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Effect of P Enrichment on Rhizodeposit Quantity and Bioavailability: a Comparison of Two Macrophyte Species

Master Thesis

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Annotation: a review on plant rhizodeposition, nutrient uptake and interactions with rhizosphere microbial community is presented. Based on reviewed findings, a study on rhizodeposition rates and rhizodeposits bioavailability of two macrophyte species with different life strategies (stress-tolerator and competitor) was conducted. The effects of P addition and sediment type on rhizodeposition and microbial activity were examined. Research was carried out in tropical marshes of Belize; results from field were supported by C partitioning mesocosm study.
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Poděkování: Děkuji v první řadě svým školitelkám Haně a Elišce, za jejich mateřské vedení a příklad. Děkuji naší katedře za přívětivé a podnětné prostředí. Děkuji své rodině, rodičům a sestrám za obrovskou trpělivost a skvělé zázemí. Děkuji svému týmu na Ktiši, bez jejichž vstřícnosti a obětavosti bych práci sotva mohla dokončit. Děkuji Aničce Stejkozové, Sirgi Saar a Seanu Allenovi, kteří práci ochotně pročetli a pomohli odstranit chyby v angličtině a úpravě. Děkuji všem přátelům, kteří mě provázeli modlitbou nebo dobrou myšlenkou.

CONTENTS

1. INTRODUCTION

Processes present in root-soil interface play an important role even in the ecosystem scale. Complex relationships between plant and rhizosphere microbial community are crucial in all terrestrial ecosystems across almost whole latitudinal range. They influence biogeochemical fluxes between lithosphere (mineral weathering), hydrosphere (leaching) and atmosphere (exchange of gasses via photosynthesis, respiration, N_2 fixation, methanogenesis etc.). Knowledge of rhizosphere interactions provides the evaluation of nutrient cycles and other biogeochemical fluxes, which could be widely applied in agriculture, ecosystem protection, ecosystem restoration, plant water treatments etc.

While the rhizosphere processes in aerated soils are studied frequently, research under wetland conditions is still rare. Understanding to soil processes and interactions is particularly important in tropical wetlands. Wetlands in tropics are the second largest after boreal wetlands, yet, they are the most productive ecosystems in the world (net primary production) and fluxes present there are intensive. In addition, tropical wetlands often serve as a source of disease infection since the agents or their vectors inhabit wetland areas (e.g. *Anopheles* mosquito, a vector of malaria). Similar to other tropical ecosystems, the biodiversity of tropical wetlands is much higher than in other latitudes and is not sufficiently studied up to now. Tropical wetlands are generally less destroyed than temperate wetlands but they are more vulnerable and endangered when concerning the political, economic and demographic situation in many tropical countries. Wetland alteration may lead to unwanted consequences such as increase of some tropical diseases, loss of drinkable water sources, loss of food sources (fishing) or even to the desertification. Furthermore, rise, one of the most important crops all over the world is cultivated in tropical wetlands.

Wetland areas in developing countries are often being eutrophicated because of more intensive agriculture. The oligotrophic marshes of northern Belize, Central America, suffer from fertilizer input caused by run off from sugar cane fields. P eutrophication resulted in vegetation change: dense covers of cyanobacterial mats with sparse stands of sedge *Eleocharis* spp. were replaced by a native but expansive cattail *Typha domingensis* Pers. Consequently, the biodiversity and biochemistry of these marshes has changed and the mosquito *Anopheles vestitipennis* (a vector of malaria) found favourable conditions for development of its larvae in dense cattail stands. A long-term experiment of P enrichment is has been cconducted in 15 marshes and various parts of the ecosystem were described: cyanobacterial mats, vegetation characteristics, the dynamics

of *Typha domingensis* Pers. expansion, litter decomposition, microbial activities in the sediment etc. While sediment and plant characteristics were studied, the rhizosphere processes remained unrevealed. Our aim was to evaluate the main processes present in the rhizosphere of two dominant macrophytes and interpret them in the respect of other findings about this ecosystem.

We studied rhizodeposition characteristics (rhizodeposition rate and rhizodeposit biodegradability) of two wetland macrophytes with distinctive life strategies (stress-tolerator and competitor). We also compared the processes under P-limited and P-enriched conditions. The effect of sediment properties (marly and peaty clay) on rhizodeposit quantity and bioavailability was examined as well. Rhizodeposit collection was carried out in the field and subsequent mineralization rates were measured in the laboratory. Field data was supported by C partitioning mesocosm experiment. Our results are interpreted in the context of already known findings about studied marshes (sediment microbial activities, litter decomposition, vegetation changes, etc.). For a broader insight into plant-microbial interactions, a general review of this topic is presented.

2. REVIEW ON THE RHIZOSPHERE

2.1 THE RHIZOSPHERE

Soil is considered to be the most complex terrestrial environment on the Earth. Soil origin and development is a biologically driven process which is influenced by a variety of abiotic factors (parent material, climate, relief, time, etc., Brady and Weil 2002). We classify soils according to their origin, composition, stratification and other characteristics (Brady and Weil 2002). However, a large heterogeneity of soil environments can be observed even within a particular soil sample (m^3). The detritusphere (a zone, where detritus decomposition is recognizably maintained); the drilosphere (a zone influenced by earthworm activity); the porosphere (a zone of soil voids - pores of various sizes); the aggregatusphere (a zone of solid soil aggregates) and the rhizosphere (a zone of root primary influence) are examples of mutually not exclusive soil spheres, which are recognized to be biologically distinctive (Giri et al 2005a). This review further focuses on the rhizosphere and the rest of soil spheres are encompassed in the term bulk soil.

The rhizosphere was first defined in 1904 when Hiltner (Vančura 1988a) described it as the zone in a close proximity to roots where microorganisms are active. Later this still fairly heterogeneous sphere was divided into three distinctive zones: (1) the endorhizosphere, which constitutes the microhabitat for microbiota living in the root interior, (2) the rhizoplane, which means soil-free root surface and finally (3) the rhizosphere itself as the soil in the close root vicinity (Vančura 1988a). In this review, the term rhizosphere is mostly used in its broader sense.

The rhizosphere is typified by an increased amount of organic substances (rhizodeposits) released by root into its surroundings. Rhizodeposits are usually composed of easily degradable compounds and therefore trigger the microbial activity in rhizosphere (Vančura 1988b). The microbial biomass is substantially higher in the rhizosphere compared to bulk soil (Giri et al. 2005b, Table 1).

Steep gradients of physical and chemical characteristics occur with both the distance from the root surface and the location along the root length (Richards 1987). O_2 concentrations and related redox potential (Blossfeld et al. 2011, Yang et al. 2012); pH values (Blossfeld et al. 2011, Jones 1998) and concentration of organic compounds (Badri and Vivanco 2009, Marschner et al. 2011) vary even within the short parts of a single root.

Despite the volume of the rhizosphere being much smaller than the volume of bulk soil, the processes present in the rhizosphere are often important in the ecosystem scale (Personeni

et al. 2005, Ström et al. 2003, Ström et al. 2012). In evolutionary perspective, rhizosphere processes are crucial for mineral weathering and soil development (Calvaruso et al. 2006, Lambers et al. 2009).

An essential role of easily available C in the evolution of roots and their rhizosphere microbial communities is depicted by Lambers et al. (2009): Because of higher concentrations of CO_2 in the atmosphere at the time when plants started to colonize the terrestrial ecosystem (in the mid-Palaeozoic era 480-360 million years ago, Kenrick and Crane 1997), plants were able to photosynthesize more effectively than nowadays. The surplus of assimilates was released by plant parts and induced the growth of microbial communities around them. Surprisingly, before real roots were formed, some of the recently known rhizosphere interactions had evolved (e.g. arbuscular mycorrhizae, Brundrett 2002).

Table 1. Microbial abundance and diversity of major groups in the rhizospheric and nonrhizospheric soils. R:S is ratio of microbial abundances in rhizosphere (R) and nonrhizosphere soil (S). Modified from Giri et al. 2005b).

Organisms	Rhizosphere soil (microbes / g dry soil)	Nonrhizosphere soil (microbes / g dry soil)	R:S ratio
Bacteria	1200×10^6	53×10^6	23
Actinomycetes	46×10^6	7×10^6	7
Fungi	12×10^6	1×10^6	12
Algae	5×10^6	27×10^6	0,2

2.2 ROLES OF THE PLANT

Plants influence and change their environment in multiple ways (e.g. altering water cycle and concentrations of O_2, CO_2 and other gases in the atmosphere). As primary producers, plants support all heterotrophs by providing organic carbon compounds. Two major inputs of organic C into the ecosystem are represented by plant litter fall and rhizodeposition. At the same time, plants need to meet their nutrition demands by nutrient uptake from the environment.

For simplicity, we further focus on the two main phenomenona (rhizodeposition and nutrient uptake), which influence plant-microbe interactions in the rhizosphere most importantly.

2.2.1 RHIZODEPOSITION

The term rhizodeposition denotes the general release of organic substances by roots. It encompasses a wide range of processes: root cap and border cell loss, death and lysis of root cells, flow of C to root-associated symbionts, gaseous losses, passive leakage of solutes from living cells and insoluble polymer secretion from living cells (mucilage). The term exudation is used in two different meanings: 1) as a synonym for rhizodeposition and 2) as a particular case of rhizodeposition – the passive release of organic C by living cells. The term rhizodeposition is recently preferred for the general phenomenon of C release (Kuzyakov and Domanski 2000). Consistent with that, the term exudation is used in its narrower sense in this study.

The rhizodeposition can consume up to 40 % of photosynthates according to more recent review by Brüggemann et al. (2011). Some studies report on even higher belowground allocation as the gross flux of recently fixed C to rhizosphere can reach up to 80 % in pasture plants (for review see Kuzyakov and Domanski 2000). The most intense rhizodeposition occurs in the growing parts of the root. Vančura (1988a) reported on rhizodeposition in the zone of extension cell growth about 300 mm behind the tip of both lateral and main roots. it occurs partly due to the vesiculation when the cytoplasmatic membrane is elongating (Ovečka et al. 2005); partly because of the absence of any efficient transport barrier such as the secondary cell wall, which is formed later in cells of rhizodermis and endodermis (Franke and Schreiber 2007).

Marschner et al. (2011) used root rhizodeposition rate, root nutrient uptake capacity and microbial densities in the rhizosphere to create a model of plant-microbe interactions in the rhizosphere in relation to nutrient (P and Fe) availability along the root axis (root tip and distal elongation zone, proximal elongation zone, root hair zone and mature root zone). Chemical com-

position (Badri and Vivanco 2009, Berg and Smalla 2009, Ström et al. 2003) and spatial distribution (Sauer et al. 2006) of rhizodeposits are species specific.

The quantity and composition of rhizodeposits change during plant ontogenesis. Vančura (1988a) described the rhizodeposition characteristics for germinating seeds, seedlings and intact roots of various ages: higher rhizodeposition was observed mostly in younger plants and seedlings. The rhizodepostion is generally higher during the day and lower at night as it is linked to photosynthetic activity (Kuzyakov and Cheng 2001). In addition, the diurnal composition of rhizodeposits may also differ (Melnitchouck et al. 2005).

Many other biotic and abiotic characteristics that influence the rhizodeposition are summarized in Table 2.

Table 2. Schematic representation of biotic (plant and soil microbiota) and abiotic (soil and environment) factors, which influence rhizodeposition. Modified from Jones et al. (2004).

