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Influence of nutrient and metal concentration on root exudation under waterlogged conditions

Einfluss der Nährstoff- und Metallkonzentration
auf die Wurzelexsudation unter staunassen Bedingungen

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For my parents Ingrid and André,

my sister Marionna and my brother Yves,

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and my boyfriend Alexis.

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Content

Abstract

The worldwide energy demand constantly increases and the search for regenerative alternatives is inevitable. A concept that exploits a bioenergy source in situ is the plant Microbial fuel cell (MFC). The plant-MFC uses the natural interplay between electrogenic bacteria in soil and plants to convert solar energy into green electricity.

The interplay between bacteria and plant is based on the rhizodeposition of organic compounds into the rhizosphere. It is known, that low phosphate (P) content in the soil as well as a high Aluminum (Al) concentration can induce an enhanced exudation of organic compounds by plants into the rhizosphere. However, so far little is known about the factors that influence the rhizodeposition under waterlogged conditions. Thus, the aim of this study was to increase the exudation of several plants into the rhizosphere under waterlogged conditions by treating the plants with low P content or Al addition. Total organic carbon and inorganic carbon analyses indicated that a reduction of P supply induced a twofold increased release of organic compounds in waterlogged *Spartina anglica,* a threefold in *Hemarthria altissima* and a fivefold in *Glyceria maxima* plants. In contrast, the addition of Al did not affect the root exudation of any screened plant species. It is likely, that the screened plants use other mechanisms to detoxify the Al in the nutrient solution such as absorption into the symplasm or pH modifications of the rhizosphere. In future studies it should be tested, if a reduction of P in the plant-MFC is a possible tool to increase the productivity of the plant-MFC without affecting plant development and health.

Zusammenfassung

Aufgrund eines kontinuierlich zunehmenden weltweiten Energiebedarfs, ist die Suche nach regenerativen Energiequellen unumgänglich. Eine mögliche Lösung ist die Entwicklung einer Brennstoffzelle die durch zusammenlebende Pflanzen und Bakterien betrieben wird. Die Pflanzen versorgen die Bakterien mit organischen Kohlenstoffen als Nahrungsgrundlage, die sie in den Boden abgeben (Exsudation) und die Bakterien liefern die Protonen und Elektronen für die Brennstoffzelle. Die Rhizodeposition ist beeinflussbar durch Umweltfaktoren, wie das Nährstoffangebot oder toxische Metalle im Boden. So können sowohl eine geringe Phosphatversorgung wie auch eine hohe Aluminiumkonzentration im Boden eine Erhöhung der Abgabe von organischen Kohlenstoffen (Exsudation) induzieren. Allerdings ist bisher wenig über die Faktoren bekannt, die eine Erhöhung der Exsudation unter staunassen Bedingungen induzieren können. Das Ziel dieser Arbeit war es daher, die Exsudation von Pflanzen durch eine Verringerung des Phosphatgehaltes in der Nährlösung oder durch Zugabe von Aluminium zu erhöhen. Die Analyse des organischen und anorganischen Kohlenstoffes zeigten deutlich, dass eine Reduzierung des Phosphatgehalts eine Erhöhung der Exsudation bei den untersuchten Pflanzen Arten, *Hemarthria altissima, Glyceria maxima* und *Spartina anglica* zur Folge hatte. Bei *Hemarthria altissima* zeigte sich eine Verdreifachung des organischen Kohlenstoffs, bei *Spartina anglica* eine Verdopplung und bei *Glyceria maxima* eine fünffache Erhöhung des anorganischen Kohlenstoffs. Im Gegensatz dazu, konnte bei Zugabe von Aluminium eine Veränderung der Exsudation bei keiner der untersuchten Pflaneznarten beobachtet werden. Vermutlich nutzen die untersuchten Pflanzenarten andere Mechanismen, um das Aluminium zu detoxifizieren wie zum Beispiel eine Aufnahme in den Symplasten oder pH-Wert Veränderungen in der Rhizosphäre. In zukünftigen Studien sollte getestet werden, ob die Produktivität der Pflanzenbrennstoffzelle durch eine Reduzierung von Phosphat in der Nährlösung gesteigert werden kann, ohne jedoch den Pflanzen zu schaden.

1 Introduction

1.1 Plant - Microbial fuel cell (MFC)

The International Energy Agency calculated that the global primary energy demand will increase by 36% between 2008 and 2035 from around 12,3000 million tons of oil equivalent (Mtoe) to over 16,700 Mtoe or 1.2% per year on average (Chow et al., 2003). Approximately 95% of this energy comes from fossil fuels (Chow et al., 2003), which are limited. In addition, the current climate change, threat by carbon dioxide (CO_2) emissions from combustion of fossil fuels, increases the urgency for alternative energy production based on reliable and renewable sources, such as solar energy and biomass. Current systems for the production of bioenergy, such as green energy or bioelectricity, bioethanol, and biodiesel still have some drawbacks, especially competition with food production (Strik et al., 2008). Wind, solar, and geothermal systems are capital-intensive and their viability is geographically limited (Chow et al., 2003) . A concept that exploits a bioenergy source in situ is the plant Microbial fuel cell (plant-MFC). The plant-MFC uses the natural cooperation between electrogenic bacteria in soil and plants to convert solar energy into green electricity. The system is based on two proven processes: Rhizodeposition of organic compounds by living plants and electricity generation from organic compounds in the plant-MFC (de Schamphelaire et al., 2008; Strik et al., 2008). The plants convert solar radiation into chemical energy (carbohydrates) for their metabolism and up to 40% of the net fixed carbon can be released by the roots (Blossfeld et al., 2010), e.g. for nutrient uptake. Thus, the principal idea is that plant rhizodeposits will be utilized as substrates by the bacteria to generate electricity in the plant-MFC. The electrochemically active bacteria in the plant-MFC use a part of the chemical energy of the substrate for their own metabolism and deliver simultaneously electrons to the anode of the electrochemical fuel cell (Strik et al., 2008). They oxidize the organic matter to CO_2 (mineralization) and produce electrons in the anode compartment. Electrons, produced by the bacteria, are transferred to the anode and flow to the cathode linked by a conductive material (Logan et al., 2006). The protons, generated by the oxidation of the organic matter diffuse over a cation permeable exchange membrane into the cathode compartment (Fig.1).

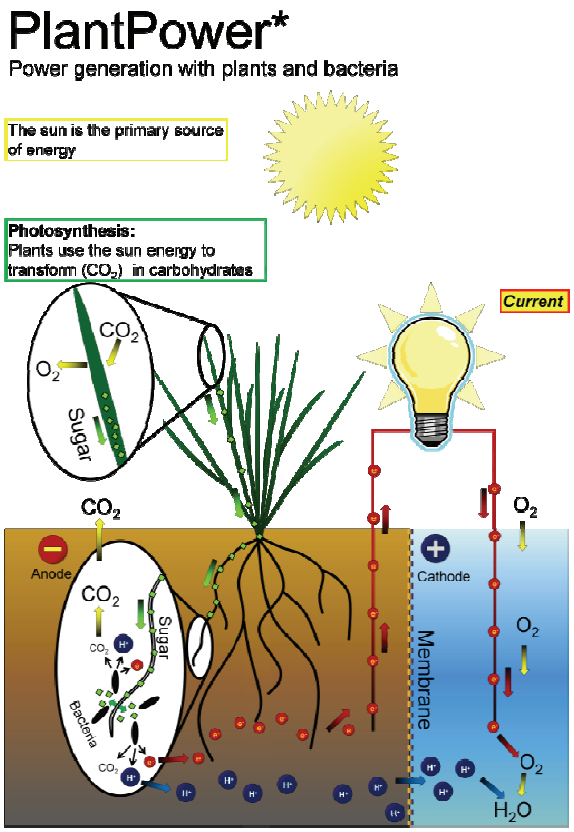

Fig. 1: Model of a plant microbial fuel cell producing electricity and driving a light source. CO_2 is assimilated and released as rhizodeposits (e.g. root exudates) by the plants and is oxidized by micro-organisms. The released electrons flow, due to the potential difference, from the anode through an electrical circuit with a load or a resistor to the cathode. To preserve electrical neutrality, protons diffuse through the membrane into the cathode, where oxygen is reduced with the protons and electrons to form water.

Up to now, the maximum electrical power achieved by one plant-MFC reached 67 mWm^{-2} (Strik et al., 2008), yet the achievable electricity production of a plant-MFC could reach 5800 kWh ha^{-1} $year^{-1}$ by (i) reducing the internal resistance of the plant-MFC by using a biocathode or similar, (ii) increasing the light intensity from approximately 15Wm^{-2} in the proof of principle up to 150 Wm^{-2} and (iii) improved plant vitality management and improved rhizodeposition by optimizing the conditions and plant selection (Strik et al., 2008). For example, De Schamphelaire demonstrated that the presence of plants in a sediment microbial fuel cell (SMFC) increased the power output with a factor of seven and the current output with a factor of 2.7 (2008).

To enhance the productivity of the plant-MFC, a project founded within the 7th framework program by the European Union (FP 7) started in 2009 (Plant Power Grant No. 226532). Several groups are working on optimizing the components, influencing the efficiency of the plant-MFC, and focusing on the construction of the MFC as well as on the biological aspects e.g. (i) screening for plants, (ii) influencing/increasing the exudation to provide better carbon supply for electrogenic bacteria and (iii) screening for electrogenic bacteria. This thesis is integrated into this EU project and focuses on the interference and optimization of root exudation under plant-MFC conditions.

1.2 The Rhizosphere

One of the most fascinating hot spots of activity and biodiversity in soils is the rhizosphere (Jones and Hinsinger, 2008).The rhizosphere is the volume of soil around living roots, which is influenced by root activity (Hartmann et al., 2008) and represents one of the most complex ecosystems on Earth with almost every root on the planet expected to have a chemically, physically and biologically unique rhizosphere. Its radial extension can range from sub-µm to supra-cm scales (Darrah, 1993). In most rhizosphere models the inner boundary is defined as the outer surface of the root (Watt et al., 2006). The rhizosphere is a very complex system because plants have to adapt on a wide range of biotic and abiotic factors and to interact with many different members of the soil microbial community to survive (Jones and Hinsinger, 2008). The adaptations are of physiological as well as chemical nature e.g. root architecture, adaptation of pH or release of organic compounds (Blossfeld and Gansert, 2007; Hinsinger et al., 2009).

1.2.1 Rhizodeposition

For over a century it has been established that plants can dramatically modify their soil environment, giving rise to the so called rhizosphere effect (Clark, 1949). Although the initial trigger of this rhizosphere effect was not identified, subsequent research has shown that it is largely induced by the release of carbon (C) from roots into the surrounding soil. Although roots can release large amounts of inorganic C (i.e. CO_2, HCO_3^-, CO_3^{2-}), which may directly affect the biogeochemistry of the soil (Cheng et al., 1993; Hinsinger et al., 2009), it is the release of organic carbon that produces the most dramatic changes in the biological and chemical nature of the soil.

This release of organic C is often termed rhizodeposition which includes root cap and border cell loss, death and lysis of root cells, flow of C to root-associated symbionts (e.g. mycorrhiza), gaseous loss, leakage of solutes from living cells (root exudates) and polymer secretion from living cells (mucilage) (Fig.2; Jones et al., 2004). Virtually, all compounds (e.g. carbohydrates, organic and amino acids) contained in root tissues can be released into the soil by various mechanisms including diffusion, secretion or cell lysis (Jones et al., 2009).

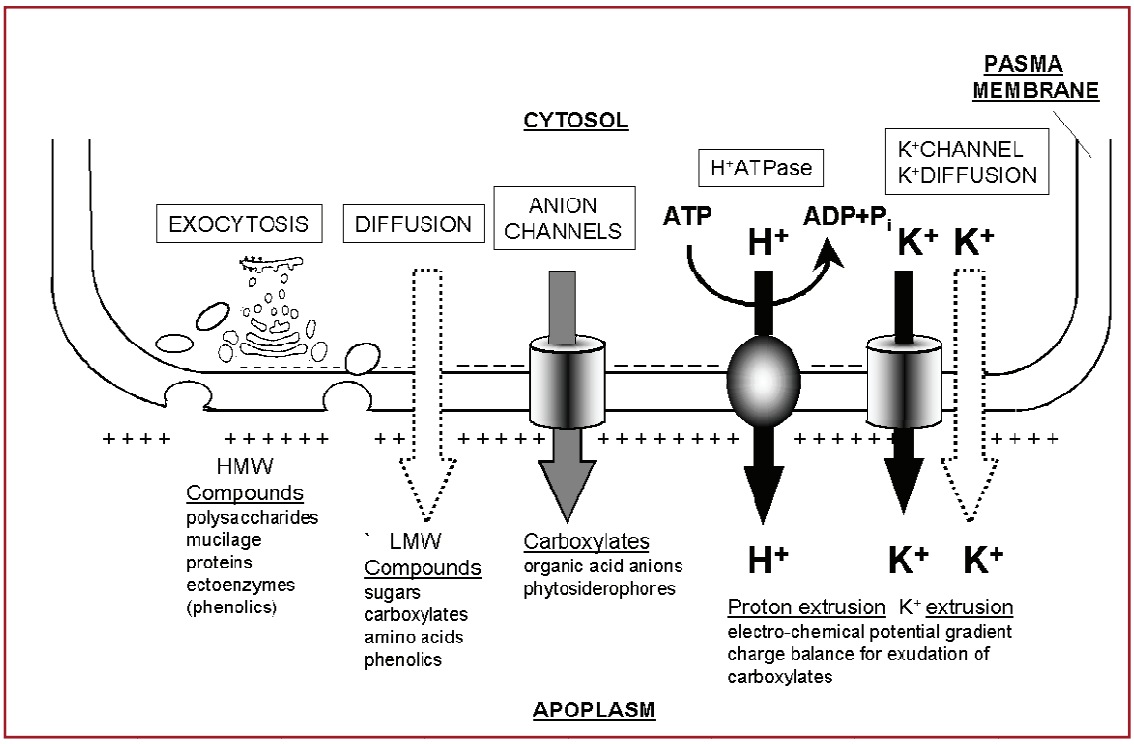

Fig. 2: Rhizodeposition can occur passive by diffusion or active by opening of channels or exocytosis. High molecular weight compounds (HMW) e.g. polysaccharides or proteins are released by exocytosis and low molecular weight compounds (LMW) e.g. organic acids by diffusion or anion channels. The pumping of H^+ and cation channels ensures the maintenance of the electro-chemical gradient.

Exudates are lost passively by the root and the rate of loss is depending upon three critical factors, namely the root-soil concentration gradient, the permeability of the plasma membrane, and the spatial location of the solutes in the root tissue (Jones et al., 2009). Typically exudates flow across the lipid bilayer at a slow rate in response to the electrochemical gradient (baseline exudation); however, efflux can be increased greatly by the opening of channels embedded in the lipid bilayer (Jones, 1998).

The theoretical net efflux of malate from a wheat root tip into the soil solution can be calculated at $3.3 \cdot 10^{-2}$ pmol mg^{-1} root fresh weight s^{-1}. This is in close agreement with experimentally derived malate efflux rates for wheat root tips which range from $1.4 \cdot 10^{-2}$ pmol mg^{-1} root FWs^{-1} under normal conditions rising up to $3.3 \cdot 10^{-1}$ pmol mg^{-1} root FWs^{-1} in the presence of toxic levels of Aluminum (Al) and when transport is probably enhanced through the opening of channel proteins (Ryan et al., 1995). Based on theoretical calculations, it can be hypothesized that malate (and citrate) release from the root will be a predominantly unidirectional passive transport process using the cell's electrochemical potential gradient. (Jones, 1998) In contrast, root influx of organic anions such as malate, citrate and oxalate at soil pHs >5.5 must be an active, thus energy-requiring, transport process (Jones and Darrah, 1995). Probably the best known example to speed up the release of organic acids is when roots experience either phosphorous (P) deficiency or high external concentrations of free toxic Al (Al^{3+}). The release of organic acids such as citrate, malate and oxalate leads to aluminum (Al^{3+}) complexes, rendering it non-toxic (Zhang et al., 2004).

Furthermore, plants can release organic compounds by exocytosis, including high molecular weight compounds, root mucilage and removal of border cells (Jones et al., 2009). Root mucilage forms a gelatinous layer surrounding the root tip, which is actively secreted by exocytosis from root cap cells (Paull and Jones, 1975). The carboxylic groups of mucilage can complex potentially toxic metals, such as Al, cadmium (Cd), zinc (Zn), copper (Cu) and enhance the soil aggregate stability (Mench et al., 1987; Czarnes et al., 2000). Of most concern is that mucilage represents a source of labile C in the soil and is consequently rapidly consumed by soil microorganisms, with a typical half-life of 3 days. (Zheng et al., 2000). Additionally, cells that detach from the external layers of the root cap (border cells), which is continuously renewed (Zhao et al., 2000), constitute a small proportion of the C entering the soil (Farrar et al., 2003).

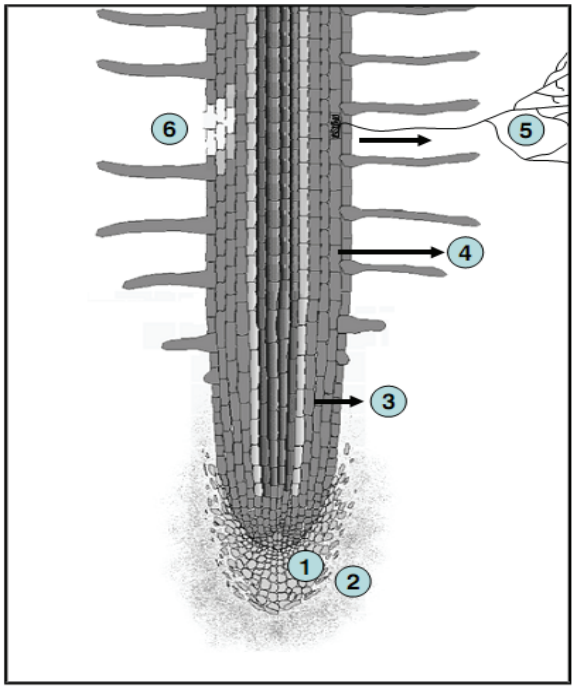

Fig.3: Schematic representation of a longitudinal section of a growing root showing the six major sites of rhizodeposition: 1 loss of root cap and border cells, 2 loss of insoluble mucilage, 3 loss of soluble root exudates, 4 loss of volatile organic C, 5 loss of C to symbionts (e.g. arbuscular mycorrhizas), and 6 loss of C due to death and lysis of root epidermal and cortical cells. (Jones et al., 2009)

1.2.2 Carbon flow

The C flow in the rhizosphere is a bi-directional mechanism. Plants release organic compounds into the rhizosphere as well as they can take them up. ^{14}C labeling studies with *Zea mays* L. showed that 30-50% of the assimilated carbon is allocated to the below-ground parts of the plants, 10–30% is accumulated in the roots, 10–20% is respired in the rhizosphere and 1–5% is accumulated as organic material and microbial cells in the rhizosphere (Merckx et al., 1986). The three main groups of organic substances exuded by roots into the rhizosphere are sugars (50–70% of total exudate), carboxylic acids (20–30% of total exudates) and amino acids (10–20% of total exudates) (Jones, 1998). Three quarters of the exuded carbon are ultimately transformed into carbon dioxide (CO_2) by microbial respiration. Reported fluxes of roots vary a lot, depending on the species and experimental approach. For example Lambers et al. (1996) reported that 50–200 nmol g^{-1} DW s^{-1} in *Cucumis sativus* (Cucumber), *Lycopersicon esculentum* (Tomato) *and Holcus lanatus* (Velvet grass) and Hinsinger et al. (2003) 100–200 nmol $plant^{-1}$ s^{-1} CO_2 in *Zea mays* (maize) were exudated.

Most plants in natural and semi-natural vegetation systems form symbiotic associations with mycorrhizal fungi and there is increasing evidence suggesting that the flow of C to and through this symbiotic interface may be of significance in many plant–soil interactions (Finlay, 2008). Mycorrhizal symbionts contribute to carbon flow in the rhizosphere in three main ways: (i) Biomass production of intra- and extra radical mycelial structures, (ii) release of a range of exudates into the mycorrhizosphere, the soil volume affected by mycorrhiza, and (iii) the mycorrhizal mycelium itself, can be used as an energy rich substrate by other organisms, resulting in respiratory loss of C as CO_2 (Leake et al., 2004; Jones et al., 2009). In contrast, a range of plant species have shown an uptake of low molecular weight solutes, such as organic acids, sugars and amino acids (Jones and Darrah, 1995). C cannot be only released by plant roots but inorganic C could although be taken up by plant roots and converted to organic acids inside the root or in mycorrhizal tissues, if it is present in a dissolved form, such as bicarbonate (HCO_3^-) , and carbonate (CO_3^-) (Amiro and Ewing, 1992; Ford et al., 2007). Besides C can be transported directly as dissolved inorganic carbon via the transpiration stream and fixed aboveground by light or anaplerotic reactions (Ford et al., 2007).

Cluster roots of *Lupinus albus* (White lupin) are one potential exception, where significant uptake and assimilation of HCO_3^- into malate and citrate occurs. These HCO_3^- derived organic acids are then exuded back into the soil to aid in phosphate (PO_4^{3-}) mobilization in the rhizosphere (Johnson, 1996). Neither inorganic C can be active taken up by plant roots but also sugars and organic nitrogen (N) compounds (e.g. amino acids, polyamines etc.) from soil. Typically, these compounds are taken up into the plant by co-transporters, which are powered by the plasma membrane H^+ – ATPases and the organic solutes are transported simultaneously with protons across the plasma membrane (Jones and Darrah, 1996; Jahn et al., 1998).

However, so far the benefit for the plants in taking up sugars and organic nitrogen (N) compounds is unknown. Hypotheses are the recapturing of lost C or prevention of C accumulation in the rhizosphere, thereby reducing the growth of the soil microbial community (Jones et al., 2009).

1.3 Organic acids

Organic acids are low-molecular weight compounds (LMW), containing carbon (C), hydrogen (H) and oxygen (O) which are characterized by the possession of one or more carboxyl groups (Jones, 1998). Further LMW are amino acids, sugars and phenols. In contrast, high molecular weight compounds (HMW) are proteins and polysaccharides (Badri and Vivanco, 2009).

Typically, roots contain different organic acids varying in chain length, with lactate, acetate, oxalate, succinate, fumarate, malate, citrate, isocitrate and aconitate being the primary anion components (Jones, 1998). In maize typically, the total concentration of organic acids inside the roots is around 10–20 mM, which corresponds to 1–4% of total dry weight. The concentrations of amino acids (approx. 10–20 mM) and sugars (approx. 90 mM) are in a similar range (Jones and Darrah, 1994) and 1000-fold greater than that present in the soil solution (approx. 0.5–10 µM) (Jones, 1998). However the composition of root exudates is highly variable and dependent on plant species, plant age and physiochemical environment (Trolldenier, 1987).

Depending on the dissociation properties and number of the carboxylic groups, organic acids can carry varying negative charge, thereby allowing the complexation of metal cations in solution and the displacement of anions from the soil matrix. For this reason, they are actors in many soil processes including the mobilization and uptake of nutrients by plants and microorganisms, like P and iron (Fe), the detoxification of metals by plants e.g., Al, microbial proliferation in the rhizosphere, and the dissolution of soil minerals leading to pedogenesis (Jones, 1998). Due to the fact that organic acid release can be influenced by reduction of the P content or Al addition they are a good tool for the plant-MFC to increase the exudation rate. A higher exudation of organic acids can lead to an increased growth of the electrogenic bacteria and a higher efficiency of the plant-MFC.

Due to the negative charge associated with their carboxyl groups, organic acids can become rapidly and readily sorbed to the soil's solid phase. The general sorption trend is P > oxalate > citrate > malate >sulfate > acetate (Jones and Darrah, 1994) but the organic acid sorption is highly pH dependent with increasing sorption with decreasing solution pH (Jones and Brassington, 1998).

In the rhizosphere, a concentration gradient of di- and tricarboxylic acids exists with an effective sphere of influence in the rhizoplane between 0.2 and 1.0 mm, depending on soil type, organic acid type and time (Darrah, 1991). However the distance for non-sorbing compounds such as glucose and monocarboxylic acids such as acetate can be much greater (>5 mm) (Darrah, 1991). Most of the organic acids (>60%) will be rapidly adsorbed to the soil's exchange phase. Depending on Al and P stress level, the resultant concentration in the soil solution is expected to be in the range of 1–50 µM (Jones et al., 1996).

1.3.1 Impact of the soil microbial activity on organic release

In the rhizosphere, a vast number of microorganisms exist with an enormous activity and C utilization capacity. Hence, the decomposition is much faster in organic surface horizons than in low organic content subsoils and the decomposition rates in rhizosphere soils are 2–3 fold faster than in bulk soils (Jones, 1998). For example, the mineralization of citrate and malate, added at realistic rhizosphere concentrations (10–100 µM), is rapidly degraded in non-rhizosphere (bulk) soil with an average half life of 2–3 h depending on soil type. Approximately 60% of the organic acids are being mineralized to CO_2 and 40% incorporated into new cell biomass (Jones and Darrah, 1994). In bacterial cultures, it has been demonstrated, that soil bacteria are capable to regulate the amount and type of transporters, required for the uptake of organic acids into the cell, based on their available C supply (Jones et al., 1996c). Jones showed an up-regulation of transport activity in response to additions of malate.

The release of large amounts of organic acids into the rhizosphere can be expected not only to induce growth of pre-existing rhizosphere bacteria, but also to act as attractants inducing the movement (chemotaxis) towards the roots of motile microbes such as flagellate bacteria and fungal hyphae. Experiments with pure cultures of rhizosphere bacteria have shown concentrations of 10 µM are sufficient to induce a chemotactic response, i.e. similar to concentrations found in rhizosphere soil solutions (Shen et al., 1996). While microbes can consume root exudates, they are also responsible for the production of a wide range of organic acids especially in situations where nutrients may be limiting (Jones, 1998). The release of large amounts of oxalate and the presence of Calcium (Ca)-oxalate crystals on the surface of fungal hyphae, both in wood and soil, is well documented (Dutton and Evans, 1996).

It has been speculated that oxalate is involved in a number of processes, including the acquisition of nutrients, such as P, Fe, free-radical formation, extracellular pH modification, Ca precipitation and subsequent pectin hydrolysis, and acid catalysis of hemicelluloses and cellulose (Green and Highley, 1997; Micales, 1997).

Certainly, soil bacteria can not only consume organic acids but other kinds of rhizodeposits, too. For example, they can consume carbohydrates exuded by the plant such as amino acids, sugars, phenols and fatty acids as well as death plant cell material e.g. border cells (Jones et al., 2009). At concentrations likely to exist in the rhizosphere (0.1–5 mM), sugars are primarily used by soil microorganisms for anabolic processes, while organic acids will be largely used for catabolic processes and amino acids are used for both (Jones et al., 2003).

1.3.2 Organic acid release by plant roots under phosphorus deficiency

Due to its insolubility and high sorption capacity in soil, P supply can be one of the major constraints to plant growth. Some plants can directly modify the rhizosphere in order to gain access to previously unavailable soil P reserves. This can include the manipulation of root hair length and density, the extra provision of C for mycorrhizal exploitation of non-rhizosphere soil, the release of phosphatases to release organically bound soil P (PO_4^{3-}), and the release of organic acids and H^+ to solubilise inorganic P (Jones, 1998). Organic acids appear to be able to induce a 2–4 fold increase of the mineral dissolution rate in comparison with rainwater alone; however, this is highly dependent on mineral type, pH, Al content, the mineral and organic acid type (Jones and Kochian, 1996). It has been demonstrated that some dicotyledonous plant roots, and especially non mycorrhizal plants such as *Lupinus albus* and *Brassica napus* (Rapeseed), are capable of releasing large amounts of organic acids into the rhizosphere in response to P deficiency, while other dicotyledonous (e.g., *Sisymbrium officinale* (Hedge mustard)) and graminaceous (wheat, maize) plants do not appear to express this trait. Besides, this mycorrhiza can enhance the uptake of a wide range of nutrients, amongst others P (Neumann and George, 2005). The primary components released by roots under P deficiency appear to be malate and citrate (Jones, 1998). This organic acid exudation under P deficiency constitutes a drain of 5–25% of the plant's photosynthetically fixed C (Krzyszowska et al., 1996) and the organic acids are leading to 10–1000-fold higher soil solution P concentrations (Fox et al., 1990).

The carboxylic acids are able to mobilize P mainly by ligand exchange, dissolution and occupation of P sorption sites (Fox et al., 1990). Citrate for example directly replaces P on ligand exchange surfaces, such as crystalline Aluminum hydroxide ($Al(OH)_3$) or Iron hydroxide ($Fe(OH)_3$). The extraction efficiency of inorganic P by the organic acids appears to follow the series citrate > oxalate > malate> acetate with P release dependent on the ability of the anion to complex Al (Jones and Darrah, 1994; Lan et al., 1995). However, rhizosphere concentrations of carboxylic acids at a level high enough to mediate desorption of significant amounts of soil PO_4^{3-} have been reported only for a limited number of plant species, mainly cluster-rooted plants such as *Lupinus albus* and members of the Proteaceae (Neumann et al., 1999). The cluster root is defined as a densely packed grouping of determinate rootlets (Skene, 2003). Therefore the release of large amounts of citric acid from specialized root clusters (proteoid roots) of P deficient *Lupinus albus* is an efficient strategy for chemical mobilization of sparingly available P sources in the rhizosphere (Neumann et al., 1999).

Depending on the dominant soil P fraction, P solubility can be increased by root induced acidification or alkalinization of the rhizosphere (Neumann and Römheld, 1999). Neumann and Römheld (1999) showed that P release is accelerated by a decrease in soil solution pH from 7.0-7.5 to 4.0-4.9 for *Lycopersicon esculentum*, cv. Moneymaker, *Cicer arietinum* and *Lupinus albus* L. cv. Amiga (Neumann and Römheld, 1999). In addition, organic acids also appear to be able to mobilize P bound in humic-metal complexes, depending on the metal-organic anion stability constant (Lan et al., 1995) or by promoting the growth of microorganisms and the subsequent mineralization of organic P (Khademi et al., 2010).

1.3.3 Release of organic acids by plant roots as a response to Al toxicity

In soils with a neutral pH Al exists as insoluble aluminosilicate or oxide. Through soil acidification, phytotoxic forms of Al can be released into the soil solution to levels that affect root and plant growth. Al rhizotoxicity can inhibit root cell elongation and to a lesser extent cell division. However, some plants appear to be able to resist concentrations of Al which are typically toxic to most plants (>5 µM). In acidic solutions (pH < 5.0), Al exists as an octahedral hexahydrate ($Al(H_2O)_6^{3+}$), which by convention is usually called Al^{3+}. Monomeric Al forms complexes with a number of ligands, and Al^{3+} interacts most strongly with oxygen donor ligands, such as carboxylate-, P-, and sulfate groups. The detoxification with organic acids happens either outside (apoplast) or inside (vacuole) the root through a complexation reaction (Kochian, 1995; Kochian et al., 2004).

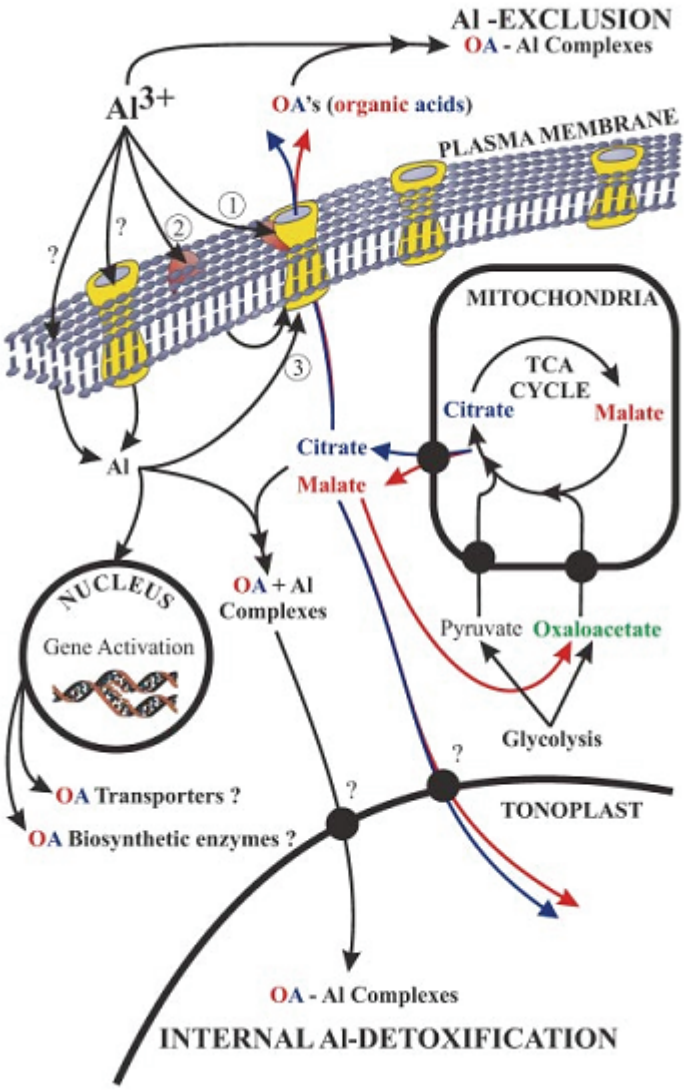

Fig. 4: Roots release either malate (Triticum aestivum) or citrate (Phaseolus vulgaris, and Zea mays) into the apoplast and external solution upon exposure to high levels of Al, with the amount released being directly proportional to the external Al concentration (0–200µM). Once these organic acids have left the root, they rapidly form Al-organic acid complexes in the apoplast and soil solution, rendering Al non-toxic and making the roots 5–20 times more resistant to Al (Delhaize et al., 1993; Ryan et al., 1995).

The release of root exudates in response to Al occurs only at the root apex (0–3 mm) which correlates well with this being the primary site of Al toxicity (Kochian, 1995; Ryan et al., 1995).

Cytosolic pH of roots ranges from 7.1 to 7.4 (Marschner, 1995). Thus, primary organic acids in the cytosol will occur in their fully dissociated forms. During passage of the organic acids across the membrane into the apoplast, a counter ion (H^+) is also required to maintain electrical neutrality. However under Al^{3+} rhizotoxic conditions, no stimulation of H^+ excretion and no change in rhizosphere pH is observed when malate is being released, indicating that H^+ is not the counter ion (Kochian, 1995). This is beneficial, as a lowering of the external pH would increase Al^{3+} solubility in the rhizosphere, thereby increasing rhizotoxicity.

1.4 Anoxic environment

In the plant-MFC plant roots are growing under anoxic waterlogged conditions to inhibit the transfer of the electrons to oxygen already in the anode compartment. In addition, electrogenic bacteria are sensitive to oxygen, e.g. *Geobacter sulfurreducens* (Logan, 2009). For plants changes in anatomy, morphology and metabolism are of vital importance for surviving in anoxic root environments (Visser et al., 1996) as oxygen diffuses 10^3-10^4 times slower in water than in air (Armstrong, 1979) and most of the available oxygen is utilized by microorganisms within the top few millimeters of soil (Teal and Kanwishe, 1966). The most common and important adaptation to survive long-term flooding is the development of a gas-space continuum, called aerenchyma, primarily in the cortical tissues, which may stretch from the stomata to the root tip (Jackson and Armstrong, 1999). This aerenchymatic tissue is present in a vast array of plant species among others in *Glyceria* (Mannagrass) species (Smirnoff and Crawford, 1983). The capacity to form porous tissues in terrestrial species is determined by the flooding conditions e.g. for *Rumex hydrolapathum* (Great water dock), occurring on almost permanently waterlogged soils, the porosity is nearly 50%, which is similar to the porosities of many water plants (Visser et al., 1996). High root porosity allows oxygen, originating from the atmosphere or from photosynthesis, to diffuse from the shoot to the root allowing aerobic root metabolism to proceed.

To a certain extent the root system of non wetland species has the capacity to adapt to waterlogging conditions by increasing the root porosity under flooding (Yu et al., 1969). During waterlogging, many old roots die and adventitious roots with well developed aerenchmya emerge (Laan et al., 1991). The aerenchyma enables oxygen transport to the roots from the shoot to the root allowing aerobic root metabolism to proceed. However, the root will also lose oxygen to the surrounding soil or sediment by means of radial oxygen loss. The rhizosphere becomes oxidized by release of oxygen; thereby mineral nutrients are oxidized and become available for plant use (Bloom, 1999).

The amount of oxygen released is not only species- and root-specific (Grosse et al., 1992), but also dependent on soil physical factors, such as compaction and redox potential (Engelaar et al., 1993; Kludze et al., 1993). Radial oxygen loss does not occur along the whole root system, but is often restricted to the young parts and apices of adventitious and secondary roots, as demonstrated for *Oryza sativa* (Rice) by Armstrong et al. (1971). The formation of aerenchyma is induced by ethylene accumulation, stimulated by auxin, and the aerenchmatic tissues are formed by cell wall separation, cell wall collapse or cell lysis (Marschner, 1995; Bloom, 1999).

Plants have developed a second strategy to tolerate hypoxic or anoxic conditions: Rapid changes in carbon metabolism and organic acid transport. For example Marschner et al. (1995) reported, that the rates of lactic acid efflux from maize root tips under anaerobic conditions has been estimated between 0.55–1.58 pmol mg^{-1} root FWs^{-1} greater than most reported rates of citrate and malate release.

1.5 Aim of this study

The motivation of this thesis was to contribute new knowledge on root exudation under waterlogged conditions and the way, how it could be influenced, in order to enhance the efficiency of the plant microbial fuel cell. The reduction of the P supply and Al toxicity are two known aspects which can enhance root exudation of plants. However, most studies are done with only a few model plants like *Lupinus albus*, *Oryza sativa* and *Solanum lycopersicum* and almost nothing is known about their influence on other plant species or plants grown under waterlogged conditions. Hence, this study has got two main aspects: Screening for plant species which perform well under MFC conditions and increasing the exudation of plants with the aim to enhance the productivity of the plant-MFC.

