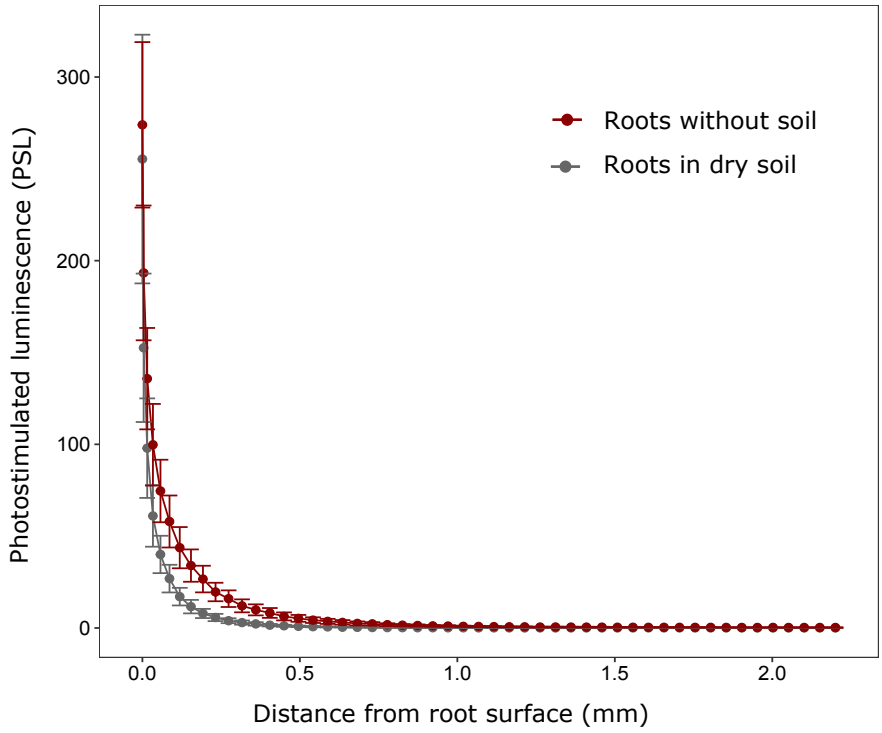

Figure 6.4: Radial profiles of dry roots removed from soil and placed on a plane surface without soil (red line) and of the same roots after they were replaced into dry soil (grey line). Variation is given as standard error, n=4. The treatments differed significantly ($\alpha = 0.05$).

calculated attenuation coefficient was 67 cm^{-1} for water and 148 cm^{-1} for soil which corresponds to the differences in the density of the materials which is 1 g cm^{-3} for water and 2.65 g cm^{-3} for quartz. The ^{14}C signal obtained during ^{14}C imaging was calculated for different scenarios with regard to the position of the root in the soil and the amount of exudates present around the root. For the first scenario we assumed that the root was placed either below the soil surface (0.1 and 0.05 mm), right at the soil surface or slightly above the soil surface (0.02 and 0.05 mm), creating an air gap between the sample imaging screen (Fig. 6.6, left). In this scenario we considered only the ^{14}C activity of the root and assumed that there were no exudates in the soil surrounding the root. The position of the root strongly influenced the distribution of the ^{14}C signal. Changing the root position from 0.1 mm below the surface to the soil surface increased the ^{14}C signal in the middle of the root from around 1000 to 4000 ^{14}C (PSL) cm^{-2}. The extends of the radial profiles were not affected by these changes and the profiles extended around 0.5 mm into the soil starting from