Plant biotic factors	Root architecture	Temperature	**Abiotic factors**
Plant species	Cytosolic concentration	Moisture	Available space
Developmental status	Membrane permeability	Humidity	Ozone
Shoot herbivory	Membrane electrochemical potential	Wind speed	Physical disturbance
Photosynthesis	Release of microbial signals	Light intensity	Fire
Supply of C from shoot to root	Allelochemical release	Elevated CO_2	Irrigation
Evapotranspiration	Mycorrhizae	Pesticides	Erosion
Nutrient Deficiency	Nodulation	Atmospheric N deposition	Altitude
Root age	**Rhizodeposition**		Latitude
Root membrane permeabilisers			Redox potential
Root herbivory			Organic matter
Mycorrhizae	Release of root signal molecules	Compaction	Cation and anion exchange
Microbial community size	Quorum sensing	Soil type	Drainage and aeration
Microbial community structure	Pathogens	Soil pH	Rooting depth
Microbial community activity	Biocontrol agents	Salinity	Soil texture
Toxin production	Phytohormon production	Metal toxicity	Soil structure
Soil biotic factors	Mesofauna	Water availability	**Soil abiotic factors**

2.2.1.1 Origin and Composition of Rhizodeposits

A terminology based on the origin of rhizodeposits and on the mechanism of their release (e.g. active and passive transport) was formed by Rovira (1979 in Richards 1987). Despite numerous other proposed nomenclatures, this one has been used most extensively. Jones et al. (2009) in their review described the main categories of rhizodeposits according to the process of their excretion: exudation, secretion, border cell detachment, senescence-derived compounds and C flow to symbionts.

Exudation and C Flow to Symbionts

This category represents low-molecular weight compounds leaking passively from roots. The plant is expected to exert little direct control over this diffusion. According to Jones et al. (2009), the critical factors, which influence the rate of passive C losses, are the root-soil concentration gradient, permeability of plasma membrane and spatial location of solutes in the root tis-

sue, and the most influencial factor in the creation of all rhizodeposits prior to their release – the rate of photosynthesis.

Exudates are usually simple or oligo – sugars, amino acids and low molecular weight organic acids. Lambers et al. (2009) reported that this passive leakage of exudates represents less than 5 % of daily fixed C. Although the C flow to symbionts probably originated as the passive exudation (Brundrett 2002), C costs directed to symbionts are much higher compared to passive C leakage: up to 20% (Tinker et al. 1994, Morgan et al. 2005) or even 30 % (Brüggemann et al. 2011) of recent photosynthates can be used by mycorrhizal fungi.

Secretion

To this category belong low and high-molecular weight compounds, which are expelled actively, at the expense of energy (e.g. by active membrane transport or by exocytosis, Badri and Vivanco 2009). Some of them have a signalling character, which promote or inhibit the growth of microbes (Bais et al. 2004, Doornbos et al. 2011, Rudrappa et al. 2008) or other plants (Bais et al. 2004, Rudrappa et al. 2007). Others are exoenzymes, which cleave specific chemical bonds to obtain nutrients from soil organic matter (Lambers et al. 2006, Adamczyk et al. 2009). Other compounds are also released in order to mobilize nutrients: Negishi et al. (2002) described the production of phytosiderophores, which are secreted by grasses in order to enhance Fe^{3+} uptake. Dacora and Phillips (2002) explained how the production of phenolics can enhance mobilization of Fe and P. Secretion of organic acids enhances the mobility of P and reduces the toxicity of Al^{3+} (Jones 1998). By secretion of secondary metabolites (e.g. salicylic acid) plants stimulate microorganisms to biodegrade xenobiotics (Singer et al. 2003).

Compared with passive exudation, the secretion is much more controlled by the plant. Ueno and Ma (2009) reported on intensive secretion of phytosiderophores under certain temperatures and Dessureault-Rompré et al. (2007) described the outburst of organic acids from cluster roots in the afternoon. One of the potential explanations of this effect might be a higher concentration of assimilated C in the plant at the second part of the day. Another reason could be the effort to avoid microbial utilization of organic acids before they manage to mobilize at least some amount of soil P.

The highest amount of organic C is usually secreted in the form of mucilage. This mixture of polysaccharides, proteins and phospholipids is released by exocytosis from the root cap cells to form a gelatinous protective layer around the root (Jones et al. 2009). The mucilage soon mixes with microbial cells and their metabolic products (e.g. polysaccharides of glycocalyx) to form

a so-called mucigel. As compiled by Vančura (1988a), the mucigel layer spans from 0.5 to 0.8 μm and is much thicker on inoculated than on sterile roots. Therefore, it is thought that a substantial portion of mucigel is produced by bacteria. This gelatinous (mucilagenous) layer provides various benefits to both plant and microbes. Carboxylic groups of the mucilage complex potentially toxic metals and protect apical meristems (e.g. immobilization of Al, Cd and Cu; Mench et al. 1986). Some specific mucilage components possess antimicrobial properties and protect the root from pathogen attacks (Sobolev et al. 2006). Furthermore, the gelatinous layer reduces frictional resistance when the root tip is moving through the soil (Hawes et al. 2003). The water-intrinsic affinity of mucilage is remarkable – its water content can be 100 000 times greater than its dry weight (McCully and Boyer 1997). This fact suggests an important role of mucigel in water supplies and in the prevention against drying out. The continual water flow towards the rhizoplane is maintained by this gelatinous layer as well (Jones et al. 2009).

Border Cells (Slough-off cells)

A small proportion of plant organic C enters the soil in the form of so-called border cells, which detach from the external layers of root cap and stay alive in the mucigel for several days (Jones et al. 2009). Border cells provide another means by which plant can reduce the frictional resistance (Hawes et al. 2003). In addition, border cells help to complex some toxic metals and produce molecular signals to inhibit pathogens or promote symbioses (Hawes et al. 1998).

Senescence-Derived Compounds

This category contains chemicals released to the rhizosphere during the degeneration of various parts of the root epidermis (e.g. root hairs and cortical cells, Jones et al. 2009). Chemical composition of senescent-derived compounds probably depends on whether the root undergoes spontaneous (necrosis) or programmed (apoptosis) cell death. However, we do not know enough about the differences between effects of these two processes on the rhizosphere (Jones et al. 2009).

Other important parts of rhizodeposition are CO_2 respiration (a relatively continuous input of inorganic C) and dead root biomass (a prevalently temporal input of organic C). The role of dead roots, as a part of plant litter, is discussed more in the chapter 2.2.3. A compiled list of organic compounds released by roots is presented in Table 3.

Table 3. A summary of organic compounds released by roots. Modified from Uren (2001).

Organic Compounds Released by Plant Roots	
Sugars and polysaccharides	Arabinose, fructose, galactose, glucose, maltose, mannose, mucilages of various compositions, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose
Amino acids	α-alanin, β-alanin, γ-aminobutyric, arginine, asparagine, aspartic, citrulline, cystathionine, cysteine, cystine, deoxymugineic, 3-epihydroxymugineic, glutamine, glutamic, glycine, homoserine, isoleucine, leucine, lysine, methionine, mugineic, ornithine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine, etc.
Organic acids	Acetic, aconitic, ascorbic, benzoic, butyric, caffeic, citric, p-coumaric, ferulic, fumaric, glutaric, glycolic, glyoxilic, malic, malonic, oxalacetic, oxalic, p-hydroxybenzoic, propionic, succinic, syringic, tartaric, valeric, vanillic
Fatty acids	Linoleic, linolenic, oleic, palmitic, stearic
Sterols	Campesterol, cholesterol, sitosterol, stigmasterol
Growth factors	p-amino benzoic acid, biotin, cholin, N.methyl nicotinic acid, niacin, pantothenic, vitamins B1 (thiamine), B2 (riboflavin), B6 (pyridoxine)
Enzymes	Amylase, invertase, peroxidase, phenolase, phosphatases, polygalacturonase, protease
Flavonons and nucleotids	Adenine, flavonone, guanine, uridine / cytidine
Other substances	Auxins, ethanol, glucosides, hydrocyanic acid, inositol, scopoletin, etc.

2.2.1.2 Biological Availability and Degradability of Rhizodeposits

Biological Availability

In a broader sense, this characteristic refers to the potential of the microbial community to interact with rhizodeposits and it is affected by temporal and spatial distribution of organic compounds and microorganisms in soil. It encompasses not only the direct bioavailability of originally released compounds but also the bioavailability of these compounds after their hydrolyzation by exoenzymes (Marschner and Kalbitz 2003). Bioavailability of dissolved organic matter (DOM) in the bulk soil depends mainly on soil characteristics (size of pores, soil aggregates, sorption activity of the soil and also on drought, Marschner and Kalbitz 2003).

The availability of rhizodeposits for microbes depends on their location in the rhizosphere. Symbiotic microorganisms receive organic compounds directly while other associative rhizosphere inhabitants compete for rhizodeposits and some of them are forced to utilize less biodegradable substrates.

Biological Degradability

The biodegradability has two meanings:

1) A microbial uptake or breakdown of the original compounds, which are then used for the biosynthesis of microbial cell material (Marschner and Kalbitz 2003). This characteristic is expressed by a microbial assimilation efficiency or yield factor calculated as C present in microbial biomass per the total C utilized (Cheng and Gershenson 2007).

   C partitioning studies show that exuded C can be quickly assimilated by microbes as it was detected in microbial DNA within three hours after labeling (Clayton et al. 2010). Root-derived C fraction in microbial biomass represents the prevalent amount of recently fixed C present in soil (Kaštovská and Šantrůčková 2007, Chaudhary et al. 2012).

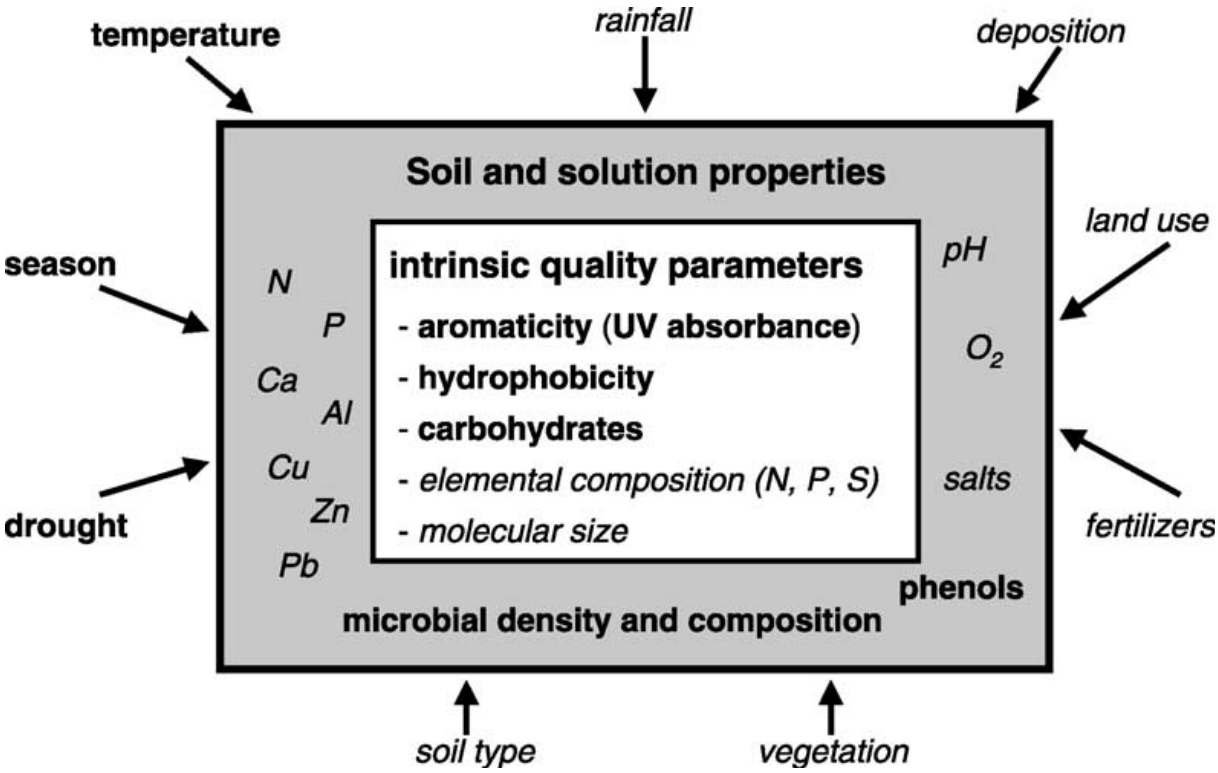
2) A complete mineralization to obtain energy and inorganic nutrients (Marschner and Kalbitz 2003).