2 Materials and methods

2.1 Analytics

2.1.1 Total organic carbon (TOC)

The bacteria decompose all organic compounds released by the plant roots as well as dead cell material. Thus, not only organic acids and sugars, released by the plant, are interesting but all organic compounds which are measurable in the nutrient solution. For this reason the amount of the total organic carbon was measured.

The used instrument was a DC-190 High-temperature TOC Analyzer, Rosemount Analytical. For the total carbon (TC) measurement the sample has to be completely oxidized to CO_2 and water (H_2O) in a combustion tube at 680 °C. The gas flow sweeps the CO_2 containing steam through a condenser and the IC reactor to the non-dispersive infrared detector (NDIR) for peak quantification.

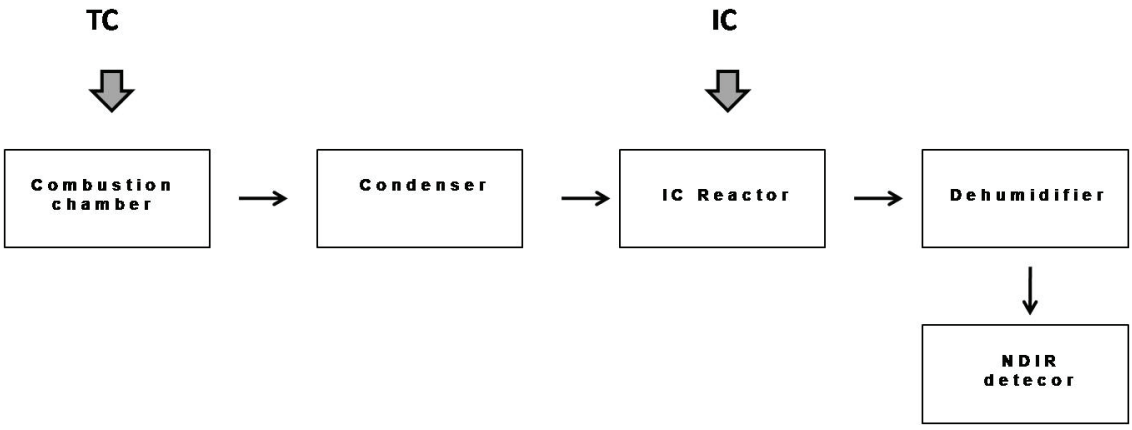

Fig. 5: Schematic figure of the TOC analysis.

For inorganic carbon (IC) measurement the sample is injected into the IC detector, where it is acidified with phosphoric acid. In an acidic solution all forms of inorganic carbon (IC) are purged out of the solution as CO_2. After drying in a dehumidifier the gas passed through a CO_2 specific non-dispersive infrared detector (NDIR) for peak quantification.

As standard for calibration Anhydrous potassium hydrogen phthalate was used.

2.1.2 Capillary electrophoresis (CE)

The analyses were done with a G1600A capillary electrophoresis (Agilent, Böblingen, Germany) containing a diode array detector. Silica capillaries (Polymicro, Phoenix, USA) with a diameter of 75 µm, a total length of 64.5 cm and an effective length of 56 cm were used. Phenylacetic acid (phenyl acetate) served as internal standard in the samples and a standard solution of the organic acids (Oxalic acid, Formic acid, Succinic acid, Malic acid, Acetic acid, Glyoxylic acid, Lactic acid and Citric acid) in concentrations of 5, 10, 20 $\mu molL^{-1}$ as external standard. The samples were measured two times after flushing the capillary with 0.1 M Sodium hydroxide (NaOH) for 5 min, water for 1 min and electrolyte for 5 min. The separation occurred at 30 kV and 25 °C.

The electrolyte solution was prepared fresh and contained 7.5 mM salicylic acid, 15 mM TRIS, 0.5 mM dodecyltrimethylammonium hydroxide and 0.3 mM calcium hydroxide.

The samples were diluted 10 times and phenyl acetate was added as internal standard. Composition of a sample for the capillary electrophoresis:

- 10 µL Sample volume
- 10 µL phenyl acetate [100µM]
- 80 µL DI water

2.1.3 Absorption measurement

The amount of sugars in the nutrient solution was calculated by photometric measurement of the optical density (OD) at 340 nm with a photometer 12550 (Anthos labtech Intruments)

A volume of 20 µL of the sampled solution was mixed with 161 µL of a mix containing 150 µL Imidazol buffer (100 mM Imidazol, 5 mM Magnesium chloride ($MgCl_2$), pH 6,9), 4,5 µL Nicotinamide adenine dinucleotide phosphate (NADP) (36 mg/mL), 4,5 µL Adenosine-triphosphate (ATP) (60 mg/mL) and 2,0 µL Glucose-6-phosphate dehydrogenase (G6PDH) and filled in 96 well plates. As soon as the OD reached its maximum 2 µL Hexokinase were added for the glucose degradation, than as soon as the OD reached its maximum again the next enzyme, Phosphoglucose Isomerase (PGI), was added for fructose degradation. After the maximum was reached again Invertase was added for Sucrose degradation.

2.1.4 Magnetic resonance imaging (MRI)

The NMR Imaging was done with a 4.7T Varian VNMRS vertical wired-bore MRI system with a quadrature transmit/receive coil and a 300mTm^{-1} gradient system. Images were obtained by a multi-slice (multi) spin echo technique with sagittal or axial orientation. The plants were larger than the field of view, thus multiple stages were recorded by shifting the plant consecutively through the MRI bore.

2.2 Plant cultivation

2.2.1 Cultivated plant species

I. Glyceria maxima

Glyceria maxima is a monocotyledonous wetland species of Poaceae. *Glyceria maxima* adapts to flooding through phenological adaptations with a seasonal metabolic tolerance of anoxia confined to winter and spring which, combined with a facility for root aeration and early spring growth, allows rapid colonization of sites with only shallow flooding (Bloom, 1999) . Furthermore, *Glyceria maxima* develops an aerenchyma under waterlogging (Studer and Brändle, 1984).

II. *Phalaris arundinacea*

Phalaris arundinacea (Reed canary grass) is one of fifteen species of the genus *Phalaris* that is distributed throughout the world, except in Antarctica and Greenland (Anderson 1961). The center of diversity for the genus is the Mediterranean region. *Phalaris arundinacea* survives prolonged flooding by possessing anoxia tolerant rhizomes (Brandle, 1983). *Phalaris arundinacea* was one of the most tolerant species tested; it tolerated the highest levels of alcohol in rhizomes. Barclay and Crawford (1983) found carbohydrate levels in *Phalaris arundinacea* rhizomes to be very stable and suggested this related to the survivability of plants during prolonged anoxic periods.

III. Spartina anglica

Spartina anglica is a cool-temperate C_4-grass species and is thought to have arisen on the south coast of England as a hybrid of *Spartina maritima* (Smith et al., 1982). As adaption on waterlogging it can develop aerenchymatic tissues (Teal and Kanwishe, 1966). Spartina grasses flourish under saline and anoxic estuarine conditions that are often uninhabitable by other plants. Due to their vigorous growth in stressful conditions, *Spartina anglica* has been introduced throughout Europe, North America, Australia, and Asia (Maricle and Lee, 2002).

IV. *Arundinella anomala*

Arundinella anomala, a grass species, grows along the riverside of Yangtze River and its branches in The Three Gorges reservoir region. Annual summer river water level rise leads to partial or complete submergence of the plants. However, the species persist, indicating high flooding tolerance (Ye, 2010).

V. *Hemarthria altissima*

Hemarthria altissima is a perennial C4 grass with long spreading stolons and short rhizomes. Plants of *Hemarthria altissima* prefer moist soils and occur along river banks, but are also capable of growing in dry land (Luo et al., 2009). *Hemarthria altissima* is able to maintain the functionality of the photosynthetic apparatus through rapid acclimation to changing O_2 and light conditions. The ability for photosynthetic acclimation may be essential for adaptation to wetland habitats in which water levels fluctuate (Luo et al., 2009).

VI. *Oryza sativa*

Oryza sativa belongs to the tribe Oryzeae of the family Poaceae. It grows worldwide, including in Asian, North and South America, European Union, Middle Eastern and African countries and is an important crop plant. *Oryza sativa* forms a fibrous root system and tillers (2005). Furthermore, *Oryza sativa* develops an aerenchyma to survive under waterlogged conditions (Irfan et al.). During the domestication of rice, natural variation resulted in a large number of distinct cultivars (2005). Two cultivars were further studied in this work *Oryza sativa Milyang 23* and *Oryza sativa IR 5440-1-1-3,* which are characterized by P deficiency tolerance (*Oryza sativa Milyang 23)* and P deficiency intolerance (*Oryza sativa IR 5440-1-1-3*).

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2.2.2 Climate chambers and green house

Green house:

Temperature 24°C day, 18°C night

Relative humidity: constant 50%

Chamber 1:

06:00 – 22:00 21 °C, 60% humidity light on (16 h)

22:00-6:00 21 °C, 60% humidity, light off (8 h)

Light intensity:

The light intensity was measured 40 cm below the lamps ≙ the averaged shoot tip high.

UVA: 0,537 W/m^2

UVB: 0,014 W/m^2

PAR: 161 µE/m^2s

Chamber 2:

07:50 – 07:55; 24 °C; 75% humidity; light on

07:55 – 08:00; 24-28 °C; 75-70% humidity; light on

08:00 – 20:00; 28 °C; 70% humidity; light on

20:00 – 20:05; 28-24 °C; 70-75% humidity; light on

20:05 – 20:10; 24 °C; 75% humidity; light on

20:10 – 07:50; 24 °C; 75% humidity; light off

Light intensity:

The light intensity was measured 40 cm below the lamps ≙ the averaged shoot tip high.

UVA: 0,619 W/m^2

UVB: 0,013 W/m^2

PAR: 195 µE/m^2s

Chamber 3:

Light intensity: 150 µmol, 16 h day

Temperature: day 20°C, night 18°C

Relative humidity: 80%

2.2.3 Composition of the Hoagland solution

100% Hoagland solution:

- Potassium nitrate (KNO_3): 5 mM
- Calcium nitrate $Ca(NO_3)_2$: 5 mM
- Magnesium sulfate ($MgSO_4$): 2 mM
- Potassium phosphate: 1 mM
- Iron-EDTA: 0.0896 mM

Micro nutrients:

- Manganese chloride ($MnCl_2$ X 4 H_2O): 0.01 mM
- Copper sulfate $CuSO_4$ X 5 H_2O: 0.001 mM
- Zinc sulfate ($ZnSO_4$ X 7 H_2O): 0.001 mM
- Boric acid (H_3BO_3): 0.05 mM
- Sodium molybdate (Na_2MoO_4 X 2 H_2O): 0.0005 mM

50% Hoagland solution with 10% potassium phosphate (KH_2PO_4):

Composition as in a 50% Hoagland but the content of phosphate *(*KH_2PO_4*)* was reduced to 0.1 mM and 0.4 mM Potassium chloride (KCl) was added to maintain the K^+ concentration.

50% Hoagland solution with 1 mM aluminum chloride (AlC_3):

Composition as in a 50% Hoagland but 1 mM $AlCl_3$ was added.

2.3 Preparation of plates and tubes

LB medium plates

9 gL^{-1} LB agar was solved in 1 L deionized (DI) water followed by sterilization in an autoclave at 120 °C for 20 min. In the flow cabinet the plates were filled with approximately 7 mL medium and air-dried until the agar was solid. Unused plates were stored in the refrigerator at 8°C for further use.

Growing tubes

The growing tubes were filled with 7 mL Murashige and Skoog (MS) Medium, closed with a cap and sterilized in an autoclave at 120°C for 20 min. The MS medium contained 7 gL^{-1} Daishin agar and 2.2 gL^{-1} MS nutrient solution. When the medium was chilled, it was covered with a thin layer of sterile LB medium (1 mL) in the flow cabinet. Afterwards the gap between cap and tube had to be closed with parafilm and the tubes were stored at 8 °C until use.

Eppendorf tubes

The Eppendorf tubes were put into test tubes and filled with 750 µL MS medium. The test tube was closed with a cap and autoclaved at 120 °C for 20 min. All further steps were carried out in a flow cabinet to ensure a sterile environment. The bottom of the test tube was filled with 1 mL sterile DI water and the MS medium covered with a layer of LB medium (150 µL). Finally the gap between test tube and cap was closed by sealing with parafilm. The tubes were stored in a refrigerator at 8 °C until use.

2.3.1 Sterilization of the seeds

Oryza sativa seeds

First the testa was removed because otherwise it would disrupt the sterilization. The seeds were incubated in 70% Ethanol in an Eppendorf tube with a volume 10 times bigger than the seed volume for 5 min. Subsequently the Ethanol was removed and the seeds were washed with sterile deionized (DI) water for 10 times, followed by incubation in a bleaching solution with Polyoxyethylen(20)-sorbitan-monolaurat for 15 min. Subsequently the seeds were rinsed with DI water again until all bleaching solution was washed off. At last the seeds were put onto the LB medium plates and stored for germination in a climate chamber (0; chamber 3)

Glyceria maxima and Phalaris arundinacea seeds

To figure out which treatment yields the best germination and the lowest infection rate six different treatments were compared.

The first treatment was performed like described for *Oryza sativa* (see above). In the second treatment the seeds were only incubated in Ethanol for 2 min. The following steps remained like described for *Oryza sativa* (see above). In the third treatment, the protocol was as described for *Oryza sativa* but the seeds were incubated for 5 min. into a bleaching solution. Treatment four was performed like the first treatment but after the bleaching the seeds were incubated into an antibiotic solution which contained Ampicillin (600 mg/L) and Streptomycin (250 mg/L).The fifth treatment correlated with the second treatment and the sixth treatment correlated with the third treatment including an incubation in an antibiotic solution (see above). All seeds were cultivated in a climate chamber (0; chamber 3).

2.3.2 Cultivation of non sterile plants

The seeds were sowed out in germination soil and transferred into sand one week after germination or directly sowed out in sand. The plants were subsequently cultivated in a green house (2.2.2)

2.3.3 Cultivation of sterile plants

After sterilization (2.3.1) the seeds were sowed out on LB medium plates (2.3), sealed with parafilm and subsequently cultivated in a climate chamber (2.2.2, chamber 3) until germination. After germination they were transferred into test tubes (2.3) and further cultivated in the climate chamber (2.2.2; *Phalaris arundinacea* and *Glyceria maxima* in chamber 3; *Oryza sativa* until an age of 3 weeks in chamber 3; further in chamber 2).

Repotting of the germinated seeds in test tubes

Rice and grass seeds were transferred into growing tubes (see above) under sterile conditions. Then the non transferred seeds on the plates and the transferred seeds in the test tubes were kept in a climate chamber (2.2.2; chamber 3).

Repotting of the germinated seeds in Eppendorf tubes

Instead of transferring the germinated seeds into test tubes, they were directly transferred into Eppendorf tubes (see above). The transfer was carried out in a flow cabinet. Finally the tubes with the germinated seeds inside were stored in a climate chamber (2.2.2; chamber 3).

Transfer of the seedling into blue caps

The plants, grown in test tubes, were transferred into empty Eppendorf tubes, pushed through the cap of a blue cap, filled with sterile 50% Hoagland solution. The tip of the Eppendorf tubes was removed so the plant roots could reach the nutrient solution in the blue cap. Finally the tubes were stored in a climate chamber (2.2.2; *Phalaris arundinacea* and *Glyceria maxima* in chamber 3; *Oryza sativa* in chamber 2).

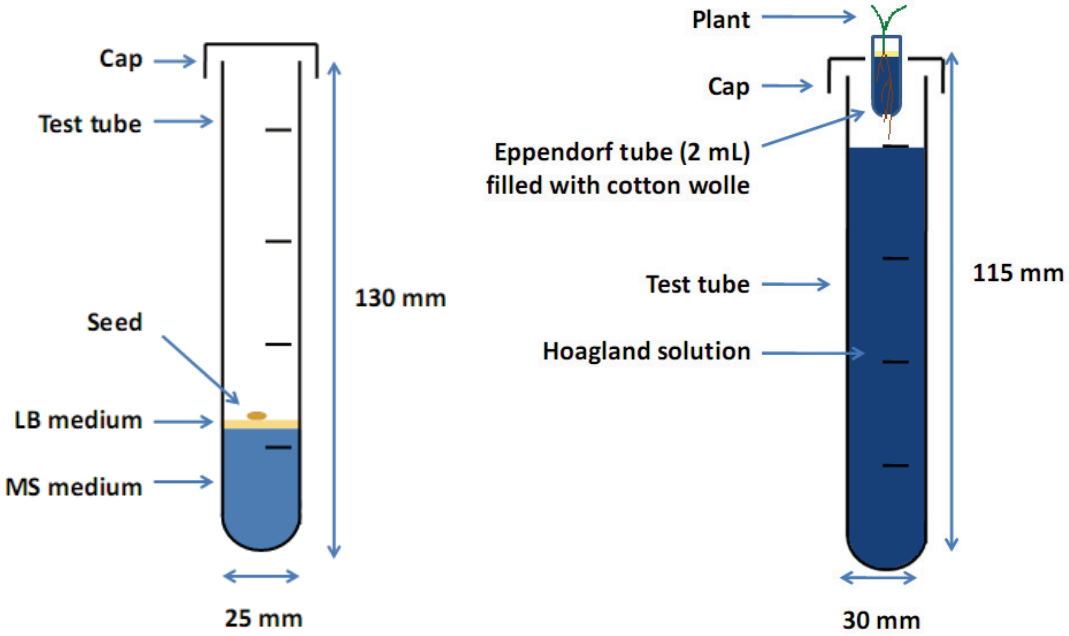


Fig. 6: Schematic figures of a growing tube (left) and the blue cap-system (right) for sterile plant cultivation.

2.4 Experimental designs

2.4.1 Absorption test of substrates

To calculate the required volume to fill the cachepots, the cachepots, with pots containing sand or graphite, were filled completely with a known volume of water and the amount of water in the cachepot was measured. The difference equaled the amount which was bound by the substrate.

Preparation of the substrate pots

All pots were cleaned with DI water and ethanol (70%) and filled with sterile sand or graphite. The sand was sterilized at 600 °C for 6 h and the graphite at 400 °C for 4 h.

Solution of organic acids and sugars

A stock solution of organic acids (Oxalic acid, formic acid, succinic acid, malic acid, acetic acid, lactic acid and citric acid) and sugars (Glucose, fructose and sucrose) was prepared with a individual concentration of 10 mM. From this stock solution a sample was taken immediately after preparation and frozen at -20 °C. The stock solution was diluted with 25% Hoagland solution (2.2.3) to concentrations of 0.1 mM and 0.6 mM to a total volume of 500 mL for each pot.

Filling of the pots

Eight pots placed in cachepots were filled up with sand or graphite, respectively, and 0.1 or 0.6 mM solution per treatment. Also eight pots filled with sand or graphite and 25% Hoagland served as control to show that there was no contamination with sugars or organic acids in the Hoagland solution.
To demonstrate that no organic acids or sugars were adsorbed at the pot surface, eight pots and four beakers per treatment were filled with 25% Hoagland, 0.1 mM solution or 0.6 mM solution of the organic acids and sugars without adding sand or graphite.
After 3, 6 and 24 h samples were taken from every pot or beaker and frozen at -20 °C. The experiment was done in a climate chamber (2.2.2; chamber 1). The resulting samples were subsequently tested for organic acids using capillary electrophoresis (CE) (2.1.2) and sugars using photometry (2.1.3).

The resulting samples had to be diluted otherwise to match requirements for the CE. The samples with a concentration of 500 µM were diluted 200 times with water and the 50 µM solutions were diluted 20 times. Furthermore phenyl acetate was added as internal standard. Samples for the CE analysis were composed as follows:

Samples with 500 µM added solution

- 0.5 µL Sample volume
- 10 µL phenyl acetate [100 µM]
- 89.5 µL DI water

Samples with 50 µM added solution

- 5 µL Sample volume
- 10 µL phenyl acetate [100 µM]
- 85 µL DI water

2.4.2 Treatment of the plants

P deficiency and Al addition

0.2 mM KH_2PO_4 and 1 mM $AlCl_3$ non sterile

Ninety days old *Glyceria maxima* and *Phalaris arundinacea* plants were repotted into sand and the *Phalaris* plants were split to enhance the number of replicates. Three d later, both species were water logged with 50% Hoagland (2.2.3) solution in a climate chamber (2.2.2; chamber 1). Per plant species 12 plants were treated. The sand was rinsed with DI water before water logging. After 5 days samples were taken with a sterile syringe (volume 10 mL) of each plant trough a sterile filter and stored at -20 °C (0 d). Afterwards the solutions were replaced against fresh 50% Hoagland, 50% Hoagland with 10% phosphate content or 50% Hoagland with a concentration of 2 mM Al (2.2.3). In each case four plants per species were treated with one of these solutions. Over a time period of two weeks samples were taken five times. The sampling occurred always between 10-10:30 a.m. The samples were analyzed with CE (2.1.2) and TOC (2.1.1).

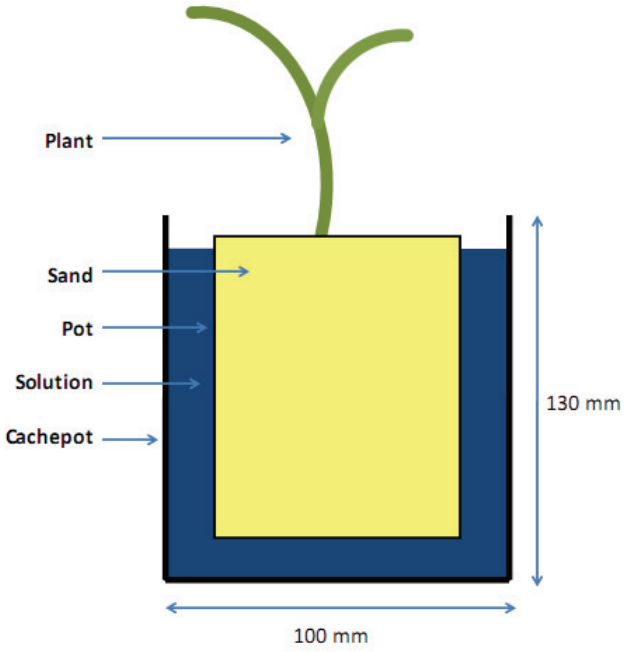


Fig. 7: The plant is growing in a pot filled with sand. This pot is standing in a second pot filled with nutrient solution to ensure the waterlogged environment.

0.1 mM KH_2PO_4 and 1 mM $AlCl_3$ non sterile

This experiment was done with four different species, *Hemartria altissima, Arundinella anomala* and *Spartina anglica*. All these plant species are adapted on waterlogged conditions but differ in strategy so survive under waterlogging, their habitat or the duration of waterlogging in the natural habitat. All plants were reproduced by splitting (*Hemarthria compressa* two individuals; *Spartina anglica* 10 individuals; *Arundinella anomala* 5 individuals).

Thirty plants were cultivated in sand in a green house (2.2.2) fed with 50% Hoagland solution (2.2.3). For the treatment they were transferred into the climate chamber and water logged in 50% Hoagland (2.2.3). One week later the sand was rinsed with DI water, each replicate transferred in a single cachepot and filled up with fresh 500 mL 50% Hoagland. Twenty four hours later samples were taken with a sterile syringe true a sterile filter between 10-10:30 a.m. In the following the solution was discharged, the sand rinsed with DI water and the pots were filled with 500 mL fresh 50% Hoagland, 50% Hoagland containing 0.1 mM phosphate or 50% Hoagland containing 1 mM Al (2.2.3) (0 d). Samples were taken with a sterile syringe (volume 10 mL) of each plant trough a sterile filter and stored at -20 °C between 10-10:30 a.m. 24 h (1 d) and 72 h (3 d) after starting of the treatment. The samples were analyzed with CE (2.1.2) and TOC (2.1.1).

0.1 mM KH_2PO_4 Oryza sativa non sterile

Comparison of two different cultivars:

Oryza sativa Milyang 23, *Oryza sativa IR 5440-1-1-3*

Oryza sativa Milyang 23 is a phosphate deficiency tolerant and *Oryza sativa IR 5440-1-1-3* a phosphate deficiency intolerant cultivar. In this experiment their ability and method to adapt on reduced phosphate supply should be studied.

Twenty plants per cultivar were cultivated in sand in a green house (2.2.2) fed with 50% Hoagland solution (2.2.3). Three month after germination they were transferred into the climate chamber (2.2.2; chamber 2) and water logged in 50% Hoagland (2.2.3). Thirteen days later the sand was rinsed with DI water, each replicate transferred in a single cachepot and filled up with fresh 500 mL 50% Hoagland. Samples were taken with a sterile syringe trough a sterile filter between 10-10:30 a.m after 3 and 7 days. In the following the solution was discharged, the sand rinsed with DI water and the pots were filled with 500 mL 50% Hoagland containing 0.1 mM KH_2PO_4 (2.2.3). Three days later samples were taken with a sterile syringe (volume 10 mL) of each plant trough a sterile filter between 10-10:30 a.m. and stored at -20 °C. In the following ten plants per cultivar got fresh nutrient solution and ten plants remained in the solution. Four days later samples were taken again between 10-10:30 a.m. and stored at -20°C. The samples were analyzed with CE (2.1.2) and TOC (2.1.1).

0.1 mM KH_2PO_4 Oryza sativa sterile

Comparison of the two rice cultivars:

Oryza sativa Milyang 23, *Oryza sativa IR 5440-1-1-3*

Oryza sativa Milyang 23 is a phosphate deficiency tolerant and *Oryza sativa IR 5440-1-1-3* a phosphate deficiency intolerant cultivar. In this experiment their ability and method to adapt on reduced phosphate supply should be studied. Furthermore, the cultivars could be successfully cultivated under sterile conditions and afford a comparison of the released organic compounds under sterile and non sterile conditions.

Both *Oryza sativa* cultivars were sterile cultivated in a climate chamber (2.2.2; chamber 2). Samples of the nutrient solution were taken with a sterile syringe (volume 10 mL) of each plant through a sterile filter and were frozen immediately in liquid nitrogen. Sampling occurred between 10-10:30 a.m. 14 days after the transfer into the blue caps (2.3.3). In the following the volume was reduced to 20 mL and the tubes were filled up with fresh sterile 50% Hoagland solution up to 40 mL.

Four days later samples were taken between 10-10:30 a.m. and afterwards the solution was changed against 50% Hoagland containing 0.1 mM KH_2PO_4 (2.2.3). After three days samples were taken, the solution discharged and fresh Hoagland containing 0.1mM KH_2PO_4 added.

The last sampling occurred 4 days later. All samples were analyzed with CE (2.1.2) and TOC (2.1.1), and absorption measurement (2.1.3).

2.4.3 Day rhythm

To figure out, if exudation is depending on the day time, samples of *Phalaris arundinacea* plants were taken during a one-day period.

The plants were waterlogged in 25% Hoagland solution (2.2.3) in a climate chamber (2.2.2; chamber 1) and after one week the solution was discharged, the sand rinsed with DI water and fresh nutrient solution (50%) added. The next day samples were taken six times (6:30; 7:30; 9:30 a.m.; 12:30; 3:30; 5:30 p.m.). The samples were analyzed with TOC (2.1.1) and CE (2.1.2) measurement.

2.4.4 Day rhythm with Al

To examine if there is a fast reaction to Al stress, *Phalaris arundinacea* plants were treated with a Hoagland solution containing 1 mM $AlCl_3$ and samples were taken during a one-day period.

The plants were waterlogged in 50% Hoagland solution (2.2.3) in a climate chamber (2.2.2; chamber 1) and after one week the solution was discharged, the sand rinsed with DI water and fresh 50% Hoagland solution added (2.2.3). The next day samples were taken six times (5:45; 7:30; 9:30 a.m.; 12:30; 3:30; 5:30 p.m.). After the first sampling the solution was discharged and 50% Hoagland solution containing 1 mM Al added (2.2.3). The samples were analyzed with TOC measurement (2.1.1).

2.5 NMR Imaging

Jahnke et al. showed that natural sand yielded the best MRI results (2009). Therefore, *Phalaris arundinacea* plants were grown from seeds in tubes of 200x55 mm (inner diameter: 49 mm) filled with sand in a green house (2.2.3). The plants were fed with 50% Hoagland solution (2.2.3) until one week after germination. Then the plants were waterlogged in 50% Hoagland solution, in 50% Hoagland containing 0.1 mM KH_2PO_4 or in 50% Hoagland containing 1 mM $AlCl_3$ (2.2.3) with three replicates per treatment in a green house (2.2.3). Non-invasive MRI measurements were done every three weeks within two months with a 4.7T Varian VNMRS vertical wired-bore MRI system. Two days before measuring, the plants were removed of the nutrient solution to dry the sand substrate, because high water contents would disturb the NMR signal. The measurement procedure took 9 min. per plant and every plant had to be measured three times. The data were analyzed with the programs IDL ver. 7.11, ITT Visual Information Solutions, MeVisLab ver. 2.1, MeVis Medical Solutions AG, Bremen, Germany) and Matlab ver. 7.11 R2010b.

2.6 Statistical analysis

For the statistical analysis two methods were combined: Repeated measurements and t-tests. The repeated measurements analyzes was done with SPSS version 11.5

The chance to get a wrong result by a pairwise comparison of groups (A to B, A to C, B to C) with t-tests would be: 1 - (0.95 x 0.95 x 0.95) = 14.3%

Thus, only if the analysis with repeated measurements indicated significant differences over the complete experimental period and between the different groups, a pairwise comparison with t-tests was done to get more detailed information e.g. at one point of time between the groups or if an analysis with repeated measurements was not possible e.g. to small number of replicates.

3 Results

It is known that the reduction of the P supply as well as Al toxicity can increase the exudation of organic acids. However, every plant species is reacting differently and experiments are usually done with model species. For a well performing plant-MFC, it is important to search for species which develop well under waterlogged conditions, as well as plants which provide optimal carbohydrate supply to the bacteria. The main focus was to measure changes of the total organic carbon content (TOC) rather than quantifying all organic compounds, found in the nutrient solution because bacteria decompose all organic compounds released by the plant roots as well as dead cell material. Thus, not only organic acids and sugars, released by the plant, are interesting but all organic compounds, which are measurable in the nutrient solution. Furthermore, the inorganic carbon content (IC) can serve as indicator for an increased microbial activity referring to enhanced exudation or an increase of root respiration.

3.1 Adsorption of the substrate

The adsorption of organic compounds on substrates varies and depends on the physical-chemical properties of the organic compounds as well as the substrate. Therefore, in this experiment the adsorption of organic acids and sugars on graphite granules (used as substrate in plant-MFCs) and sand (used in the experiments) was compared.

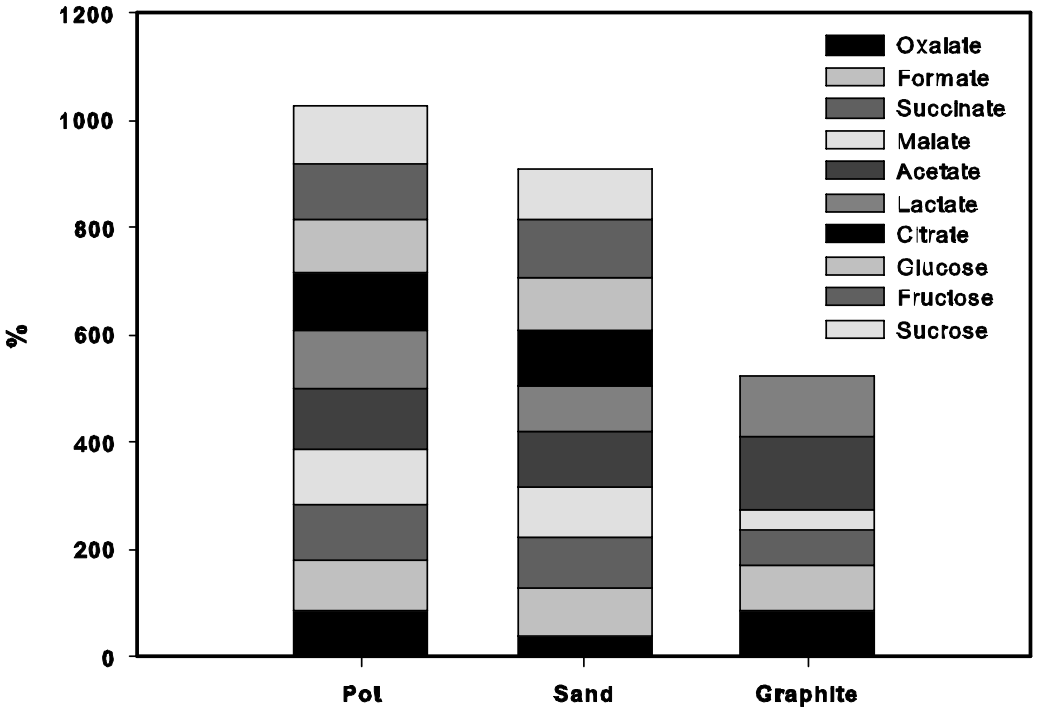


Fig. 8: Percentage of recovered organic acids (oxalate, formate, succinate, malate, acetate, lactate and citrate) and sugars (glucose, fructose and sucrose) in the nutrient solution filled in either empty plastic flower pots, flower pots filled with sand or flower pots filled with graphite granules. The values correspond to the amount detected in beaker glasses filled with the same nutrient solution.

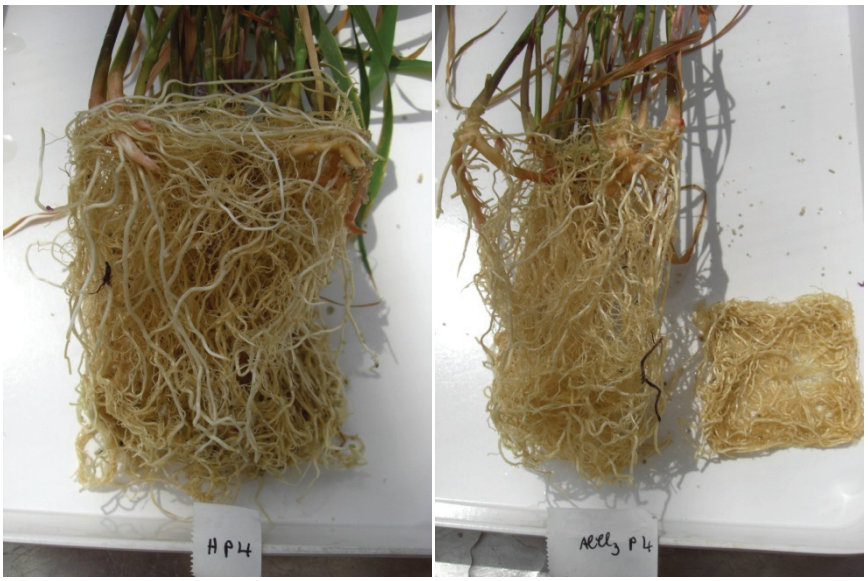
Tab. 1: Percentage of recovered organic acids (oxalate, formate, succinate, malate, acetate, lactate and citrate) and sugars (glucose, fructose and sucrose) in the nutrient solution filled in either empty plastic flower pots, flower pots filled with sand or flower pots filled with graphite granules. The values correspond to the amount detected in beaker glasses filled with the same nutrient solution.

% of recovery beaker glas	Mean pot	STW pot	Mean sand	STD sand	Mean graphite	STD graphite
oxalate	**88.21**	0.63	**98,43**	106.06	**87.57**	133.00
formate	**93.76**	12.86	**89,25**	84.65	**83.59**	51.53
succinate	**102.89**	2.21	**92,50**	85.69	**67.30**	75.72
malate	**100.49**	4.97	**94,83**	92.06	**35.25**	8.42
acetate	**113.25**	25.23	**103,97**	100.18	**134.47**	125.41
lactate	**109.14**	20.57	**85,27**	71.07	**113.23**	41.61
citrate	**109.32**	8.62	**102,04**	91.10	**0.00**	0.00
glucose	**96.19**	9.16	**100,48**	13.91	**n.m.**	n.m.
fructose	**107.76**	8.6	**106,9**	10.43	**n.m.**	n.m.
sucrose	**106.02**	20.26	**96,99**	12.96	**n.m.**	n.m.
SUM	**104.19**	10.73	**95,18**	90.11	**74.49**	62.24

Fig. 8 and Tab. 1 depict the amount of recovered organic acids (oxalate, formate, succinate, malate, acetate, lactate and citrate) and sugars (glucose, fructose and sucrose) in the nutrient solution filled in either empty plastic flower pots, flower pots filled with sand or flower pots filled with graphite granules. The values correspond to the amount detected in beaker glasses filled with the same nutrient solution. The nutrient solution samples were taken 3 h after the solution was added. In the plastic pots the concentrations of solved organic acids and sugars were not reduced compared to the beaker glass control. All values ranged around 100 %. The lowest concentrated organic acid was oxalate with a range of 88 ± 1% and the highest acetate with a range of 113 ± 19 %. Furthermore, on the sand substrate a low adsorption of the organic acids and sugars occurred and the recovery rate ranged around 95%. Oxalate was the lowest concentrated organic acid with a range of 39% but with a wide spread (56%). The other acids and sugars alternated around 100%. The strongest adsorption showed the graphite granules but also with a wide spread e.g. malate 35 ± 50% and citrate was not detectable. The sugars were not analyzed for graphite. The amount of total recovered organic compounds was 75%.