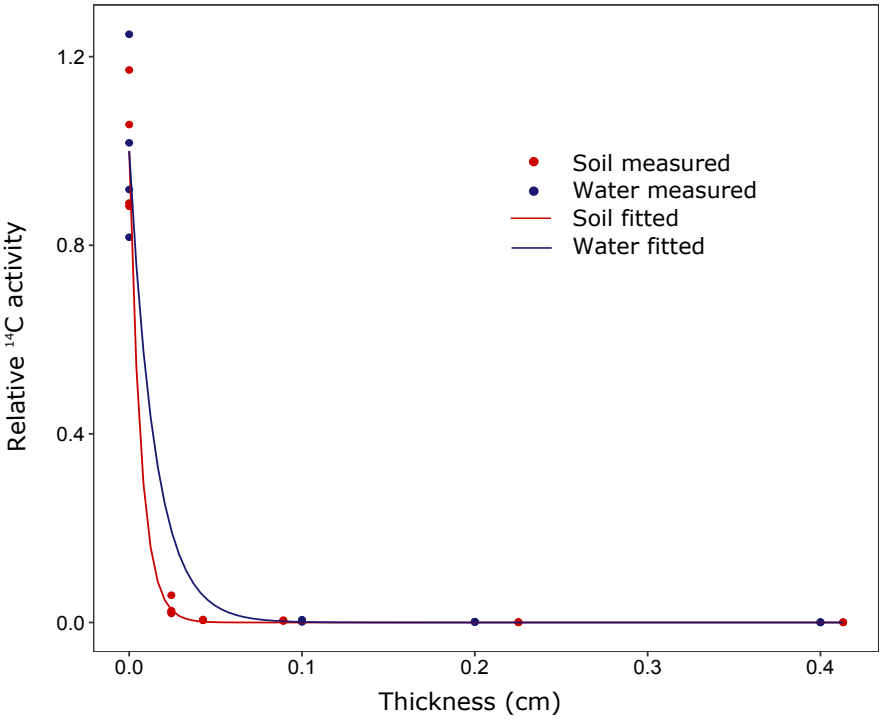

Figure 6.5: Attenuation of ^{14}C signal by soil and water of increasing thickness. The x axis shows the thickness of soil and/or water while the y axis depicts the normalized ^{14}C activity: 1=^{14}C activity without soil (thickness = 0). Variation is given as standard error, n=4.

the middle of the root (i.e. x = 1.0 mm). However, assuming that the root was placed slightly above the soil surface strongly increased the radial extend of the ^{14}C profiles. Placing the root 0.05 mm above the soil surface resulted in an extension of the ^{14}C profile of around 1 mm (Fig. 6.6). These results prove that the signal of ^{14}C extends beyond the root surface, because soil does not completely attenuates ^{14}C. This makes the interpretation of the images difficult. Additionally, the root position is very important for the interpretation of ^{14}C images. Particularly the existence of air gaps between the sample and the imaging screen strongly broadens the profiles of ^{14}C around roots.

In the second scenario, we considered different amounts of root exudates in the soil around the root which correspond to 5 - 30% of C allocated to the roots (Holz et al., 2017b; Jones et al., 2004; Nguyen, 2003). The amount of root exudates did not influence the ^{14}C signal obtained close to the root but did have a strong influence on the radial extend of the profiles. For the lowest concentration of exudates, which