   The final product of rhizodeposit C mineralization is CO_2. Approximately one third of belowground C allocation is consequently respired (Jones et al. 2009). Rhizosphere respiration encompasses root respiration and rhizomicrobial respiration (of rhizodeposits and dead roots) and represents the mayor CO_2 flux from grassland soil (75 % of total soil respiration on average, Kuzyakov and Cheng 2001).

In comparison to bioavailability, the biodegradability is controlled by four distinctive groups of factors (Marschner and Kalbitz 2003): 1) by the chemical character of DOM (molecular size, chemical structure, polarity and acidity), 2) by soil and solution properties (redox potential, pH, content of salts, nutrients, metals and toxic organic compounds and on the composition of the microbial community), 3) by external factors (e.g. seasonality in temperature, moisture, input of organic matter, etc.) and 4) by microbial density and composition. The groups of factors, which influence the biodegradability of rhizodeposits, are shown in Figure 1.

Composition of microbial community and development of particular functional groups might be affected by rhizodeposition of particular compounds. Concerning the fact that the composition of rhizodeposits is species specific, the composition of plant species at a particular site may strongly influence the local microbial diversity and consequently also biogeochemical cycles. For instance, the results of Ström et al. (2012) showed that cotton grass (*Eriophorum scheuchzeri*) induces the activity of methanogens associated with its roots via the higher release of acetate.

Figure 1. Summary of parameters, which have been identified as controlling factors for DOM biodegradability. Bold: verified in several studies; italic: with conflicting or circumstantial evidence in some studies or assumed factor (Marschner and Kalbitz 2003).



Biological availability of rhizodeposits influences in an essential way the rhizosphere microbial community. However, ^{13}C label incorporated in microbial biomass can soon be detected also at higher trophic levels, e.g. in earthworms and Collembola rather than in Acari and Enchytraeidae (Brüggemann et al. 2011).

2.2.1.3 Other Biological Roles of Rhizodeposits

Besides being the substrate for rhizosphere microbiota, rhizodeposits maintain a variety of other functions. Their role in nutrient uptake is summarized in chapter 2.2.2.

Plants are able to "cultivate" their own species-specific microbial community in the rhizosphere (Berg and Smalla 2009, Marchner et al. 2001) by promoting or inhibiting particular microbial groups or species. Plant families (e.g. cruciferous plants, which release high levels of S-rich compounds), genera (e.g. rhizobial legumes: *Medicago*, *Melilotus*, *Pisum*, etc.) and even cultivars of the same species (e.g. T4 potato genetically modified cultivars) shape specific microbial community in their rhizosphere (Berg and Smalla 2009). Invasive plant species are reported to interact with the new microbial community differently than the native plants and they also interact differently with the same microbes in their native and non-native ranges (Rout and Callaway 2012). The ollowing text briefly deals with the complex role of rhizodeposits as signalling compounds.

Positive Communication

The role of rhizodeposits in plant-plant or plant-microbe communication has been intensively studied; however, our understanding of these complex processes is limited (Bais et al. 2004, Hartmann et al. 2009). Most information has been obtained on the rhizobial nodule formation. A legume root produces flavonoids and betaines to communicate with *Rhizobia*. The signalling compound enters bacterial cells and binds with a bacterial gene product, which interacts with the gene promotor for *Nod* genes. The products of these genes (*Nod* factors) induce division of cortical cells and curling of root hair, which becomes the initial part of nodule (Lambers et al. 2009). Flavonoids also act as the signal molecules for formation of AM (arbuscular mycorrhiza) and they are likely to play a role in ECM (ectomycorrhiza) formation (Neumann and Römheld 2001). In addition, when AM plants suffer from lack of P, they produce strigolactones (sesquiterpene lactones), which induce extensive hyphal branching in germinating spores of their fungal symbiont (Bouwmeester et al. 2007). Furthermore, some of plant secondary metabolites (e.g. salicylate) are known to trigger microbial biodegradation of xenobiotics in polluted soils (Singer et al. 2003).

Other interesting types of signalization are various tritrophic interactions (Badri and Vivanco 2009, Rassman and Turlings 2008). For instance, grazing on plant roots induces the pro-

duction of volatile compounds by roots. These chemicals attract entomopathogenic nematodes, which prey on soil herbivorous larvae (Rassman and Turlings 2008).

Negative Communication

Leaving aside other negative interactions such as competition for resources, plants can inhibit the growth of microorganisms (Bais et al. 2005, Doornbos et al. 2012), other plant species (allelopathy, described on *Phragmites australis*, Rudrappa et al. 2007) and even the species itself (autotoxicity, Zhang et al. 2009) by secretion of species-specific substances. The inhibition of microbes functions directly (Singer et al. 2003) or indirectly by promoting of microbial and mycorrhizal symbionts, which produce pathogen-inhibiting compounds (Martin et al. 2001).

A common feature of most of allelopathic compounds (e.g. benzoic and cinnamic acids, artemisinin, juglons, monoterpenes, etc.) is their ability to induce the formation of reactive oxygen species in the soil (Weston and Duke 2003). It leads to an oxidative stress from which neighbouring plants suffer. However, it is not easy to evaluate the importance of allelopathy. In many cases the experiments were set up in a way that did not correspond with the natural conditions (e.g. study on *Typha domingensis* allelopathy, Gallardo-Williams et al. 2002). The persistence of allelochemical compounds in soils depends on soil characteristics (e.g. sorption capacity, Tharayil et al. 2008). Interestingly, some allelochemical compounds are reported to persist in soils for a longer time (allelochemicals derived from decomposing litter, Rashid et al. 2010).

Autotoxicity is a phenomenon mostly visible in agriculture. For instance, cucurbite crops (e.g. *Cucumis sativus,* Zhang et al. 2009) release several autotoxic compounds (e.g. cinnamic acid). Crop rotation is then a part of an efficient management to avoid gradual declines in crop yields (Lambers et al. 2009). Autotoxicity could be also involved in root growth regulation. In response to obstructions present in the soil, some oligotrophic grass species secrete autotoxic compounds to avoid further growth in the same direction (Semchenko et al. 2008).

Roots secrete antimicrobial compounds such as rosmarinic acid to inhibit multiple soilborne microorganisms (Bais et al. 2004). Secretion of some organic acids (e.g. malic acid) attracts biocontrol microorganisms (e.g. *Bacillus subtilis* FB17) which inhibit pathogens (e.g. leaf pathogen *Pseudomonas syringae* pv. *tomato*, Rudrappa et al. 2008). An efficient and wide spread strategy of plants is to promote microorganisms which produce secondary metabolites such as antibiotics, biosurfactants or lytic enzymes to suppress deleterious microorganisms (Doornbos et al. 2012).

2.2.1.4 Research on Rhizodeposits

The first studies on the rhizosphere appeared in the first decades of twentieth century (the term rhizosphere was defined by Hiltner in 1904, Vančura 1988a). Since then, the quantity and composition of rhizodeposits, the mechanisms of rhizodeposition, the microbial activities in the rhizosphere, symbiotic relationships and other characteristics have been studied profoundly. However, obtaining reasonable scientific data is still difficult since the rhizosphere microenvironment is very complex and dynamic. In spite of numerous experimental challenges, the rhizosphere has been widely studied as declared by more than 14.500 publications on this topic in the Web of Science database (number to December 2012, internet reference 1).

2.3 DIFFICULTIES OF RHIZODEPOSITION STUDIES

Three main obstacles for rhizodeposition research were described by Kuzyakov and Domanski (2000): *i)* low concentration of root-derived organic substances in the soil compared to the content of other organic substances, *ii)* fast decomposition of root-derived compounds by soil microorganisms ($T_{1/2}$ = 0.5-10 days) and *iii)* appearance of rhizodeposits in the narrow zone of soil adhering to the root surface.

Phillips et al. (2008) summarized a set of common challenges, which need to be resolved when using all 'traditional' methods: 1) the capturing of released C before it is assimilated by microbes, 2) the selection of a medium that does not affect root physiology and exudate recovery and 3) the distinguishing of exuded compounds from other soluble C compounds in the solution.

1) Roots growing under sterile conditions do not provide representative data on plant rhizodeposition. Generally, the results are underestimated because the flow of C from root is not driven by steep gradient caused by continual microbial uptake of rhizodeposits. The increase of organic C in the solution then slows down the rhizodeposition. Vančura (1988a) compared the rhizodeposition of sterile and inoculated plant roots. When microflora is present on the root, the study is more comparable to natural conditions. Yet, it is impossible to measure the amount of released C qualitatively and quantitatively; as its large part is immediately metabolized by microbes (see point 3). Some studies used antibiotics to suppress rhizosphere microbiota. This method has probably many unknown side-effects and the results depend significantly on the type and concentration

of antibiotical compounds used and on the plant species studied (Neumann and Römheld 2001).

Vančura (1988a) commented on the choice of sampling period. Frequent sampling intervals (e.g. every two days) resulted in root length shortening (about 20 %) and in root biomass decrease (about 11 %; Vančura and Přikryl 1980 in Vančura 1988a). Thus, the percolating (non-static) systems of rhizodeposit trap solution should be preferred to the static ones in the culture-based experiments (Phillips 2008).

2) The root system changes its architecture when grown in the solution without any mechanical support (Lavelle and Spain 2001). Some kind of solid substrate should be, therefore, used for the cultivation. However, it is not an easy choice. Working with sand or glass beads (ballotini) is the easier way, but the sharp edges of sand grains can injure roots and, therefore, bias rhizodeposition data. Sand, even acid-washed, can act both as a source and as a sink for C (Phillips unpublished data in Phillips 2008). The best way is to sample root rhizodeposits in *situ*. For qualitative in *situ* research, various microsuction cups were developed (Dessureault-Rompré et al. 2007). Agar, specialized resins and filter papers were used for microscale research of rhizodeposits (Gregory and Hinsinger 1999). Phillips et al. (2008) proposed a new method of quantitative rhizodeposit collection in *situ*. The rhizodeposits were collected by syringes filled with acid-washed glass beads and C-free nutient solution. Prior that, roots were carefully cleared of soil and left for 2-3 days to recover from this cleaning. A longer time-scale was another positive aspect to this experiment.

3) For the purposes of qualitative measurements, axenic laboratory experiments are acceptable. Various methods of isotope labeling are widely used for qualitative and quantitative identification of rhizodeposits (see below: *Isotope Tracer Techniques*). As mentioned in point 1), on non-sterile roots, the rhizodeposits are immediately metabolized by microbes. Thus, it is very important to adjust the time of sampling accordingly when we try to distinguish whether detected labeled compounds are real rhizodeposits or are previously modified microbial products.

Quite reasonably, there is a tendency to move the rhizosphere experiments to the field. This fact is accompanied by other issues: A) the laboratory methods (so far inaccurate with respect to the points above) should be further modified for field use. B) In most cases the roots

must be temporarily removed from the soil to be studied. This inevitably leads to modifications of root environment and to consequent alteration of measured characteristics. C) Roots and rhizosphere processes are very variable in space and time. It brings difficulties in capturing this variability and adjusting the experiment to an ecosystem-scale (Phillips et al. 2008).