3.2 Influence of Al addition on root growth

Most plant species already react to low concentrations of Al (70-185 µM) in the soil solution e.g. *Zea mays* but there are also other species which can resist concentrations of Al up to 1000-6400 µM as shown for C*amellia sinensis* (Marschner, 1995). The initial and most dramatic symptom of Al toxicity is inhibition of root elongation, which can occur within 1-2 h after exposure to Al (Kochian, 1995). For this reason reduced root growth is a good indicator for a toxic Al concentration. In a preliminary test, a strong decrease after Al treatment (2mM) was detectable for *Phalaris arundinacea* (Fig. 9) as well as *Glyceria maxima.* Thus, in the next series the concentration was reduced to 1mM Al and no strong reduction of biomass occurred (data not shown).



Control (50% Hoagland) 2 mM Al

Fig. 9: Roots of *Phalaris arundinacea plants after treatment with 50% Hoagland solution (left) or 50% Hoagland solution containing 2 mM Al (right) for six weeks.*

3.3 Influence of reduced P content and Al addition on rhizodeposition

Several plant species were cultivated in sand pots under waterlogged conditions. The pots were waterlogged with 50% Hoagland solution containing reduced phosphate content (0.2 mM or 0.1 mM) or accessorily Al (1 mM) and samples of the nutrient solutions were taken and analyzed via TOC measurement for the organic (TOC) and inorganic carbon (IC) content, CE analysis for the content of organic acids or adsorption measurement for determination of the sugars.

3.3.1 0.2 mM P content and 1mM Al

The first experiment was done with *Phalaris arundinacea* and *Glyceria maxima* grown in 50% Hoagland solution, 50% Hoagland solution containing reduced phosphate content (0.2 mM) or accessorily Al (1 mM). The experiment was running for two weeks. During this time, samples were taken and TOC, IC and organic acids were analyzed. Samples of all plants were taken in Hoagland solution (0 d) and afterwards the solutions were changed against the different treatments. Sampling under treatment occurred 2 d, 5 d, and 7 d after reduction of the phosphate content or aluminum addition.

The TOC and IC contents of the stock solutions were measured and were deducted from the sample values. Therefore, negative TOC values occurred if the consumption of the organic material by the bacteria was too fast. Negative values for the IC content occurred because under acidic conditions most of the inorganic carbon is released into the atmosphere and does not solve into water. Thus, if the amount of consumed organic material is low and with it the produced inorganic carbon, a negative IC content could occur.

3.3.2 *Phalaris arundinacea*

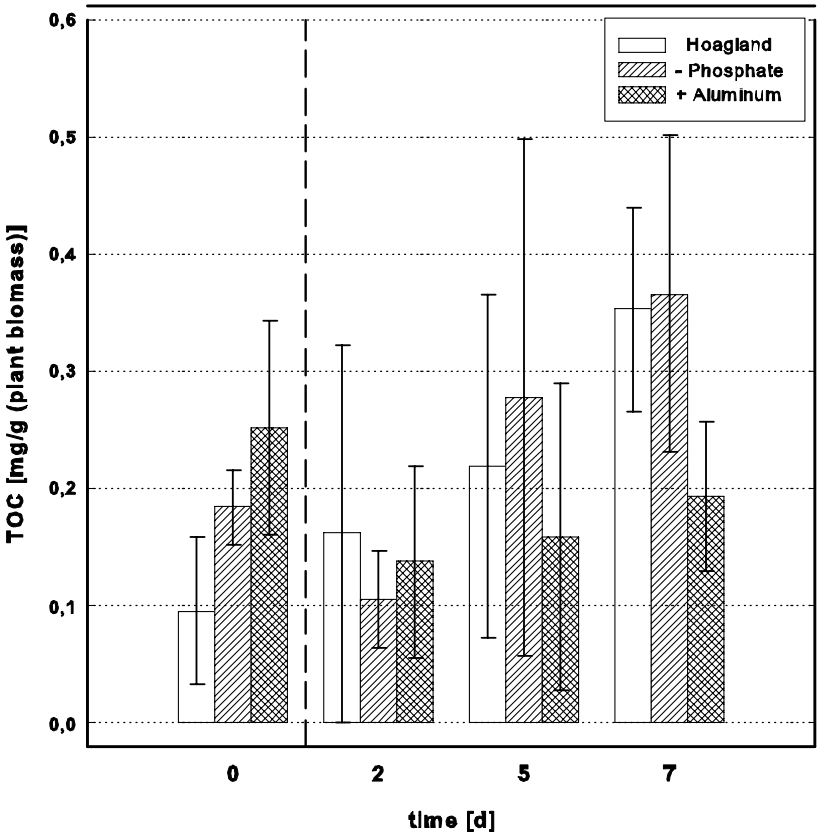


Fig. 10: Total organic carbon (TOC) (mg) content in the nutrient solution of waterlogged Phalaris arundinacea plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of four replicates (n=4). Significances tested with repeated measurements $p<0.05$ after log transformation.

Fig. 10 depicts the TOC content in the nutrient solution of waterlogged *Phalaris arundinacea* plants. At the beginning of the experiment (0 d) all plants were measured in 50% Hoagland solution and afterwards the solution was changed against fresh 50% Hoagland solution (i.e. control), 50% Hoagland solution with reduced phosphate content (0.2 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3). During the experiment no significant changes in TOC content for each treatment ($p=0.107$) or between the treatments ($p=0.737$) were measurable.

The group of *Phalaris arundinacea* plants, which were waterlogged in 50% Hoagland solution with reduced phosphate (0.2 mM) content, reveal a higher TOC content of 0.18 ± 0.03 [mg/g (plant dry weight)] than the control plants in 50% Hoagland solution with a TOC content of 0.1 ± 0.03 [mg/g (plant dry weight)] before starting of the treatment (0 d). Also the TOC content of the group with aluminum treatment (0.25 ± 0.03 [mg/g (plant dry weight)]) was higher than for the control plants before starting of the experiment (0 d). Furthermore, a reduced TOC content for plants treated with aluminum addition (0.19 ± 0.13 [mg/g (plant dry weight)]) was measurable after 7 days compared to the control plants (0.35 ± 0.09 [mg/g (plant dry weight)]). The TOC content of the plants with phosphate reduction decreased after the solution change from 0.18 ± 0.03 (0 d) to 0.11 ± 0.04 (2 d) [mg/g (plant dry weight)].

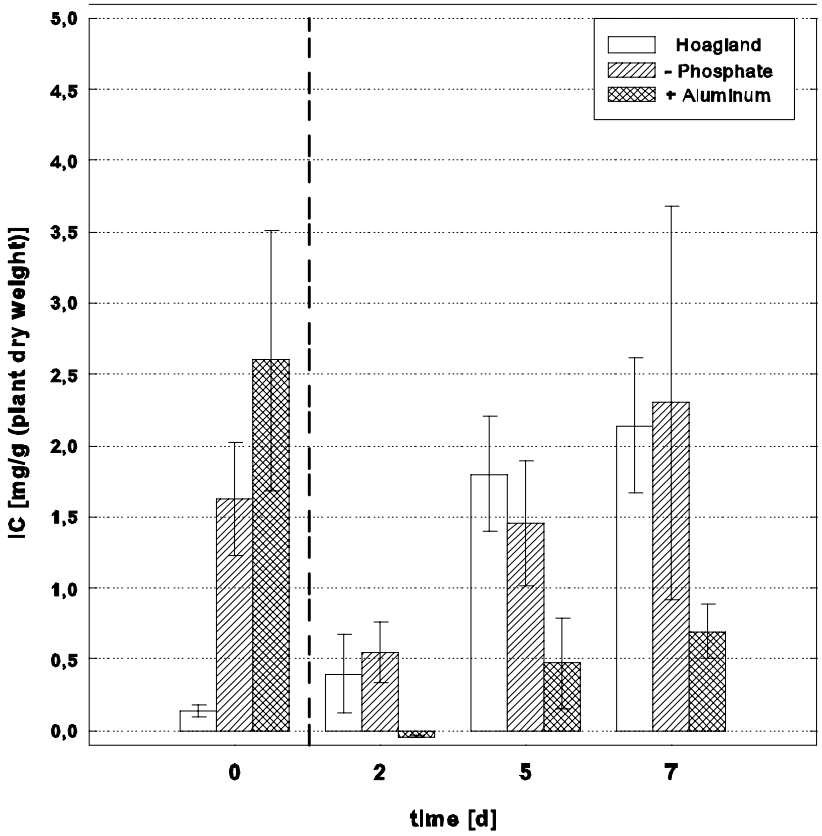


Fig. 11: Inorganic carbon content (IC) (mg) in the nutrient solution of waterlogged Phalaris arundinacea plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of four replicates (n=4). Significances tested with repeated measurements $p<0.05$.

Fig. 11 depicts the IC content in the nutrient solution of waterlogged *Phalaris arundinacea* plants. During the experiment significant changes in IC content for each treatment (p=0.04) and between the treatments (p=0.01) were measurable. The first sample was taken from all plants in 50% Hoagland solution. The first sampling revealed a significantly lower amount of IC in the samples of the control group 0.13 ± 0.05 mg/g (plant dry weight) (0 d) than in the both groups, which were treated in the following (P reduction group 1.63 ± 0.39 (p=0.004); Al addition group 2.6 ± 0.92 [mg/g (plant dry weight)] (p=0.03)). Afterwards the solution was discharged and fresh solution was added (dashed vertical line), either fresh 50% Hoagland solution (i.e. control) or 50% Hoagland solution with reduced phosphate (0.2 mM) content or 50% Hoagland solution containing 1mM Al (2.2.3). After 5 d (1.80 ± 0.4 mg/g (plant dry weight); p= 0.001) and 7 days the IC content in the samples of the control plants was significantly higher than at 0 d and 2 d (0.39 ± 0.28 [mg/g (plant dry weight)]). Plants treated with reduced P content did show a significant decrease in IC content after the solution change (0.55 ± 0.21 [mg/g (plant dry weight)]; p=0.002) (2 d) and a significant increase after 5 d (1.46 ± 0.44 mg/g [(plant dry weight)]; p=0.017). The IC content of plants treated with Al addition displayed a significant decrease of IC in the nutrient solution after the changing (2 d) (-0.04 ± 0.009 [mg/g (plant dry weight)]) and a significant increase after 7 d (0.69 ± 0.2 [mg/g (plant dry weight)].

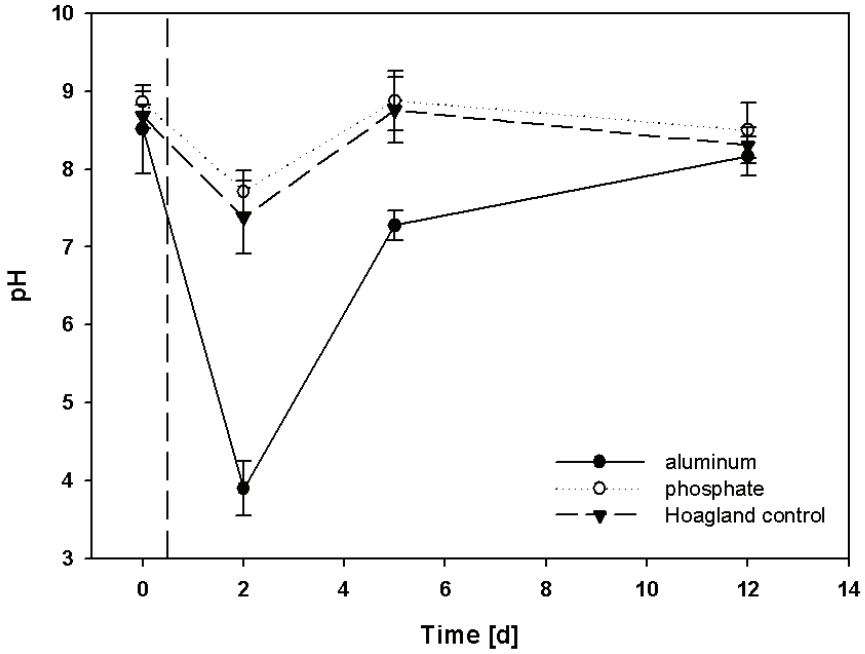


Fig. 12: pH dynamics of Phalaris arundinacea plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.2 mM) and 50% Hoagland solution containing Al (1 mM) (2.2.3). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of four replicates (n=4)

Fig. 12 depicts the pH dynamics of *Phalaris arundinacea* plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.2 mM) and 50% Hoagland solution containing Al (1 mM). The pH of plants grown only in 50% Hoagland solutions ranged from 7.38 ± 0.47 to 8.76 ± 0.42. After the addition of fresh Hoagland solution, a short decrease was detectable (2 d). Also plants in 50% Hoagland solution with reduced phosphate content (0.2 mM) showed the same dynamic like the control group. The pH ranged from 7.71 ± 0.27 to 8.88 ± 0.38. After addition of the 50% Hoagland solution, containing Al (1 mM), the pH dropped to 3.90 (2 d), but it already reached a neutral pH of 7.28 ± 0.19 after 4 d and 8.17 ± 0.25 after 12 d. Control pots filled with sand and nutrient solution (50% Hoagland, 50% Hoagland with reduced phosphate (0.1 mM) and 50% Hoagland containing 1mM Al but without plant showed no changes in pH (see VI).

Organic acids

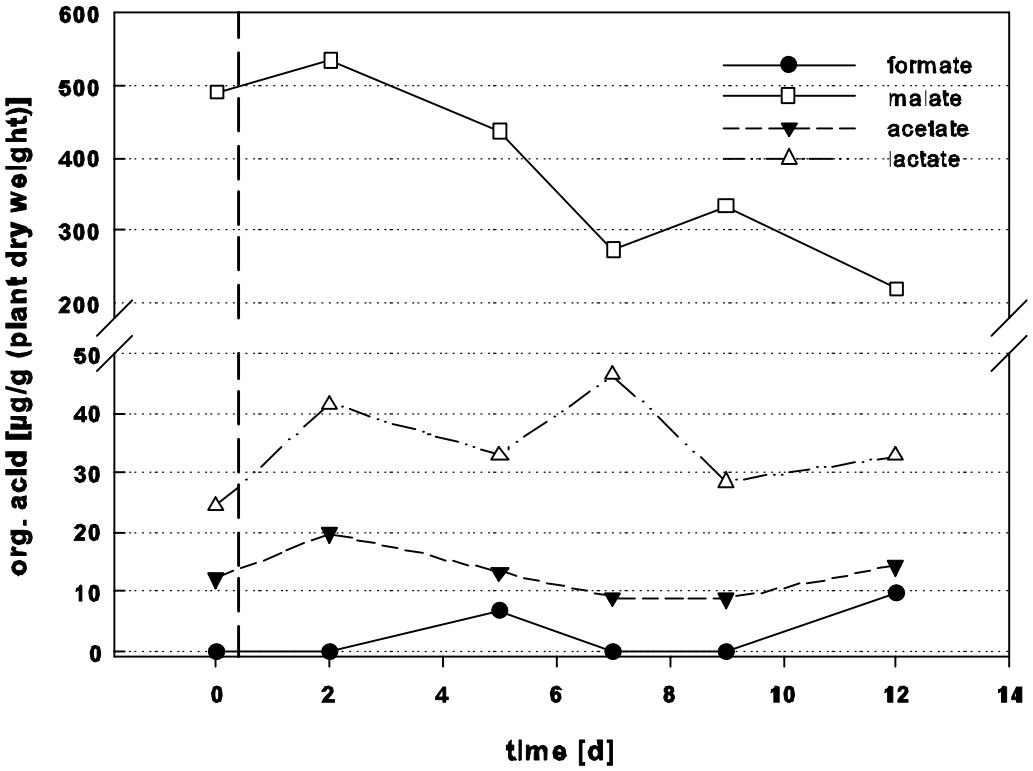


Fig. 13: Organic acid content (µg) per plant dry weight (g) in the nutrient solution of Phalaris arundinacea plants grown waterlogged in 50% Hoagland solution (2.2.3) for 12 d. The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. At the beginning of the experiment (0 d) plants were 24 h in the Hoagland solution. The samples of four replicates per time point were pooled before CE analysis.

Fig. 13 depicts the organic acid content [µg/g (plant dry weight)] in the samples of the nutrient solution of waterlogged *Phalaris arundinacea* plants, remaining in 50% Hoagland solution (control group), over a period of 12 d. Before CE analysis the sampling replicates of each time point (n=4) were pooled to reduce the number of samples to measure. No difference between the sampling before and after the solution change (dashed vertical line) was visible for acetate, lactate and formate. Acetate ranged between 8.81 and 19.85 [µg/g (plant dry weight)] and formate was only measurable on day two (6.9 [µg/g (plant dry weight)]) and 12 (9.85 [µg/g (plant dry weight)]) in low amounts. Lactate content varied from 24.0 to 46.37 [µg/g (plant dry weight)]. The detectable amounts of acetate and formate were low compared to lactate and malate. In contrast, the malate concentration decreased over time.

The content of malate declined about 60% from 219 (2 d) to 535 (12 d) [µg/g (plant dry weight)] during the period.

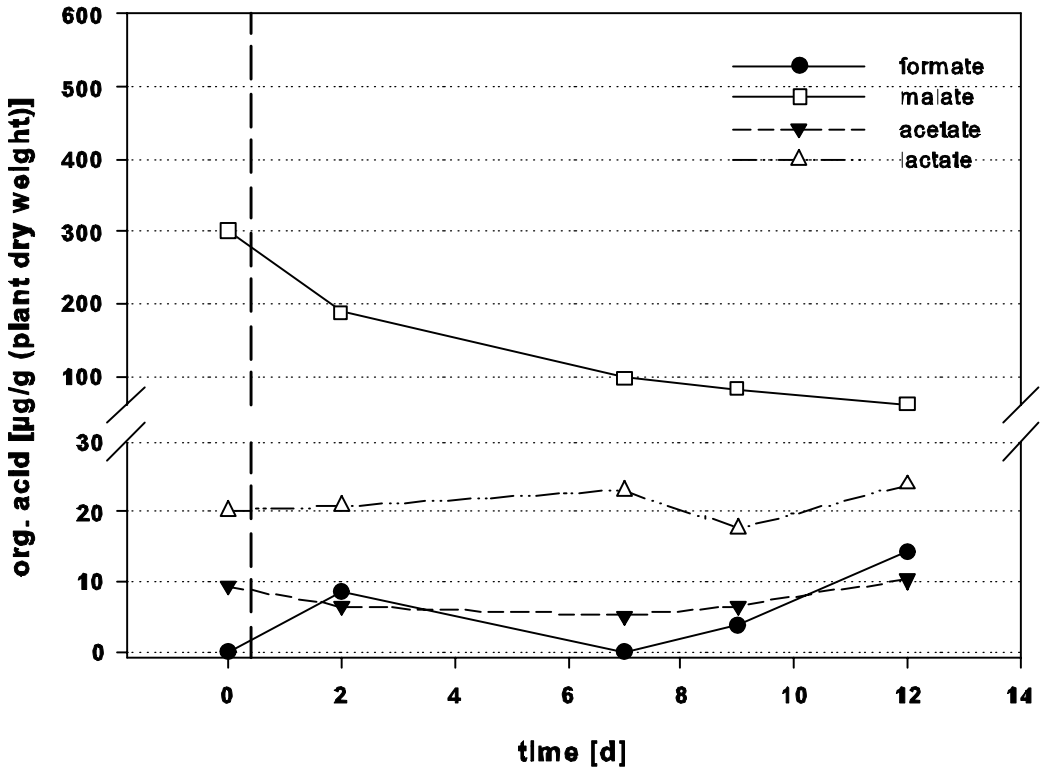


Fig. 14: Organic acid content (µg) per plant dry weight (g) in the nutrient solution of Phalaris arundinacea plants grown waterlogged in 50% Hoagland solution with reduced P content (0.2 mM) (2.2.3) for 12 d. The dashed vertical line represents the point of time when the 50% Hoagland nutrient solution was discharged and fresh solution with reduced P content (0.2 mM) was added. The samples of four replicates were pooled before CE analysis.

Fig. 14 depicts the organic acid content [µg/g (plant dry weight)] in the samples of the nutrient solution of waterlogged *Phalaris arundinacea* plants, with low P treatment, over a period of 12 d. Before CE analysis, the sampling replicates of each time point (n=4) were pooled to reduce the number of samples to measure. The first sample was taken in 50% Hoagland solution (2.2.3 d) and afterwards the solution was changed against 50% Hoagland solution with reduced phosphate content (0.2 mM) (dashed vertical line) (2.2.3). No significant difference between the sampling before and after the solution change was visible for acetate, lactate and formate. All acids showed a similar progress like in Hoagland solution (Fig. 13).

The amount of acetate alternated 0 from to 10.32 [µg/g (plant dry weight)], formate from 0 to 14.26 [µg/g (plant dry weight)] and lactate from 17.60 to 23.02 [µg/g (plant dry weight)]. Only the malate content decreased after solution change about 80% from 300 to 62 [µg/g (plant dry weight)], whereas the malate concentration of the control declined about 60%.

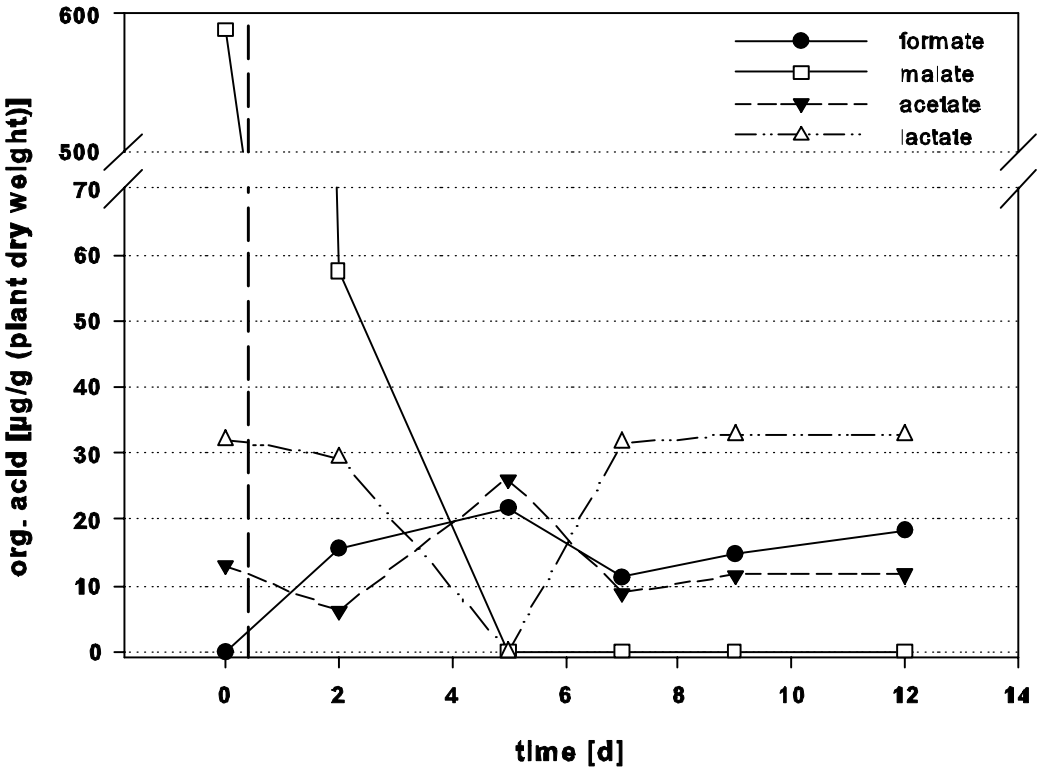


Fig. 15: Organic acid content (µg) per plant dry weight (g) in the nutrient solution of Phalaris arundinacea plants grown waterlogged in 50% Hoagland solution, containing Al (1 mM) (2.2.3), for 12 d. The dashed vertical line represents the point of time when the 50% Hoagland nutrient solution was discharged and fresh solution, containing Al (1 mM), was added. The samples of four replicates per time point were pooled before CE analysis.

Fig. 15 displays the organic acid content in the samples of the nutrient solution of waterlogged *Phalaris arundinacea* plants, with Al treatment, over a period of 12 d. At the beginning of the experiment (0 d) plants are sampled in 50% Hoagland solution. Afterwards the solution was changed against 50% Hoagland containing 1 mM Al (dashed vertical line).

Before CE analysis the sampling replicates of each time point (n=4) were pooled to reduce the number of samples to measure. The amounts of acetate and formate varied over the time and both reached their maximum five days after the solution was changed. Acetate content varied from 6.21 up to 26.10 [µg/g (plant dry weight)]. After the solution change it decreased from 12.96 to 6.21 [µg/g (plant dry weight)]. The dynamic of the formate content is similar to acetate and varied from 0 up to 21.78 [µg/g (plant dry weight)]. At the fifth day lactate showed a decrease to zero but during the remaining time it alternated between 29.40 to 32.80 [µg/g (plant dry weight)].

Malate decreased faster after addition of the Al (1mM) than in the P reduction treatment (Fig. 14) and by the control plants (Fig. 13). Before the treatment (0 d), the amount was 589 [µg/g (plant dry weight)] but after the solution change it decreased about 90% to 58 [µg/g (plant dry weight)] (2 d) and in the following to zero (5 to12 d).

Both treatments showed a stronger decrease in malate concentration in comparison to the control plants (control 60%, P reduction 80% and Al addition 90%). In acetate, formate and lactate concentration the plants treated with P reduction did not differ compared to the control group. In contrast, the plants treated with Al showed an increase of formate and acetate and a decrease of lactate content after 5 d. Acetate increased 4.3 fold, formate 1.3 fold and lactate decreased from 29 to 0 [µg/g (plant dry weight)].

3.3.3 Glyceria maxima

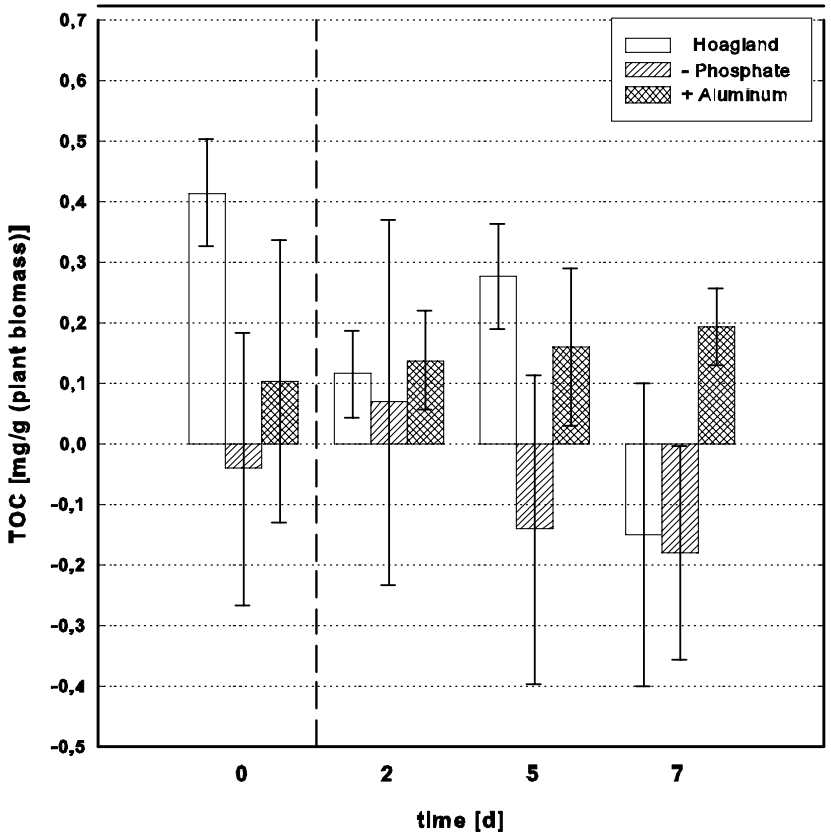


Fig. 16: Total organic carbon (TOC) (mg) content in the nutrient solution of waterlogged Glyceria maxima plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of four replicates (n=4). Significances tested with repeated measurements $p<0.05$.

Fig. 16 depicts the TOC content in the nutrient solution of waterlogged *Glyceria maxima* plants. During the experiment no significant changes in TOC content for each treatment ($p=0.278$) but between the treatments ($p=0.007$) were measurable. The TOC content of the control plants was significantly higher than of the plants treated with phosphate reduction ($p=0.032$). First, the TOC content of the control plants was higher than of the two treatment groups (control 0.41 ± 0.09, P reduction -0.04 ± 0.22, Al 0.10 ± 0.23 [mg/g (plant dry weight)] (0 d)). After 2 d the TOC content of the control group was similar as the two treatments and after 7 d the TOC content of control group as well as of the P reduction group decreased (control -0.15 ± 0.25, P reduction -0.18 ± 0.18 [mg/g (plant dry weight)] (7 d)). In contrast, the TOC content in the Al treatments remained constant during the experiment.

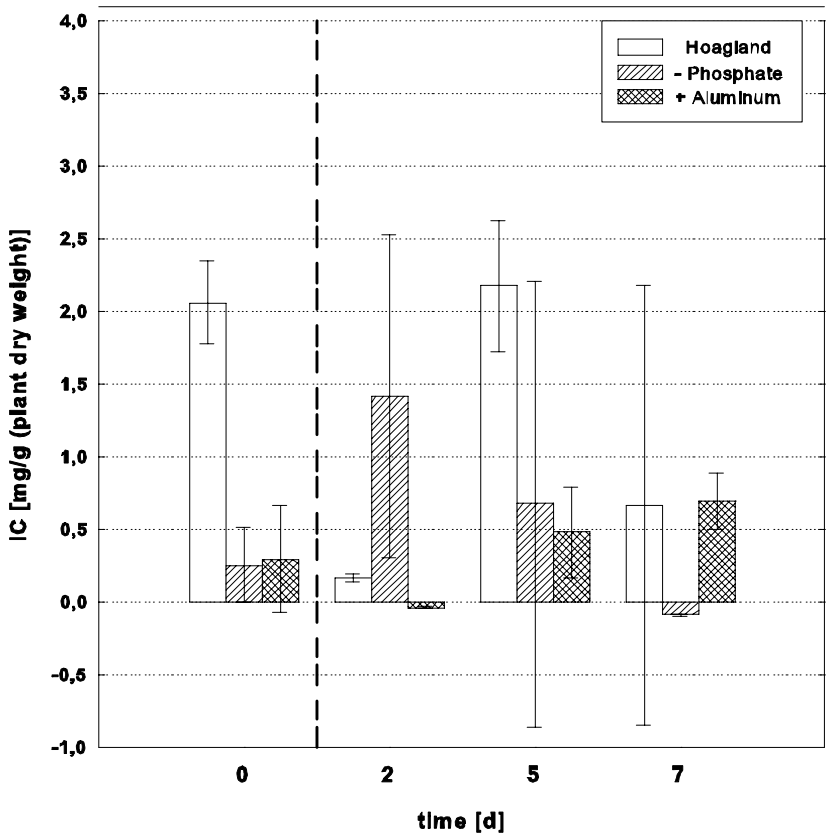


Fig. 17: Inorganic carbon content (IC) (mg) in the nutrient solution of waterlogged Glyceria maxima plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of four replicates (n=4). Significances tested with repeated measurements $p<0.05$.

Fig. 17 depicts the IC content in the nutrient solution of waterlogged *Glyceria maxima* plants. During the experiment no significant changes in IC content for each treatment (p=0.062) but between the treatments (p=0.01) were measurable. The first sampling revealed a significantly higher amount of inorganic carbon in the samples of the control group 2.06 ± 0.28 [mg/g (plant dry weight)] than in the group treated with P reduction 0.29 ± 0.37 [mg/g (plant dry weight)] (p=0) or the group with Al addition 0.25 ± 0.25 [mg/g (plant dry weight)] (p=0) (0 d). After 2 d the IC content in the samples of the control plants was significantly lower than at 0 d (0.16 ± 0.03 [mg/g (plant dry weight)]) (p=0) and increased significantly again after 5d to 2.17 ± 0.45 [mg/g (plant dry weight)] (p=0.003).

Plants treated with reduced P content showed an increase in IC content after the solution change to 1.41 ± 1.11 [mg/g (plant dry weight)] (2 d), followed by a decrease up to -0.09 ± 0.01 [mg/g (plant dry weight)] (7 d). The IC content of plants treated with Al addition displayed a decrease of IC in the nutrient solution after the changing to -0.04 ± 0.01 [mg/g (plant dry weight)] (2 d) and a significant increase after 5 d up to 0.48 ± 0.32 [mg/g (plant dry weight)] (p=0.014). Furthermore, the IC content was significantly lower in solutions containing Al than in the control group (p=0.054).

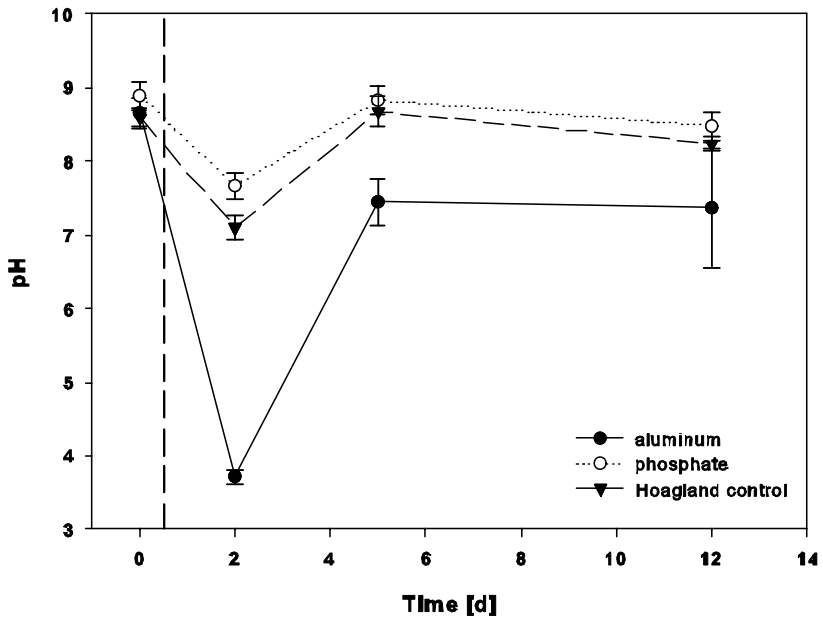


Fig. 18: pH dynamics of Glyceria maxima plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.2 mM) and 50% Hoagland solution containing Al (1 mM) (2.2.3). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. At the begin of the experiment (0 d) all plants were measured in 50% Hoagland solution and afterwards the solution was changed against fresh 50% Hoagland solution (i.e. control), 50% Hoagland solution with reduced phosphate content (0.2 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3). Error bars represent ± standard deviation of four replicates (n=4)

Fig. 18 depicts the pH dynamics of *Glyceria maxima* plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.2 mM) and 50% Hoagland solution containing Al (1 mM). The pH of plants grown only in 50% Hoagland solution (i.e. control) ranged from 7.10 ± 0.17 to 8.68 ± 0.21. After the addition of fresh Hoagland solution, a short decrease was detectable (2 d). Also plants in 50% Hoagland solution with reduced phosphate content (0.2 mM) show the same dynamic like the control group. The pH ranged from 7.67 ± 0.17 to 8.89 ± 0.20. After addition of the 50% Hoagland solution, containing Al (1 mM) the pH dropped to 3.72 (2 d) but it already reaches a neutral pH of 7.45 ± 0.32 after 4 d.

The pH of *Glyceria maxima* showed the same dynamic like *Phalaris arundinacea* and the values were in the same range.

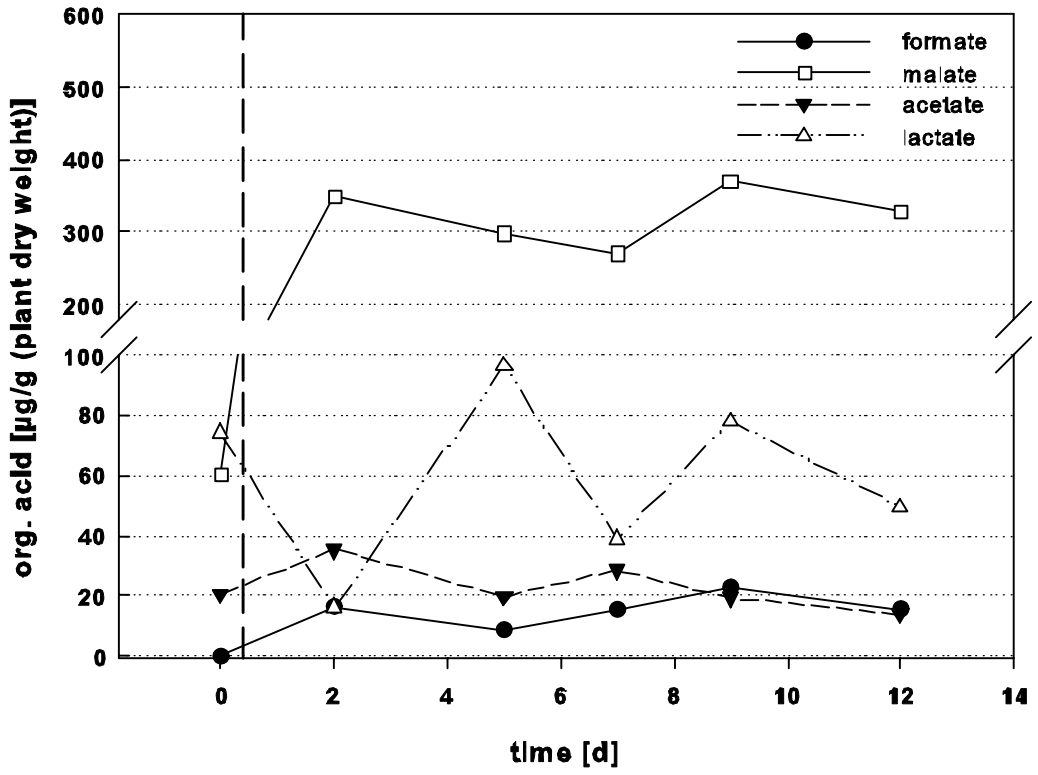


Fig. 19: Organic acid content (µg) in the nutrient solution per plant dry weight (g) of Glyceria maxima plants grown waterlogged in 50% Hoagland solution (2.2.3) for 12 d. The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. At the beginning of the experiment (0 d) plants were 24 h in the Hoagland solution. The samples of four replicates per time point were pooled before CE analysis.