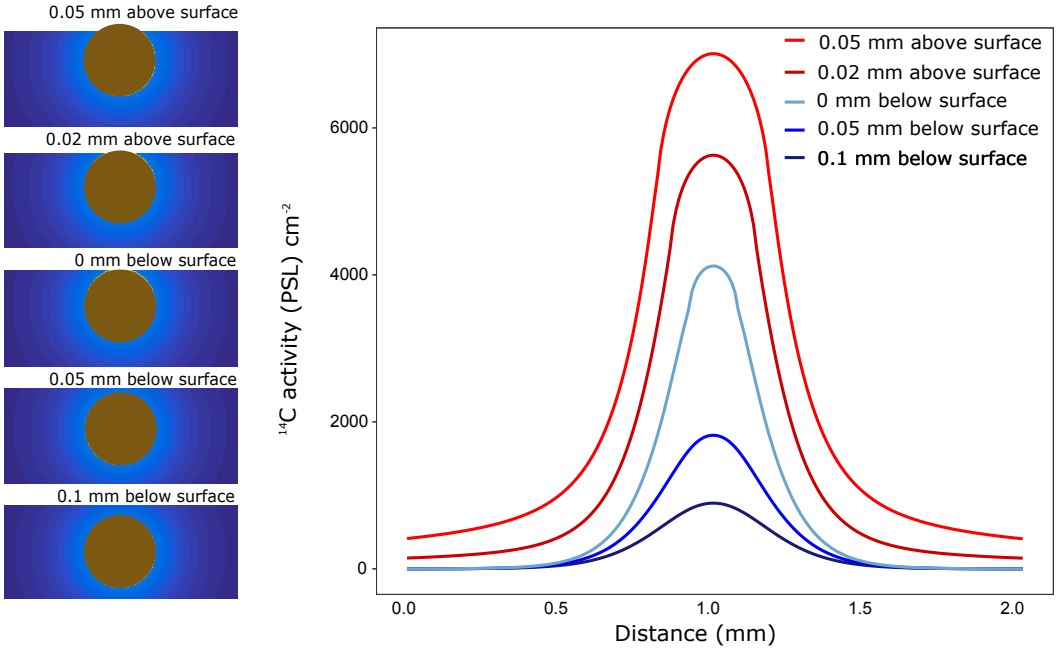

Figure 6.6: Calculated profiles of ^{14}C activity around roots in soil. The roots were assumed to be in five different positions in the soil, either below the soil surface (blue lines), right at the soil surface (light blue line) or above the soil surface, assuming an air gap between the sample and the imaging screen (red lines).

corresponds to 5% of ^{14}C in the root, the profile nearly corresponded to that obtained assuming that only the root contains ^{14}C (Fig. 6.7a, black dashed line). Doubling the amount of root exudates resulted in an increase of the radial extend of ^{14}C from 0.5 mm (low exudation) to around 0.75 mm for medium exudation (Fig. 6.7a). Doubling the amount of exudates again, increased the radial profile of ^{14}C up to 1 mm from the middle of the root (i.e. x = 1.0 mm). The amount of root exudates placed into soil affected the relative contribution of root exudates to the total ^{14}C signal which is displayed in Figure 6.7b. When considering rather low root exudation, even far from the root the contribution of root exudates to the total ^{14}C signal was relatively low and reached only 60%. In contrast, assuming high exudation, the contribution of root exudates to total ^{14}C reached almost 1 already at a distance of 0.5 mm from the root surface. These results highlight the importance of the amount of root exudates released from roots for interpretation of ^{14}C images. For high root exudation it may be possible to calculate back the contribution of exudates from the profiles of ^{14}C because