Isotope Tracer Techniques

An elegant tool to overcome some of the issues mentioned above is the use of isotope tracers (stable and radioactive isotopes). These techniques allow conducting experiments in *situ* without or with only a minor modification of natural conditions. Using them, we are able to distinguish plant-derived C in various pools of the system. Kuzyakov and Domanski (2000) reviewed the isotope techniques of belowground C allocation studies. Three general methods are used for different purposes: pulse labeling, stable labeling and natural ^{13}C abundance.

Pulse labeling is the most widely used approach up to now. In this method, the shoots assimilate labeled CO_2 for only a short period in an airtight chamber. The obtained data refering to the recent photosynthate distribution can be used for kinetic investigations of C fluxes to the soil and corresponds to the relative distribution of assimilates at the moment of labeling (Chaudhary et al. 2012, Clayton et al. 2010, Kaštovská and Šantrůčková 2007, Sauer et al. 2006). All of the rare C isotopes (^{11}C, ^{13}C and ^{14}C) are suitable for pulse-labeling. A short half-lifetime of ^{11}C (20.4 min) allows repeated labeling of the same plants (Kuzyakov and Domanski 2000). The limit of pulse-labeling method is the fact that the results are not representative of the whole growth period. For these purposes, a series of labelings in regular intervals during the plant growth period provides reasonable estimates of belowground C input (Kuzyakov and Domanski 2000).

Stable (continuous) labeling is more demanding as shoots are exposed to the labeled CO_2 atmosphere for a longer period. The method is expensive and requires special equipment for plant incubation and thus has been used less often than pulse-labeling. The provided data is relevant for total C distribution during the labeling period (usually between the emergences of the first leaf to the sampling time) and can be applied for the estimates of total C allocated belowground (Brüggemann et al. 2011). Root-derived and SOM-derived CO_2 fluxes can be distinguished by this method (Kuzyakov and Domanski 2000). ^{13}C and ^{14}C isotopes could be both used for continuous labeling.

Although the work with ^{11}C and ^{14}C (radioactive) isotopes is more complicated than the work with ^{13}C (stable) isotope, their detection limits are more sensitive: 10^{-19} mol, 10^{-13} mol and

10^{-7} mol for ^{11}C, ^{14}C and ^{13}C respectively (Kuzyakov and Domanski 2000). Some studies combine two different tracer techniques or the use of two different isotopes to describe the fluxes and their dynamics better: e.g. a combination of simultaneous ^{14}C labeling with the natural abundance ^{13}C tracer method was used to link photosynthesis with rhizosphere respiration and organic matter decomposition (Kuzyakov and Cheng 2001). Natural ^{13}C depletion in *Lolium perenne* litter together with ^{15}N-labelled fertilizer were used to describe how three grassland species varying in competitiveness influence the soil C and N cycles under different N availability (Personeni et al. 2005).

The natural ^{13}C abundance method is based on the natural discrimination of ^{13}C compared to ^{12}C isotope during various processes (e.g. photosynthesis, post-carboxylic transformations, etc.; Brüggemann et al. 2011). C_3 plants, fixing CO_2 directly to Rubisco enzyme, are relatively more ^{13}C depleted (-27 ‰) than C_4 plants, which fix CO_2 by phosphoenol pyruvate carboxylase first (-13 ‰, Kuzyakov and Domanski 2000). This fact can be used to estimate C translocations by plants and to determine original substrates for CO_2 emissions from soil. When C_3 plants are cultivated on a C_4 soil (a soil where C_4 plants were grown previously), or vice versa, the relative participation of SOM decomposition and rhizodeposit utilization on C cycling in soil could be distinguished (Personeni et al. 2005). This method is relatively easy to conduct; however, the cultivation of C_3 plants on C_4 soil (or vice versa) is not natural (Kuzyakov and Domanski 2000). Brüggemann et al. (2011) reviewed the available data on C isotope fluxes and fractionation in the plant-soil-atmosphere continuum.

High-resolution and highly sensitive analyses are required for isotope studies (Kuzyakov and Domanski 2000). The combinations of liquid or gass chromatography with isotope-ratio mass spectrometry enable to describe the dynamics and fates of assimilated C (Brüggemann et al. 2011). NanoSIMS, i.e. secondary ion mass spectrometry, enables to study isotope ratios at the nanometer scale, which could bring a deeper insight into rhizosphere processes (Brüggemann et al. 2011).

2.2.2 PLANT NUTRIENT UPTAKE

Plant ability to acquire nutrients was thought to be restrained to only few, mostly inorganic, chemical compounds. Recently, the evidence of root ability to uptake organic compounds has been reported (Jones et al. 2005, Schimel and Bennett 2004). Furthermore, Jones et al. (2009) suggested four explanations why the C flow in the rhizosphere is bidirectional: 1) direct exudate recapture (from the soil back to the root), 2) indirect exudate recapture (from apoplast back

to symplast), 3) organic nutrient (e.g. amino acid) capture from soil and 4) transfer of chemicals involved in inter-root and root-microbial communication pathways.

Throughout the terrestrial ecosystems, plants are mostly limited by P or by N, following this pattern: tropical regions are mainly limited by P in comparison to boreal and arctic regions where N limitation prevales (Martinelli et al. 1999). It could be partly explained by the old age of tropical soils and partly by the temperature optimum for nitrogenase activity. This enzymatic activity reaches maximum at 26°C, hence the lower N_2 fixation occurs in colder areas (Houlton et al. 2008). Lambers et al. (2009) described how N limitation could be switched to P limitation in the course of primary and secondary succession: N_2 fixers incorporate more and more N into the system and at the same time the source of P (the maternal bedrock) is slowly getting exhausted. The very old soils thus mostly result in P limitation.

The limitation of more than one resource (i.e. co-limitation) is not rare in natural environments. Arrigo (2005) described three different types of co-limitation in a simple system with two resources (two nutrients and two phytoplankton species consuming them). A multi-nutrient co-limitation is present when both nutrients are at the levels too low for uptake. Both nutrients need to be added to increase the species growth in this case. A biochemical co-limitation occurs when the uptake of one nutrient depends on the availability of the latter one (e.g. the secretion of exoenzymes, see chapter 2.2.2.3). The experimental addition of both nutrients separately would then cause a different response. A community co-limitation appears when members of the community are each limited by a different nutrient. Examples of co-limitation and cross-talk between nutrients (S, N, P and Fe), their acquisition and utilization in various metabolic pathways, are compiled by Ohkama-Ohtsu and Wasaki (2010).

The type of vegetation can substantially influence the availability and cycling of particular nutrients. Oelmann et al. (2011) described how plant species composition and diversity influence P cycling: different plant species possess different strategies of P acquisition and reuse. Because of that, the species-rich grasslands are better prepared for a possible P limitation than are less diverse communities. Plant species composition in grasslands influences C and N cycling as well (Personeni et al. 2005). Personeni and Loiseau (2005) compared the impact of two grassland species differing in their competitiveness for N: the stronger competitor (*Lolium perenne*) induced a "complete" N cycle between plant stand and SOM, while more conservative species (*Dactylis glomerata*) induced a "shorter" N cycle between plant stand and root litter.

Plants have developed various strategies to sustain their life under nutrient limited conditions, such as changes in root morphology and functions, secretion of chemically active compounds and various symbioses (Figure 2). An interesting strategy has been developed by carnivorous plants, which absorb nutrients from the decomposing prey (Allison 2006). However, the wide-spread and also most-studied adaptations are symbiotical interactions (plants and fungi, plants and N_2 fixers), which are discussed in chapter 2.3.1.

2.2.2.1 Changes in Root Morphology

Root hairs, root caps and the zones between them are known as the root parts capable of effective absorption of water and nutrients (Lavelle and Spain 2001). When the plant is limited by nutrients, which are taken up by roots, the root / shoot ratio (expressed in biomass dry weight) usually increases (Vančura 1988a). The plant invests relatively more in the enlargement of its absorption surface. Besides the increase of root biomass, Lambers et al. (2006) in their review commented on other four morphological changes observed under nutrient limited conditions: 1) root architecture changes (spatial configuration of roots of different orders and ages, 2) root length increase, 3) specific root length (SRL) increase coupled with the decrease of root diameter and 4) formation of more and longer root hairs.

Specialized structures (e.g. cluster roots) improve P uptake efficiency (Lambers et al. 2006). So far, we are not aware of any specialized root structures for enhacement of N uptake, except for nodular symbiotic structures. This is explained by much higher mobility of N nutrients than of P compounds (Brady and Weil 2002). Root morphological adaptations in symbiotic relationships are more (nodules and short ECM roots) or less (AM intracellular arbuscules) apparent but definitely the best-studied.

2.2.2.2 Nitrogen Uptake

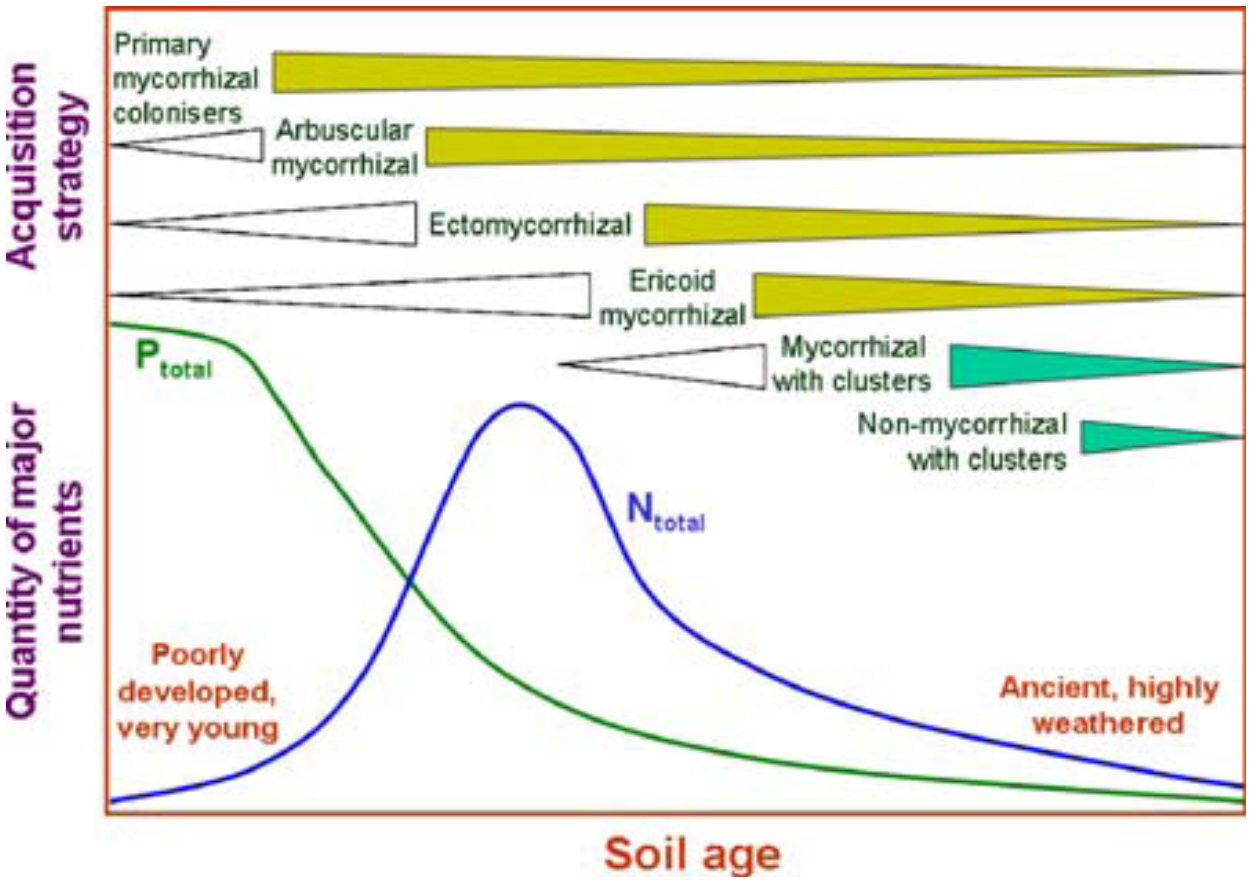
Plants are able to acquire N in both inorganic (NH_4^+ and NO_3^-) and organic (amino acids) form (Jones et al. 2005). Nevertheless, plants are not able to fix the ubiquitous N_2 from the atmosphere. This is the reason why they have evolved symbioses with prokaryotic N_2 fixers (chapter 2.3.1). Rhizobial nodules formed by *Rhizobium* bacteria are typical for legumes (Vessey et al. 2005). Actinorhizal nodules (rhizothamnia) formed by the actinomycete *Frankia* are common within eight angiosperm families (e.g. Casuarinaceae, Rhamnaceae, Betulaceae and *Myricacea*, Hocher et al. 2009). Some other plants have evolved symbioses with N_2-fixing cyanobacteria (Rai et al. 2000): peat and feather mosses (e.g. *Sphagnum* spp., *Pleurozium schreberi*, Gentili