Fig. 19 depicts the organic acid content [µg/g (plant dry weight)] in the samples of the nutrient solution of waterlogged *Glyceria maxima* plants, remaining in 50% Hoagland solution (i.e. control group), over a period of 12 d. Before CE analysis the sampling replicates of each time point (n=4) were pooled to reduce the number of samples to measure. No difference between the sampling before and after the solution change (dashed vertical line) was visible for acetate, lactate and formate but the malate concentration decreased. The detectable amounts of acetate and formate were low compared to lactate and malate. Acetate varied from 14 to 36 [µg/g (plant dry weight)] and formate from 9 to 22 [µg/g (plant dry weight)]). Lactate content varied from 16 to 78 [µg/g (plant dry weight)]. The minimum of lactate content (16 [µg/g (plant dry weight)]) was reached after the solution change (2 d) and the maxima of lactate content arose after 9 d (78 [µg/g (plant dry weight)]). The content of malate rose from 60 (0 d) to 370 (9 d) [µg/g (plant dry weight)] during the experimental period.

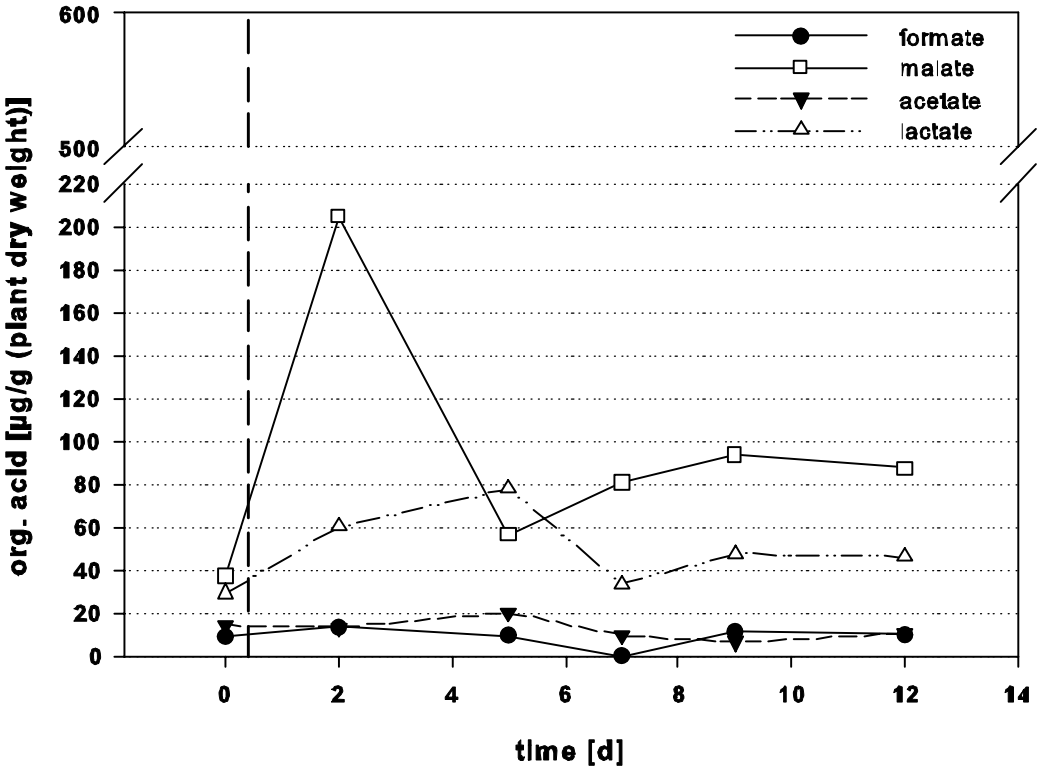


Fig. 20: Organic acid content (µg) per plant dry weight (g) in the nutrient solution of Glyceria maxima plants grown waterlogged in 50% Hoagland solution with reduced P content (0.2 mM) (2.2.3) for 12 d. The dashed vertical line represents the point of time when the 50% Hoagland nutrient solution was discharged and fresh solution with reduced P content (0.2 mM) was added. The samples of four replicates per time point were pooled before CE analysis.

Fig. 20 depicts the organic acid content [µg/g (plant dry weight)] in the samples of the nutrient solution of waterlogged *Glyceria maxima* plants, with low P treatment, over a period of 12 d. Before CE analysis the sampling replicates of each time point (n=4) were pooled to reduce the number of samples to measure. The first sample was taken in 50% Hoagland solution (2.2.3 d) and afterwards the solution was changed against 50% Hoagland solution with reduced phosphate content (0.2 mM) (dashed vertical line). No difference between the sampling before and after the solution change was visible for acetate and formate. Both acids showed a similar progress like in the untreated Hoagland solution (Fig. 19). The amount of acetate alternated from 6 to 14 [µg/g (plant dry weight)] and formate from 0 to 13 [µg/g (plant dry weight)] after the solution change. The lactate content increased from 29 (0 d) to 78 (5 d) [µg/g (plant dry weight)] and decreased after 7d again to 33 [µg/g (plant dry weight)]. Similar to the control plants, the malate content of the treated plants increased strong after the solution change from 37 (0 d) to 204 [µg/g (plant dry weight)] but it decreased again after 5 d to 56 [µg/g (plant dry weight)].

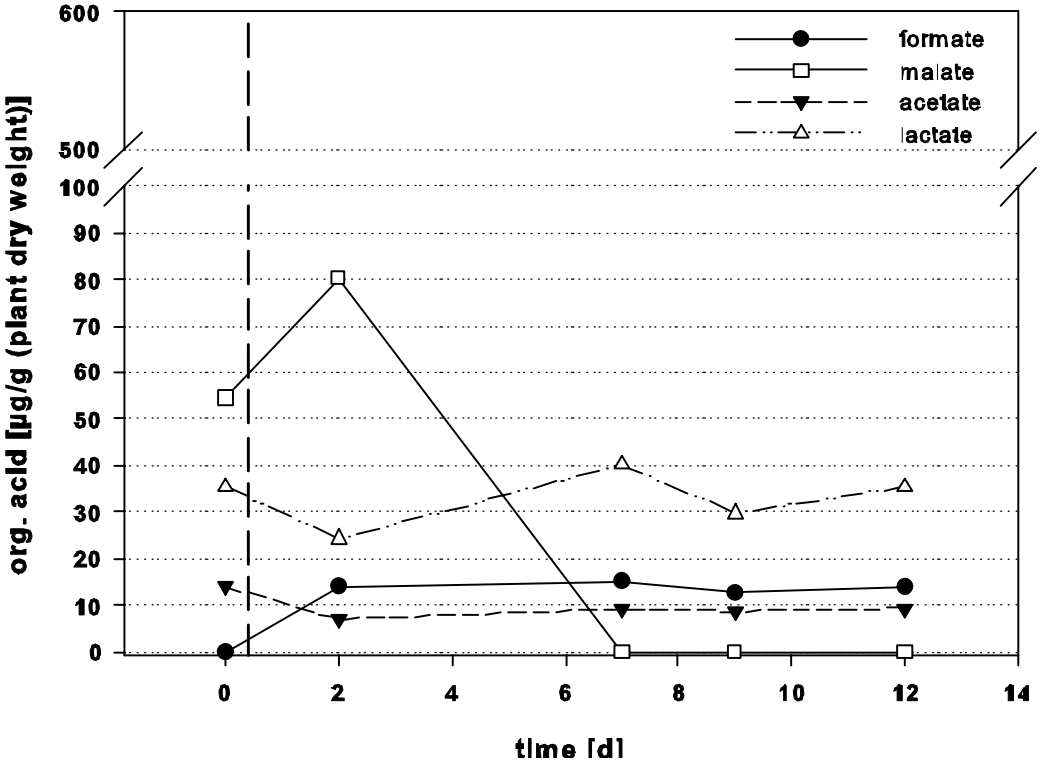


Fig. 21: Organic acid content (µg) per plant dry weight (g) in the nutrient solution of Glyceria maxima plants grown waterlogged in 50% Hoagland solution, containing Al (1 mM) (2.2.3), for 12 d. The dashed vertical line represents the point of time when the 50% Hoagland nutrient solution was discharged and fresh solution, containing Al (1 mM), was added. The samples of four replicates per time point were pooled before CE analysis.

Fig. 21 depicts the organic acid content in the nutrient solution of waterlogged *Glyceria maxima* plants, with Al treatment, over a period of 12 d. At the beginning of the experiment (0 d) plants are sampled after 24 h in 50% Hoagland solution. Afterwards the solution was changed against 50% Hoagland containing 1 mM Al (dashed vertical line). Before CE analysis the sampling replicates (n=4) were pooled to reduce the number of samples to measure. The amounts of acetate, formate and lactate remained stable after the solution change. Acetate content varied from 7 up to 9 [µg/g (plant dry weight)]. After the solution change it decreased from 14 to 7 [µg/g (plant dry weight)]. The dynamic of the formate content was similar to acetate and alternated between 0 and 15 [µg/g (plant dry weight)].

Also the lactate content remained stable from 24 to 40 [µg/g (plant dry weight)]. Malate content did not strong increase after addition of the Al (1 mM) in comparison to the P reduction treatment (Fig. 20) or the Hoagland control (Fig. 19). In 50% Hoagland solution the amount was 55 [µg/g (plant dry weight)] and after the solution change it increased to 80 [µg/g (plant dry weight)] (2 d) and in the following it decreased to zero (5 to12 d).

Both treatments and the control showed an increase of malate after the solution change, but both treatments showed a strong decrease in malate concentration after 5 d. In contrast the content remained high in the control samples. The acetate and formate concentration of all treatments remained low and did not change during the experiment. But only plants treated with P reduction showed an increase of lactate content after 2 d.

The control plants of *Phalaris arundinacea* and *Glyceria maxima* showed a similar dynamic for all measured organic acids. The malate concentration strongly decreased after the solution change and remained high by the control plants during the experiment. Acetate, lactate and formate concentration remained low and stable. Also the plants treated with phosphate reduction of both cultivars reacted similar. First, the concentration of malate was increased but it decreased after 5 d again. For acetate and formate no concentration changes were measurable during the experiment. However, just *Glyceria maxima* plants showed an increase of lactate exudation after 5 d of low P treatment. The aluminum treated plants of *Phalaris arundinacea* and *Glyceria maxima* showed a contrary reaction in malate exudation. *Phalaris arundinacea* showed a decrease already 2 d after the solution change up to zero and the concentration remained low, but in the case of *Glyceria maxima* the malate concentration increased after the solution change (2 d) first and decreased again (5 d). Furthermore, the lactate concentration in the nutrient solution of *Glyceria maxima* did not decrease after 5 d in comparison to *Phalaris arundinacea* with Al treatment.

3.4 0.1 mM P content and 1 mM Al

The first experimental series with *Phalaris arundinacea* and *Glyceria maxima* showed no significant increase in exudation due to the treatment. Therefore, the phosphate content was reduced to 0.1 mM in further experiments. The Al concentration was not changed because higher concentrations reduced the root growth (3.2). The treatment duration in the past experiments was 2-3 weeks but problems occurred in differences in biomass between treated and non treated plants and algae growth in the nutrient solutions. Thus, the running time of the series was reduced to one week. Additionally, more species were tested in this new experimental series.

3.4.1 *Arundinella anomala*

Arundinella anomala was treated with 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3).

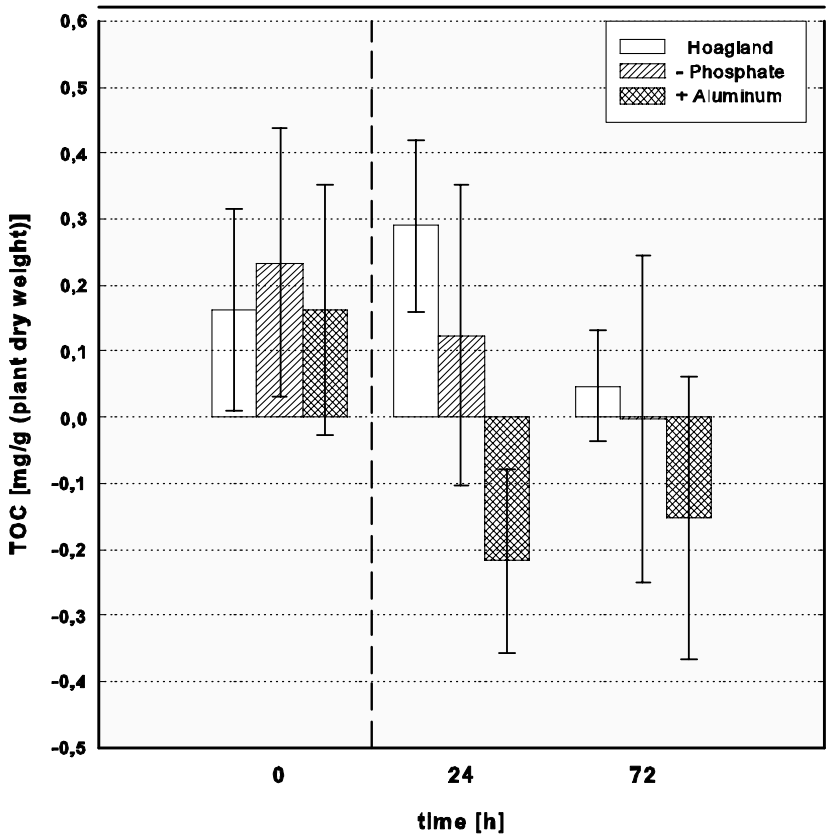


Fig. 22: Total organic carbon (TOC) content (mg) in the nutrient solution of waterlogged Arundinella anomala plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of ten replicates (n=10). Significances tested with repeated measurements p<0.05.

Fig. 22 depicts the TOC content in the nutrient solution of waterlogged *Arundinella anomala* plants. During the experiment significant changes in TOC content for each treatment (p=0) and between the treatments (p=0.012) were measurable. The first sampling revealed no significant differences in nutrient solution's TOC content between the three groups (0 h) (control group 0.16 ± 0.15; P reduction group 0.23 ± 0.20; Al addition group 0.16 ±0.19 [mg/g (plant dry weight)]).

But after the solution change, the TOC content of the Al treatment (-0.21 ± 0.14 [mg/g (plant dry weight)]) was significant lower in comparison to the control (0.29 ± 0.13 [mg/g (plant dry weight)]; p=0) and in comparison to P reduction (0.12 ± 0.23 [mg/g (plant dry weight)]; p=0.048)). Between the Hoagland control and the P reduction treatment no significant differences occurred.

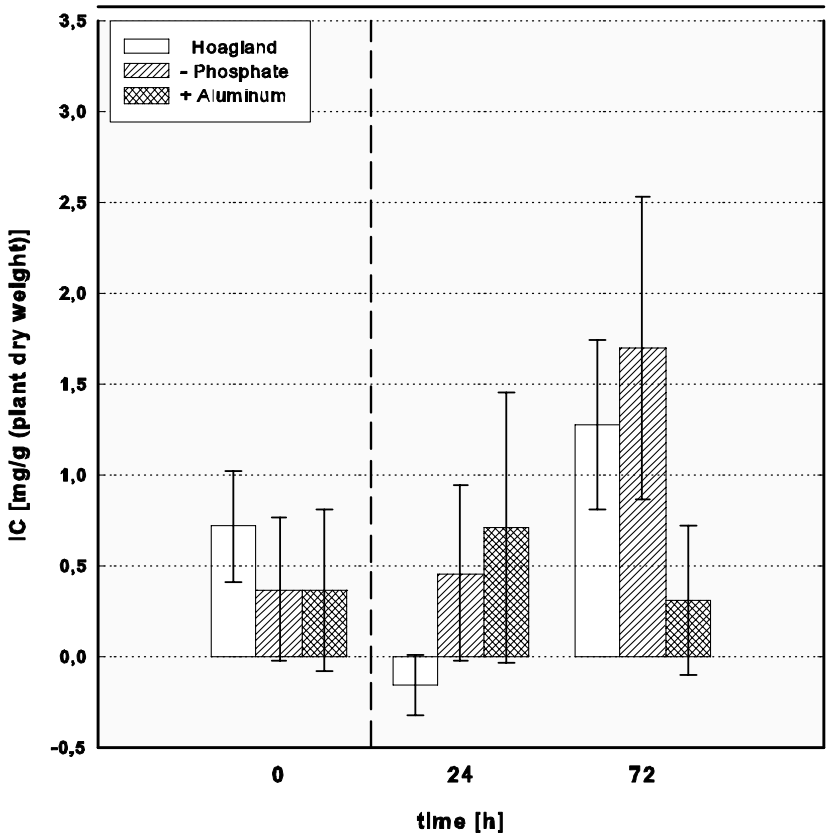


Fig. 23: Inorganic carbon content (mg) in the nutrient solution of waterlogged Arundinella anomala plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution added. Error bars represent ± standard deviation of ten replicates (n=10). Significances tested with repeated measurements p<0.05.

Fig. 23 depicts the IC content in the nutrient solution of waterlogged *Arundinella anomala* plants. During the experiment significant changes in IC content for each treatment (p=0.015) and between the treatments (p=0.017) were measurable. The first sampling revealed no significant differences in nutrient solution's IC content between the three groups (control group 0.71 ± 0.31; P reduction group 0.37 ± 0.39; Al addition group 0.36 ±0.44 [mg/g (plant dry weight)]) (0 h).

After 24 h the IC content in the nutrient solution of the control plants was significantly lower than before changing the solution (-0.16 ± 0.17 [mg/g (plant dry weight)]; p=0). The IC content in the nutrient solutions of the treated plants with 1mM Al (0.71 ± 0.75 [mg/g (plant dry weight)]; p=0.005) or reduced P content (0.1 mM) (0.45 ± 0.48 [mg/g (plant dry weight)]; p=0.003) was significantly higher than for the samples containing 50% Hoagland solution. After 72 h a significant increase in IC content was measurable for the control group (1.27 ± 0.47 [mg/g (plant dry weight)]; p=0) and the treatment with reduced phosphate (1.69 ± 0.84 [mg/g (plant dry weight)]; p=0.001) but no change for the treatment with Al (0.31 ± 0.41 [mg/g (plant dry weight)]; p=0.15). No differences between the control plants in 50% Hoagland solution and the plants 50% Hoagland solution with reduced phosphate (0.1 mM) content occurred after 72 h. In contrast, after 72 h the IC content of the plants in 50% Hoagland solution containing 1mM Al revealed a significantly lower IC content than the plants in 50% Hoagland solution (p=0) or 50% Hoagland solution with reduced phosphate (0.1 mM) content (p=0).

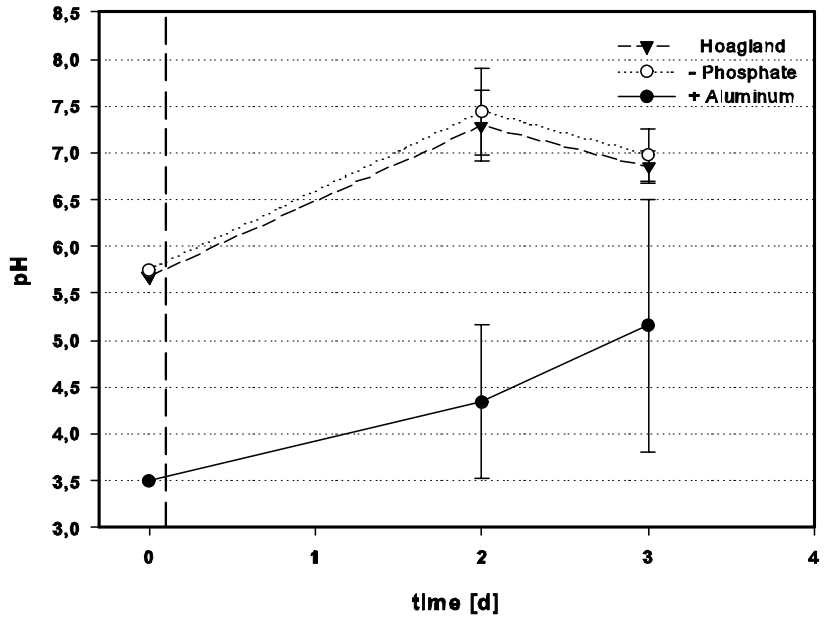


Fig. 24: pH dynamics of Arundinella anomala plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) and 50% Hoagland solution containing Al (1 mM) (2.2.3). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of ten replicates (n=10)

Fig. 24 depicts the pH curves of *Arundinella anomala* plants grown in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) and 50% Hoagland solution containing Al (1 mM) (2.2.3).

The values of the point of time 0 d refer to the stock solutions concentrations. Two days after the fresh solutions were added the nutrient solutions of plants grown in 50% Hoagland solution or 50% Hoagland solution with reduced phosphate content (0.1 mM) reached a neutral pH value (Hoagland control 6.85 ± 0.17 to 7.29 ± 0.38; reduced P 6.98 ± 0.27 to 7.44 ± 0.47). In the nutrient solutions containing 1mM Al the pH raised from 3.5 (Hoagland 1 mM Al stock solution) to 4.34 ± 0.82 (2 d) and 5.16 ± 1.35 (3 d).

3.4.2 Hemarthria altissima

Hemarthria altissima was also treated with 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3).

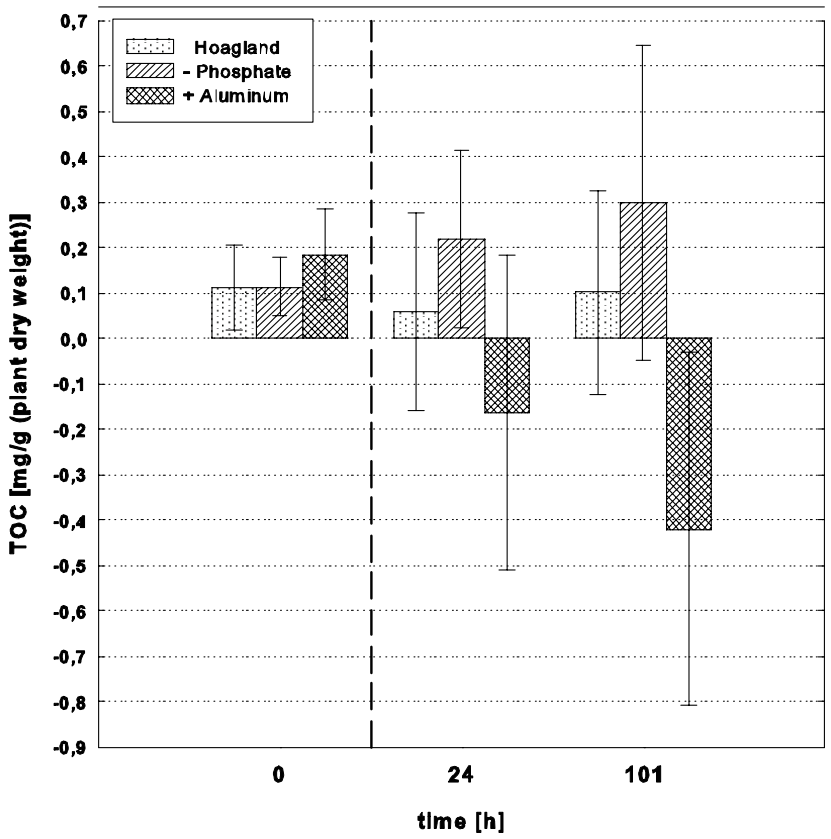


Fig. 25: Total organic carbon (TOC) content (mg) in the nutrient solution of waterlogged Hemarthria altissima plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution added. Error bars represent ± standard deviation of ten replicates (n=10). Significances tested with repeated measurements p<0.05.

Fig. 25 depicts the TOC content in the nutrient solution of waterlogged *Hemarthria altissima* plants. During the experiment no significant changes in TOC content for each treatment (p=0.184) but between the treatments (p=0) were measurable. Before the treatments no significant differences occurred between the compared groups (control group 0.11 ± 0.09; P reduction group 0.11± 0.06; Al addition group 0.19 ± 0.1 [mg/g (plant dry weight)]).

After the solution change significant differences between all treatments were measurable (24 h and 101 h). The P reduction treatment induced a significant increase of TOC content to 0.22 ± 0.2 [mg/g (plant dry weight)] compared to the Al addition group (-0.16 ± 0.35 [mg/g (plant dry weight)]; p=0) but not compared to the control group (0.06 ± 0.22 [mg/g (plant dry weight)]) (24 h). Especially after 101 h the Al treatment group showed a strong decrease of TOC (-0.42 ± 0.39 [mg/g (plant dry weight)]) content compared to the control group (0.28 ± 0.22 [mg/g (plant dry weight)]; p=0.005) and the P reduction treatment (0.30 ± 0.35 [mg/g (plant dry weight)]; p=0). Furthermore, the difference between the control group and the P reduction group was significant (p=0) after 101 h.

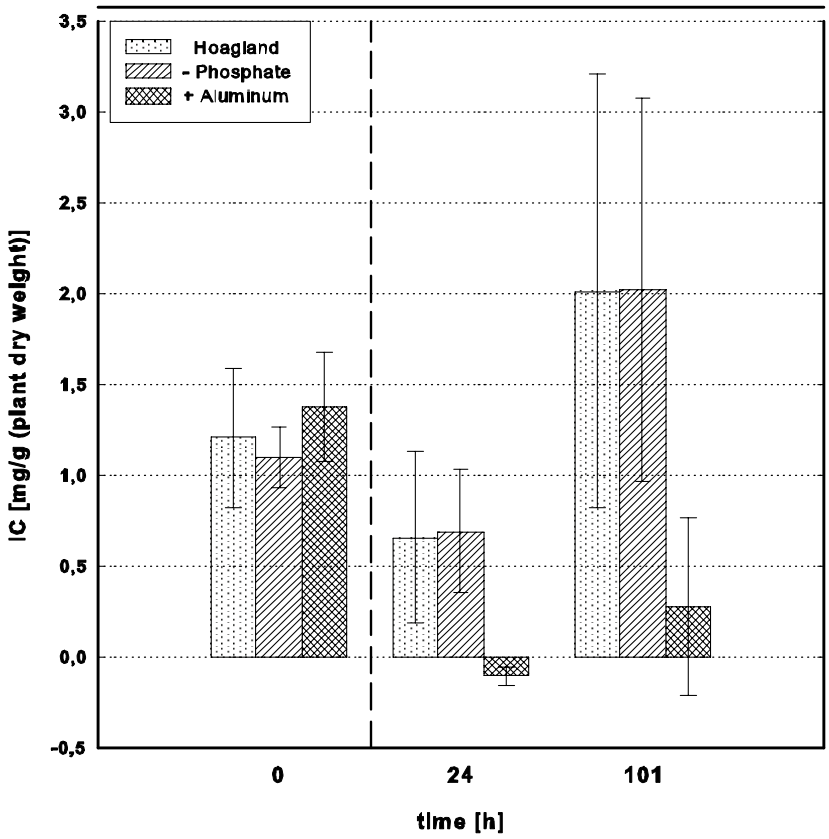


Fig. 26: Inorganic carbon content (mg) in the nutrient solution of waterlogged Hemarthria altissima plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of ten replicates (n=10). Significances tested with repeated measurements $p<0.05$.

Fig. 26 depicts the IC content in the nutrient solution of waterlogged *Hemarthria altissima* plants. During the experiment significant changes in IC content for each treatment (p=0) and between the treatments (p=0.001) were measurable. Before the treatments no significant differences occurred between the compared groups (control group 1.2 ± 0.38; P reduction group 1.1 ± 0.17; Al addition group 1.38 ± 0.3 [mg/g (plant dry weight)]). After 24 h the IC content in the nutrient solution was significantly lower for all treatments than before the solution changing (control group 0.66 ± 0.47; p=0.01, P reduction group 0.69 ± 0.34; p=0.01 and Al addition group -0.11 ± 0.05; p=0.01 [mg/g (plant dry weight)]). Furthermore, the IC content of the Al treatment was significant lower as the control group (p=0) and the P reduction group (p=0). After 101 h a significant increase in IC content was measurable for all treatments (p=0.001). Between the first (0 h) and the last sampling (72 h) no significant difference occurred. Furthermore, the IC content of the samples containing 1mM Al (p=0) and for the samples with reduced P content (p=0) was significantly lower, than for the samples containing 50% Hoagland solution.

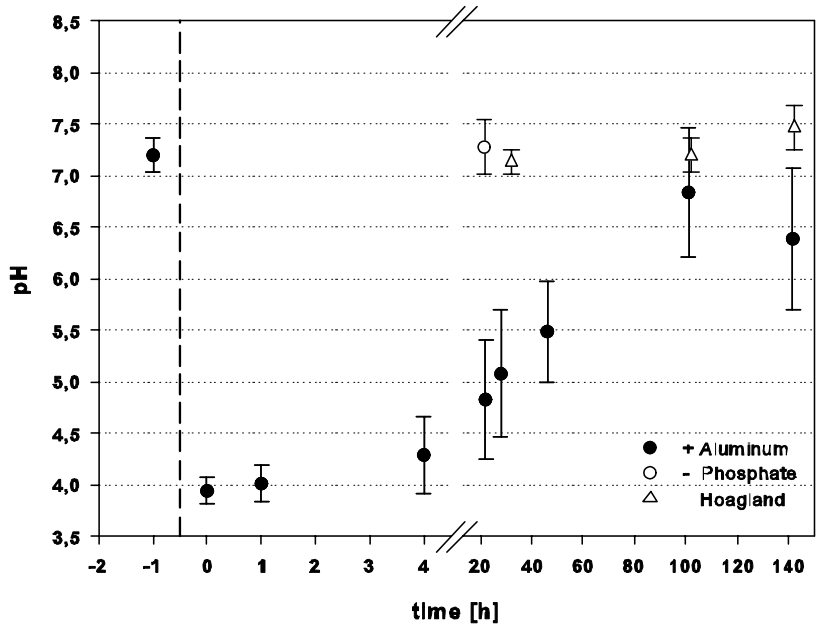


Fig. 27: pH dynamics of Hemarthria altissima plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) and 50% Hoagland solution containing Al (1 mM) (2.2.3). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. At the beginning of the experiment (0 d) all plants were measured in 50% Hoagland solution and afterwards the solution was changed against 50% Hoagland solution containing Al (1 mM) ((2.2.3). Error bars represent ± standard deviation of ten replicates (n=10)

The time depending pH curve (Fig.27) depicts that the pH in the Al containing nutrient solution of waterlogged *Hemarthria altissima* plants increased already four hours after replacing the 50% Hoagland solution against 50% Hoagland solution containing 1 mM Al (2.2.3). After 46 h, the pH raised from 3.94 ± 0.13 to 5.5 ± 0.49 and after 101 h the pH reached the maximal value of 6.84 ± 0.62. The pH of control plants grown in 50% Hoagland solution remained between 7.14 ± 0.12 and 7.47 ± 0.21 and plants grown in 50% Hoagland solution containing 0.1 mM phosphate reached a pH of 7.28 ± 0.07.

3.4.3 Spartina anglica

Spartina anglica was also treated with 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3).

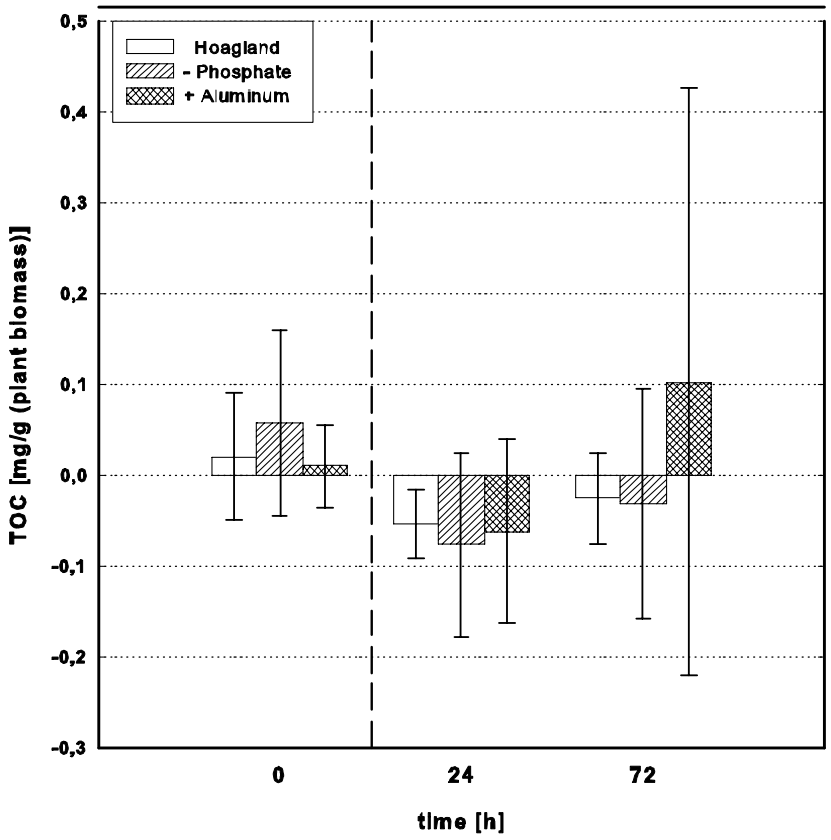


Fig. 28: Total organic carbon (TOC) content (mg) in the nutrient solution of waterlogged Spartina anglica plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of ten replicates (n=10). Significances tested with repeated measurements p<0.05.

Fig. 28 depicts the TOC content in the nutrient solution of waterlogged *Spartina anglica* plants. During the experiment significant changes in TOC content for each treatment (p=0.032) but not between the treatments (p=0.306) were measurable. *Spartina anglica* plants waterlogged in 50% Hoagland solution with reduced phosphate (0.1 mM) content or in 50% Hoagland solution containing 1 mM Al did not reveal a higher or lower TOC content than the control plants in 50% Hoagland solution after 0 h (control group 0.2 ± 0.07; P reduction group 0.06 ± 0.1; Al addition group 0.01 ± 0.05 [mg/g (plant dry weight)]).

Also after 24 h the treated plants did not reveal a different TOC content as the control plants (control group -0.05 ± 0.04; P reduction group -0.08 ± 0.1; Al addition group -0.06 ± 0.1 [mg/g (plant dry weight)]) and after 72 h (control group -0.02 ± 0.05; P reduction group -0.03 ± 0.13; Al addition group 0.1 ± 0.32 [mg/g (plant dry weight)]), but all groups showed a significant decrease after 24 h (p=0.013).

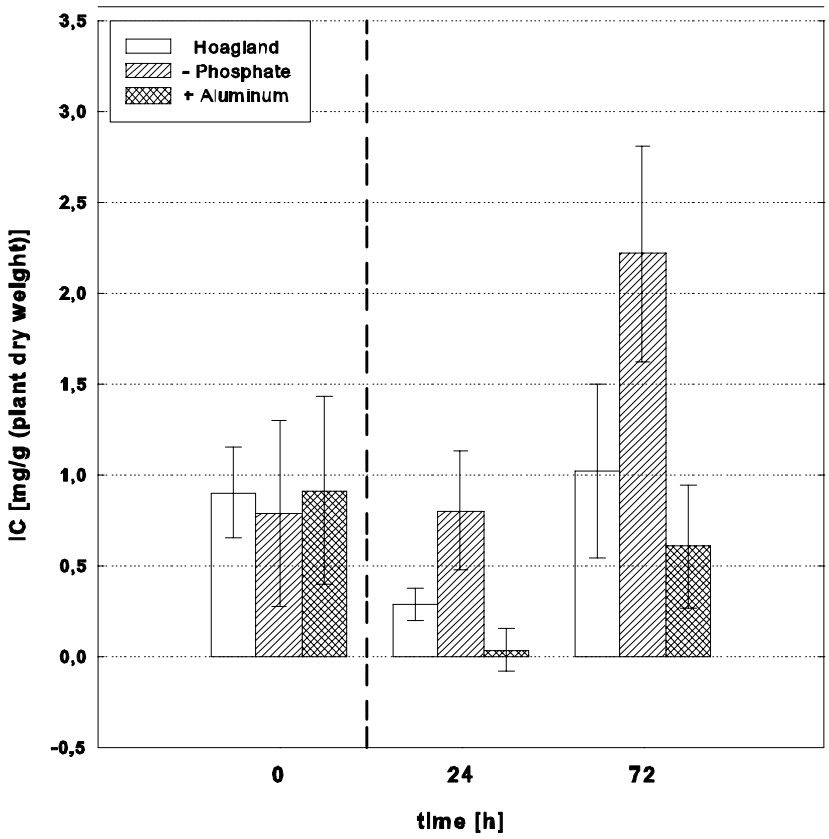


Fig. 29: Inorganic carbon content (mg) in the nutrient solution of waterlogged Spartina anglica plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. Error bars represent ± standard deviation of ten replicates (n=10). Significances tested with repeated measurements p<0.05.

Fig. 29 depicts the IC content in the nutrient solution of waterlogged *Spartina anglica* plants. During the experiment significant changes in IC content for each treatment (p=0) and between the treatments (p=0) were measurable.

The first sampling revealed no significant differences in nutrient solution's IC content between the three groups (control group 0.9 ± 0.25; P reduction group 0.8 ± 0.51; Al addition group 0.91 ± 0.51 [mg/g (plant dry weight)]).