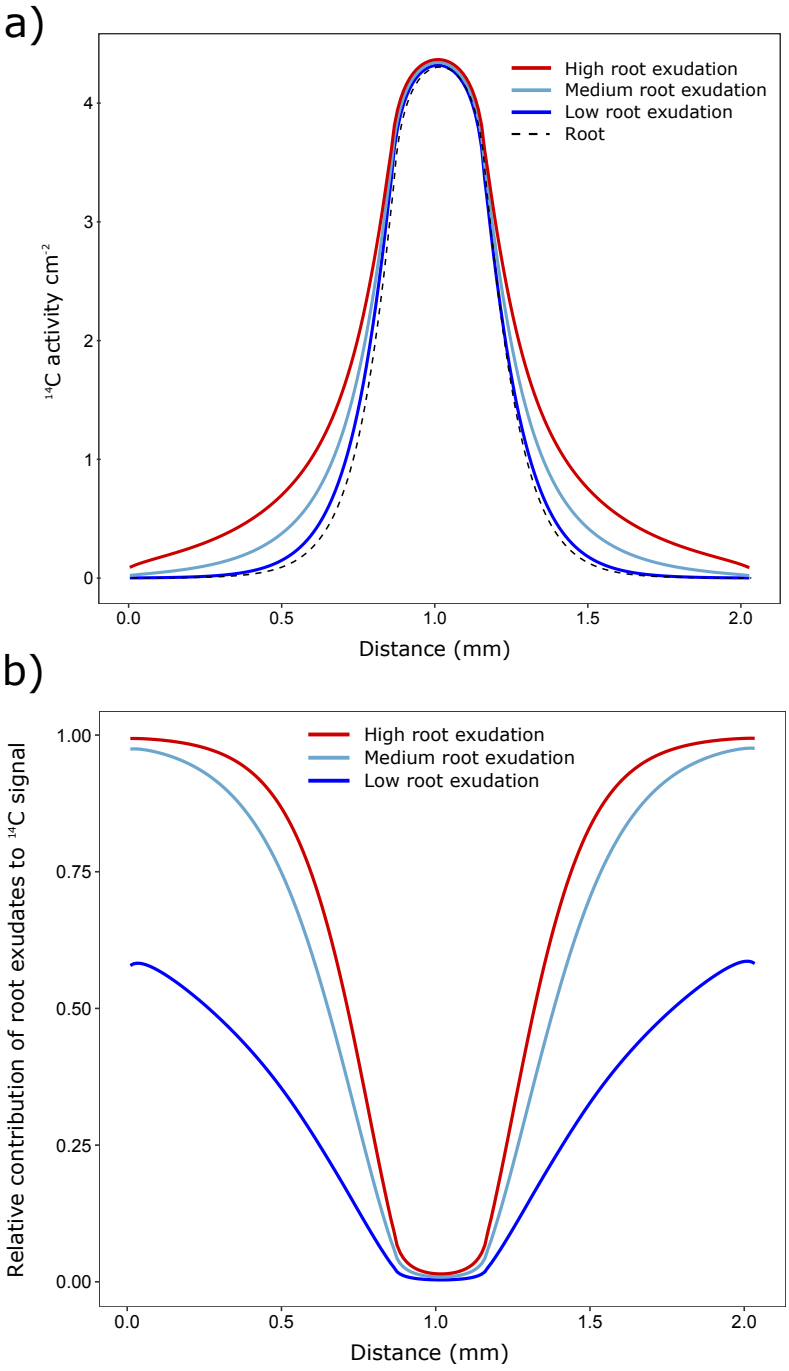

Figure 6.7: Calculated profiles of ^{14}C activity around roots in soil assuming low, medium and high root exudation. The black dashed line in a) corresponds to the ^{14}C activity by the root, assuming no root exudates in soil.

the contribution of root exudates to the total signal is relatively high (Fig. 6.5b). However, assuming low exudation, this calculation may be prone to errors because the contribution of root exudates to the ^{14}C signal will be negligible. Figure 6.8

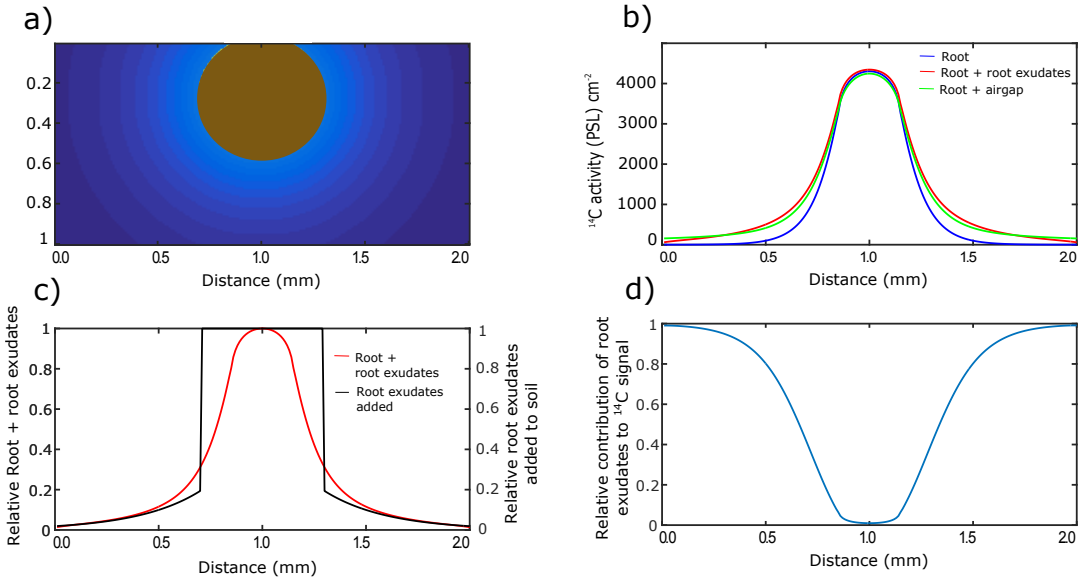

Figure 6.8: b) Calculated profiles of ^{14}C activity around roots. The profiles were calculated for three scenarios, first, assuming that only the root is radioactive (blue line), second, assuming that the root and root exudates in the soil around the root are radioactive (red line) and third, assuming that only the root is radioactive and that there is an airgap of 0.03 mm between the root and the imaging screen (green line). c) shows the distribution of the actual root exudates in soil (black line) compared to the signal that is seen after imaging root and root exudates (red line). d) displays the relative contribution of root exudates to the ^{14}C signal measured. 1 indicates that 100% of the signal is caused by root exudates, while 0 indicates that 100% of the signal is caused by the activity of the root.