et al. 2005), ferns (*Azolla*), gymnosperms (cycads, e.g. *Macrozamia*) and the only angiosperm genus *Gunnera*. In traditional and modern agriculture "N_2-fixing plants" are used to fertilize the soil. For instance, *Azolla* fern is applied as a biological fertilizer to rice fields (Choudhury and Kennedy 2004).

Associative N_2 fixing microbes in the rhizosphere (e.g. *Azotobacter*, *Rhizobium*) could be also important for plant N-budget (Houlton et al. 2008). They do not supply Nto the plant directly as do the symbionts: the organic N must be first released to the soil via microbial turnover and only then (usually after an additional mineralization) can it be absorbed by roots. Ectomycorrhiza and ericoid mycorrhizae are reported to enhance plant N uptake as well (Lambers 2009).

Some plants are known to secrete extracellular proteases; enzymes, which cleave amin-opeptide bonds in soil organic matter and thus make N more accesible (Adamczyk et al. 2009). In fact, it might be a counter-productive strategy to uptake N at the expense of N-rich enzymes production. This can perhaps explain why the secretion of proteases seems to be less common among plants compared to phosphatases or phases.

Figure 2. Changes in total N and total P as a function of soil age and in nutrient-acquisition strategies. The soil age scales from 'poorly developed very young soils' (e.g. soils resulting from recent volcanic eruptions) to 'ancient weathered soils' (i.e. soils that have been above sea level and have not been rejuvenated by glaciation over several millions of years). Some mycorrhizal species may co-occur with non-mycorrhizal cluster-bearing species in severely P-impoverished soils, but they never become dominant. The width of the triangles reffering to the different ecological strategies of nutrient acquisition provides a (relative) measure of the abundance of these strategies as dependent on soil age. The total P level in soils range from 30 to 800 mg kg-1, while N levels range from < 5 to 8,000 mg kg-1 (Lambers et al. 2009).



2.2.2.3 Phosphorus Uptake

Sources of P

In contrast to C and N, which are fixed biologically and their main source is the atmosphere, parent soil material (bedrock) serves as the primary source of P. Relatively young soils and igneous rocks contain, in general, higher amounts of P (Figure 2), however, this non-renewable source can become exploited in the course of time (Lambers et al. 2009).

P availability is not directly influenced by redox potential changes, except for P released from ferric insoluble complexes when they are reduced to ferrous cations. Therefore, in wetland soils, where redox potential is generally lower, the P is more mobile and accessible (Mitsch and Gosselink 2000). P availability changes substantially according to pH values and it is the most available at near neutral pH values (Brady and Weil 2002). in alkaline (basal) soils, P is trapped into insoluble complexes with Ca. as a result, calciphilous plants generally secrete higher amounts of organic acids in order to mobilize this unavailable P (Jones 1998). In acid soils, P is bound in al and Fe sesquioxides and has lower availability when fixed by Fe sesquioxides at pH lower than 3 (Brady and Weil 2002).

Particular forms of P compounds act differently under various environmental conditions. For our purposes, it is useful to distinguish between low and high-turnover P forms (Adams 1992). Orthophosphate in the soil solution, its proportion reversibly attached to the soil particles (exchangeable P) and some P-containing organic compounds, undertakes rapid turnover. On the other hand, primary (apatite) and secondary (insoluble complexes with Fe, Ca and Al) minerals are more stable and P could be slowly released from them by the process of mineral weathering. Occluded inorganic P (physically encapsuled by minerals) and some other organic P compounds are also involved in the low turnover-rate processes. on a shorter time scale, most of the P is acquired from dead organic material and microbial biomass is one of the most important organic P pools (from 3 to 90 % but mostly between from 30 to 50 % of P in soil is organic, Haider and Schäffer 2009).

Rhizodeposition of Low Molecular-Weight Organic Acids

A common strategy to enhance P acquisition is to secrete low molecular-weight organic acids (e.g. citrate, malate, oxalate, etc., Jones 1998). Their carboxylic groups serve as complexing agents for metal cations present in soil solution. Coupled with that, a displacement of anions bound in soil matrix happens. Thus, not only P but also Fe and other micronutrients are mobilized by organic acids. On the other hand, toxic elements are affected in the similar way and al and Ni, particularly, are converted to forms, which are toxic for plant growth (Ahonen-Jonnarth et al. 2000). Some plant species from typically non-mycorrhizal families such as Brassicaceae, Caryophyllaceae, and Proteaceae maintain mycorrhiza in P-impoverished but Ni-rich soils (e.g. serpentines, Jones et al. 2009). Boulet and Lambers (2005) suggested that those species have retained the ability to form AM mycorrhiza to gain P as well as to avoid Ni toxicity,

which would be triggered by organic acid release. Last but not least, organic acids belong to important weathering agents in the process of pedogenesis (Jones 1998).

Cluster Roots

Although these specialized roots carry out many other functions (e.g. plant hormone metabolism etc. Lambers et al. 2006), the secretion of low molecular-weight organic acids is the most studied characteristic. This secretion is often not continuous but occurs in the exudative outbursts (Dessureault-Rompré et al. 2007). Lambers et al. (2006) presented a view of known types of cluster roots: 1) "bottle-brushed" proteoid roots of Proteaceae and proteoid-like roots of some Fabaceae (proteoid roots can be either "simple" or "compound" into multiples of "simple" root clusters with tendency to form dense root-mats); 2) dauciform ("carrot-like") roots of Cyperaceae and 3) "capillaroid roots" of Restionaceae.

Secretion of Exoenzymes

P in organic matter accounts for 30 - 80 % of total P present in the soil (Adams 1992). For this reason, both roots and rhizosphere microbes release extracellular enzymes to cleave the organic matter and make P available (Lambers et al. 2006). Phosphatases are reported to hydrolyse various organic compounds, mostly phosphate of monoesters (mononucleotides and inositol phosphates) and diesters (nucleic acids and phospholipids, Adams 1992, Rejmánková et al. 2011). Phosphomonoester forms of organic P are considered to be more available and they require only phosphomonoesterases for hydrolysis. Phosphodiesters must be hydrolysed by both phosphodiesterase and phosphomonoesterase to release phosphate. Rejmánková et al. (2011) reported that both types of enzymes are active on roots of various wetland plants. Phytases are efficient in phytate (= *myo*-inositol penta- and hexa-phosphates) hydrolysis. Mycorrhizal fungi are known to secrete high amounts of phosphatases (Ahonen-Jonnarth et al. 2000), which is important for mycorrhizal plants.

The production of exoenzymes is costly; therefore the secretion of phosphatases is triggered only under P deficiency (Houlton et al. 2008, Lambers et al. 2006, Rejmánková et al. 2011). As exoenzymes are N-rich protein molecules, N-limitation can also result in a decrease of exoenzyme secretion. Hence, the activity of N_2 fixers can indirectly influence the production of exoenzymes (Houlton et al. 2008). The N / P ratio in plant tissues serves as a good predictor of phosphatase activity (above 10, the secretion of mono- and diesterases increases, Rejmánková et al. 2011).

In addition to the strategies described above, Lambers et al. (2006) completed the list of P acquiring strategies with 1) the hydraulic redistribution: this redistribution of water within roots

(upwards, downwards and horizontally) helps to transport soluble P compounds from soil to roots, which is of particular importance in arid soils; 2) the secretion of phenolics and mucilage, an often neglected factor of plant P acquisition; 3) the increased expression of membrane high-affinity P_i transporters.

2.2.3 NUTRIENT STORAGE AND LITTER DECOMPOSITION

Besides various nutrient uptake strategies, plants have other ways to improve their nutrient budget. Once the nutrients are present in the plant, they can be reabsorbed from senescing tissues and reused or stored. Common percentage of N and P reuse by plants is around 50% for each of them (Aerts 1996). However, this proportion is species specific and fluctuates according to nutrient availability in the environment. For instance, the sedge (*Eleocharis* spp.) can recycle up to 80 % of its P content under P limitation (Rejmánková and Snyder 2008). In contrast, C content in the senescing tissues remains almost unchanged because C is usually incorporated into structural materials.

The ability to translocate nutrients from senescing plant material also reflects on the quality of plant litter, which is, together with rhizodeposition, the most important C input to the soil. It is crucial to know how much this organic material is available for soil microbiota and subsequently for all other organisms. Litter bioavailability is primarily determined by its chemical composition: a nutrient-rich litter triggers the activity of microbial communities (Richards 1987). Chemical recalcitrance is higher in plant structural material (Lavelle and Spain 2001). Particularly, aromatic compounds (e.g. lignin and phenolics) persist in the ecosystem for long periods and, thus, keep larger amounts of C and nutrients immobilized (Berg and Claugherty 2008). An extreme example are more than 3000 years old undecomposed trunks of *Sequoiadendron giganteum* in the forest understory, internet reference 2). Some plants (e.g. peat mosses) are known to produce biological conservants such as polyuronic acid, which to inhibit microbial activity (Aerts et al. 1999) and results in the accumulation of peat (partly decomposed organic material).

Wetlands are known to accumulate organic matter, because the decomposition is inhibited by lack of oxygen (Mitsch and Gosselink 2000). The rate of litter decomposition does not depend only on the chemical composition of litter but also on the composition and activity of microbial community. The activities of decomposers are influenced by multiple environmental conditions (Kuzyakov and Cheng 2001, Personeni and Loiseau 2005, Rejmánková and Sirová 2007). Generally, the decomposition is faster in warmer than in colder climate, under wet than under dry conditions and under aerobic than in anoxic conditions (Brady and Weil 2002).

2.4 ROLES OF RHIZOSPHERE MICROFLORA

Soil is the most biologically diverse terrestrial environment. Without living creatures, it is not soil any more but only a substrate, which misses the great potential of various biogeochemical processes. Plants and microbial communities in their rhizosphere have been closely coupled since plants colonized terrestrial ecosystems and evolved roots (Brundrett 2002).

Generally, positive and negative interactions between plants and microbes are distinguished, both in direct and indirect way. These interactions may have either loose (associative organisms) or tight (symbionts, parasites and pathogens) character. For the plant, the positive relationships are represented by three main effects – 1) nutrient and water acquisition; 2) plant-growth promotion and 3) protection against pathogens. Negative effects of rhizosphere microbiota on the plant could be summarized as 1) plant-growth inhibition; 2) colonization of parasites and pathogens.