After 24 h the IC content in the nutrient solution of the Al treated plants (0.033 ± 0.11 [mg/g (plant dry weight)]) was significantly lower than the both treatments (in comparison to P reduction 0.80 ± 0.32 [mg/g (plant dry weight)]; p=0, compared to Hoagland 0.29 ± 0.09 [mg/g (plant dry weight)]; p=0). Furthermore, the IC content in the nutrient solution of the control plants was significant lower as before the solution changing (p=0) and significant different as both treatments (in comparison to P reduction p=0; compared to Al addition p=0). The IC content in the nutrient solutions of the treated plants with reduced P content (0.1 mM) was significantly higher than for the samples containing 50% Hoagland solution (p=0) or 50% Hoagland solution with 1 mM Al (p=0) (24 h). After 72 h a significant increase in IC content was measurable for all treatments. The samples of the treatment with reduced phosphate (2.21 ± 0.59 [mg/g (plant dry weight)]) depicted a significant higher IC content than the Al treatment (0.6 ± 0.34 [mg/g (plant dry weight)]; p=0) and the control group 1.02 ± 0.48 [mg/g (plant dry weight)]; p=0.04).

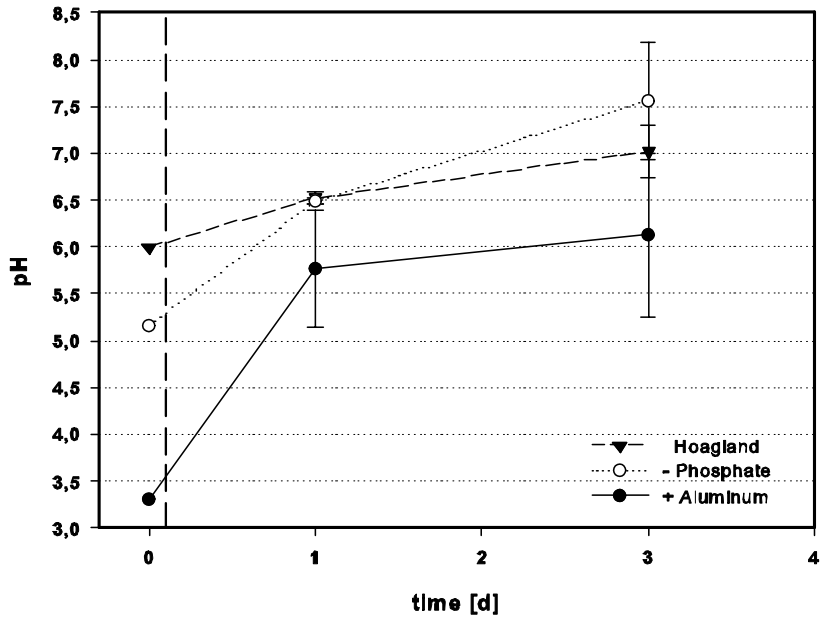


Fig. 30: pH dynamics of Spartina anglica plants grown waterlogged in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. At the beginning of the experiment (0 d) the measured value referred to the stock solutions (50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) or 50% Hoagland solution containing Al (1 mM) (2.2.3)). Error bars represent ± standard deviation of ten replicates (n=10)

Fig. 30 depicts the pH curves of *Spartina anglica* plants grown in 50% Hoagland solution, 50% Hoagland solution with reduced phosphate content (0.1 mM) and 50% Hoagland solution containing Al (1 mM) (2.2.3). The values of the point of time 0 d refer to the stock solutions concentrations and not to the soil solution samples. The pH of nutrient solutions of plants grown in 50% Hoagland solution or 50% Hoagland solution with reduced phosphate content (0.1 mM) remained neutral after 3 d treatment (Hoagland control 7.02 ± 0.28 (3 d); Hoagland with reduced P content 7.56 ± 0.62 (3 d)). In the nutrient solutions containing 1 mM Al the pH rose from 3.31 (stock solution) to 5.77 ± 0.62 and 6.13 ± 0.89 after 2 d and 3d, respectively.

3.4.4 Oryza sativa

Two rice cultivars (*Oryza sativa* Milyang 23 (phosphate deficiency tolerant) and *Oryza sativa* IR 5440-1-1-3 (phosphate deficiency intolerant)) were treated with 50% Hoagland solution or 50% Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3) for one week to study their ability to adapt on reduced phosphate supply. Furthermore, the cultivation under sterile and non sterile conditions was compared.

3.4.4.1 Non sterile *Oryza sativa* plants

The plants were cultivated in sand pots and samples were taken two times in Hoagland solution (13 d and 17 d since start of waterlogging) and two times in Hoagland solution with reduced phosphate content (0.1 mM) (20 d and 24 d since start of waterlogging). This procedure was the same like for the other non sterile plants (discussed in the chapters 3.3.1 to 3.4.2).

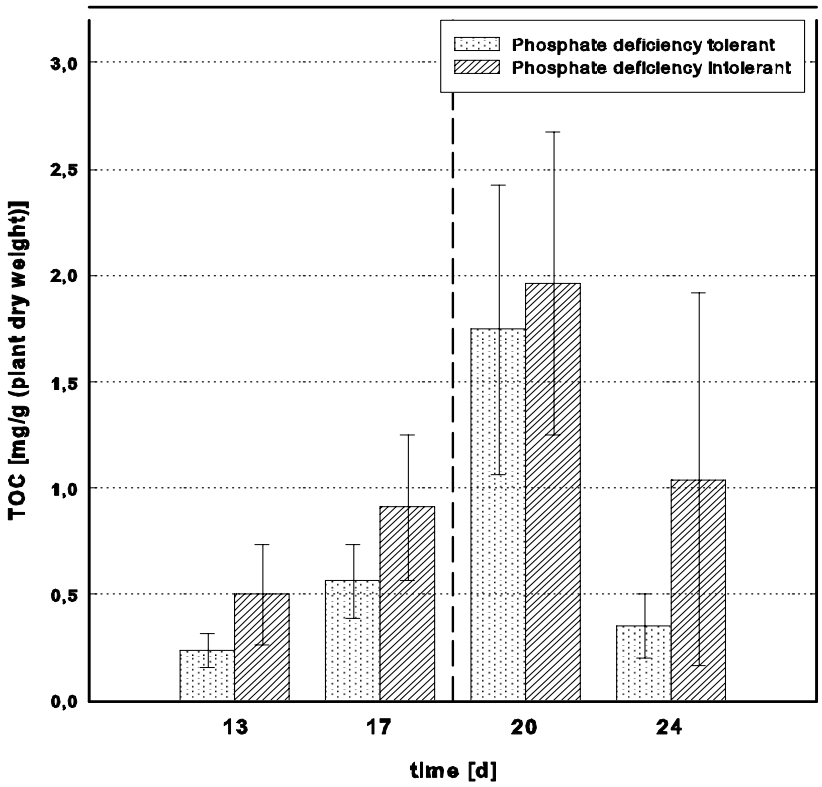


Fig. 31: Total organic carbon (TOC) content (mg) in the nutrient solution of waterlogged non sterile Oryza sativa plants (Oryza sativa Milyang 23 (phosphate deficiency tolerant) and Oryza sativa IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution with reduced P content was added. Error bars represent ± standard deviation of twenty replicates (n=20) after 13 d, 17 d and 20 d; (n=10) after 24 d. Significances tested with t-test p<0.05.

Fig. 31 depicts the TOC content in the nutrient solution of waterlogged *Oryza sativa* Milyang 23 (phosphate deficiency tolerant) and *Oryza sativa* IR 5440-1-1-3 (phosphate deficiency intolerant) plants. During the experiment significant changes in TOC content for each cultivar and between the cultivars were measurable. The first samples were taken in 50% Hoagland solution (Milyang 23 0.56 ± 0.17 [mg/g (plant dry weight)]; IR 5440-1-1-3 0.91 ± 0.34 [mg/g (plant dry weight)] after 17 d). Afterwards the solution was discharged and fresh 50% Hoagland solution with reduced phosphate (0.1 mM) content was added (dashed vertical line).

The TOC content of *Oryza sativa* Milyang 23 increased significantly during the experiment until 20 d (1.75 ± 0.68 [mg/g (plant dry weight)]; p=0); (13 d / 17 d p=0; 17 d/20 d p= 0) but after 24 d the TOC content significantly decreased to 0.35 ± 0.15 [mg/g (plant dry weight)] (20 d / 24 d p=0) to a similar value as at 13 d. A similar development occurred for *Oryza sativa* IR 5440-1-1-3. The TOC content of *Oryza sativa* IR 5440-1-1-3 was significantly different between the both samplings in Hoagland (13 d / 17 d p=0) and significantly increased after the change of the solution until 20 d (1.96 ± 0.71 [mg/g (plant dry weight)]; 17 d / 20 d p=0.006). After 24 d the TOC content significantly decreased to 1.04 ± 0.88 (20 d / 24 d p=0) to a similar value as at 13 d.

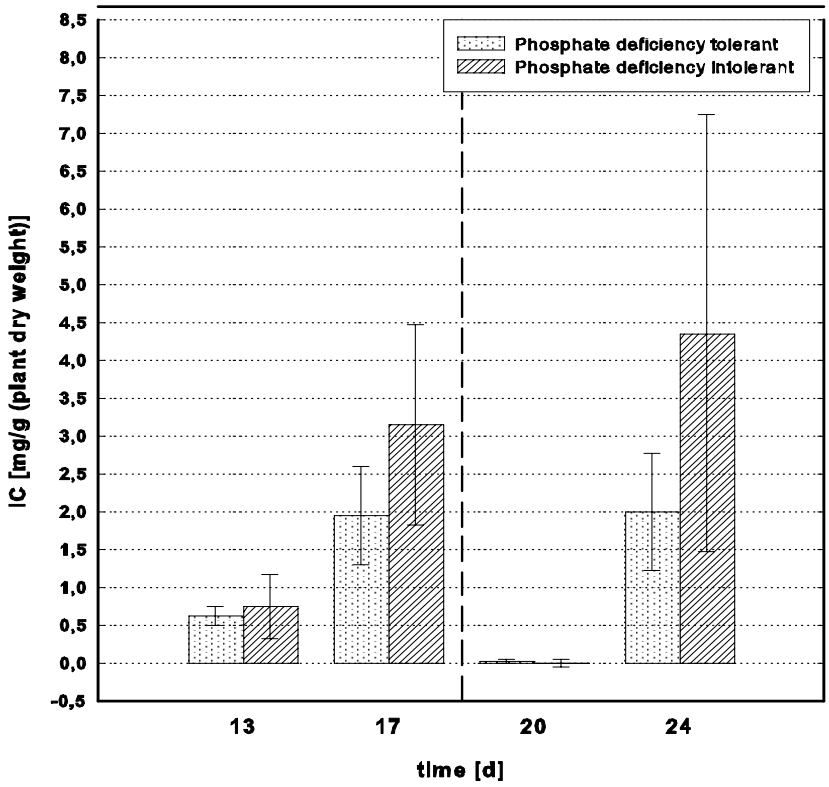


Fig. 32: Inorganic carbon content (mg) in the nutrient solution of waterlogged non sterile Oryza sativa plants (Oryza sativa Milyang 23 (phosphate deficiency tolerant) and Oryza sativa IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution with reduced P content was added. Error bars represent ± standard deviation of twenty replicates (n=20) after 13 d, 17 d and 20 d; (n=10) after 24 d. Significances tested with repeated measurements p<0.05.

Fig. 32 depicts the Inorganic carbon (IC) content (mg) in the nutrient solution of waterlogged non sterile *Oryza sativa* plants per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. The first samples were taken in 50% Hoagland solution.

The IC content after 17 d (Milyang 23 1.95 ± 0.65 [mg/g (plant dry weight)]; IR 5440-1-1-3 3.15 ± 1.32 [mg/g (plant dry weight)] (17 d)) was significant higher (p=0) than after 13 d (Milyang 23 0.61 ± 0.13 [mg/g (plant dry weight)]; IR 5440-1-1-3 0.75 ± 0.42 [mg/g (plant dry weight)] (13 d)). Afterwards the solution was discharged and fresh 50% Hoagland solution with reduced phosphate (0.1 mM) content was added (dashed vertical line). The IC content of *Oryza sativa* Milyang 23 decreased after the solution change (0.02 ± 0.03 [mg/g (plant dry weight)]; p=0) but after 24 d the IC content increased again (1.99 ± 0.77 [mg/g (plant dry weight)]; p=0). A similar development occurred for *Oryza sativa* IR 5440-1-1-3. The IC content of *Oryza sativa* IR 5440-1-1-3 increased significantly after 17 d in Hoagland (3.15 ± 1.32 [mg/g (plant dry weight)]; p=0) but decreased after the change of the solution (0 ± 0.04 [mg/g (plant dry weight)]; p=0). After 24 d the IC content increased to 4.35 ± 2.89 [mg/g (plant dry weight)] (p=0).

The nutrient solution of ten plants was completely changed again after 20 d and ten plants remained in the old solution. This allows a comparison with the sterile rice plants, as their solution was also changed after 20 d (see chapter 3.4.5.2).

Tab. 2: Comparison of the total organic carbon (TOC) and inorganic carbon content (mg) in the nutrient solution of waterlogged non sterile Oryza sativa plants (Oryza sativa Milyang 23 (phosphate deficiency tolerant) and Oryza sativa IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g) after 24 d. The solution of the plants was either changed after 20 d or not.

24 d Milyang 23	TOC mg/g (plant dry weight)	STD TOC ± mg/g (plant dry weight)	IC mg/g (plant dry weight)	STD IC ± mg/g (plant dry weight)
no change	0.35	0.15	1.99	0.77
change	0.24	0.06	1.73	0.44

24 d IR 5440-1-1-3	TOC mg/g (plant dry weight)	STD TOC ± mg/g (plant dry weight)	IC mg/g (plant dry weight)	STD IC ± mg/g (plant dry weight)
no change	1.04	0.88	4.35	2.89
change	0.49	0.40	2.90	1.83

Tab. 2 depicts the TOC and IC content in the nutrient solution of waterlogged *Oryza sativa* Milyang 23 (phosphate deficiency tolerant) and *Oryza sativa* IR 5440-1-1-3 (phosphate deficiency intolerant) plants after 24 d. After 20 d the 50% Hoagland solution with reduced P content (0.1 mM) of 10 replicates was discharged and fresh solution added and 10 replicates remained in the old solution. The TOC content as well as the IC content of the plants which remained in their solution was higher than for the plants whose solution was changed. For example, the measured TOC content of the cultivar Milyang 23 was 0.35 ± 0.15 [mg/g (plant dry weight)] without solution change and 0.24 ± 1.73 [mg/g (plant dry weight)] with a solution change.

3.4.4.2 Sterile *Oryza sativa* plants

Rice was the only plant which could be successfully cultivated in a sterile system and afford a comparison of the released organic compounds under sterile and non sterile conditions. This made it possible, to quantify the organic acids and sugars released by the plants without any interference by soil microorganisms.

At the beginning of the experiment (13 d and 17 d) all plants were measured in 50% Hoagland solution and afterwards the solution was changed against 50% Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3). After 20 d the solution was discharged and fresh Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3) added.

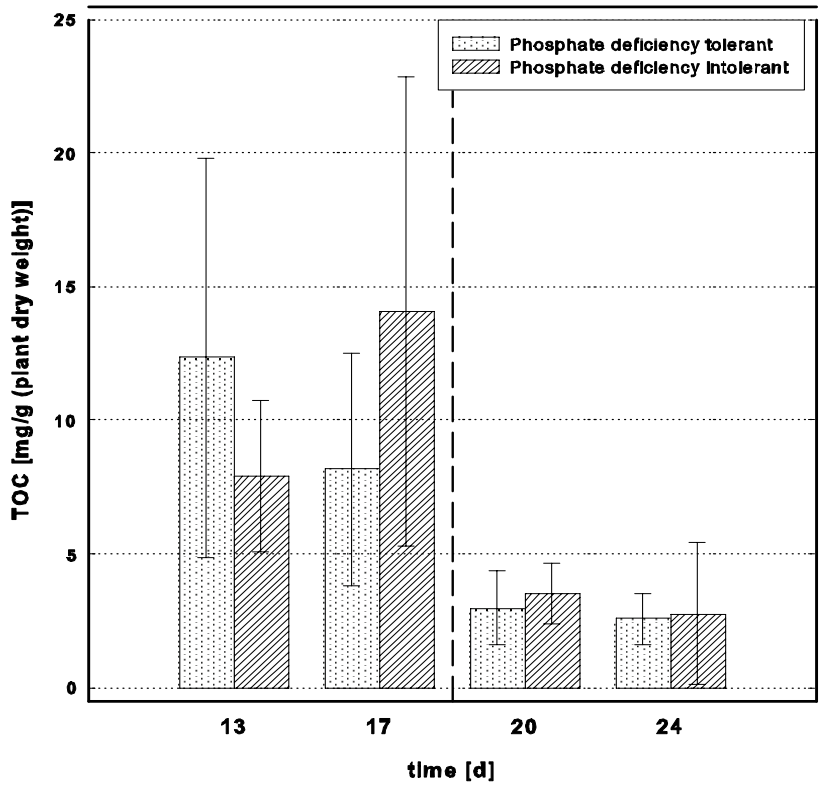


Fig. 33: Total organic carbon (TOC) content (mg) in the nutrient solution of waterlogged sterile Oryza sativa plants (Oryza sativa Milyang 23 (phosphate deficiency tolerant) and Oryza sativa IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution with reduced P content was added. Error bars represent ± standard deviation of six replicates (n=6). Significances tested with repeated measurements $p<0.05$ and t-test.

Fig. 33 depicts the Total organic carbon (TOC) content (mg) in the nutrient solution of waterlogged sterile *Oryza sativa* plants (*Oryza sativa* Milyang 23 (phosphate deficiency tolerant) and *Oryza sativa* IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. For both cultivars the TOC content decreased after the solution change (20 d and 24 d) but these changes were not significant. The TOC content of Milyang 23 was around 12.35 ± 7.46 [mg/g (plant dry weight)] after 13 d in 50% Hoagland solution and decreased after the solution change to 2.58 ± 0.94 [mg/g (plant dry weight)], 24 d after the experiment started. The TOC content in the sampling solution of the cultivar IR 5440-1-1-3 showed a similar development. After 13 d the TOC content was around 7.87 ± 2.84 [mg/g (plant dry weight)] and decreased to 2.75 ± 2.65 [mg/g (plant dry weight)] after 24 d.

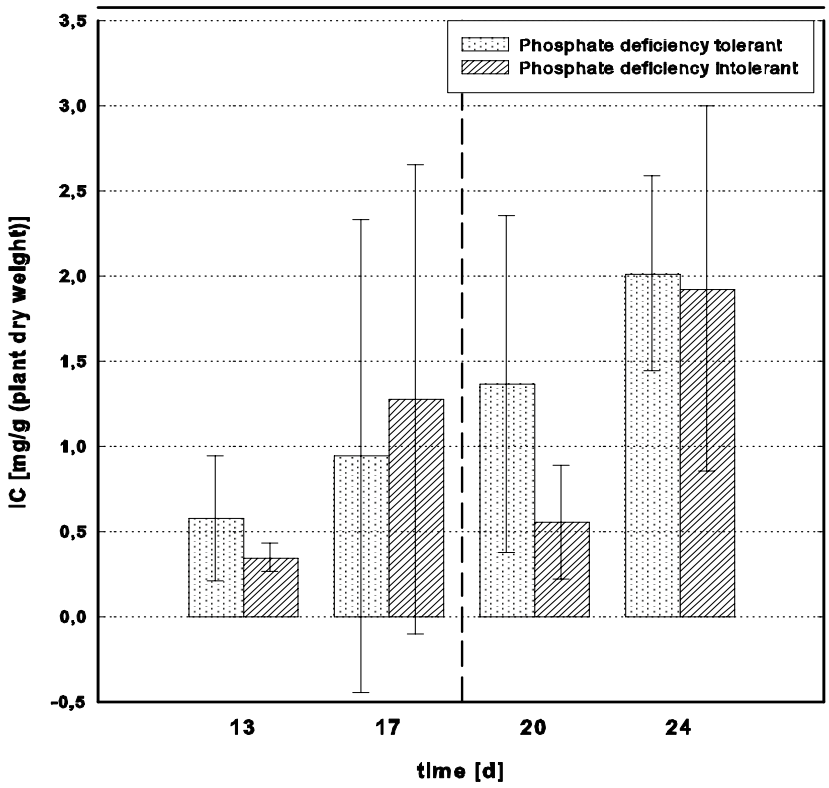


Fig. 34: Inorganic carbon (IC) content (mg) in the nutrient solution of waterlogged sterile Oryza sativa plants (Oryza sativa Milyang 23 (phosphate deficiency tolerant) and Oryza sativa IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution with reduced P content was added. Error bars represent ± standard deviation of six replicates (n=6). Significances tested with repeated measurements and t-test p<0.05.

Fig. 34 depicts the Inorganic carbon (IC) content (mg) in the nutrient solution of waterlogged sterile *Oryza sativa* plants (*Oryza sativa* Milyang 23 (phosphate deficiency tolerant) and *Oryza sativa* IR 5440-1-1-3 (phosphate deficiency intolerant)) per plant dry weight (g). The dashed vertical line represents the point of time when the nutrient solution was discharged and fresh solution was added. During the experiment no significant changes in IC content occurred for the cultivar Milyang 23. The IC content increased during the experiment in 50% Hoagland solution 0.58 ± 0.37 (13 d) and 0.94 ± 1.39 [mg/g (plant dry weight)] (17 d)) as well as in 50% Hoagland with reduced P content (0.1 mM) 1.37 ± 0.99 (20 d) and 2.01± 0.57 (24 d) [mg/g (plant dry weight)]). In contrast, the cultivar IR 5440-1-1-3 also showed no significant changes in 50% Hoagland solution (0.35 ± 0.08 (13 d) and 1.28 ± 1.38 [mg/g (plant dry weight)] (17 d)) but a significant increase of the IC content after 24 d (0.55 ± 0.33 (20 d) and 1.93 ± 1.08 [mg/g (plant dry weight)] (24 d); p=0.01).

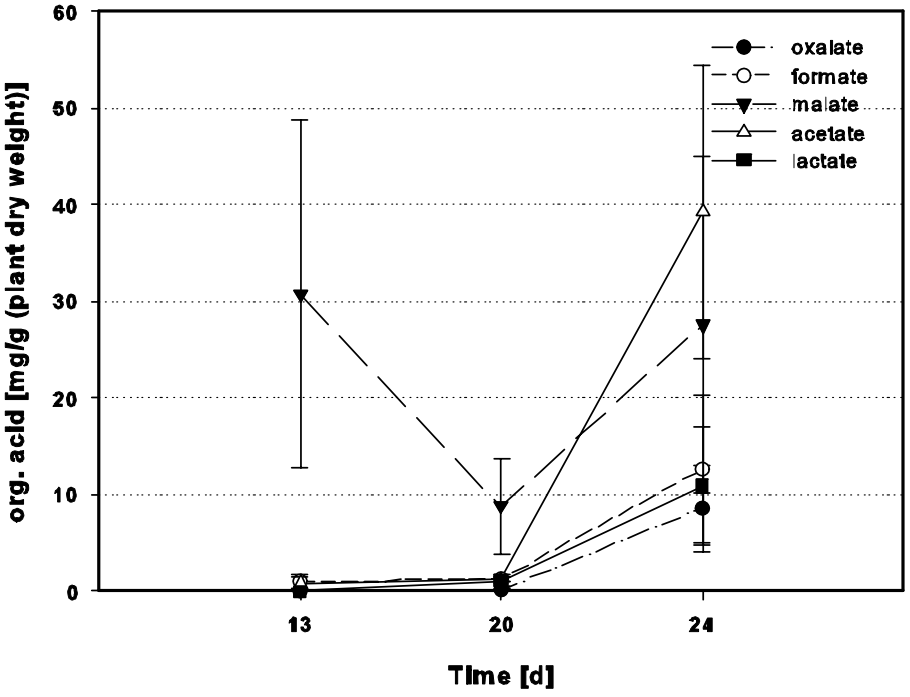


Fig. 35: Concentration of oxalate, formate, malate, acetate and lacate [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3) (20 d and 24 d) of sterile Oryza sativa Milyang 23 plants (*phosphate deficiency tolerant). The dashed vertical line represents the point of time, when the 50% Hoagland solution (i.e. control) was discharged and 50% Hoagland solution with reduced phosphate content (0.1 mM) was added. Error bars represent ± standard deviation of six replicates (n=6). Significances tested with t-test p<0.05.*

Fig. 35 depicts the concentration of oxalate, formate, malate, acetate and lacate [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3) (20 d and 24 d) of sterile *Oryza sativa* Milyang 23 plants (phosphate deficiency tolerant). The dashed vertical line represents the point of time, when the 50% Hoagland solution (i.e. control) was discharged and 50% Hoagland solution with reduced phosphate content (0.1 mM) was added. The acetate, oxalate content as well as the formate content remained stable between 13 d and 20 d but increased after 24 d. Acetate increased from 1.22 ± 0.17 (17 d) up to 39.29 ± 15.3 (24 d) [mg/g (plant dry weight)] (p=0.002), oxalate from 0.18 ± 0.4 (17 d) up to 8.6 ± 4.4 (24 d) [mg/g (plant dry weight)] (p=0.005) and formate from 1.06 ± 0.7 (20 d) to 12.65 ± 7.68 (24 d) [mg/g (plant dry weight)] (p=0.01). The malate content decreased after 20 d and increased after 24 d again. First it decreased from 30.75 ± 17.96 (13 d) to 8.84 ± 5.01 (17 d) (p=0) and then it increased to 27.64 ± 17.48 (24 d) [mg/g (plant dry weight)]. The lactate content increased from 0 (13 d) to 1.1 ± 0.66 (20 d) [mg/g (plant dry weight)] and to 10.94 ± 6.09 (24 d) [mg/g (plant dry weight)] (p=0.01).

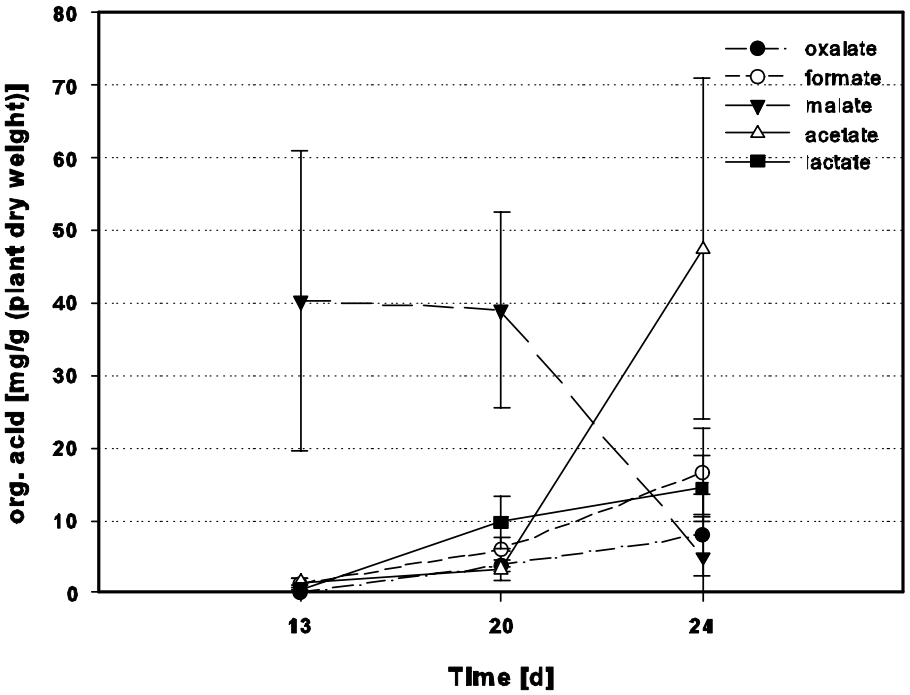


Fig. 36: Concentration of oxalate, formate, malate, acetate and lacate [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3) (20 d and 24 d) of sterile Oryza sativa IR 5440-1-1-3 plants *(phosphate deficiency intolerant). The dashed vertical line represents the point of time, when the 50% Hoagland solution (i.e. control) was discharged and 50% Hoagland solution with reduced phosphate content (0.1 mM) was added. Error bars represent ± standard deviation of six replicates (n=6). Significances tested with t-test p<0.05.*

Fig. 36 depicts the concentration of oxalate, formate, malate, acetate and lacate [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (2.2.3) (20 d and 24 d) of sterile *Oryza sativa* IR 5440-1-1-3 plants (phosphate deficiency intolerant). The dashed vertical line represents the point of time, when the 50% Hoagland solution (i.e. control) was discharged and 50% Hoagland solution with reduced phosphate content (0.1 mM) was added. Lactate, oxalate and formate showed an increase during the experiment. The formate content increased from 1.2 ± 0.7 (13 d) and 6 ± 1.55 (20 d) to 16.59 ± 6.22 (24 d) [mg/g (plant dry weight)] (p=0.04), oxalate from 0.04 ± 0.11 (13 d) and 3.79 ± 1.99 (20 d) to 8.01 ± 5.54 (24 d) [mg/g (plant dry weight)] and lactate from 0.58 ± 0.61 (13 d) and 9.71 ± 3.73 (20 d) to 14.46 ± 4.51 (24 d) [mg/g (plant dry weight)] (p=0.04).

Acetate showed a significant increase from 1.47 ± 0.6 (13 d) and 3.33 ± 0.38 (20 d) to 47.42 ± 23.5 (24 d) [mg/g (plant dry weight)] (p=0.03). In contrast, the malate content decreased after the solution change from 40.31 ± 20.78 (13 d) and 39.01 ± 13.55 (20 d) to 4.93 ± 4.51 [mg/g (plant dry weight)] (p=0.001).

The dynamics of the two cultivars differed during the experiment but only for malate and formate a significant difference occurred after 20 d. The malate content of the cultivar Milyang 23 was significant lower than for the cultivar IR 5440-1-1-3 (p=0.004) and the formate content was significant higher by the cultivar Milyang 23 than by IR 5440-1-1-3 (p=0.002) after 20 d. The phosphate deficiency tolerant cultivar Milyang 23 showed an increase of the concentration of all measured organic acids after the P reduction. In contrast, the phosphate deficiency intolerant cultivar IR 5440-1-1-3 showed a strong decrease of the malate content and a strong increase of the acetate content after the P reduction.

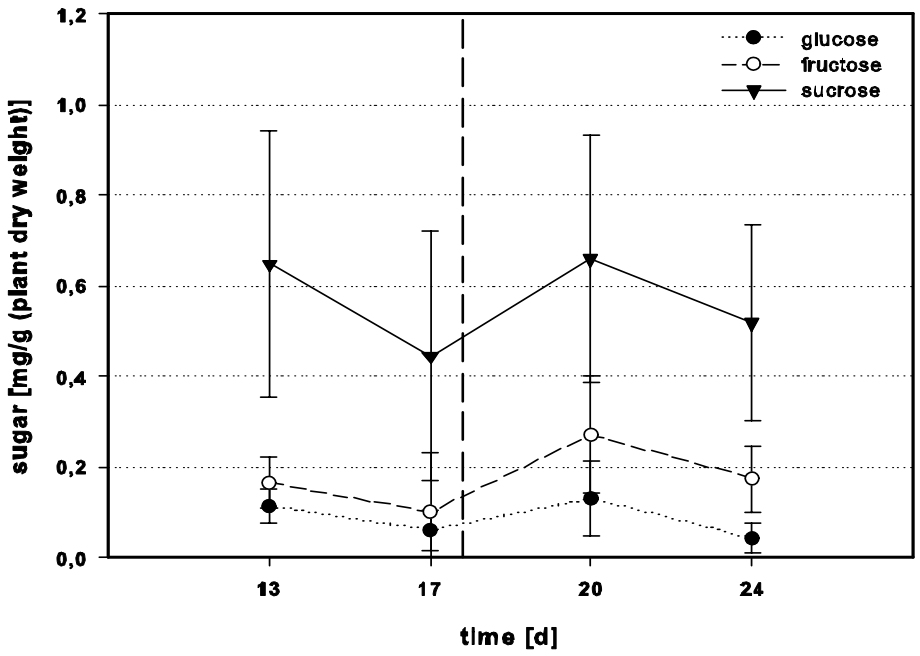


Fig. 37: Concentration of glucose, fructose and sucrose [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d and 17 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (20 d and 24 d) of sterile Oryza sativa Milyang 23 plants (phosphate deficiency tolerant). The dashed vertical line represents the point of time, when the 50% Hoagland solution (i.e. control) was discharged and 50% Hoagland solution with reduced phosphate content (0.1 mM) was added. Error bars represent ± standard deviation of four replicates (n=4). Significances tested with t-test p<0.05.

Fig. 37 depicts the amount of sugars (glucose, fructose and sucrose) [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d and 17 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (20 d and 24 d) of sterile *Oryza sativa* Milyang 23 plants (phosphate deficiency tolerant). The first samples were taken in 50% Hoagland solution (i.e. control) (13 d and 17 d) and no difference between the samples was detectable, yet after the solution change (17 d) the concentration increased. After 17 d the concentration of glucose was 0.06 ± 0.04 [mg/g (plant dry weight)], fructose was 0.1 ± 0.13 [mg/g (plant dry weight)] and sucrose was 0.45 ± 0.27 [mg/g (plant dry weight)]. But after the solution change the glucose concentration increased with a factor of 2.14 to 0.13 ± 0.08 (p=0.056), fructose with a factor of 2.68 to 0.27 ± 0.13 (p=0.001) and sucrose with a factor of 1.48 to 0.66 ± 0.27 (p=0.052) [mg/g (plant dry weight)]. Four days later (24 d) the concentrations decreased (glucose 0.04 ± 0.03 (p=0.02); fructose 0.18 ± 0.07 (p=0.089) and sucrose 0.52 ± 0.22 (p=0.27) [mg/g (plant dry weight)]).

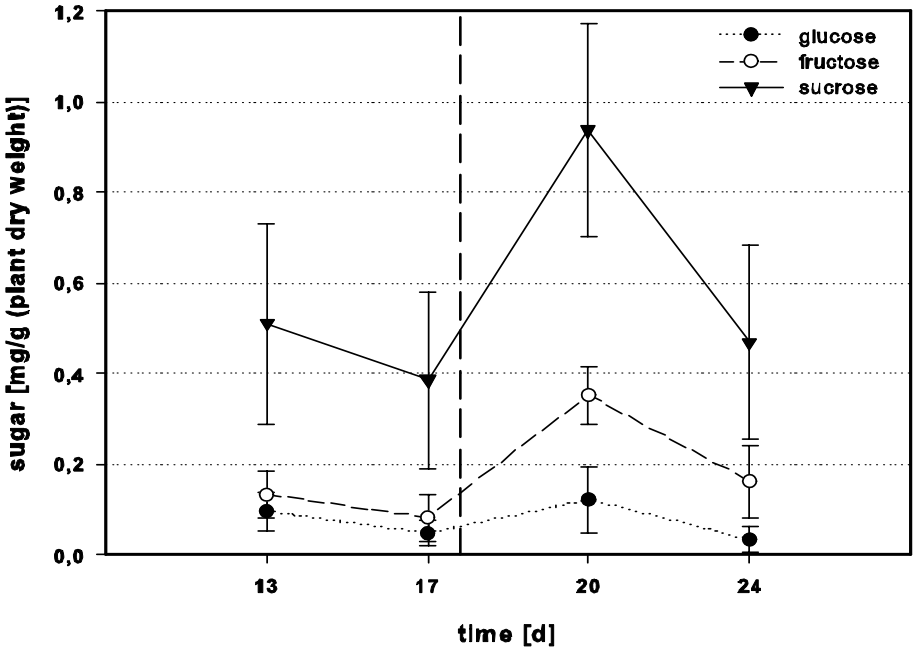


Fig. 38: Concentration of glucose, fructose and sucrose [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d and 17 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (20 d and 24 d) of sterile Oryza sativa IR 5440-1-1-3 plants *(phosphate deficiency intolerant). The dashed vertical line represents the point of time, when the 50% Hoagland solution was discharged and 50% Hoagland solution with reduced phosphate content (0.1 mM) was added. Error bars represent ± standard deviation of four replicates (n=4). Significances tested with t-test p<0.05.*

Fig. 38 depicts the amount of sugars (glucose, fructose and sucrose) [mg/g (plant dry weight)] in 50% Hoagland solution (2.2.3) (13 d and 17 d) and 50% Hoagland solution with reduced phosphate content (0.1 mM) (20 d and 24 d) of sterile *Oryza sativa* IR 5440-1-1-3 plants. The first samples were taken in 50% Hoagland solution (i.e. control) (13 d and 17 d) and no difference between the samples was detectable, yet after the solution change the concentration increased. After 17 d the concentration of glucose was 0.05 ± 0.03, fructose was 0.08 ± 0.05 and sucrose was 0.39 ± 0.20 [mg/g (plant dry weight)]. But after the solution change the glucose concentration increased with a factor of 2.55 to 0.12 ± 0.07 ($p=0.08$), fructose with a factor of 4.25 to 0.36 ± 0.06 ($p=0$) and sucrose with a factor of 2.41 to 0.93 ± 0.24 ($p=0.001$) [mg/g (plant dry weight)]. Four days later (24 d) the concentrations decreased (glucose 0.03 ± 0.03 ($p=0.02$); fructose 0.16 ± 0.08 ($p=0.001$) and sucrose 0.48 ± 0.21 ($p=0.007$) [mg/g (plant dry weight)]). In comparison to the rice cultivar *Oryza sativa* Milyang 23 no significant differences occurred.

3.5 Day rhythm

To figure out, if exudation changed during one day, nutrient solution samples of *Phalaris arundinacea* plants were taken six times during one day.

Tab. 3: Total organic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Plants were sampled six times. Error bars represent ± standard deviation of five replicates (n=5). Significances tested with repeated measurements p<0.05.