sums up the results for three different scenarios of ^{14}C activity. We assumed that either only the root contained radioactive material, emitting β- radiation or that both, root and root exudates emitted β- radiation. The last scenario considered only the root to be radioactive, however, a small airgap of 0.03 mm was assumed to be in between the sample and the imaging screen (Fig. 6.8b). Comparing the radial profile of ^{14}C that considers root and root exudates to be radioactive, with those one where only the root is radioactive but there is a small air gap in between the imaging screen, it is noticeable that both lines show an almost similar curve shape. This result confirms that the interpretation of ^{14}C signal around roots as root exudates is only possible if any air gaps around the roots can be excluded. This is because the low

attenuation of β- radiation in the air leads to an extreme blurring of the ^{14}C signal. Figure 6.8c compares the radial profile of ^{14}C assuming the root and root exudates to be radioactive with the actual distribution of root exudates in soil. While for distances farther than 0.75 mm from the root surface, both profiles correspond well, close to the root the profile of the actual root exudates in soil was below that profile of ^{14}C that would be seen after imaging. This finding corresponds well with the distribution of the contribution of root exudates to the total ^{14}C signal (Fig. 6.8d) which shows that with increasing distance from the root, the relative contribution of root exudates to the total ^{14}C signal increases. The interpretation of ^{14}C signal around roots as root exudates may therefore be possible if relatively high root exudation can be expected and will be more precise with increasing distance from the root. Close to the root, the calculation will be prone to errors because the contribution of ^{14}C signal originating from the root is high compared to the signal originating actually from root exudates. However, root exudates follow a concentration gradient from the root surface and have high concentrations close to the root surface that decrease with increasing distance from the root. The fact that the interpretation of ^{14}C signal is particularly difficult close to the root surface where the concentration of root exudates is actually high, is a severe drawback of the method.

6.4 Conclusions

We showed that the interpretation of profiles of ^{14}C around roots measured by ^{14}C imaging as profiles of root exudates can be confounded by several factors. In general, the profiles of ^{14}C extended beyond the root surface. This is because the ^{14}C signal originating from the root is not completely attenuated by the soil surrounding the root and hits the imaging screen beyond the root surface, thereby blurring the profiles. Knowing the root radius, the shape of the root, its exact position in soil and the ^{14}C activity in the root, it would be possible to calculate the contribution of root exudates to the profiles of ^{14}C signal. This calculation is realistic assuming a high root exudation and is more reliable with increasing distance from the root surface. However, in practice, it is unlikely to measure all the needed information for the calculation precisely. Particularly in rhizobox experiments it would be unrealistic to assume a cylindrical root shape as roots in rhizoboxes are commonly slightly flat. Additionally, the presence of air gaps between soil and imaging screen or regions with

high porosity around the roots leads to extreme blurring of the images. As potential air gaps between the sample and the imaging screen and the soil porosity around the roots are barely quantifiable, the interpretation of ^{14}C profiles as root exudates is questionable.

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A Mucilage exudation facilitates root water uptake in dry soils[1]

written by Mutez A. Ahmed, Eva Kröner, Maire Holz, Mohsen Zarebanadkouki and Andrea Carminati

published in Journal of Functional Plant Biology (Ahmed et al., 2014)

Abstract

As plant roots take up water and the soil dries, water depletion is expected to occur in the rhizosphere. However, recent experiments showed that the rhizosphere was wetter than the bulk soil during root water uptake. We hypothesise that the increased water content in the rhizosphere was caused by mucilage exuded by roots. It is probably that the higher water content in the rhizosphere results in higher hydraulic conductivity of the rootsoil interface. In this case, mucilage exudation would favour the uptake of water in dry soils.

To test this hypothesis, we covered a suction cup, referred to as an artificial root, with mucilage. We placed it in soil with a water content of 0.03 cm^3 cm^{-3}, and used the root pressure probe technique to measure the hydraulic conductivity of the rootsoil continuum. The results were compared with measurements with roots not covered with mucilage.

The root pressure relaxation curves were fitted with a model of root water uptake including rhizosphere dynamics. The results demonstrated that when mucilage is added to the root surface, it keeps the soil near the roots wet and hydraulically well conductive, facilitating the water flow from dry soils towards the root surface. Mucilage exudation seems to be an optimal plant trait that favours the capture of water when water is scarce.