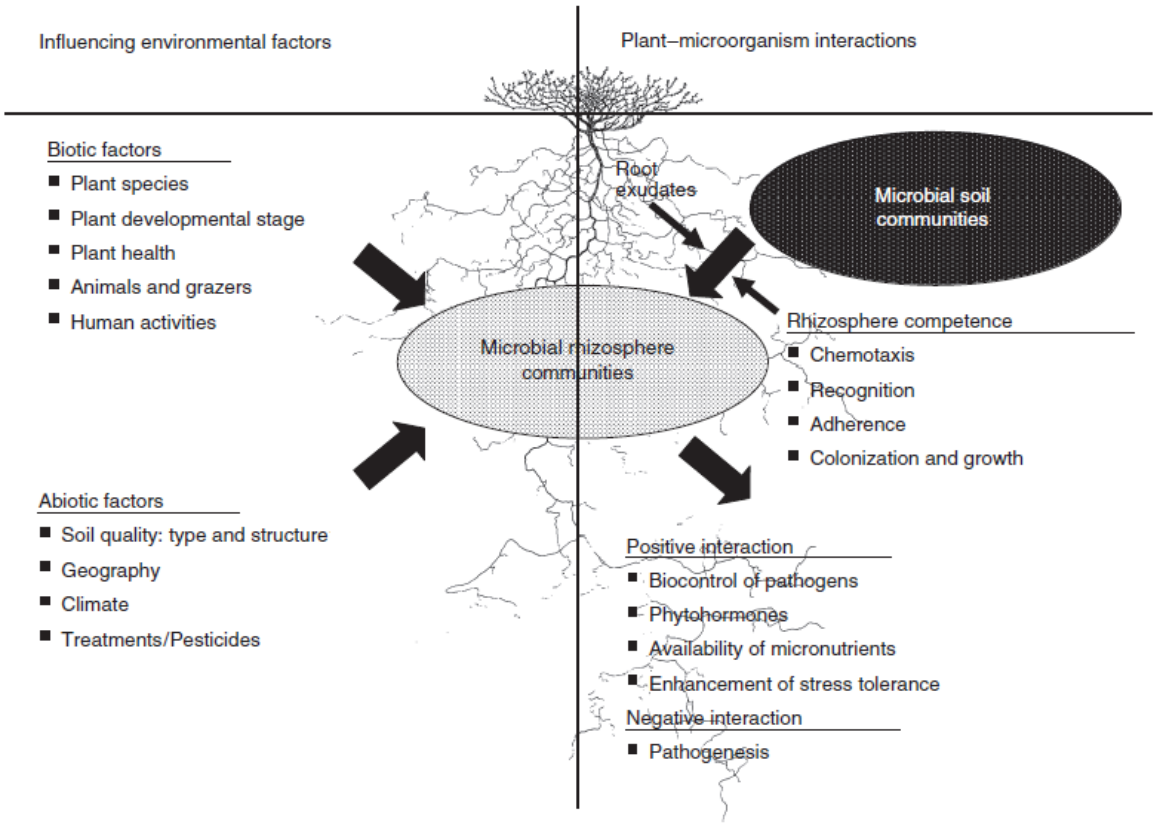
Benefits and drawbacks in the opposite direction, the plant impact on microbial community, are much more difficult to evaluate. Berg and Smalla (2009) outlined multiple factors, which influence the rhizosphere microbial community, its origin and composition (Figure 3). They also reviewed the effects of plant species (see chapter 2.2.1) and soil type on the composition and function of rhizosphere microbial community. Although a substantial effect of plant species was documented in the majority of reviewed studies, other studies reported that soil type could be even more important for the formation of rhizosphere microbial community than plant species (Berg and Smalla 2009). In general, soil pH value is the most important characteristic influencing the diversity of soil microbial communities throughout the soil environments (Fierer and Jackson 2006 in Doornbos et al. 2011). The results of Marschner et al. (2001) showed that the composition of rhizosphere microbial community is most probably affected by a complex interaction of soil type (sandy soil, sandy loam and clay), plant species (*Cicer arietinum*, *Brassica napus* and *Sorghum bicolor*) and the location within the root zone (root tip and mature root at the site of lateral root emergence). Interestingly, higher diversity of microbes was observed in the rhizosphere of mature root zone than in the zone of root tip in clay and sandy soils (Marschner et al. 2001).

Rhizosphere microorganisms generally behave as typical R-strategists/copiotrophs (Fierer et al. 2007), compete for continuously supplied rhizodeposits, immobilize them quickly and exert a fast turnover (Kaštovská and Šantrůčková 2007, Clayton et al. 2010, Chaudhary et al. 2012).

Motile forms of microorganisms are preferred for the chemical cross talk and signalling with plant (Lugtenberg et al. 2002 in Berg and Smalla 2009).

Some other positive and negative interactions occur in the rhizosphere: both plant and microbes contribute to the development of environment favourable for their life (e.g. production of mucigel, nutrient mineralization, pedogenesis, etc.). But in the same time, their competition for water and nutrients is a strong constraint. The line between negative and positive influences is usually very thin and intricate. For instance, both symbiotic and parasitic fungi evolved from root endophytes and the transition between symbiosis and parasitism is sometimes blurred (Saikkonen et al. (1998).

Figure 3. Influencing factors of rhizosphere microbial communities and model how microbial communities were selected from soil: by rhizodeposits and their rhizosphere competence (Berg and Smalla 2009).



2.3.1 SYMBIONTS

Mycorrhizal and nodular symbioses are the most studied relationships in the rhizosphere. Although there are plenty of mycorrhizal plants and only a few groups of nodular plants, these two phenomenons do not appear separately. Plants with nodular symbioses often maintain AM as well (Lambers et al. 2009).

2.3.1.1 Mycorrhizae

Several types of mycorrhizae are distingiushed: arbuscular (AM), ectomycorrhiza (ECM), orchideoid mycorrhiza and at least three types of mycorrhizae in Ericales (ericoid, arbutoid and monotropoid mycorrhiza). Plants support their symbionts by low molecular-weight carbohydrates ranging from 4 to 30 % of their daily fixed C (Morgan et al. 2005, Brüggemann et al. 2011). Fungi, in turn, provide water and nutrients to the plant as they are able to uptake these substances more efficiently. Fungal hyphae increase the exchangeable surface with soil (mycorrhizosphere), reach further and grow faster than roots. In addition, hyphae can access to water and nutrients present in much smaller soil pores (Coleman and Crossley 1996). Fungi release a variety of exoenzymes to decompose complex organic substances and they are also able to absorb some organic compounds, which would have, otherwise, remained unavailable for plants.

A second important effect of mycorrhizal fungi is the protection against plant pathogens. Besides the competition with pathogens for space and resources, mycorrhizal fungi also produce pathogen-inhibiting compounds (e.g. antibiotics, Manka 2009). However, all of these benefits are not always warranted. When the soil is fertilized or when the light is reduced, the costs exceed the benefits for the plant and symbiosis easily shifts to parasitism (Lambers et al. 2009).

Arbuscular Mycorrhiza (AM)

The oldest known evidence of root symbiotic structure is AM-like structure in the “roots” of bryophyte-like plants, dated approximately 400 million years back (Brundrett 2002). The ancient origin of AM reflects on its abundance: 92 % of known plant families (80 % of known plant species) maintain this kind of symbiosis (Brundrett 2009). In addition, all AM fungi belong to one ancient lineage Glomeromycota (Redecker et al. 2000). These unique fungi are obligate biotrophs, in other words, they are not able to survive without their host plant (Jones et al. 2004). Brundrett (2002) suggested that all known non-mycorrhizal species used to have an AM ancestor. The groups of non-mycorrhizal plants (Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae, Juncaceae, Proteaceae and *Lupinus* and *Kennedia* from Fabaceae, Morgan 2005) generally

inhabit harsh (e.g. arid, wet or saline) habitats and have evolved other strategies of P obtaining (Brundrett 2002).

Ectomycorrhiza (ECM)

In comparison to AM, only a little proportion of ECM fungi is fully dependent on its plant host (Brundrett 2002). Ectomycorrhizal fungi encompass several fungal groups (foremost Basidiomycota and Ascomycota) and according to Hibbert et al. (2000, in Lambers 2009), their diversification continues to these days. On the contrary to AM, there is much lower number of plant species involved in ECM. All of them belong to woody gymnosperms and dicotyledons (except for one monocotyledonous species). ECM is substantially younger than AM (Figure 2, Lambers et al. 2009); its origins are dated about 100 million years back. At that time, the initial development of flowering plants occurred as well (Brundrett 2002). ECM fungi are reported to not only supply the plant with P and micronutrients (e.g. K, Mg and Ca), but also to considerably contribute to the plant N uptake (Johnson and Gehring 2007).

Eriocoid Types of Mycorrhiza

At least three types of endomycorrhizae (ericoid, arbutoid and monotropoid) are known for Ericaceae and related families (Johnson and Gehring 2007). Interestingly, some fungi involved in ericoid mycorrhiza are typical representatives of ECM (Vralstad 2004). These fungi exert different properties when they are in the relationship with Ericaceae. For instance, they are able to acquire N from recalcitrant phenolic complexes, Bending and Read 1996).

Orchideoid Mycorrhiza

Orchideoid mycorrhiza on the roots of Orchideaceae encompasses a wide range of relationships with Basidiomycota (Rasmussen 2002). All orchids are fully dependent on their fungal symbionts because their tiny seeds are not able to germinate succesfully without the support of fungi (Johnson and Gehring 2007). However, it is not always advantagaeous for the fungi; some orchid species lack chlorophyll and thrive on assimilates moved by fungus from other photosynthetical plants (exploited mycorrhiza, Merckx et al. 2009).

2.3.1.2 Nodular Symbioses

As far as we know, nodular symbiosis is restricted to only one cosmopolite group of flowering plants (Fabids) and to two genera of N_2 fixing microorganisms (*Rhizobium* spp. and *Frankia* spp.). Its origin has been estimated about 55 million years back. In that period, CO_2 con-

centration in the atmosphere increased substantially and plants were more likely limited by N (Lambers et al. 2009).

Rhizobial nodules are formed by free living soil bacteria *Rhizobium* spp. and by plants from the group Leguminosae (except for Caesalpinioidae) and with the genus *Parasponia* from the family Ulmaceae (Vessey et al. 2005). Actinorhizal nodules (rhizothamnia) are created by coopration of actinomycetes *Frankia* spp. with at least 12 plant genera from 7 families (Betulaceae, Casuarinaceae, Coriariaceae, Eleagnaceae, Myricaceae, Rhamnaceae and Rosaceae; Squartini 2001). „N_2 fixing plants" are succesful colonizers of N-impoverished environments (e.g. lava fields) during the primary succesion (Lambers et al. 2009).

2.3.2 PATHOGENS AND PARASITES

Morgan et al. (2005) pointed out that if plants would not need rhizosphere microorganisms, they would simply produce antibiotics to repell them as pathogens. On the contrary, plants rather undergo the risk of pathogen infection than get completely rid of other microbiota. Plant – pathogen interactions are, in general, host specific and influenced by rhizodeposits (Brimecombe et al. 2001). Rhizodeposits can both directly stimulate or supress pathogens (Richards 1987). An efficient plant strategy is to promote the growth of particular microorganisms, which, consequently supress plant pathogens (chapter 2.3.2).

In comparison to airborne parasites, which have evolved specific gene-for-gene response, plant resistance or tolerance to soilborne parasites is predominantly controlled by complex genetic determinants (i.e. polygenic effects, Lambers et al. 2009).