	Plants sampled six times	
	TOC	STD
Time	mg/g (plant dry weight)	± mg/g (plant dry weight)
06:30	1.300	0.470
07:30	0.977	0.410
09:30	1.227	0.789
12:30	0.902	0.325
15:30	1.113	0.399
17:30	1.109	0.526

The TOC content in the nutrient solution samples of *Phalaris arundinacea* plants (n=5), which were sampled six times during the day, did not significant change during the day. The measured content of TOC alternated between 0.90 ± 0.33 and 1.3 ± 0.47 [mg/g (plant dry weight)].

Tab. 4: Total organic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Plants were sampled once. Error bars represent ± standard deviation of five replicates (n=5). Significances tested with repeated measurements p<0.05.

	Plants sampled once	
	TOC	STD
Time	mg/g (plant dry weight)	± mg/g (plant dry weight)
06:30	0.846	0.237
07:30	0.678	0.424
09:30	0.851	0.790
12:30	0.987	0.375
15:30	0.875	0.257
17:30	1.307	0.580

The TOC content in the nutrient solution samples of *Phalaris arundinacea* plants (n=5), which were sampled once during the day, did not significant change during the day. The measured content of TOC alternated between 0.68 ± 0.42 and 1.31 ± 0.58 [mg/g (plant dry weight)].

Tab. 5: Inorganic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Plants were sampled six times. Error bars represent ± standard deviation of five replicates (n=5). Significances tested with repeated measurements p<0.05.

	Plants sampled six times	
	IC	STD
Time	mg/g (plant dry weight)	± mg/g (plant dry weight)
06:30	7.026	1.471
07:30	13.653	9.312
09:30	6.200	0.827
12:30	4.966	0.565
15:30	6.575	1.156
17:30	6.602	1.075

The IC content in the nutrient solution samples of *Phalaris arundinacea* plants (n=5), which were sampled six times during the day, did not significant change during the day. The

measured content of IC alternated between 4.97 ± 0.57 and 13.65 ± 9.31 [mg/g (plant dry weight)].

Tab. 6: Inorganic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Plants were sampled once. Error bars represent ± standard deviation of five replicates (n=5). Significances tested with repeated measurements p<0.05.

	Plants sampled once	
Time	IC mg/g (plant dry weight)	STD ± mg/g (plant dry weight)
06:30	9.353	8.233
07:30	8.076	1.328
09:30	8.279	6.279
12:30	5.782	2.436
15:30	8.063	1.349
17:30	8.241	2.715

The IC content in the nutrient solution samples of *Phalaris arundinacea* plants (n=5), which were sampled once during the day, did not significant change during the day. The measured content of IC alternated between 5.78 ± 2.44 and 9.35 ± 8.23 [mg/g (plant dry weight)].

Tab. 2, 3, 4 and 5 depict the TOC and IC content (mg) per plant dry weight (g) in the nutrient solution of waterlogged *Phalaris arundinacea* plants in 50% Hoagland solution during the day. The TOC and IC content of plants, which were sampled once (n=5), and plants, which were sampled six times (n=5) during the day, are compared. No significant difference occurs between the experimental approaches (p=0.577) and furthermore no significant changes during the day (p=0.525) were measurable.

Organic acids

Furthermore, the dynamic of the organic acid content in the nutrient solution was analyzed of the plants, which were sampled six times as well as the plants which were samples once. Between the both sampling procedures no significant differences occurred, thus only the data of the plants sampled six times are shown.

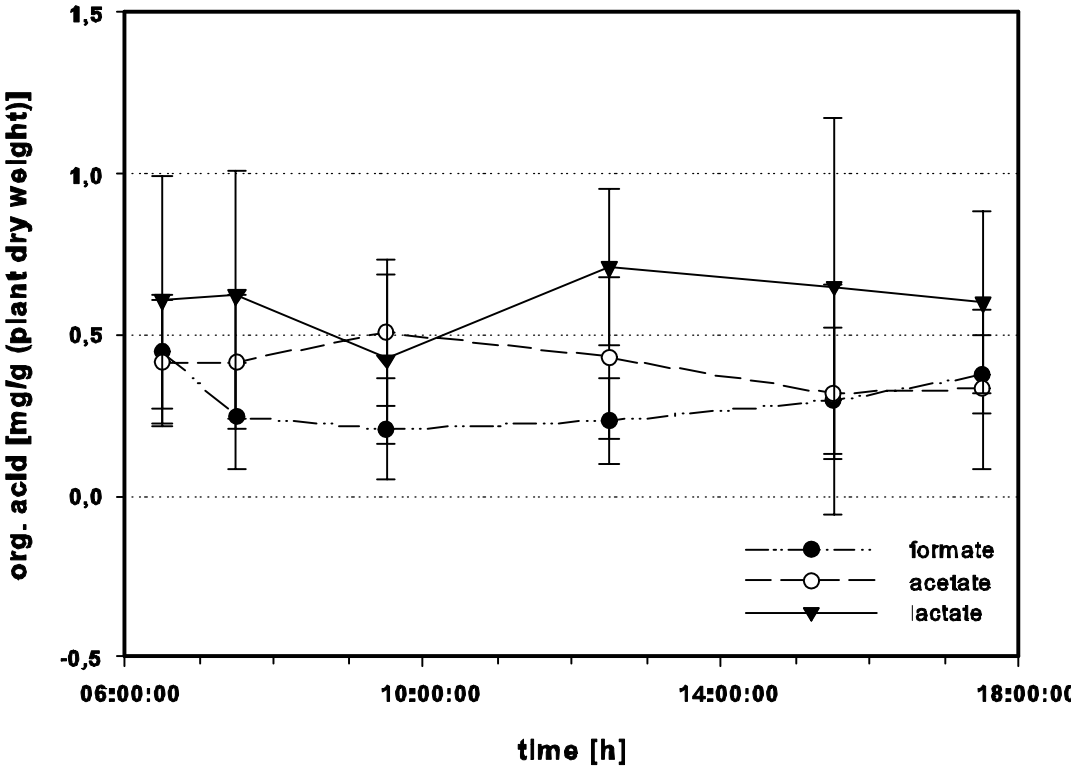


Fig. 39: Content of the organic acids formate, acetate and lactate (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Every plant was sampled six times. Error bars represent ± standard deviation of five replicates (n=5).

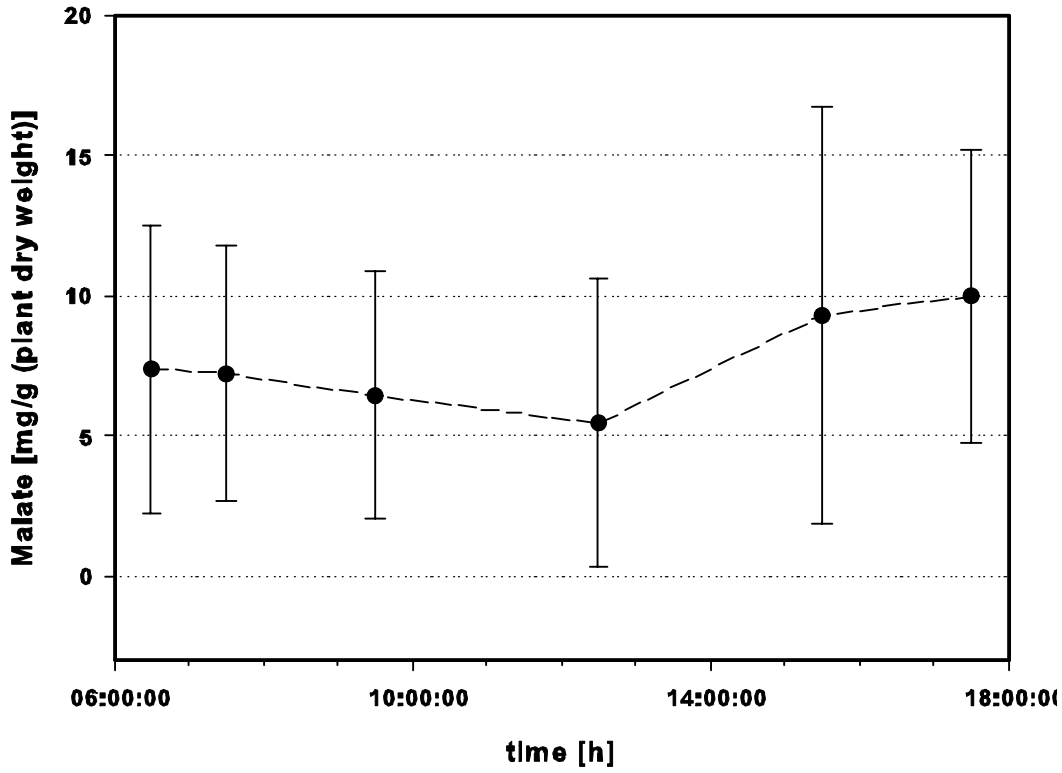


Fig. 40: Content of malate (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Every plant was sampled six times. Error bars represent ± standard deviation of five replicates (n=5)

Fig. 39 and Fig. 40 depict the content of the organic acids acetate, formate, lactate and malate in 50% Hoagland nutrient solution during the day. During the day no significant changes occurred. The content of formate alternated between 0.21 ± 0.16 and 0.45 ± 0.18 [mg/g (plant dry weight)], acetate between 0.32 ± 0.21 and 0.51 ± 0.23 [mg/g (plant dry weight)] and lactate between 0.43 ± 0.26 and 0.65 ± 0.52 [mg/g (plant dry weight)]. The amount of malate in the nutrient solution was higher than of the other acids (5.45 ± 5.10 to 9.99 ± 5.26 [mg/g (plant dry weight)]) but no change in the concentration was detectable.

Furthermore, there were no significant differences between the plants, which were sampled six times and the plants which were sampled once during the day, detectable. The plants sampled once showed no significant changes in the content of the organic acids acetate, formate, lactate and malate in the 50% Hoagland nutrient solution during the day. The content of formate alternated between 0.19 ± 0.12 and 0.40 ± 0.09 [mg/g (plant dry weight)], acetate between 0.20 ± 0.24 and 0.40 ± 0.15 [mg/g (plant dry weight)] and lactate between 0.31 ± 0.29 and 0.84 ± 0.48 [mg/g (plant dry weight)] (see VI).

The amount of malate in the nutrient solution was higher than of the other acids (6.13 ± 8.53 to 9.99 ± 2.75 [mg/g (plant dry weight)]) but also no change in concentration was detectable (see VI).

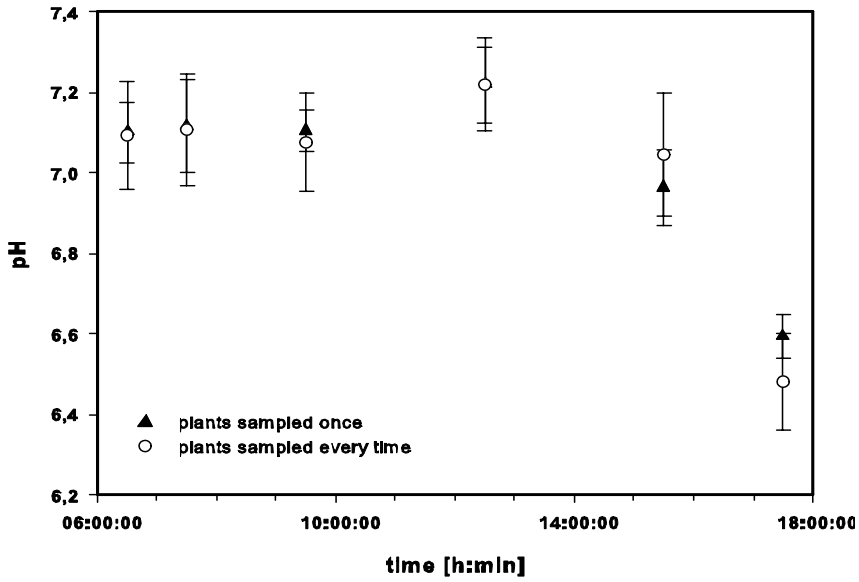


Fig. 41: pH dynamic in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Comparison between plants which were sampled once (n=5) and plants which were sampled six times (n=5). Error bars represent ± standard deviation of five replicates (n=5)

Fig. 41 depicts the pH dynamics in the nutrient solution of waterlogged *Phalaris arundinacea* plants in 50% Hoagland solution (2.2.3) during the day. Plants, which were sampled once, and plants, which were sampled six times, were compared. Plants in both experimental approaches displayed a decrease of the pH in the afternoon. From 6.30 a.m. to 12.30 p.m the pH ranged between 7.10 and 7.22 but in the afternoon it dropped to 6.48 (plants sampled six times) or 6.60 (plants sampled once) (17.30 p.m.)

3.5.1 Al day rhythm

To figure it out, if *Phalaris arundinacea* reacts on Al toxicity with a fast release of organic compounds, plants were grown waterlogged in 50% Hoagland solution. One week later the solution was exchanged against a 50% Hoagland solution containing 1 mM Al. During the day samples were taken six times and analyzed with TOC measurement. An analysis of the organic acids with CE analysis was not possible because the Al blocked the capillary.

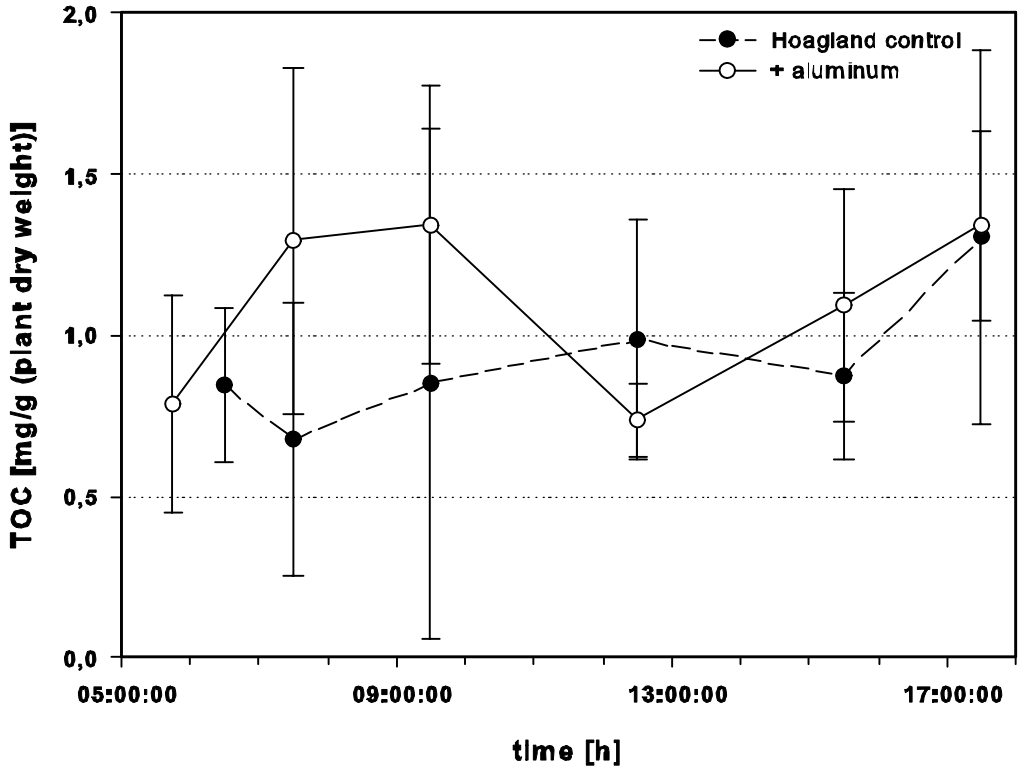


Fig. 42: Organic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) or 50% Hoagland solution containing 1mM Al (2.2.3) during the day. Error bars represent ± standard deviation of five replicates (n=5). Significances tested with repeated measurements p<0.05.

Fig. 42 depicts the TOC content (mg) per plant dry weight (g) in the nutrient solution of waterlogged *Phalaris arundinacea* plants in 50% Hoagland solution (i.e. control) (2.2.3) or 50% Hoagland solution containing 1mM Al (2.2.3) during the day.

During the experiment no significant changes in TOC content for each treatment (p=0.703) and between the treatments (p=0.903) were measurable. The TOC content of the control plants varied between 0.67 ± 0.42 and 1.31 ± 0.56 [mg/g (plant dry weight)] and of the plants treated with Al between 0.73 ± 0.11 and 1.34 ± 0.29 [mg/g (plant dry weight)].

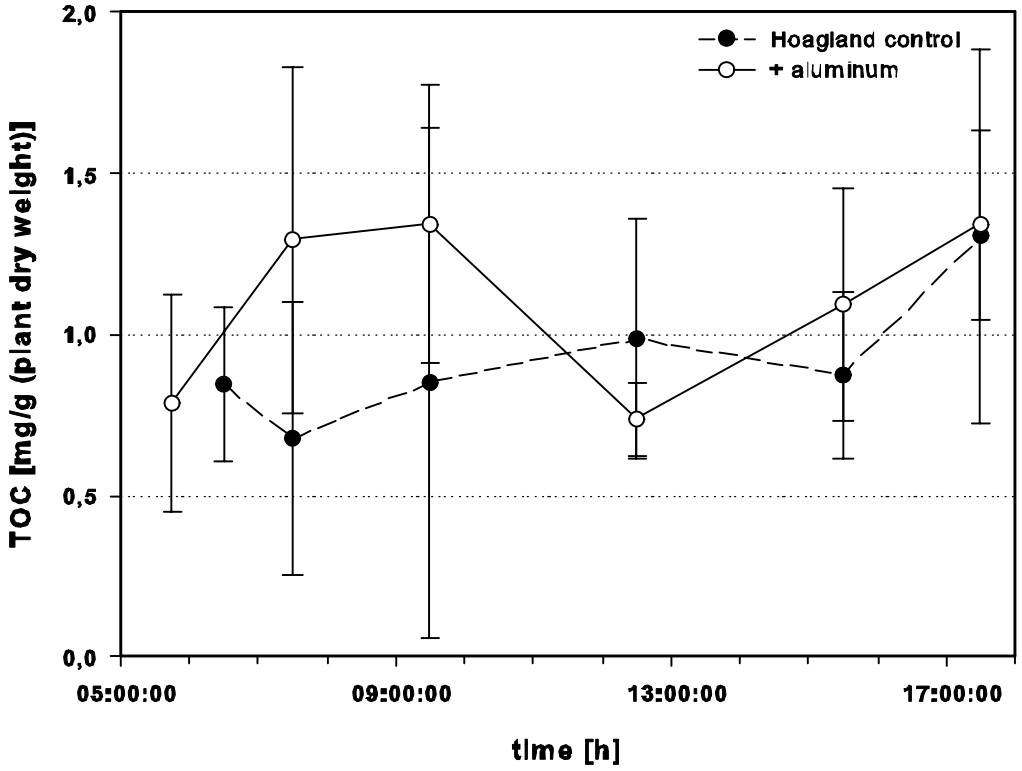


Fig. 43: Inorganic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) or 50% Hoagland solution containing 1mM Al (2.2.3) during the day. Error bars represent ± standard deviation of five replicates (n=5). Significances tested with repeated measurements p<0.05.

Fig. 43 depicts the inorganic carbon content (mg) per plant dry weight (g) in the nutrient solution of waterlogged *Phalaris arundinacea* plants in 50% Hoagland solution (2.2.3) or 50% Hoagland solution containing 1mM Al (2.2.3) during the day.

No significant differences during the day were visible (p=0.241) but the IC content of the Hoagland control plants was significant higher (p=0.001) than of the plants in Hoagland solution containing 1 mM Al. The IC content of the control plants varied between 5.78 ± 2.44 and 9.35 ± 8.23 [mg/g (plant dry weight)] and of the plants treated with Al between 0.06 ± 0.01 and 1.32 ± 0.27 [mg/g (plant dry weight)].

The photosynthetic activity of a treated and a non treated plant were measured during the experiment and no differences arise neither within the treated nor the non treated plant during the day (see VIII).

3.6 NMR Imaging

To test, how strong the root growth is influenced by a reduction of the P supply or through Al addition, *Phalaris arundinacea* plants were cultivated waterlogged in 50% Hoagland (i.e. control), 50% Hoagland with reduced phosphate (0.1 mM) content or 50% Hoagland containing 1 mM Al. After 3, 6 and 9 weeks NMR Imaging was done to document the development of the root systems (n=3).

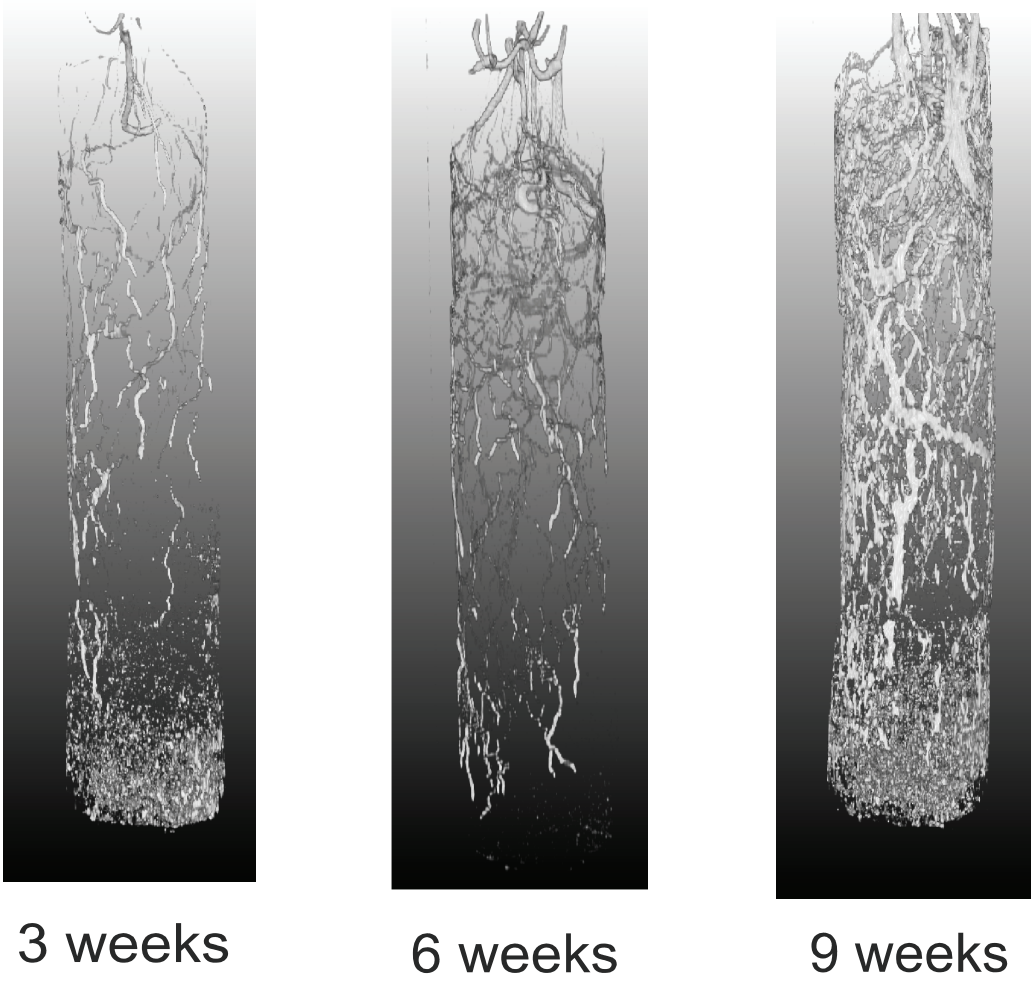


Fig. 44: Exemplary Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

Fig. 44 displays the development of an exemplary *Phalaris arundinacea* plant grown in 50% Hoagland solution for 2 month (n=1). After three weeks of waterlogging (four weeks after germination) the development of a root system started and the roots already reach the ground of the tube. The strong signal in the bottom is water that could not be removed before measuring. Three weeks later the image gave the impression that more and thicker roots were in the tube than after three weeks. After nine weeks the tube was completely filled with roots. Interestingly, the roots preferred to grow at the tube wall.

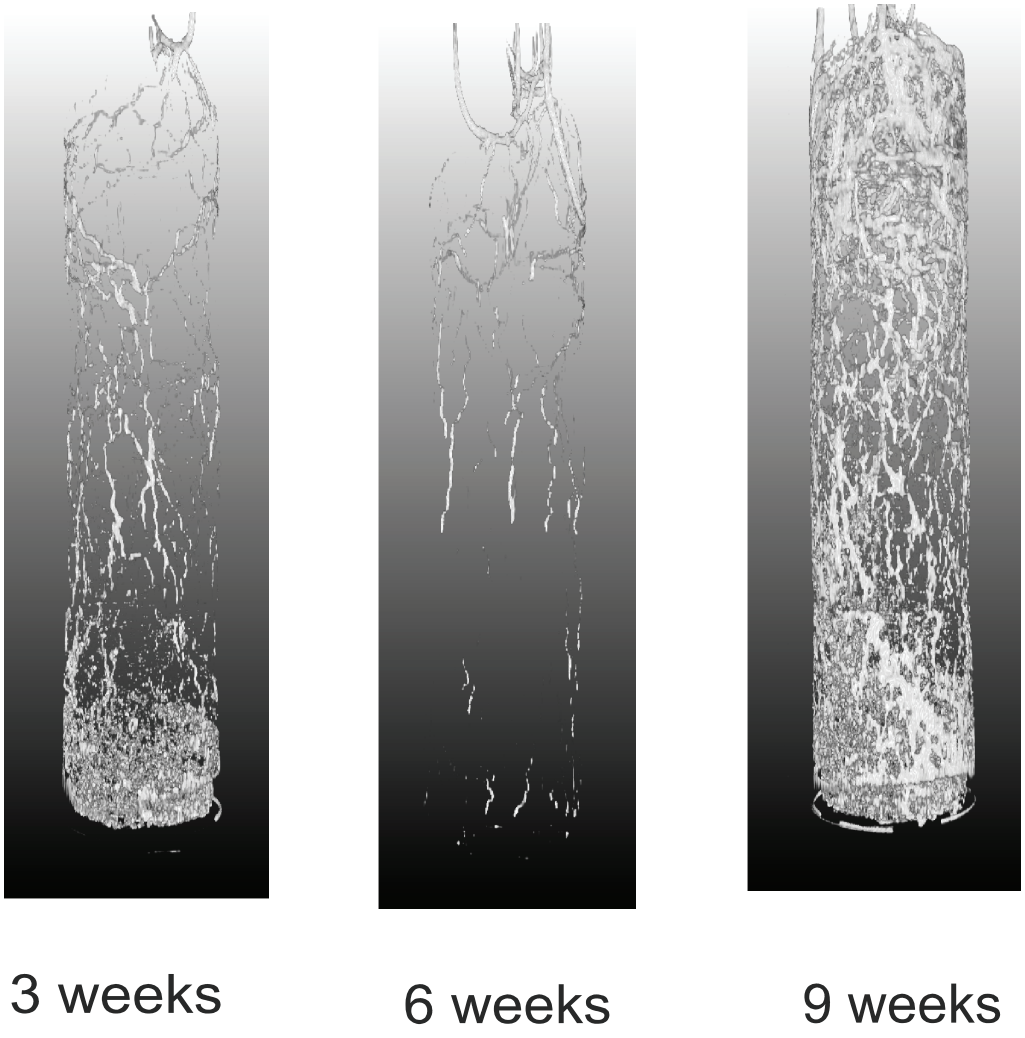


Fig. 45: Exemplary Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution with reduced phosphate content (0.1 mM) for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

Fig. 45 displays the development of an exemplary *Phalaris arundinacea* plant grown in 50% Hoagland solution with reduced phosphate content (0.1 mM) for 2 month (n=1). After three weeks of waterlogging (four weeks after germination) only a few roots were developed, but they already reach the ground of the tube. The strong signal in the bottom is water that could not be removed before measuring. Three weeks later the image gave the impression that less roots were in the tube than after three weeks growing. This is caused by the fact that the plants were kept one day longer not waterlogged and dried out. Therefore, the measurable water content in the roots was lower than during the first measurement. After nine weeks the tube was completely filled with roots. Interestingly, the roots preferred to grow at the tube wall. In comparison to the control plants waterlogged in 50% Hoagland solution (Fig. 44) the development of the root system seems to be delayed and less intensive at all time points.

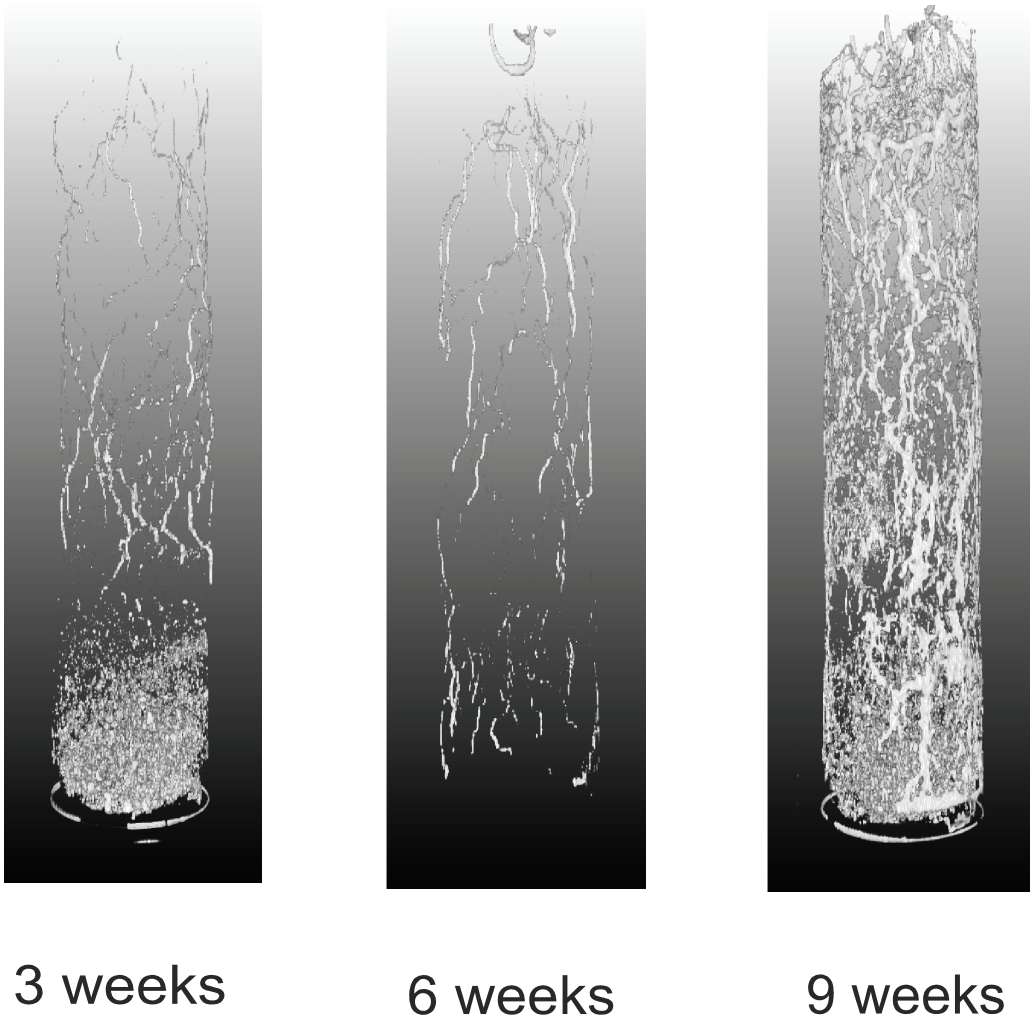


Fig. 46: Exemplary Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution containing 1 mM Al for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

Fig. 46 displays the development of an exemplary *Phalaris arundinacea* plant grown in 50% Hoagland solution containing 1 mM Al for 2 month (n=1). After three weeks of waterlogging (four weeks after germination) only a few roots were developed but they already reach the ground of the tube. The strong signal in the bottom is water that could not be removed before measuring. Three weeks later the image gave the impression that a similar amount of roots were in the tube than after three weeks. This is caused by the fact that the plants were kept one day longer not waterlogged and dried out. Therefore, the measurable water content in the roots was lower than during the first measurement. After nine weeks the tube was completely filled with roots. Interestingly, the roots preferred to grow at the tube wall.

In comparison to the control plants waterlogged in 50% Hoagland solution (Fig. 44) the development of the root system seems to be delayed and less intensive at all time points.

Tab. 7: Fresh and dry weight and root/shoot ratio of Phalaris arundinacea plants grown in NMR tubes for two month. The colored rows refer to the presented plants in Fig. 44, 45 and 46. H1-H3 plants cultivated in 50% Hoagland, P1-P2 plants cultivated in 50% Hoagland with reduced P content (0.1 mM) and A1-A3 plants cultivated in 50% Hoagland containing 1 mM Al.

PLANT	shoot wet (g)	root wet (g)	shoot dry (g)	root dry (g)	root/ shoot (wet)	root/ shoot (dry)
H1	29,67	28,54	6,30	10,28	0,96	1,63
H2	28,75	18,8	4,58	4,01	0,65	0,87
H3	30,80	20,3	4,67	3,98	0,65	0,85
P1	6,93	13,88	1,64	2,38	2,00	1,44
P2	8,75	17,10	1,66	3,01	1,95	1,81
P3	8,90	16,73	2,27	3,29	1,87	1,44
A1	8,30	12,82	1,99	2,67	1,54	1,34
A2	7,29	12,17	1,77	1,81	1,67	1,02
A3	5,56	4,11	1,33	0,40	0,73	0,29

Furthermore, the treated plants (P reduction as well as Al addition) developed less plant biomass than the control group. Especially the shoot biomass was strongly reduced and the root/shoot ratio was increased by the treated plants (Tab. 6). The replicates showed a similar development than the exemplary pictured plants above but there were plant specific differences (V).

4 Discussion

4.1 Adsorption of the substrate

The experimental conditions of this thesis should be similar to plant-MFC conditions to allow an application in the plant-MFC. Thus, all experiments were done under waterlogged conditions and sand served as substrate. However, the plants in the microbial fuel cell are growing in graphite granules but the adsorption capacity of graphite (75% recovered) for organic acids and sugars is higher than for sand (91 % recovered), thus graphite would have hindered the analysis of the organic compounds (3.1). The advantage of sand to other soil types is, that in sand the amount of plant available nutrients is low, therby it is possible to control the supply of nutrients by feeding with nutrient solutions.

4.2 Morphological reactions to water logging

Plants growing under submerged conditions often experience oxygen deficiency in root tissues (Jackson and Armstrong, 1999) as oxygen diffuses 10^3-10^4 times slower in water than in air (Armstrong, 1979) and most available oxygen is utilized by microorganisms within the top few millimeters of soil (Teal and Kanwishe, 1966). Many flooding-tolerant and wetland plants counter oxygen deficiency by forming aerenchyma, that enhance metabolic efficiency and facilitates internal oxygen transport (Armstrong, 1979; Jackson and Armstrong, 1999). Thus in a preliminary test, all plant species were tested for their ability to grow waterlogged and to form aerenchyma. Only one plant species, *Lythrum salicaria,* indicated wilting and root rot as reaction to waterlogging. Plant species, which are not adapted on waterlogged conditions, react to the lack of oxygen with wilting, leaf senescence and epinasty after several days (Drew, 1990). In contrast the plant species *Glyceria maxima*, *Phalaris arundinacea, Arundinella anomala, Spartina anglica and Hemarthria altissima* performed well under waterlogging as they develop an aerenchyma in reaction to flooding (Yu et al., 1969; Studer and Brändle, 1984; Rozema et al., 1985; Maricle and Lee, 2002; Chen, 2009). Hence, these plant species excluding *Lythrum salicaria* were suitable for waterlogged experiments and for growing in a plant- MFC.

4.3 NMR Imaging

To study the development of the root system under waterlogging, non-invasive NMR Imaging of the roots was done exemplary with *Phalaris arundinacea* over a period of 2 month. The NMR Imaging allows studying the root growth of plants in a non-invasive manner without damaging the natural environment. Thus, this technique enables long term studies under natural conditions. The *Phalaris arundinacea* plants did not show any kind of root damaging during two months waterlogging in 50% Hoagland, thus, *Phalaris arundinacea* can perform well under waterlogged conditions. But the treatment with reduced P supply inhibited the root as well as the shoot growth of *Phalaris arundinacea (*Fig. 45*)*. The plants developed less root as well as shoot biomass compared to the control plants in 50% Hoagland solution (Fig. 44). Furthermore, the root/shoot ratio increased with decreased P supply, caused by an increase of the root biomass. It is known, that a low P content in the soil induces reduced shoot and root growth and an increase of the root/shoot ratio (Forde and Lorenzo, 2001). Changes in the root structure or thickness induced by the P supply could not be observed by NMR Imaging due to technical problems. However, in earlier studies Forde and Lorenzo (2001) showed that plants grown under nutrient deficiency often develop fine roots and Keerthisinghe et al. (1998) showed that plants often develop cluster roots to deal with P deficiency. Probably, for the development of cluster roots as well as for the development of fine roots the P content was still too high or *Phalaris arundinacea* does not develop cluster roots.

Likewise, Al addition inhibited the shoot and root growth (Fig. 46) compared to the control plants, but the root/shoot ratio was not strong infected compared to the control plants. However, the diversity between the three Al treated replicates was high. One plant showed a really strong reduction of the root/shoot ratio (0.3) but the two other replicates had ratios of 1.34 and 1.0, thus they are more similar to the control plants with a root/shoot ratio of 0.85; 0.87 and 1.63 than to the Al treated plant with the strong reduction in root growth. Thus, these data did not supply a clear result. It is known that Al ions are toxic for plant roots and can inhibit root growth (Kochian et al., 2004) and Haling et al. (2011) showed for *Phalaris aquatica* that fewer lateral roots were grown on axial roots and seminal roots were shorter under high Al^{3+} conditions. However, further studies are necessary to verify this effect in the case of *Phalaris arundinacea*.