[1]M.H. was responsible for the measurement of the drying rate of mucilage in soil

B Effect of soil drying on mucilage exudation and its water repellency: A new method to collect mucilage[2]

written by Mutez A. Ahmed, Maire Holz, Susanne K. Woche, Jörg Bachmann and Andrea Carminati

published in Journal of Plant Nutrition and Soil Science (Ahmed et al., 2015)

Abstract

Despite the importance of mucilage for soilplant relations, little is known about the effect of soil drying on mucilage exudation. We introduce a method to collect mucilage from maize growing in wet and dry soils. Mucilage was collected from brace roots. The amount of mucilage exuded did not change with soil water content and transpiration rate. Mucilage exuded in dry soils had a higher degree of hydrophobicity, suggesting that the wetting properties of mucilage change in response to soil drying.

[2]M.H. helped to develop the setup for the collection of mucilage and partly conducted the mucilage collection

C Warming increases hotspot areas of enzyme activity and shortens the duration of hot moments in the root-detritusphere[3]

written by Xiaomin Ma, Bahar S. Razavi, Maire Holz, Evgenia Bladodatskaya and Yakov Kuzyakov

published in Soil Biology and Biochemistry (Ma et al., 2017)

Abstract

Temperature effects on enzyme kinetics and on the spatial distribution of microbial hotspots are important because they are crucial to soil organic matter decomposition.

We used soil zymography (in situ method for the two dimensional quantification of enzyme activities) to study the spatial distributions of enzymes responsible for P (phosphatase), C (cellobiohydrolase) and N (leucine-aminopeptidase) cycles in the rhizosphere (living roots of maize) and root-detritusphere (7 and 14 days after cutting shoots). Soil zymography was coupled with enzyme kinetics to test temperature effects (10, 20, 30 and 40 °C) on the dynamics and localization of these three enzymes in the root-detritusphere.

The percentage area of enzyme activity hotspots was 1.9 - 7.9 times larger and their extension was broader in the root-detritusphere compared to rhizosphere. From 10 to 30 °C, the hotspot areas enlarged by a factor of 2 - 24 and Vmax increased by 1.5 - 6.6 times; both, however, decreased at 40 °C. For the first time, we found a close positive correlation between Vmax and the areas of enzyme activity hotspots, indicating that maximum reaction rate is coupled with hotspot formation. The substrate turnover time at 30 °C were 1.7 - 6.7-fold faster than at 10 °C. The Km of cellobiohydrolase and phosphatase significantly increased at 30 and 40 °C, indicating low affinity between enzyme and substrate at warm temperatures.

We conclude that soil warming (at least up to 30 °C) increases hotspot areas of enzyme activity and the maximum reaction rate (Vmax) in the root-detritusphere. This, in turn, leads to faster substrate exhaustion and shortens the duration of hot moments.

[3]M.H. partly supervised the experiment and helped with the data analysis and the writing of the manuscript

D Effects of mucilage on rhizosphere hydraulic funtions depend on soil particle size[4]

written by Eva Kroener, Maire Holz, Mohsen Zarebanadkouki, Mutez Ahmed and Andrea Carminati

published in Vadose Zone Journal (Kroener et al., 2017)

Abstract

Mucilage secreted by roots alters hydraulic properties of soil close to the roots. Although existing models are able to mimic the effect of mucilage on soil hydraulic properties for specific soils, it has not yet been explored how the effects of mucilage on macroscopic soil hydraulic properties depend on soil particle size.

Here, we propose a conceptual model of how mechanistic pore scale interactions of mucilage, water and soil depend on pore size and mucilage concentration and how these pore scale characteristics result in changes of macroscopic soil hydraulic properties. Water retention and saturated hydraulic conductivity of soils of different ranges of particle sizes mixed with various mucilage concentrations were measured and used to validate the conceptual model.

We found that (a) at low mucilage concentrations the saturated conductivity of a coarse sand was a few orders of magnitude higher than in a silt; (b) at an intermediate concentration, the hydraulic conductivity in a fine sand was lower than in a coarse sand and in a silt; and (c) at a high concentration, all soils had a hydraulic conductivity of same magnitude. At low matric potentials mucilage increased the water content in all soils, with higher mucilage concentrations being needed in coarser soils to induce an increase in water content of > 0.05 g g^{-1}.

This study shows how pore-scale interactions between mucilage, water and soil particles affect bulk soil hydraulic properties in a way that depends on soil particle size. Including such effects in quantitative models of root water uptake remains challenging.

[4]M.H. conducted the measurements of the water retention curves together with E.K.

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