Although plant – patogenes/parasites interactions are of crucial importance and extensive literature is available, they are not further discussed as it is beyond the topic of this thesis

2.3.3 ASSOCIATIVE MICROORGANISMS

Microbes living in the rhizosphere without any tight (symbiotic, pathogenic or parasitic) relationship also influence the plant in miscellaneous ways. Positive and negative interactions overlap with the benefits and constraints mentioned in previous the two chapters (2.3.1 and 2.3.2). Although some benefits and drawbacks are listed only in this chapter, they are probably of general importance and occur also in the tighter interactions (symbiosis and parasitism).

Positive Interactions

Associative N_2-fixers indirectly (via microbial turnover) improve plant N-budget. Other microbes, called plant growth promoting rhizobacteria (PGPR), release compounds to promote plant growth (e.g. phytohormones, Benizri et al. 2001, Brimecombe et al. 2001). Some microbes secrete substances to enhance the development of plant symbionts (e.g. mycorrhization helper bacteria, MHB, Johnson and Gehring 2007) or to eliminate plant pathogens. These microorganisms most often antagonize a specific pathogen species or genus of bacteria or fungi (Weller et al. 2002 in Doornbos).

Brimecombe et al.(2001) compiled a list of ways how rhizosphere microorganisms can control pathogens: by production of various compounds:

1) antibiotics, which inhibit pathogens;
2) siderophores, which cause the $Fe3^+$ limitation;
3) volatile substances (e.g. ammonia, cyanide), which are believed to serve as a biocontrol.

Pathogens are further controlled by competition for 4) nutrients and 5) for ecological niche. They are also restrained by 6) their own parasites and pathogens and by 7) plant induced systematic resistance (ISR). ISR to particular pathogens has to be previously induced by infection of other microbes. The mechanisms 2), 3) and 4) do not supress only pathogens but may have a negative effect on the host plant as well.

The presence of Protozoa and bacteriophage Nematodes in the rhizosphere is thought to have a positive impact on plants (Rasmann and Turlings 2008) because the microbial growth is controlled by both top-down (predators) and bottom-up (source of C) regulation.

Negative Interactions

Loose negative interactions between microbes and plants more or less overlap with already mentioned phenomena: competition for nutrients, inhibition of PGPR and other benefitial bacteria and symbionts, by competition for rhizodeposits, nutrients and niche. Although tight pathogen interactions are the most studied negative relationships, also free-living bacteria and fungi may produce a wide range of secondary metabolites acting as phyto- and mycotoxins (Karlovsky 2008).

Brimecombe et al. (2001) described another functional group of associative microbes – deleterious rhizobacteria (DRB). Deleterious rhizobacteria produce substances, which inhibit root growth without any visual symptoms. The substances could be either phytotoxins (e.g. cyanide)

or phytohormons (e.g. IAA). Deleterious rhizobacteria can also reduce plant fitness by inhibiting the formation of mycorrhizae and by counteracting the effect of N_2-fixers in the rhizosphere (Brimecombe et al. 2001). Similarly to tighter negative interactions, DRB are host-specific (Nehl et al. 1996).

2.5 THE SPECIFICS OF WETLAND ECOSYSTEM

Wetlands are transitional lands between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water (Cowardin et al. 1978). For wetlands, hydric soils and hydrophytes (plants adapted to the life under wet conditions) are typical.

Wetlands are the most productive ecosystems on the Earth: (8,000 g m^{-2} yr^{-1} is the net primary production in salt marshes, internet reference 3). Despite they cover only minor part of Earth surface (7 to 9 million km^2, 1.3-1.7 %, Mitsch and Gosselink 2000), they are important transformers, sinks and sources of biogeochemical cycles. Human activities resulted in modifications (e.g. introduction of invasive species, eutrophication etc.) or in loss (e.g. of more than 90 % in New Zealand and Europe) of large wetland areas (Mitsch and Gosselink 2000).

This chapter aims to bring a quick overview of the main constraints for plants and sediment microorganisms inhabiting wetland ecosystems and of the mechanisms how do these organisms cope with particular limitations.

Microbial adaptations

Flooded conditions substantially decrease the diffusion of atmospheric gases. When O_2 is exhausted in the environment, aerobic metabolism is replaced by other metabolic pathways. NO_3^-, Mn_4^+, Fe_3^+, SO_4^{2-} and CO_2 or organic acids (respectively according to their redox potential) serve as electron acceptors for microbial catabolism (Mitsch and Gosselink 2000). These processes are relatively less profitable than aerobic respiration as the gain of energy is much lower. Gibbs energy (i.e. the free enthalpy, $\Delta G^{0'}$) released by the reactions varies between -219.07 for aerobic respiration and -14.58 kJ / $2e^-$ for methanogenesis (Kim and Gadd 2008).

Owing to this fact, the mineralization (decomposition) of organic matter is slower and the undecomposed material accumulates in wetlands (Lavelle and Spain 2001). on the other hand, the unique metabolic pathways present in flooded sediments (e.g. methanogenesis, denitrification, etc.) cause wetland ecosystems important transformers in many biogeochemical cycles (Mitsch and Gosselink 2000).

Plant adaptations

Wetland plants also have to deal with O_2 shortage. They are divided into two major categories according to their adaptations.

1) **Plants avoiding anoxia** by creation of specialized morphological structures – root or trunk modifications (e.g. mangrove prop roots, pneumatophores, Taxodium knee roots and buttresses, willow adventive roots, Mitsch and Gosselink 2000) or by better direction of O2 to their flooded parts (denser lenticels and aerenchyma tissues, Busch et al. 2006). This adaptation was further improved in some emergent macrophytes by development of pressurized ventilation. an enhanced flow of air can circulate through the individual plant (e.g. *Typha domingensis*, White et al. 2007) or even through the whole ramete connected by rhizomes (e.g. Phragmites australis, Armstrong et al. 1992). This pressurized air flow is caused by different pressures and/or temperatures above young and older leaves and depends on the size of stomata. The different mechanisms were well-described by Grosse et al. (1996).
2) **Plants tolerating anoxia** evolved various metabolic adaptations. For instance, the diversification of end products of glycolysis in order to avoid the accumulation of toxic metabolites or the synthesis of antioxidants and enzymes to minimize the post-anoxic stress after the water level drops down again (Mitsch and Gosselink 2000). in addition, the life under anoxia is often maintained by large carbohydrate reserves. Therefore, wetland plants usually form various storage organs (e.g. rhizomes; Lavelle and Spain 2001).

Submerged plants face the lack of CO_2 needed for photosynthesis. Some of them evolved sophisticated strategies to saturate their CO_2 demands. Winkel and Borum (2009) presented a list of submersed plant species capable of CO_2 uptake from the sediment by their roots (e.g. Isoëtida, *Lobelia dotrmanna*, etc.). Other aquatic species (e.g. *Myriophyllum tenellum* and *Juncus pelocarpus*) possess the ability to acquire CO_2 from sediment bicarbonates (Pagano and Titus 2007). Other submersed plants maintain CAM or C_4 photosynthesis (Keeley 1999). Some macrophyte species are able to switch between C_3 and C_4 photosynthesis according to their temporal submergence or emmergence (*Eleocharis vivipara*, Ueno 2001).

Because of toxic Fe^{2+} and Mn^{2+}, which are mobilized under lower redox potential, wetland plants develope tolerance to higher Fe^{2+} and Mn^{2} concentrations (Armstrong et al. 1992).

In terms of nutrient uptake, wetland plants usually do not form mycorrhizal symbiosis as the mycorrhizal fungi are not adapted to flooded (anoxic) conditions (Lambers et al. 2009). Some wetland plants maintain symbiosis with N_2 fixers (*Alnus* spp. with *Frankia* actinomycetes, *Azolla* fern with cyanobacterium *Anabaena azollae* cyanobacteria Choudhury and Kennedy 2004).

As mentioned above, wetlands cover only a negligible part of the Earth surface. Their distribution is azonal with the largest areas in the boreal and tropical zones (Mitsch and Gosselink 2000). Wetlands differ in their primary producers, net primary production and decomposition in response to climate and other abiotic conditions. Tropical wetlands such as mangroves, salt marshes, river floodplains and deltas show the highest primary production (internet reference 3) and the highest biodiversity (Bacon 1997). Hence, Ramsar and other conventions are concerned about wetland protection (internet reference 4).

In populated areas, tropical wetlands are indangered by melioration and eutrophication. Eutrophication, together with the invasion of non-native species, may alter the ecosystem completely (e.g. Everglades, Brix et al. 2010). Many examples of biological disasters show that tropical wetlands are vulnerable ecosystems and their alteration may lead to unwanted and sometimes even unexpected consequences (internet reference 4). For more details see Appendix.

3. RESULTS AND DISCUSSION

Based on the reviewed findings, the hypotheses for our research were formulated. We expected that plants with different life strategies (stress-tolerating sedge *Eleocharis* spp. and competitive *Typha domingensis* Pers.) will differ in rhizodeposition rates and rhizodeposit bioavailability. We also assumed that microbial activity in collected rhizodeposits will be influenced by studied rhizodeposit characteristics. Further, we expected that P enrichment and sediment type will reflect on both rhizodeposition and microbial activity in the rhizosphere.

Eleocharis spp. released markedly higher contents of DOC than *Typha domingensis* Pers., an evidence observed both in the field and mesocosm experiments. This fact was proved for relative (related to the amount of recently assimilated C), quantitative (per root biomass or surface) and square (per m^2 of macrophyte stand) rhizodeposition rates. Substantial DON rhizodeposition was observed in *Eleocharis* spp.treatments in the field experiment. Consistent with that higher microbial activities (C respiration, N mineralization) were measured in *Eleocharis* spp. rhizodeposits. C partitionig results showed faster and higher immobilization of *Eleocharis* spp. rhizodeposits into microbial cells, contrary to *Typha domingensis* Pers., which invested more assimilates into its own metabolism (growth and respiration). P enrichment increased rhizodeposition and microbial activity in the rhizosphere of *Eleocharis* spp., while decreased them in the rhizodeposits of *Typha domingensis* Pers. Nevertheless, the effect of P was much weaker than the effect of plant species. Marly clay sediment was generally less favourable for both plants and microbial communities.

Our findings enabled us to understand better the dynamics of *Typha domingensis* Pers. expansion and the consequences of species composition change on the biogeochemistry of studied marshes. This study contributes to still increasing knowledge of C partitioning and plant-microbial relationships in the rhizosphere and belongs to still scarce wetland studies on rhizodeposition conducted in the field.

For detailed information see Appendix.