The measurements showed that the root development of *Phalaris arundinacea* plants was not affected by the waterlogging. Furthermore, the NMR Imaging is suggestive of a delayed development of the root system under P reduction as well as Al addition treatment. After three and six weeks the root and the shoot biomass of the treated plants seemed to be less than the biomass of the control plants. In contrast, after nine weeks the visible differences were less distinctive, but still present. However, a delayed or less intense development is not a problem for the application in a plant-MFC as far as the plants develop well under treatment conditions. If the root exudation per root biomass would be increased through the treatment, a delayed development would be even an advantage for the plant-MFC. A plant-MFC is running over a long period and a delayed development would imply the formation of young roots with a high exudation rate over a longer time period and it is known that the exudation of younger plants is higher than of older once (Gransee and Wittenmayer, 2000).

So far, the NMR Imaging in this thesis did not allow a statement about the root thickness or structure because the water content in the roots as well as in the tubes differed too much between the measurements. To quantify the data, the method must be improved first to reduce the disturbing signals and to ensure that the water content in the tubes and roots is comparable between the measurements.

4.4 Day rhythm

Exudation of organic compounds does not occur continuous but can underlie fluctuations. Dessureault-Rompré et al. (2007) showed for *Lupinus albus* that the overall temporal patterns during the lifetime of cluster roots were overlaid by a diurnal pattern. In most cases, the exudation burst consisted of one or more peaks occurring in the afternoon. This phenomenon, of a strong release of organic compounds, is called exudative burst (Dessureault-Rompré, 2007). Thus, the concentration of organic compounds is not only depending on physical-chemical factors e.g. soil compaction and nutrient supply, but can also be influenced by the day time.

To figure out, if the exudation under waterlogging underlies a diurnal rhythm, exemplary an experiment was done with *Phalaris arundinacea* to investigate, if this species showed diurnal exudation. However, neither in TOC, IC nor organic acid content significant changes of exudation were observed during the day.

While no change in exudation of *Phalaris arundincea* could be detected during the day, a pH drop from 7.22 to 6.60 in the afternoon was measured. The pH drop could be an indication for changes in exudation or a result of an active acidification of the rhizosphere by the plant e.g. for nutrient uptake (Hinsinger et al., 2009). As Blossfeld et al. (2007; 2011) showed, that plants temporary acidify or alkalize the rhizosphere. For example, *Juncus effesus* temporary induced a rhizospheric acidification (0.4 units) in an acidic soil and an alkalization in an alkaline soil during night-time. Further work with other *Juncus spec*. species suggested, that the dynamics of rhizospheric pH changes are species specific. While by *Juncus effusus* indeed showed a slight oxidative acidification of the strongly oxidized rhizosphere, *Juncus articulatus* even revealed an alkalinization of the oxidized rhizosphere (Blossfeld et al., 2011).

To summarize, *Phalaris arundinacea* did not show a diurnal rhythm or an exudative burst during the day. Probably, the exudation of *Phalaris arundinacea* was not influenced by diurnal rhythm. But the fact, that no diurnal pattern for *Phalaris arundinacea* could be detected, does not include, that it does not exist for other plant species. Thus, to guarantee the same sampling conditions for all plant species, the sampling occurred always at the same day time.

4.5 Influence of Al on root exudation

At mildly acidic or neutral soil pH values (pH 5-7) Al exists primarily in the form of insoluble aluminosilicates or oxides. However, as soils become more acidic (pH<5), phytotoxic forms of Al are released into the soil solution to levels that affect root (and plant) growth.

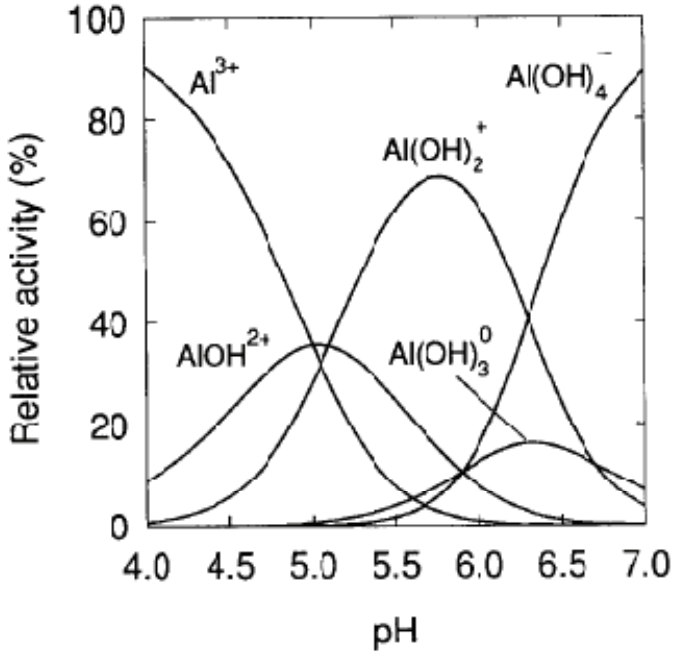


Fig. 47: Relative activities of mononuclear Al species (Kinraide, 1991)

The initial and most dramatic symptom of Al toxicity is inhibition of root elongation, which can occur within 1-2 hours after exposure to Al (Kochian, 1995). For this reason reduced root growth is a good indicator for a toxic Al concentration in the soil solution. However, the phytotoxic concentration differs for every plant species and can vary between the range of µM to mM concentrations (Marschner, 1995). Therefore, the plant growth limiting concentration of Al in the nutrient solution was tested for two plant species, *Phalaris arundinacea* and *Glyceria maxima*. For both plant species is known, that they resist high soil pH levels, up to pH 5-6 (Peeters, 2004). After a preliminary toxicity test with a nutrient solution containing 2 mM Al a strong decrease of root growth was detectable by *Phalaris arundinacea* and *Glyceria maxima* plants but in the next series with a nutrient solution containing 1 mM Al no strong reduction in biomass occurred by *Phalaris arundinacea* and *Glyceria maxima* as well as by the other model species.

For a species of the same genus, i.e. *Phalaris aquatica,* Wheeler et al. (1992) showed in a hydroponic system, that this species is moderately tolerant to Al in comparison to 33 other plant species (87 cultivars). *Phalaris aquatica* showed a reduction of the shoot yield of 50% at an Al concentration of 5-10 µM. Hence, it is possible that there exists a trait of aluminum tolerance in the genus of *Phalaris*. However, the tolerance mechanism of *Phalaris spec.* to Al toxicity is not known. It should be mentioned, that the Al concentration in the added nutrient solution does probably not correspond to the actual concentration of soluble Al^{3+} in the sand substrate and in the nutrient solution. Probably, Al^{3+} ions adsorb to the substrate and form low molecular weight compounds with oxygen donor ligands as carboxylates, phosphates and sulfates (Kochian, 1995). As a consequence, to make a statement about the plant available concentration of Al, the concentration in the nutrient solution and in the sand substrate during the experiment should be analyzed in future experiments.

So far, there are different types of Al tolerance mechanisms known:

- Those that operate to exclude Al from the root apex surface and those that allow the plant to tolerate Al accumulation in the root and shoot symplasm (Barcelo and Poschenrieder, 2002). Since this was not the focus of the present study, no analysis of the Al content in shoots and roots occurred.

- Furthermore, Al tolerant species tend to increase the soil pH (Mugwira and Patel, 1977). For an Al-tolerant *Arabidopsis thaliana* mutant it was shown that Al tolerance was correlated with an Al-activated root apical H^+ influx, resulted in an alkalinization of the rhizosphere at the surface of the root apex, which was large enough to significantly decrease the Al^{3+} activity around the root tip and lead to improved root growth (Degenhardt et al., 1998).

- For resistant plant species a well known strategy to inhibit uptake of Al is an increased release of organic acids. The exudation of malate and citrate to bind Al in the rhizosphere is documented. The exudation of these acids can increase up to10-fold as reaction on Al toxicity (Kochian et al., 2004).

However, the tested plant species (*Phalaris arundinacea, Arundinella anomala, Hemarthria altissima and Spartina anglica*) showed no increase in Total organic carbon (TOC) content after addition of Al to the nutrient solution compared to the control plants.

Either the plants do not react with an increasing exudation on Al toxicity or the released organic compounds are consumed too fast by the bacteria to identify any increase e.g. Jones et al. (1996) showed for the decomposition of malate, that this organic acid has got a half life time of 1.7 h in soil at 25°C. It is also possible, that only close to the root apex a strong exudation occurred to secure the root apex, because the root apex is the Al toxicity sensitive part of the root (Kochian et al., 2004). As consequence the amount of solved organic compounds in the nutrient solution could have been too low to detect any increase as reaction on the treatment. The TOC includes all organic compounds in the nutrient solution. Thus, it is a sum of all organic compounds as organic acids, sugars, amino acids and proteins and does not allow any statement about changes of one of these components. For the question of this thesis it was an interesting tool, because all these compounds can serve as food for the bacteria. Therefore, no detailed analysis of all components in the nutrient solutions of all plant species was intended. The missing increase in the sum of all organic compounds (TOC) does not exclude the possibility of a change in the composition of the released compounds. For example, a well known reaction on high Al concentrations is the exudation of organic acids, to complex the free Al^{3+} ions (Delhaize, 2001). Therefore, exemplarily the organic acids in the nutrient solution of *Phalaris arundinacea* and *Glyceria maxima* were analyzed. Both species are known to tolerate acidic conditions but the mechanism is unknown so far (Peeters, 2004). Under acidic conditions the solubility of phytotoxic Al forms in the soil solution is higher than at neutral pH values (Kinraide, 1991). Five days after Al addition the formate concentration was increased 20 folds and acetate concentration 4 folds in the nutrient solution 5 days after Al addition to *Phalaris arundinacea*. However, the concentrations of formate and acetate remained stable for *Glyceria maxima.* In contrast, no citrate was detectable and the malate concentration decreased during the treatment by both plant species. Thus, one explanation for the missing increase of malate concentration is that the potentially formed malate is already consumed by bacteria (Koku et al., 2003). Formate and acetate could be the fermentation products of the malate consumption during methanogenisis. (Westermann et al., 1989; Peters et al., 1999; Diekert, 2002; Duddleston et al., 2002). Besides, the increase of formate and acetate exudation could also be a reaction on the Al treatment by *Phalaris arundinacea* itself. Another explanation for the increase of formate could be the effect of waterlogging on the plants, because under anoxic conditions fermentation pathways are active in plants and it could be shown that the concentration of the enzyme formate dehydrogenase in plant cells is increased under waterlogging conditions (Dennis et al., 2000).

The experiments discussed above were long-term treatments i.e. the first sampling occurred 24 hours after the treatment began. To test, if Al addition induced a fast response by enhanced release of organic compounds, Al was added in the morning to waterlogged *Phalaris arundinacea* plants and samples were taken during the day. But no enhanced exudation of organic compounds was detectable by measuring the TOC content. Furthermore, there were no significant differences in the dynamic of TOC content compared to plants kept in 50% Hoagland solution without Al. The results suggest that *Phalaris arundinacea* does not react with an increasing exudation of chelating compounds e.g. malate on Al treatment, neither in short, nor in long term experiments (see above). Probably, the tested plant species accumulate Al in the vacuole detoxified by chelation with organic acids and do not exude the organic acids. It is known that oxalate as well as citrate can form complexes with Al in the cytoplasm (Ma et al., 2001). Furthermore it is shown that in the roots and leaves, most of the Al is complexed with oxalate in a 1:3 Al:oxalate complex (Ma et al., 1998). In contrast in the xylem stream Al is complexed with citrate and not with oxalate (Ma et al., 1998; Ma and Hiradate, 2000). Thereby, the Al is detoxified and can accumulate in high amounts without damaging the cell. For example, Buckwheat accumulates Al as high as 15,000 ppm in leaves when grown on acid soils (Ma et al., 2001). Hence, to verify this hypothesis it would be necessary to analyze the Al content in the tissues of treated and non treated plants in future experiments.

To keep the Al outside the symplasm another possibility could be the increase of the rhizosphere pH, because in acid conditions (pH<5) Al^{3+} dominates whereas the less toxic forms $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ as the pH increases. At neutral pH the solid phase $Al(OH)_3$ occurs (Delhaize and Ryan, 1995). It is known, that Al tolerant species tend to increase the pH in nutrient solutions (Mugwira and Patel, 1977) e.g. with an Al-activated root apical H^+ influx, resulted in an alkalinization of the rhizosphere at the surface of the root apex (Degenhardt et al., 1998). At the beginning of the treatment the pH in the nutrient solution of all plant species ranged between 3.31-3.9 due to the solved $AlCl_3$. All screened plant species induced an increase of the pH until it reached a neutral value, but there were of course inter-specific differences. *Arundinella anomala* induced the lowest pH increase (1.66 pH units); to a maximum pH 5.16 ± 1.36 (after 3 days)) in comparison to *Spartina anglica* (2.82 units; maximum pH 6.13 ± 0.89 (after 3 days)), *Hemarthria compressa* (2.9 units; maximum pH 6.84 ± 0.62 (after 4 days)) or *Phalaris arundinacea* (3.38 fold; 7.28 ± 0.19 (after 2 days)). *Phalaris arundinacea* reached even a 4.27 units increase (8.17 ± 0.25) after 10 days.

But, there are also other explanations for the pH increase. One reason could be the age of the plants during the experiment e.g. Huetsch et al. (2002) showed that the exudation of organic compounds decreases with increasing plant age. Thus, the number of chelating compounds in solution would be lower than by younger plants. Other reasons for more/less exudation could be differentness in biomass or in adaption on low light intensity (Kuzyakov, 2002). Furthermore, plants release H^+, OH^- or HCO_3^- depending on the nitrogen (N) source. It is known that plants supplied with NO^-_3 will counterbalance the corresponding excess of negative charges by releasing equivalent amounts of OH^- or HCO_3^- into the rhizosphere, thereby increasing rhizosphere pH (Jarvis and Robson, 1983; Gahoonia et al., 1992; Imas et al., 1997). In this study, NO_3^- was the used N-source in the Hoagland solution (2.2.3), thus the pH increase could arise from NO_3^- as N-supply.

However, the strong and fast pH increase in the nutrient solutions of all sampled plant species indicates a reaction on the Al treatment, instead of a reaction to the N-source. Most likely, the pH increase arose from an increase of proton influx from the rhizosphere into the roots in order to detoxify the Al^{3+} ions as discussed above.

After consumption of the organic compounds by the bacteria the resulting inorganic product is CO_2, which is also measurable with IC analyzes. Hence, the IC content is an opportunity for an indirect verification of an enhanced exudation. But, not all CO_2 is from microbial metabolism but also from plant respiration. An opportunity to differentiate between CO_2 from microbial metabolism or plant respiration would be to combine the IC measurement with ^{11}C or ^{14}C labeling techniques (Kuzyakov, 2002).

The fact that the IC content dramatically decreased after addition of Al to the Hoagland solution in all experiments is due to the pH dependency of the solubility of CO_2 in water (Fig. 48). At a low pH the equilibrium is on the side of CO_2, which diffuses into the atmosphere, because of the strong diffusion gradient from the soil solution to the atmosphere (Greenway et al., 2006). Thus, this proportion of the IC is not detectable in the nutrient solution anymore. In contrast, at pH values from 6-10 the IC is available as bicarbonate (HCO_3^-) and at a pH higher than 10 as carbonate (CO_3^{2-}) (Greenway et al., 2006).

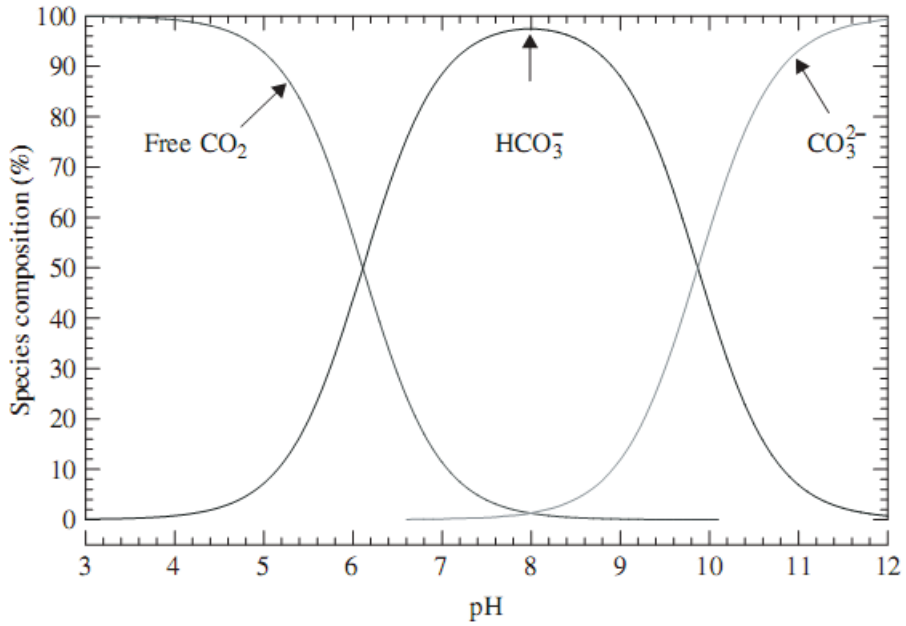


Fig. 48: Solubility of Carbon dioxide (CO_2) in water depends on the pH value. In an acidic pH range most CO_2 exists as CO_2 and becomes lost to the atmosphere but CO_2 exists predominantly at a pH range from 6-8 as HCO_3^- (pKs=6.36; 25°C) and at pH range >10 as CO_3^{2-} (pKs=10.25; 25°C) solved in the solution. (Greenway et al., 2006)

At the beginning of the Al addition experiment the pH was acidic but after 2-3 days the pH increased and reached values up to pH 7 (see above). Samples taken after three days in Al solution already revealed a significantly higher IC content than the samples taken 24 h after the beginning of the treatment due to the increase of pH. However, with IC measurement in the nutrient solution it is difficult to make a statement about the influence of Al on the root exudation because an identification of the total accumulated IC caused by the respiration of plants and bacteria is not possible.

Furthermore, the low IC content could also be a result of reduced bacterial activity, because bacterial growth can be reduced by the aluminum addition (Gristina et al., 1976). Yokoyama et al. (Yokoyama et al., 1993) showed that even bacteria species, which were tolerant to acidic pH ranges, were affected by aluminum toxicity. Consequential, the TOC concentration in the solution should increase if the studied plant species react on the aluminum addition with an increase in exudation, due to the fact that the number of bacteria decreases and as a result the decomposition, too. As far as the pH increases Al loses its toxicity to plants and bacteria because it becomes bound to chelating agents or to the soil (Yokoyama et al., 1993{Kochian, 1995 #60)}.

For future experiments, it would be interesting to conduct an experiment in a sterile hydroponic system, thus the organic compounds would not be consumed by bacteria and the released organic compounds by the roots as a reaction to the Al addition could be analyzed. However, roots grown under sterile and hydroponic conditions can be morphologically and physiologically very different from those growing in a real soil (Kuzyakov, 2002). Furthermore, it would be interesting to sample directly at the root apex because probably the amount of released compounds is too low to allow a detection of an increase in the nutrient solution or the compounds are too fast consumed by bacteria in the rhizosphere. One opportunity would be a setup with micro-suction-cups that allow the sampling of soil solutions at a defined position in a rhizobox (Puschenreiter et al., 2005; Blossfeld et al., 2011). Another possibility is the work with long and short-term labeling e.g. CO_2 (Neumann et al., 2009). The measurement of local changes of pH, O_2 or CO_2 concentration by optodes would also be a possible tool for non-invasive studies of the rhizosphere (Blossfeld and Gansert, 2007).

The analysis of TOC, IC and organic acids in the nutrient solution did not answer the question how the screened plant species *Phalaris arundinacea, Glyceria maxima, Arundinella anomala, Spartina anglica and Hemarthria altissima* handle with the addition of Al. However, an increase of the soil solution pH was observable for all plant species that indicates an exudation by the plants into the rhizosphere or an uptake of H^+ or Al^{3+} ions into the symplasm. Further studies will be necessary to quantify the potential released compounds i.e. under sterile conditions or by a more focused sampling at the side of the exudation, the root. To clarify the questions if a transport over the membrane occurred, an analysis of the root tissues or measurements of the ion flow over the membrane are necessary.

4.6 Influence of P reduction on root exudation

Phosphate is an essential micro nutrient and a lack of phosphate is a crucial problem for plants. Thus, plants developed several strategies to tolerate low P contents in the soil but so far, little is known about the adaptation to low P under waterlogging conditions. The most common strategies to colonize a soil with low P content are:

- Symbiotic association of roots with arbuscular mycorrhizal (AM) fungi is a very widespread strategy by which plants facilitate their acquisition of mineral elements e.g. P from the soil (Neumann and George, 2010)

- One strategy for chemical mobilization of sparingly available P sources in the rhizosphere is the formation of cluster roots as it is known for e.g. *Lupinus albus* (Neumann et al., 1999). It is known that *Lupinus albus* exudes large amounts of citric and malic acid from cluster roots in concentrations sufficient to mobilize significant amounts of phosphorus from P sources as Ca-, Al- and Fe-phosphates by ligand exchange (Gerke et al., 1994). Cluster root formation is even under waterlogged conditions possible, as Lamont et al. (1972) showed for *Viminaria juncea.* Reddell et al. (1997) suggested, that the formation of cluster roots could be a suitable alternative for enhancing P uptake under waterlogged conditions, as the development of mycorrhizas is poor.

- Furthermore, another opportunity to handle with a low P content is a higher exudation in general.

- Plants initiate symbiosis with bacteria to enhance the uptake of nitrogen or likely nutrients from the soil. The interaction is initiated by flavonoids or isoflavonoids. These compounds may initially assist rhizosphere colonization by acting as chemoattractants or less likely, as growth enhancers for rhizobia (Garg and Geetanjali, 2007).

Organic acids may help to release phosphate from inorganic phases by ligand exchange or ligand-enhanced dissolution (Johnson and Loeppert, 2006), as they compete with phosphate groups for binding sites in the soil and they form stronger complexes with Al, Fe and Ca than phosphate (Ryan et al., 2001).

The first experimental series was done with *Phalaris arundinacea* and *Glyceria maxima*, because both plants species are well adapted on waterlogged conditions as preliminary tests showed. The phosphate content in the nutrient solution of the plants treated with P reduction was 60% reduced compared to the nutrient solution of the control plants. However, neither *Phalaris arundinacea* nor *Glyceria maxima* showed an increase of the TOC content in the nutrient solution during the experiment. Either the available P concentration was still high enough to allow an adequate supply or the treatment period was too short to induce a deficiency reaction. Also possible is that the plants did not react with an increasing exudation on P deficiency or the released organic compounds were too fast consumed by the bacteria to identify any increase in exudation (Jones et al., 1996). Furthermore, both plant species did not show an increase in root/shoot ratio or a reduced growth due to the P reduction. This is a further indication that the available P amount was still high enough to allow the maintenance of the metabolism, as Forde and Lorenzo (2001) showed, that a low P content in the soil induced a reduced shoot and root growth and an increase of the root/shoot ratio.

Thus, the consequence was that in the following the P content was further reduced about 80% compared to the control plants and further plant species were tested. Likewise, the tested plant species *Arundinella anomala and Spartina anglica* showed no increase in TOC content after the P reduction in the nutrient solution compared to the control plants. But in contrast, *Hemarthria altissima* showed an increase in TOC content under P reduction treatment compared to the control plants. This reaction indicates an increase in exudation as reaction to a low P supply. A detailed analyses of the exuded compounds as organic acids, could prove, which compounds are responsible for the adaption.

The missing increase in the sum of all organic compounds (TOC) does not exclude the possibility of a change in the composition of the released compounds e.g. from sugar to organic acids. Especially for malate it is known, that the exudation increases, if the available P content is low (Neumann and Römheld, 1999). Therefore, exemplarily the organic acids in the nutrient solution of *Phalaris arundinacea* and *Glyceria maxima* were analyzed. After 2 d the *Glyceria maxima* plants with low P as well as the control plants showed a strong increase in malate exudation. Probably, the increase of malate exudation (5.5 fold) is a reaction to the solution change rather than to the P reduction, as the control plants (6 fold) also react with an increase. After 5 days the malate concentration of the low P plants decreased again to the same level as before the treatment.

In contrast, the malate concentration of the control plants of *Glyceria maxima* remained high during the experimental period. However, this increase of malate could be a stress reaction to the P reduction as also the Al treatment induced a strong decrease in malate exudation. Another explanation for the strong increase of malate would be a decrease of the microbial community due to the solution change. The number of anoxic bacteria can be expected to be reduced because the fresh solution was not deoxygenated and therefore the malate was not transformed to formate and acetate during fermentation (Westermann et al., 1989; Peters et al., 1999; Diekert, 2002; Duddleston et al., 2002). Probably, after 5 days the bacteria population recovered and consumed the malate released by the plants (Koku et al., 2003). Barclay and Crawford (1983) reported for *Glyceria maxima* a significantly decrease of carbohydrates in the roots after four days anaerobiosis. This coincides with the observation of an increase of lacate exudation by *Glyceria maxima* under P reduction, as the lactate could be a result of lactic acid fermentation under anaerob conditions, because under anoxic conditions, the pyruvate, generated in the glycolysis, is transformed to lactate or ethanol and could be released into the soil (Schopfer and Brennicke, 2006). For example, Shen et al. (2006) showed for *Zea mays* an increase in lactate exudation under anoxic conditions. Furthermore, it is known, that lactate can mobilize P in sandy soils as well as citrate, acetate and malate (Lambers et al., 2002). Lambers et al. (2002) showed that lactate had the highest mobilization capacity of these acids. Hence, the increase of lactate exudation of *Glyceria maxima* could be a reaction on the low P content. But it could also be a fermentation product of bacteria in the soil. To verify the hypothesis, that the origin of lactate is exudation and not fermentation, it would be useful to compare a sterile and a non sterile experimental setup.

In contrast, *Phalaris arundinacea* reacted neither to the solution change nor to the P reduction in the nutrient solution. Probably *Phalaris arundinacea* reacts with a reduced metabolism activity on waterlogging. This is in agreement with the findings of Barclay and Crawford (1983), who showed that *Phalaris arundinacea* as well as *Glyceria maxima* has got large reserves of carbohydrates in their rhizomes. These reserves allow surviving under waterlogged conditions. For *Phalaris arundinacea* the concentration of the analyzed sugars in the root tissues did not significantly change after four days waterlogging (Barclay and Crawford, 1983).

The fast microbial break down is a crucial problem for the analysis of organic compounds in a non-sterile system. The bacteria consume the released organic compounds and produce CO_2, which is measurable with IC analyzes. Hence, the IC content is an opportunity for an indirect verification of an enhanced exudation.

The IC content in the nutrient solution of *Glyceria maxima* and *Spartina anglica* strongly increased after the change to low P in comparison to the control plants. The IC increase is an indication that *Glyceria maxima* and *Spartina anglica* react with a stronger exudation of organic compounds to P reduction. Probably the organic compounds are too fast decomposed by bacteria to detect an increase of the TOC content (Jones et al., 1996). Besides, the IC could also be a plant respiration product resulting from the waterlogging conditions. In contrast, for *Phalaris arundinacea, Hemarthria altissima* and *Arundinella anomala* no increase in IC content was measurable. Maybe these species have got other strategies to deal with low P content e.g. cluster root formation. However, a formation of cluster roots could not be detected for the investigated plant species under waterlogged conditions and reduced P supply. Probably, the amount of available phosphate was still too high to induce a cluster root formation or the treatment time was too short. The P concentration which induces phosphate deficiency symptoms is highly plant and setup depend and varies from µM to mM scale. For example, Hoffland et al. (2006) used a nutrient solution with a P content of 4 mM for rice plants cultivated in sand pots, but only 1.5 mM P in hydro culture to induce P deficiency. In contrast, Johnson et al. (1996) worked with *Lupinus albus*, a plant which is well adapted on low P, and used a nutrient solution without P to induce an effect.

A further experimental series was done with two *Oryza sativa* cultivars. *Oryza sativa* is an interesting species for the plant-MFC, because it develops well under waterlogging and enormous number of different cultivars exists (Armstrong et al., 1994). One of the studied cultivars was phosphate deficiency tolerant (Milyang 23) and one was phosphate deficiency intolerant (IR 5440-1-1-3). Both cultivars showed an increase in TOC content 3 d after the P content in the nutrient solution was reduced, but the TOC content of the phosphate deficiency tolerant cultivar was decreased after 7 d of treatment to a similar value like in Hoagland solution. Probably, the strong increase in exudation after the solution change of both cultivars was a stress reaction of the plants or of the bacterial community to the solution change. Furthermore, no IC was measurable 3 d after the solution change. This is a further indication for a disturbance of the microbial community. The decomposition rate was reduced and therefore less IC produced. The lower TOC and IC content of the phosphate deficiency tolerant cultivar after 7 d of P reduction indicates that the plants do not deal with the low P content with an increasing exudation. Instead the tolerant cultivar developed more root and shoot biomass than the intolerant cultivar.

A higher root biomass is an indication for a higher root surface that allows the plant to take up more P from the solution. This is in close agreement with Wissuwa (2003), who showed that phosphate deficiency tolerant rice cultivars react with an increase in root and shoot biomass to low P contents. To conclude, the tolerance of the rice cultivar Milyang 23 to phosphate deficiency is probably induced by an increased biomass production and accordingly root surface and not by an increased exudation.

Like already mentioned, the bacterial break down makes it difficult to quantify the organic compounds released by the plant under natural conditions. Therefore, *Oryza sativa* plants were cultivated under non sterile conditions and the TOC and IC content, the organic acids and the sugars in the nutrient solution were analyzed.

The plants grown under the sterile system showed a different reaction on P reduction than the plants grown under non sterile conditions. Both cultivars showed a decrease in TOC content after the P reduction compared to the sampling in 50% Hoagland solution under sterile conditions and compared to the non sterile setup. Probably, the difference in exudation arose from the sterile hydroponic growing conditions, as roots grown under sterile and hydroponic conditions can be morphologically and physiologically very different from those growing in a real soil (Kuzyakov, 2002). Furthermore, the cultivars showed differences in IC content. The measurable IC is assumedly a product of plant respiration (Schopfer and Brennicke, 2006). The fact, that the total measured amount of IC in the sterile setup was lower than in the non sterile setup, is a further indication that the measured IC is a plant respiration product and not of bacterial origin under sterile conditions. The phosphate deficiency intolerant cultivar showed a decrease in IC content 3 d after P reduction, which could be a stress reaction to the low P content. After 7 d the IC content was even higher than in 50% Hoagland solution. The plant metabolism was obviously disturbed after the solution change but recovered a few days later and was even increased compared to the control. Presumably, the plant metabolism rose as reaction to the low P content. In contrast, there was no difference in IC content between control and low P treatment in the solution of the tolerant cultivar after 7 d of treatment.

The sterile growing conditions allow a more detailed analysis of the organic compounds released by the plant roots e.g. the organic acids and sugars. These are not the only components released by plants into the soil, but for organic acids e.g. malate and citrate it is known, that they can enhance the P uptake of plants (Neumann and Römheld, 1999).

Interestingly, both cultivars showed an increase in exudation of the organic acids oxalate, formate, lactate, acetate after the P reduction in the nutrient solution.

This is in close agreement with Hoffland et al. (2006), who showed that under P deficiency the exudation of organic acids of rice is increased. All these organic acids can mobilize bounded P. The extraction efficiency of inorganic P by the organic acids appears to follow the series citrate > oxalate > malate> acetate (Jones and Darrah, 1994; Lan et al., 1995). Besides, the phosphate deficiency intolerant cultivar IR 5440-1-1-3 showed a strong decrease of the malate content and a strong increase of the acetate content after the P reduction. Probably, the citrate cycle and the respiratory chain were inhibited by the low P supply, thus the acetate could not be converted to malate.

Another possibility for plants to increase the available P in the soil is to cooperate with the microbial community e.g. formation of mycorrhiza (Neumann and George, 2010) or the interaction with soil bacteria. Organic acids released by the bacteria or mycorrhiza could increase the amount of soluble P. Plants could release e.g. sugars into the rhizosphere to increase the activity of the soil microbiota, as sugars are the basic material for metabolism. The results of the sugar analysis in the nutrient solution agree with this hypothesis, because the exudation of sugars was increased under low P conditions by both cultivars. However, the concentration in the solution of the P deficiency tolerant cultivar is lower than in the tolerant cultivar. Maybe, the tolerant cultivar keeps more sugars for its own metabolism to maintain the enhanced plant growth as response to P reduction.

The question how plants react on a low P content is not generally answerable, as the reaction of plants on low P supply are chameleonic and plant species specific. The TOC and IC results of *Glyceria maxima*, *Hemarthria altissima* and *Spartina anglica* indicate that these species react with an increase in exudation on a reduction of the P content in the nutrient solution. In contrary, the mechanism, how *Arundinella anomala* and *Phalaris arundinacea* deal with a reduction of the P supply or if they are tolerant to lower P levels is unknown so far. Further studies with lower P supply are necessary to answer this question. Besides, the studies with sterile and non sterile rice cultivars indicate, that the phosphate deficiency tolerant cultivar is characterized by a stronger formation of biomass as reaction on low P supply. In contrast, the intolerant cultivar seems to react with an increase in exudation of organic acids and sugars on a low P content.

4.7 Conclusion

The interplay between bacteria and plant in the plant-MFC is based on the rhizodeposition of organic compounds into the rhizosphere. Rhizodeposition can be influenced by physical, chemical as well as biological factors. Thus, the aim was to increase the exudation of several plants into the rhizosphere under waterlogged conditions by treating the plants with low phosphate (P) content or Aluminum (Al) addition. Total organic carbon and inorganic carbon analyses indicated that a reduction of the P supply can increase the release of organic compounds of waterlogged *Glyceria maxima*, *Spartina anglica* and *Hemarthria altissima* plants. *Hemarthria altissima* showed a threefold increase of TOC content and the IC content in the nutrient solution of *Spartina anglica* increased twofold and of *Glyceria maxima* fivefold after the change to low P in comparison to the control plants. In contrast, the addition of Al did not affect the root exudation of any screened plant species. It is likely, that the screened plants use other mechanisms to detoxify the Al in the nutrient solution such as absorption into the symplasm or pH shift. Besides, NMR Imaging indicated that the development of the plant roots was delayed under P reduction or Al addition treatment compared to the control plants. Though, a delayed or less intense development is not a problem for the application in a plant-MFC as far as the plants develop well under treatment conditions and so far the ratio between increased exudation and reduction of root surface allow a higher C flow into the rhizosphere. The results indicated that the reduction of the P content in the nutrient solution increased the exudation of organic compounds and could be a possible tool to enhance the productivity of the plant-MFC.

4.8 Outlook

In future studies it should be tested, if a reduction of P in the plant-MFC is a possible tool to increase the productivity of the plant-MFC without affecting the plant development and health. So far, experiments were done under waterlogged and not under MFC conditions. Furthermore, other strategies should be pursued to influence the root exudation. Presumably, variations of factors that are conducive to plant growth such as the environmental and root temperature, light intensity or pH should be considered in this regard. It should be tested, how far a wide range of environmental factors influence the exudation and the functioning of plant-MFC.

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6 Appendix

I. Material

I Chemicals

- Acetic acid [$C_2H_4O_2$] MERCK
- Boric acid [H_3BO_3] MERCK
- Calcium nitrate tetrahydrate [$Ca(NO_3)_2$] MERCK
- Calcium hydroxide [$CaOH_2$] MERCK
- Chloroform [$CHcl_2$] MERCK
- Citric acid [$C_6H_8O_7$] MERCK
- Copper(II)sulfate pentahydrate [CuO_4S] MERCK
- Daishin agar Duchefa
- D(-)- Fructose [$C_6H_{12}O_6$] FLUKA
- D(+)-Glucose [$C_6H_{12}O_6$] MERCK
- Di-Sodium oxalate [$C_2Na_2O_4$] MERCK
- Dodecyltrimethylammonium hydroxide MERCK
- Ethanol [C_2H_5OH] MERCK
- Ethylenediaminetetraacetic acid [$C_{10}H_{16}N_2O_8$] OptiChem
- Formic acid [CH_2O_2] MERCK
- Glyoxylic acid [$C_2H_2O_3$] MERCK
- LB agar Duchefa

- L-(+)-Lithium lactate [$C_3H_6O_4$] MERCK
- Malic acid [$C_4H_8O_5$] MERCK
- Magnesium sulfate heptahydrate [$MgSO_4$] AppliChem
- Manganese(II)chloride tetrahydrate [$MnCl_2$] SIGMA
- Murashige and Skoog (MS) medium Duchefa
- Oxalic acid [$C_2H_2O_4$] MERCK
- Polyoxyethylen(20)-sorbitan-monolaurat TWEEN
- Potassium phosphate [KH_2PO_4] MERCK
- Lactic acid [$C_3H_6O_3$] MERCK
- Liquid nitrogen
- Potassium nitrate [KNO_3] MERCK
- Salicylic acid MERCK
- Sodium acetate [$C_2H_3NaO_2$] MERCK
- Sodium formate [HCOONa] MERCK
- Sodium hydroxide [NaOH] MERCK
- Sodium molybdate lactate [$C_3H_5LiO_3$] MERCK
- Succinic acid [$C_4H_6O_4$] MERCK
- Sucrose [$C_{12}H_{22}O_{11}$] MERCK
- Zinc sulfate heptahydrate [$ZnSO_4$] MERCK

II Consumables

• 1.5 mL tubes	Eppendorf
• 2. mL tubes	Eppendorf
• Pipette tips	Eppendorf
• Parafilm	Brand
• tubes (40 mL)	Greiner
• tubes (15 mL)	Sarstedt
• Syringes (40 mL, 10 mL)	Braun
• Needles 0.9X70 mm	Sterican
• Plastic tubes 200X55 mm (inner diameter 49mm)	
• Box 100X100X130 mm	Hünersdorf
• Plastic ballon (5 L, 25 L)	Hünersdorf
• silica capillary	Polymicro, Phoenix, USA
• Snaps polypropylene (1 mL)	Agilent
• Snap caps polyethylene	Agilent
• Syringe filter (13 mm) Bulk GHP	Agrodisc
• 0.45 µm GHP membrane	PALL
• Flower pot 145 mmX125mm	Göttinger
• Flower pot 90X90X95 mm	Göttinger

III Instruments

- AG 11 guard column
- Autoclave FVS/3 4109E IBS Integra Bioscience
- Balance Mettler Toledo PG503-S
- CarboPac PA100 4- 3 250-mm column (Dionex) preceded by a guard column (CarboPac PA100, 4 3 50 mm)
- Centrifuge 5415 d Eppendorf
- Clean bench Hera Safe 0620-U-11 LGBA-0002 Heraeus
- DC-190 High-temperature TOC Analyzer, Rosemount Analytical Inc.
- Dionex ICS2500 HPLC system
- G1600A capillary electrophoresis Agilent, Böblingen, Germany
- LI 6400XT Licor
- Oven UT6760 Thermo Electron Corporation
- Photometer 12550 Anthos labtech Intruments
- Pipette (5, 10, 20, 50, 100, 200, 1000, 5000 µL) Eppendorf
- ProfiLine pH/mV meter pH197/pH197-S
- Thermomixer Comfort Eppendorf
- 4.7T Varian VNMRS vertical wired-bore MRI system

II. Data

I *Influence of Al treatment on root growth*

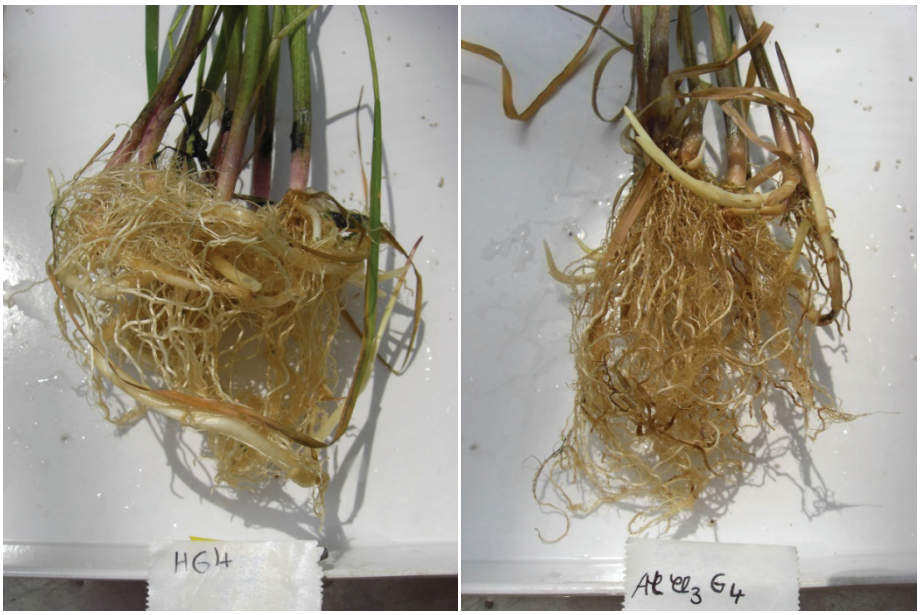


Fig. 49: Roots of Glyceria maxima plants after treatment with 50% Hoagland solution (left) or 50% Hoagland solution containing 2 mM Al (right) for six weeks.

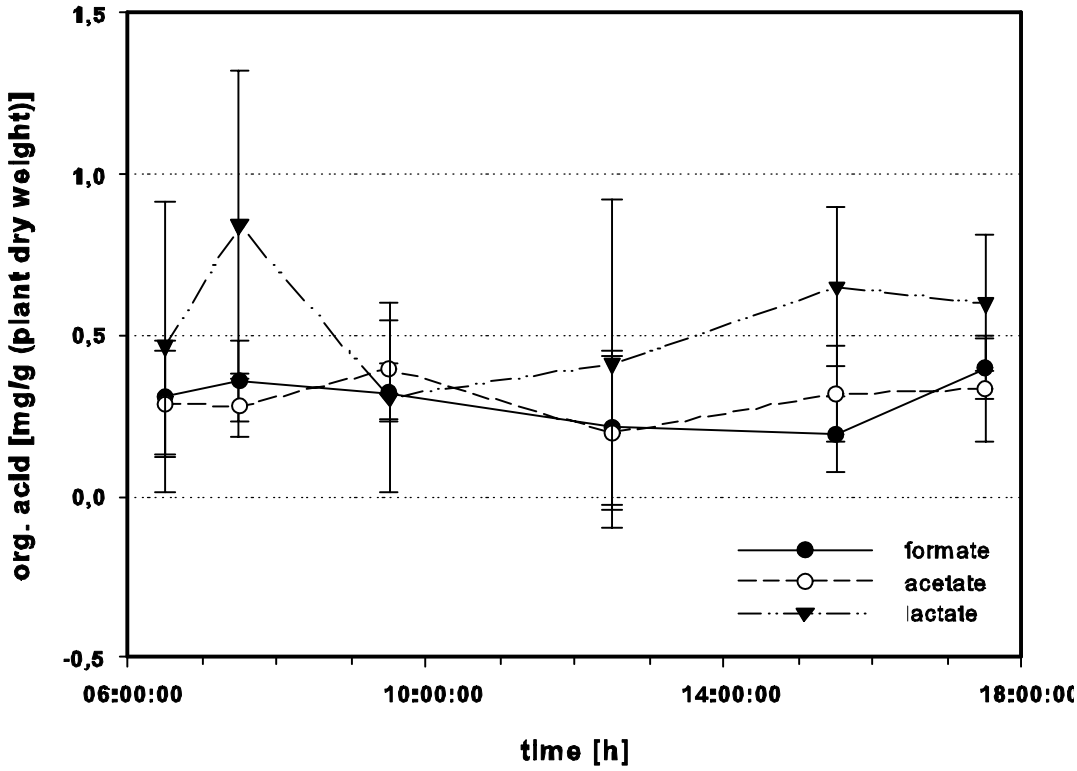
Fig. 50: Roots of Glyceria maxima plants after treatment with 50% Hoagland solution (left) or 50% Hoagland solution containing 1 mM Al (right) for six weeks.

II pH values

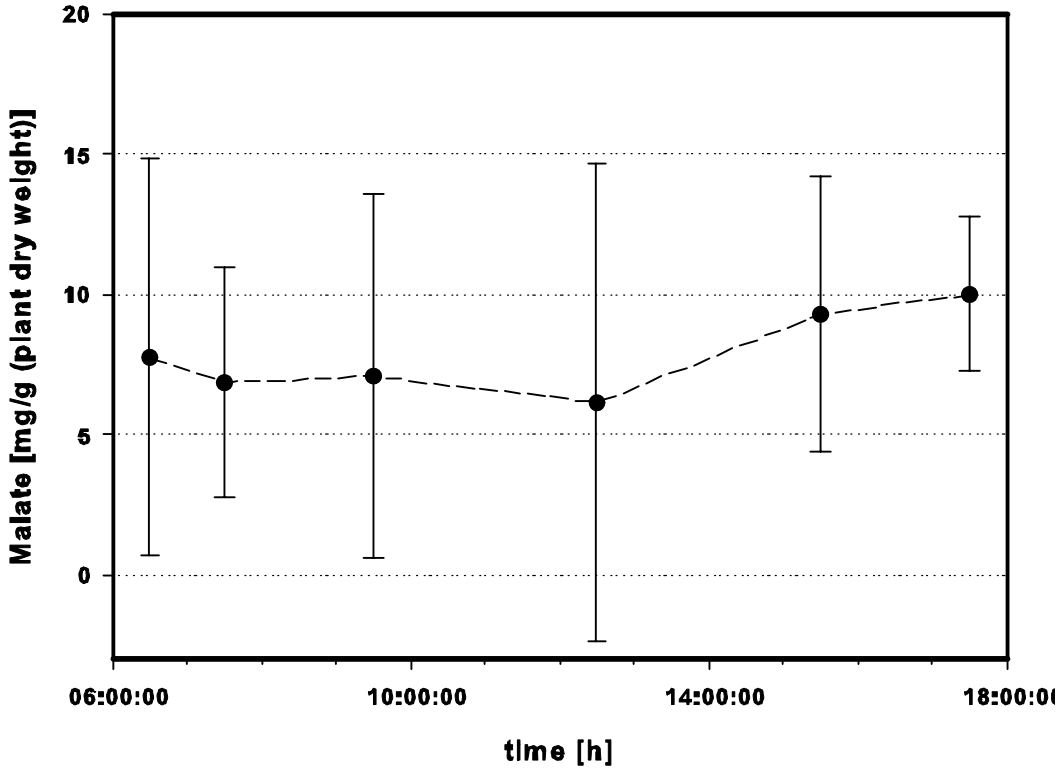
Tab. 1: pH values measured in sand pots filled with 50% Hoagland solution, 50% Hoagland solution with reduced P content (0.1 mM) or sand pots filled with Hoagland solution containing 1 mM Al.

Sample	Time [d]	pH	Time [d]	pH	Time [d]	pH
Hoagland	2	6,39	5	6,43	12	6,41
Hoagland	2	6,63	5	6,31	12	6,45
		6,51		**6,37**		**6,43**
-Phosphate	2	6,7	5	6,35	12	
-Phosphate	2	6,53	5	6,6	12	7,15
		6,615		**6,475**		**7,15**
+ Aluminum	2	3,62	5	3,8	12	3,62
+ Aluminum	2	3,61	5	3,65	12	
		3,615		**3,725**		**3,62**

III Day rhythm

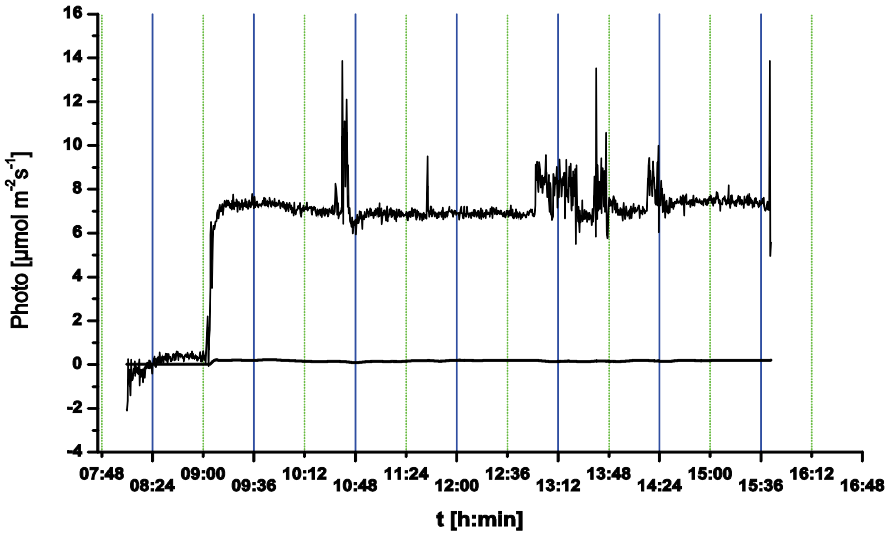


Content of the organic acids formate, acetate and lactate (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Every plant was sampled once. Error bars represent ± standard deviation of five replicates (n=5)

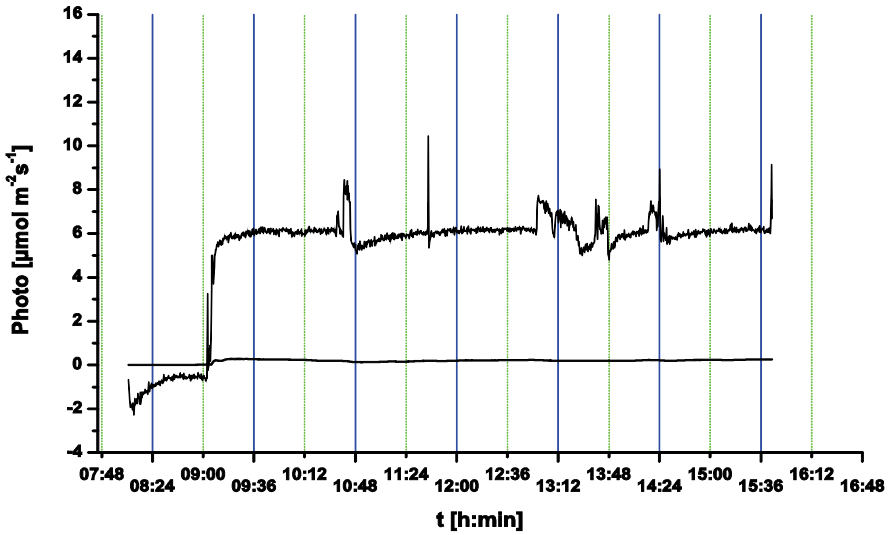


Content of malate (mg) per plant dry weight (g) in the nutrient solution of waterlogged Phalaris arundinacea plants in 50% Hoagland solution (2.2.3) during the day. Every plant was sampled once. Error bars represent ± standard deviation of five replicates (n=5)

IV Al day rhythm



Photosynthetic rate and conductivity of a plant waterlogged in 50% Hoagland solution containing 1mM Al.



Photosynthetic rate and conductivity of a control plant waterlogged in Hoagland solution.

V NMR Imaging

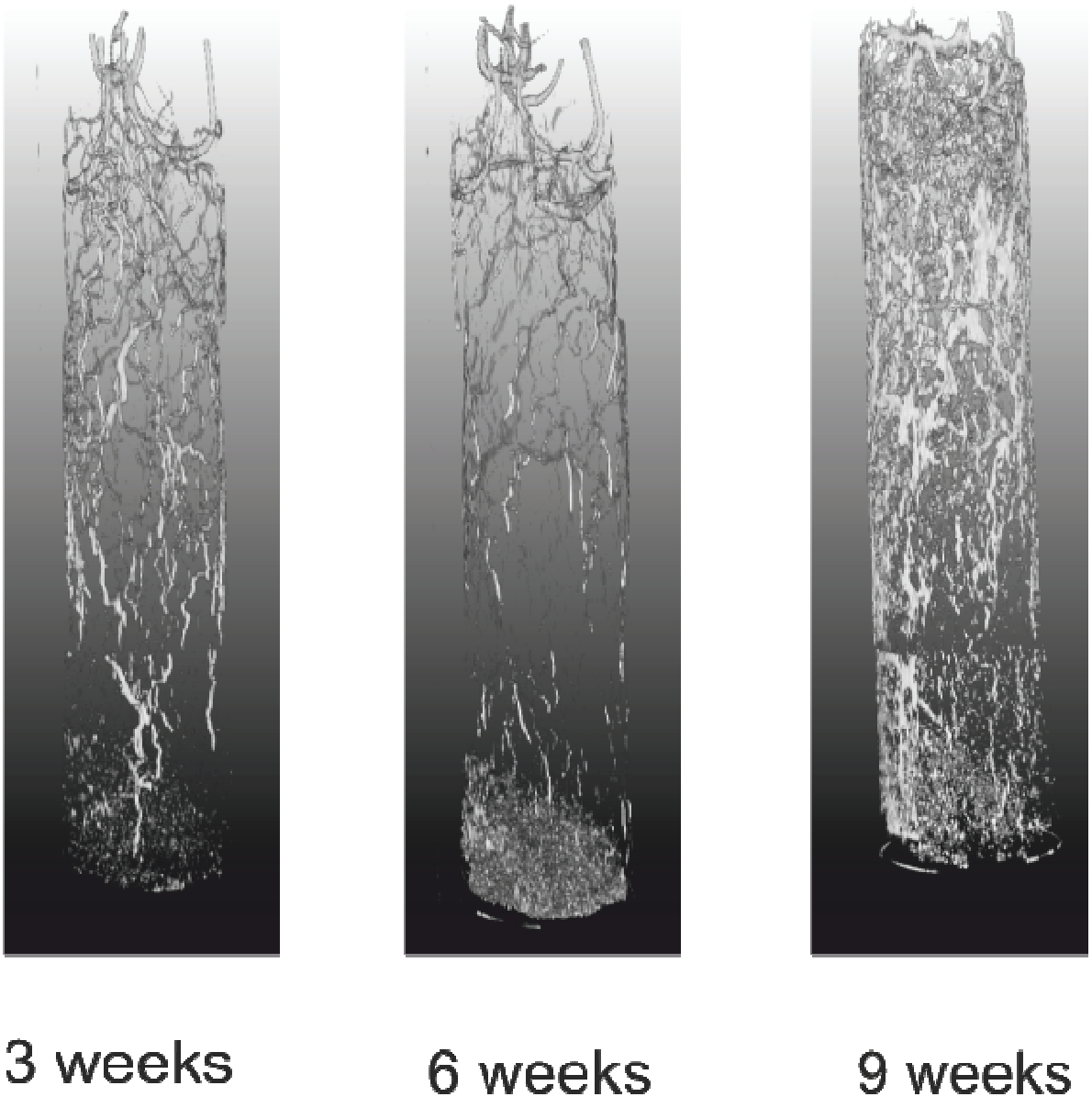


Fig. 51: Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

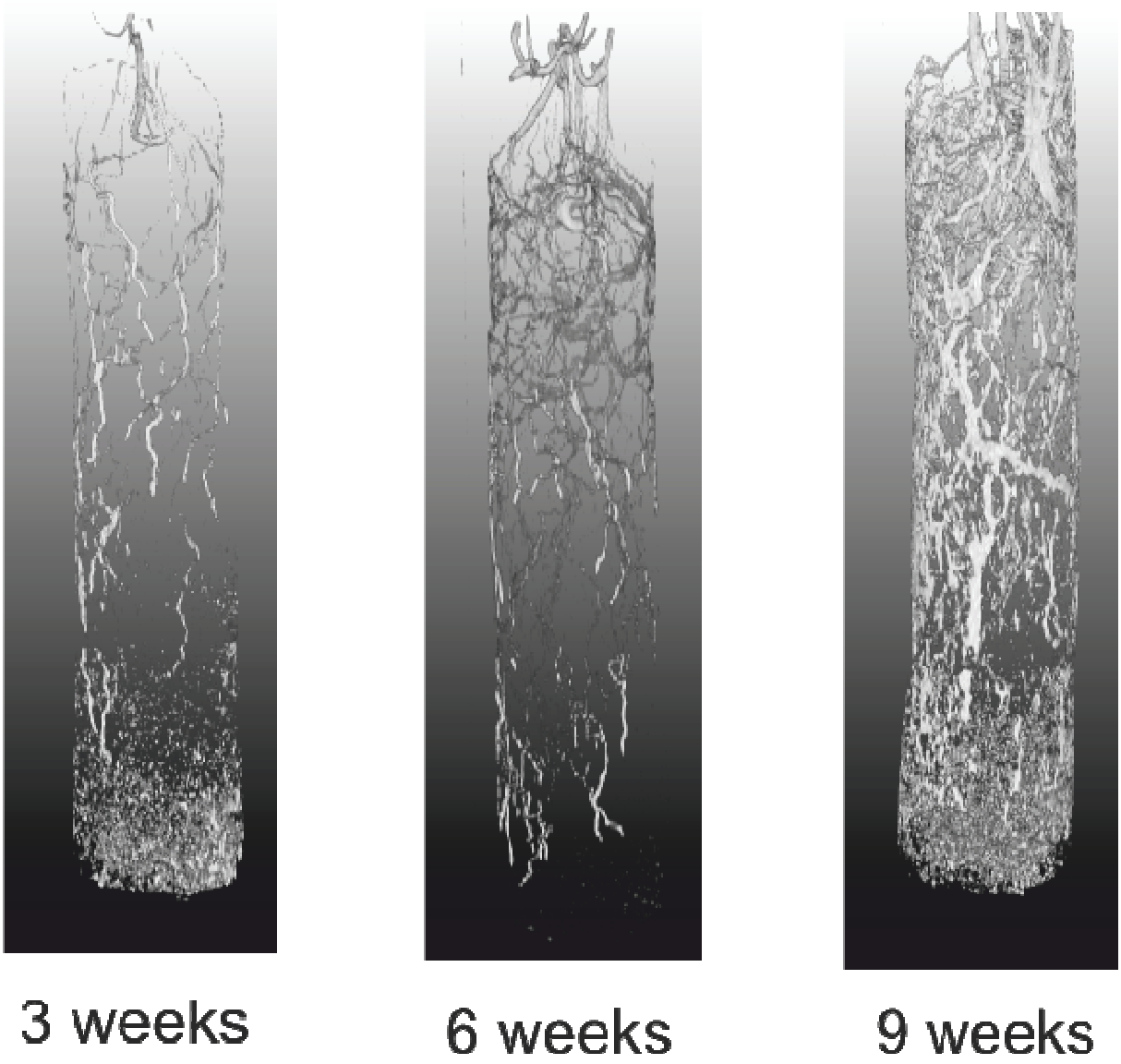


Fig. 52: Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

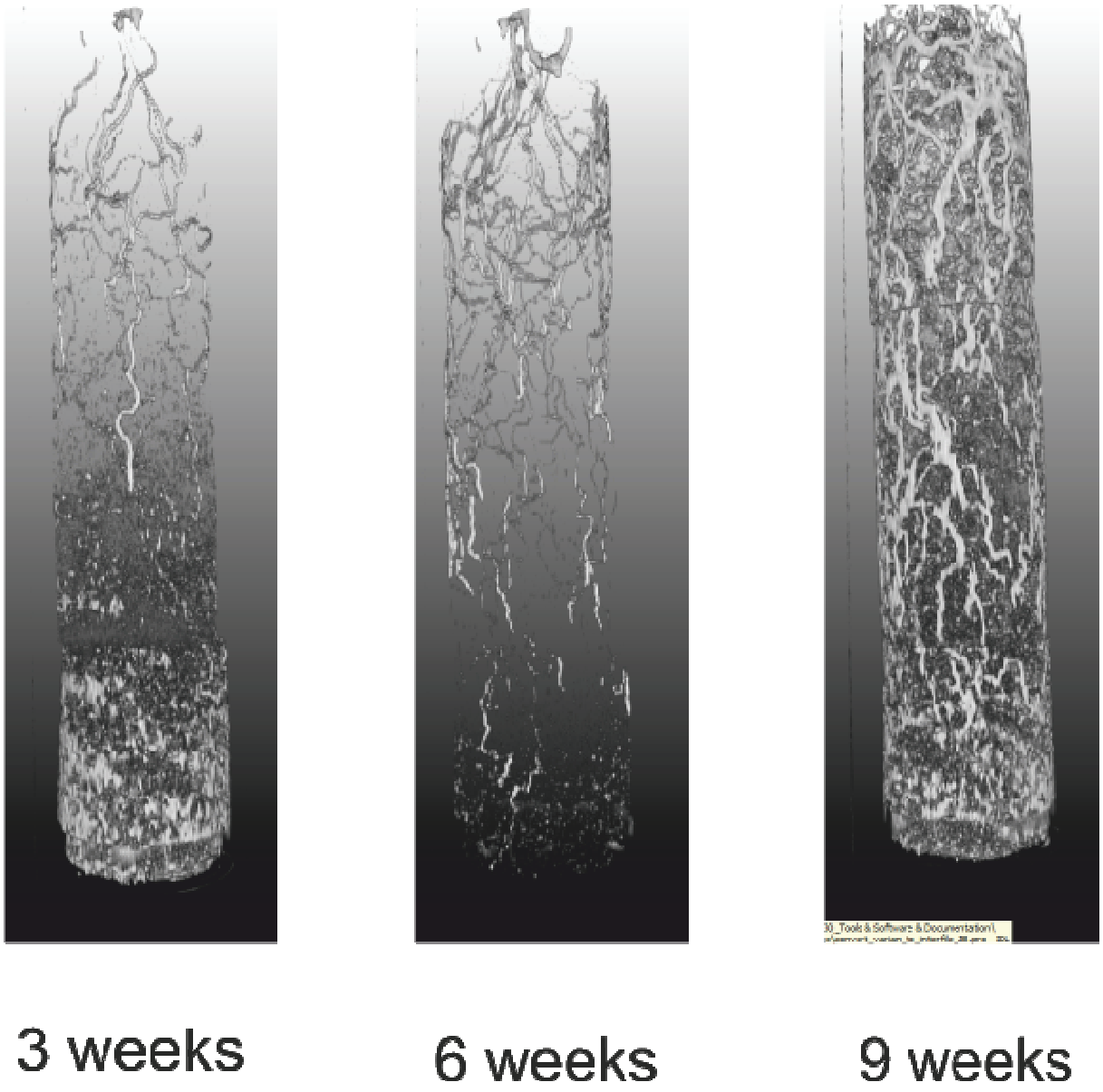


Fig. 53: Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution with reduced phosphate content (0.1 mM) for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

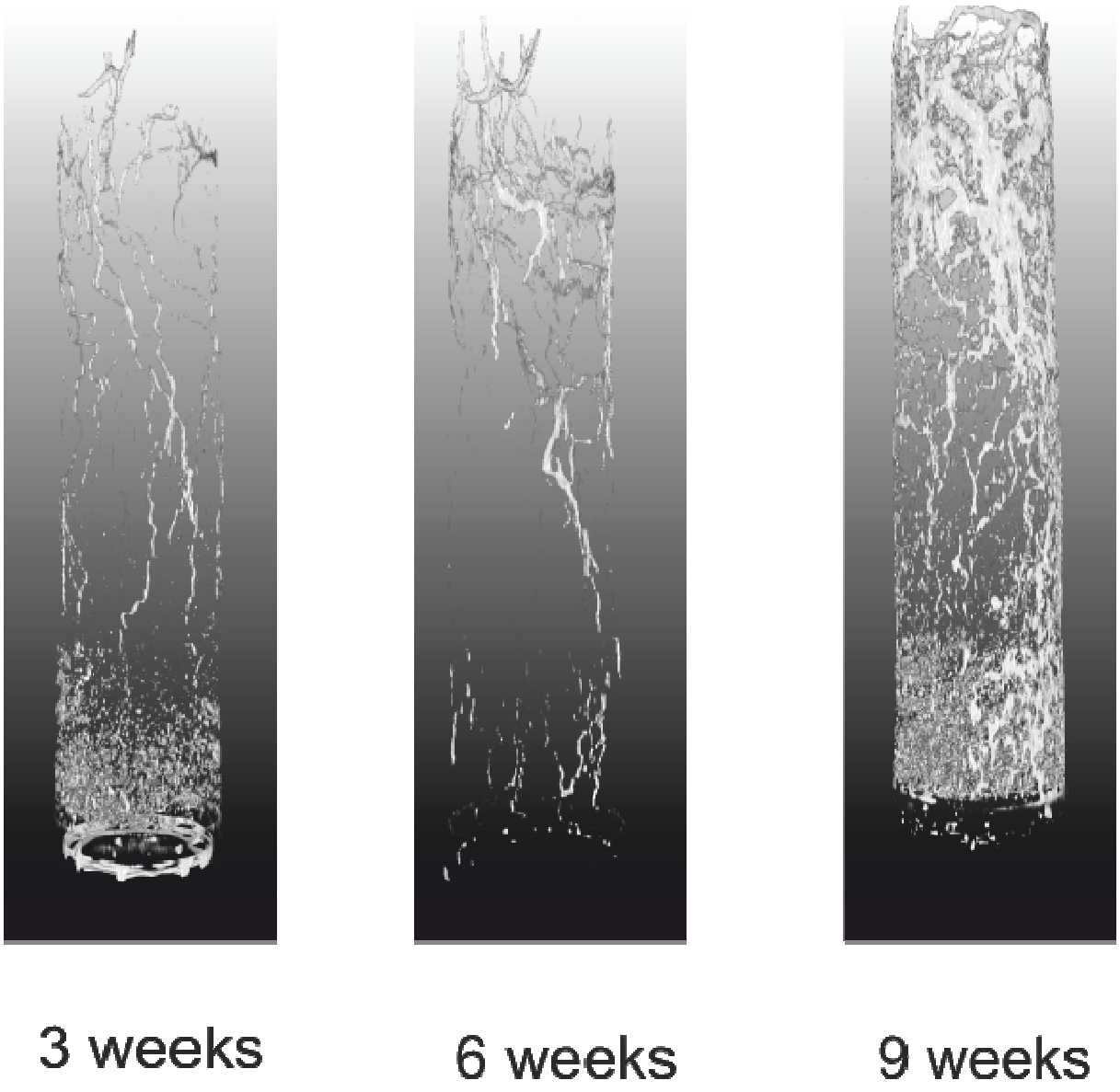


Fig. 54: Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland solution with reduced phosphate content (0.1 mM) for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

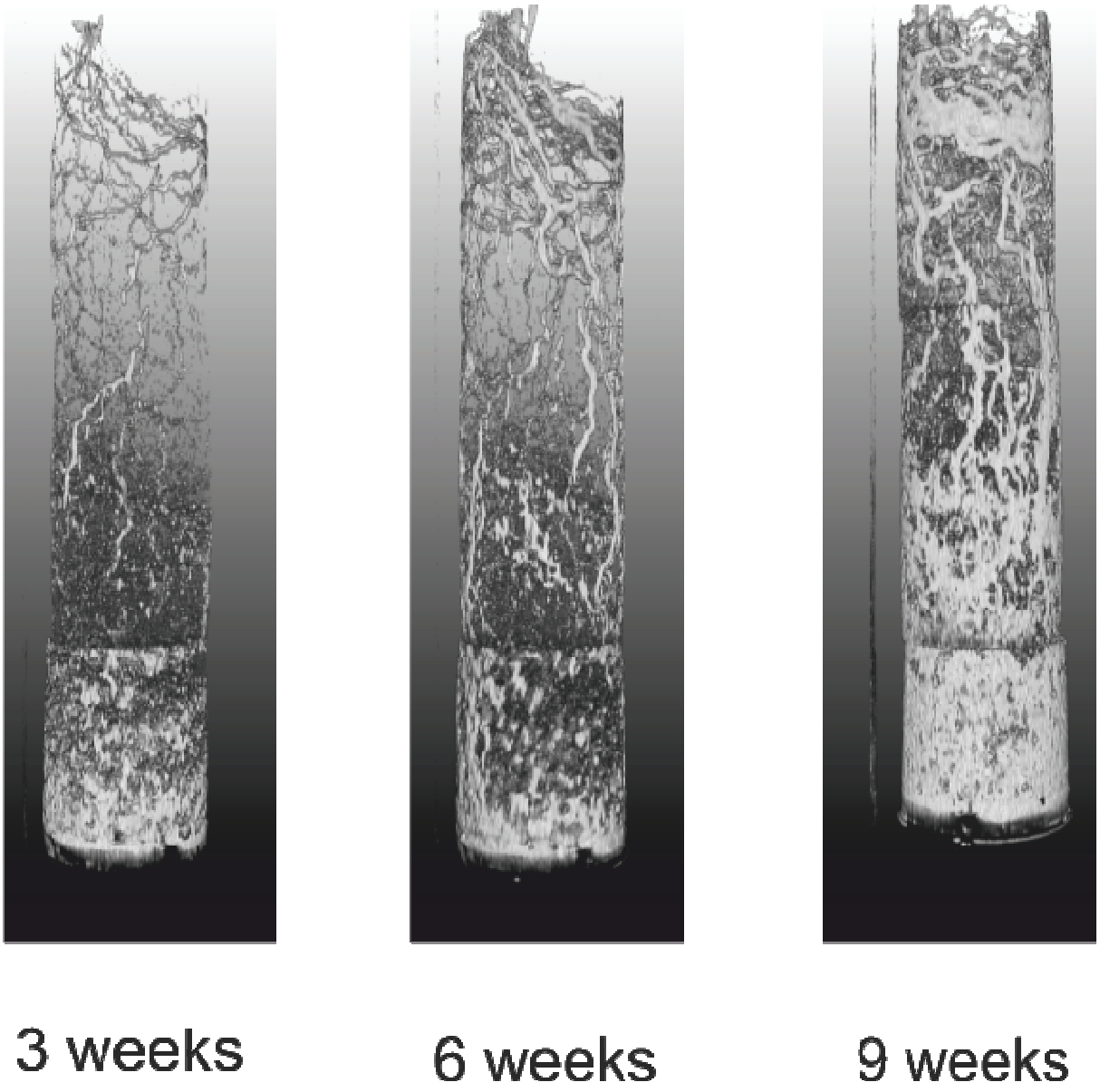


Fig. 55: Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland containing 1 mM $AlCl_3$ for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1).

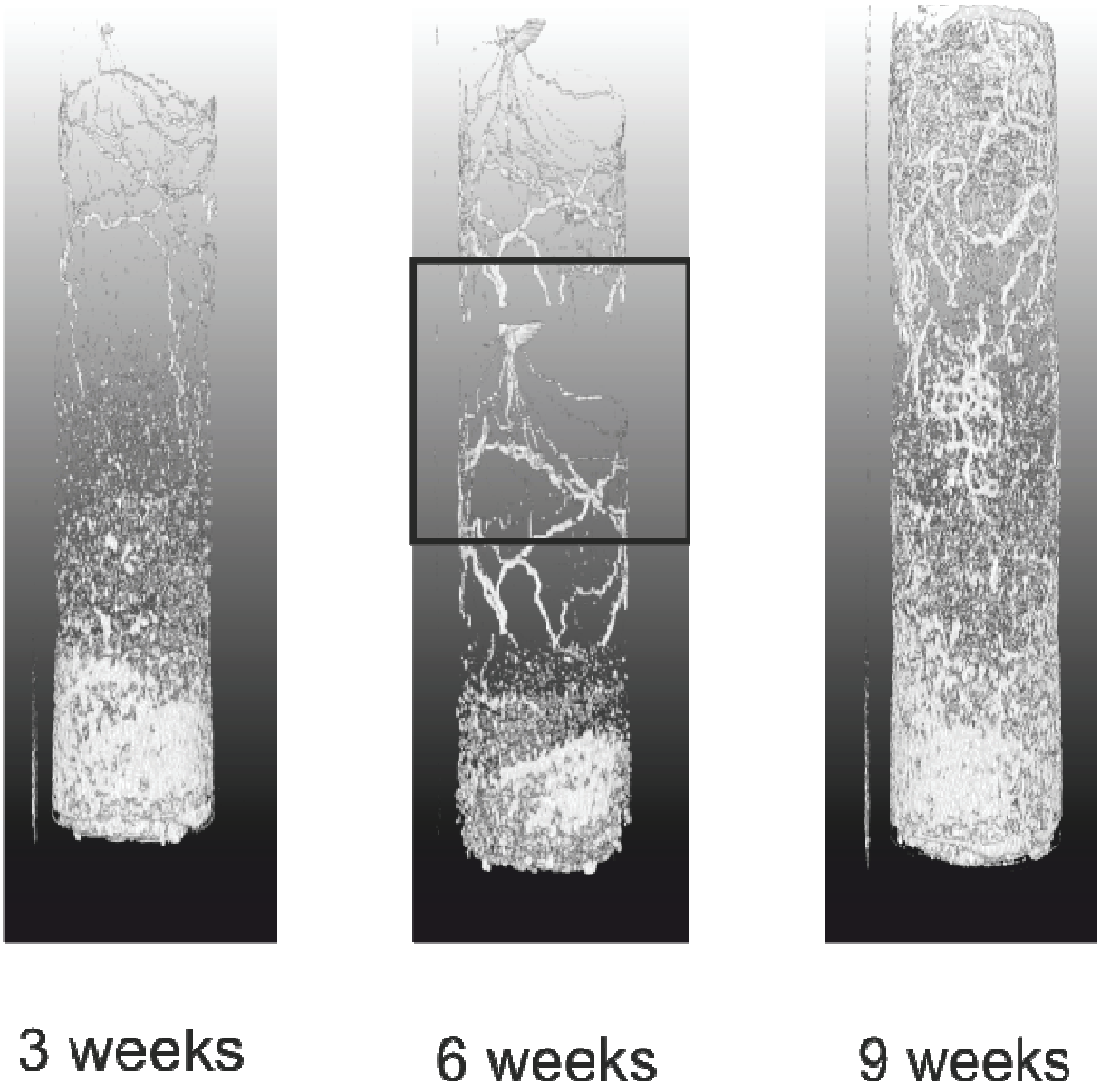


Fig. 56: Phalaris arundinacea plant cultivated waterlogged in 50% Hoagland containing 1 mM $AlCl_3$ for 2 month. NMR Imaging was done after 3, 6 and 9 weeks of waterlogging (n=1). After six weeks the upper part was measured twice instead of the middle part.

VI Abbreviations

Al	Aluminum
$Al(OH)_3$	Aluminum hydroxide
Ca	Calcium
C	Carbon
CO_2	Carbon dioxide
Cd	Cadmium
Cu	Copper
Fe	Iron
$Fe(OH)_3$	Iron hydroxide
HMW	High molecular weight compounds
H	Hydrogen
IC	Inorganic carbon
LMW	Low molecular weight compounds
MFC	Microbial fuel cell
$MgCl_2$	Magnesium chloride
Na	Sodium hydroxide
N	Nitrogen
O	Oxygen

Plant-MFC	Plant microbial fuel cell
P	Phosphate
SMFC	Sediment microbial fuel cell
TOC	Total organic carbon
Zn	Zinc

Ich (Chantal Le Marié) versichere, dass ich die vorliegende schriftliche Arbeit selbstständig verfasst und ich keine anderen als die angegeben Hilfsmittel verwendet habe. Die Stellen der Arbeit, die anderen Werken dem Wortlaut oder dem Sinne nach entnommen worden sind, wurden unter der Angabe der Quelle kenntlich gemacht. Dies gilt auch für beigegebene Zeichnungen, bildliche Darstellungen, Skizzen und dergleichen.

Aachen, den 04.04.11

Chantal Le Marié