REFERENCES

- Adamczyk B, Godlewski M, Smolander A, Kitunen V (2009) Degradation of proteins by enzymes exuded by Allium porrum roots-A potentially important strategy for acquiring organic nitrogen by plants. Plant Physiology and Biochemistry 47, 919-925
- Adams MA (1992) Phosphatase activity and phosphorus fractions in Karri (Eucalyptus diversicolor F. Muell) forest soils. Biology and Fertility of Soils 14, 200-204
- Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? Journal of Ecology 84, 597-608
- Aerts R, Verhoeven JTA, Whigham DF (1999) Plant-mediated controls on nutrient cycling in temperate fens and bogs. Ecology 80, 2170-2181
- Ahonen-Jonnarth U, Van Hees PAW, Lundstr”om US, Finlay RD (2000) Organic acids produced by mycorrhizal Pinus silvestris exposed to elevated aluminium and heavy metal concentrations. New Phytologist 146, 557-567
- Allison AM (2006) Nutrient limitation and stoichiometry of carnivorous plants. Plant Biology 8, 740-747
- Armstrong J, Armstrong W, Beckett PM (1992) *Phragmites australis*: Venturi- and humidity-induced pressure flows inhance rhizome aeration and rhizosphere oxidation. New Phytologist 120, 197-207
- Bacon P (1997) Wetlands and Biodiversity.In: Hails AJ (Eds.) Wetlands, Biodiversity and the Ramsar Convention..Ramsar Convention Bureau, Gland, Schwitzerland
- Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant, Cell and Environment 32, 666-681
- Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. TRENDS in Plant Science 9 (1), 26-32
- Bais HP, Prithiviraj B, Jha AK, Ausubel FM, Vivanco JM (2005) Mediation of pathogen resistance by exudation of antimicrobials from roots. Nature 434, 217-221
- Bending GD, Read DJ (1996) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil Biology and Biochemistry 28, 1603-1612
- Benizri E, Baudoin E, Guckert a (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Science and Technology 11, 557-574
- Berg B, McClaugherty C (2008) Plant Litter. Decomposition, Humus Formation, Carbon Sequestration. Springer-Verlag Berlin Heidelberg
- Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. Minireview. Microbial Ecology 68, 1-13
- Blossfeld S, Gansert D, Thiele B, Kuhn JA, Lösch R (2011): The dynamics of oxygen concentration, pH value and organic acids in the rhizosphere of *Juncus* spp. Soil Biology & Biochemistry 43 , 1186-1197
- Boulet FM, Lambers H (2005) Characterisation of arbuscular mycorrhizal fungi colonization in cluster roots of *Hakea verrucosa* F. Muell (Proteaceae), and its effect on growth and nutrient acquisition in ultramafic soil. Plant and Soil 269, 357-367
- Bouwmeester JR, Roux C, Lopez-Raez JA, Bécard G (2007) TRENDS in Plant Science 12, 224-230
- Brady NC, Weil RR (2002) The Soils around Us. In: Brady The Nature and Properties of Soils. Prentice Hall, New Jersey

- Brimecombe MJ, De Leij FA, Lynch JM (2001) The effect of root exudates on rhizosphere microbial populations. In: Pinton R., Varanini Z. and Nannipieri P. (eds), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 95-140
- Brix H, Lorenzen B, Mendelssohn IA, McKee KL, Miao SL (2010) Can differences in phosphorus uptake kinetics explain the distribution of cattail and sawgrass in the Florida Everglades? Plant Biology 10 (23), p. 1-14
- Brüggemann N, Gessler A, Kayler Z, Keel SG, Badeck F, Barthel M, Boeckx P, Buchmann N, Brugnoli E, Esperschütz J, Gavrichkova O, Ghashghaie J, Gomez-Casanovas N, Keitel C, Knohl A, Kuptz D, Palacio S, Salmon Y, Uchida Y, Bahn M (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosciences 8, 3457-3489
- Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytologist 154, 275-304
- Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320, 37-77
- Busch J, Mendelssohn IA, Lorenzen B, Brix H, Miao SL (2006) a rhizotron to study root growth under flooded conditions tested with two wetland Cyperaceae. Flora 201, 429-439
- Calvaruso C, Turpault MP, Frey-Klett P (2006) Root associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. Applied and Environmental Microbiology 72 (2), 1258-1266
- Chaudhary DR, Saxena J, Lorenz N, Dick RP (2012) Distribution pattern of recently fixed photosynthate in switchgrass plant-soil system. Plant, Soil and Environment 58 (6), 249-255
- Cheng W, Gershenson a (2007) Carbon fluxes in the rhizosphere. In: Cardon Z.G. and Whitbeck J. L. (eds), The Rhizosphere: an Ecological Perspective. Elsevier Academic Press, pp. 31-56
- Clayton SJ, Clegg CD, Bristow AW, Gregory PJ, Headon DM, Murray PJ (2010) Movement of newly assimilated ^{13}C carbon in the grass *Lolium perenne* and its incorporation into rhizosphere microbial DNA. Rapid Communications in Mass Spectrometry 24, 535-540
- Coleman DC, Crossley DA (1996) Fundametals of Soil Ecology. Academic Press, London
- Choudhury ATMA, Kennedy IR (2004) Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biology and Fertility of Soils 39, 219-227
- Cowardin LM (1978) Wetland classification in united states. Journal of Forestry 76, 666-668
- Dacora FD, Phillips DA (2002) Root exudates as mediators in mineral acquisition in low nutrient environments. Plant and Soil 245, 35-47
- Dessureault-Rompré J, Nowack B, Schulin R, Luster J (2007) Spatial and temporal variation in organic acid anion exudation and nutrient anion uptake in the rhizosphere of *Lupinis albus* L. Plant and Soil 301, 123-134
- Doornbos RF, Cornelis van Loon L, Bakker PAHM (2011) Impact of root exudates and plant defense signalling on bacterial communities in the rhizosphere. a review. Agronomy of Sustainable Development 32, 227-243
- Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88 (6), 1354-1364
- Franke R, Schreiber L (2007) Suberin – a biopolyester forming apoplastic plant interfaces. Current Opinion in Plant Biology 10, 252-259
- Gallardo-Williams MT, Barton RL, Dooris PM, Martin DF (2002) Inhibition of onion germination and root growth by cattail extracts involves reduced cell proliferation and disruption of cell wall integrity. Journal of Aquatic Plant Management 40, 105-109
- Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt a (2005) Physiological and molecular diversity of feather moss associativeN_2-fixing cyanobacteria. Journal of Experimental Botany 56, 3121-3127

- Giri B, Giang HP, Kumari R, Prasad R, Varma a (2005a): Microbial Diversity in Soils. In: Buscot F, Varma a (Eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag Berlin Heidelberg, p. 19-55
- Giri B, Sachdev M, Giang PH, Kumari R, Garg AP, Oelmüller R, Varma a (2005b) Mycorrhizosphere: Strategies and Functions. In: Buscot F, Varma a (Eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag Berlin Heidelberg, p. 19-55
- Gregory PJ, Hinsinger P (1999) New approaches to studying chemical and physical changes in the rhizosphere: an overview. Plant and Soil 211, 1-9
- Grosse W, Armstrong J, Armstrong W (1996) The history of pressurized gass flow studies in plants. Aquatic Botany 54, 87-100
- Haider K, Schäffer a (2009) Soil Biochemistry. Science Publishers
- Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant and Soil 321, 235-257
- Hawes MC, Bengough G, Cassab G, Ponce G (2003) Root caps and rhizosphere. Journal of Plant Growth Regulation 21, 352-367
- Hawes MC, Brigham MA, Wen F, Woo HH, Zhu Z (1998) Functions of root border cells in plant health: Pioneers in the rhizosphere. Annual Review of Phytopathology 36, 311-327
- Hocher V, Auguy F, Bogusz D, Doumas P, Franche C, Gherbi H, Laplaze L, Obertello M, Svistoonoff S (2009) Actinorhizal nitrogen fixing symbiosis: an example of adaptation against soil abiotic stresses. Cahiers Agricultures 18, 498-505
- Houlton BZ, Wang YP, Vitousek PM, Field CB (2008) a unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454, 327-334
- Johnson NC, Gehring CA (2007) Mycorrhizas: Symbiotic mediators of rhizosphere and ecosystem processes. In: Cardon Z.G. and Whitbeck J. L. (eds), The Rhizosphere: an Ecological Perspective. Elsevier Academic Press, pp. 73-100
- Jones DL (1998) Organic acids in the rhizosphere – a critical review. Plant and Soil 205, 25-44
- Jones DL, Healey JR, Willett VB, Farrar JF, Hodge a (2005) Dissolved organic nitrogen uptake by plants – an important N uptake pathway? Soil Biology & Biochemistry 37, 413-423
- Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163, 459-480 (Tansley reviews)
- Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321, 5-33
- Karlovsky P (2008) Secondary metabolites in soil ecology. In: Karlovsky P (Eds.) Secondary Metabolites in Soil Ecology. Springer Verlag Berlin Heildelberg
- Kaštovská E, Šantrůčková H (2007) Fate and dynamics of recently fixed C in pasture plant-soil system under field conditions. Plant and Soil 300, 61-69
- Keeley JE (1999) Photosynthetic pathway diversity in a seasonal pool community. Functional Ecology 13, 106-118
- Kenrick P, Crane PR (1997) The origin and early evolution of plants on land. Nature 389, 33-39
- Kim BH, Gadd GM (2008): Bacterial Physiology and Metabolism. Cambridge University Press
- Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431

- Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915-1925
- Lambers H, Mougel C, Jaillard B, Hinsiger P (2009) Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant and Soil 321, 83-115
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Annals of Botany 98, 693-713
- Lavelle P, Spain AV (2001) Soil Ecology. Kluwer Academic Publishers
- Litton CM, Giardina CP (2008) Below-ground carbon flux and partitioning: global patterns and response to temperature. Functional Ecology 22, 941-954
- Manka M (2009) Mycorrhizae and protection of forest tree species against deseases. Sylwan 153, 27-30
- Marschner B, Kalbitz K (2003) Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113, 211-235
- Marschner P, Crowley D, Rengel Z (2011) Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis – model and research methods. Soil Biology & Biochemistry 43, 883-894
- Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology & Biochemistry33, 1437-1445
- Martin FM, Perotto S, Bonfante P (2001) Mycorrhizal fungi: a fungal community at the interface between soil and roots. In: Pinton R., Varanini Z. and Nannipieri P. (eds), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 263-296
- Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, McDowell W, Robertson GP, Santos OC, Treseder K (1999) Nitrogen stable isotope composition of leaves and soil:Tropical versus temperate forests. Biogeochemistry 46 (1), 45-65
- McCully ME, Boyer JS (1997) The expansion of maize root-cap mucilage during hydration. 3. Changes in water potential and water content. Physiologia Plantarum 99, 169-177
- Melnitchouck A, Leinweber P, Eckhardt UK, Beese R (2005) Qualitative differences between day- and nighttime rhizodeposition in maiz (Zea mays L.) as investigated by pyrolisys-field ionization mass spectrometry. Soil Biology & Biochemistry 37, 155-162
- Mench M, Morel JL, Guckert a (1986) Metal binding properties of high molecular weight soluble exudates from maize (*Zea mays* L.) roots. Biology and Fertility of Soils 2, 29-34
- Merckx V, Bidartondo MI, Hynson NA (2009) Myco-heterotrophy: when fungi host plants. Annals of Botany 104, 1255-1261
- Mitsch WJ, Gosselink JG (2000) Wetlands. John Wiley & Sons, Inc
- Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant-microbe interactions in the rhizosphere. Journal of Experimental Botany 56, 1729-1739
- Negishi T, Nakanishi H, Yazaki J, Kishimoto N, Fujii F, Shimbo K, Yamamoto K, Sakata K, Sasaki T, Kikuchi S, Mori S, Nishizawa NK (2002) cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophore secretion in Fe-deficient barley roots. The Plant Journal 30, 83-94
- Nehl NB, Allen SJ, Brown JF (1996) Deleterious rhizosphere bacteria: an integrating perspective. Applied Soil Ecology 5, 1-20

- Neumann G, Römheld V (2001) The release of root exudates as affected by the plant´s physiological status. In: Pinton R., Varanini Z. and Nannipieri P. (eds), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 41-94
- Oelmann Y, Richter AK, Roscher C, Rosenkranz S, Temperton VM, Weisser WW, Wilcke W (2011) Does plant diversity influence phosphorus cycling in experimental grasslands? Geoderma 167-168, 178-187
- Ohkama-Ohtsu N, Wasaki J (2010) Recent progress in plant nutrition research: cross-talk between nutrients, plant physiology and soil microorganisms. Plant & Cell Physiology 51 (8), 1255-1264
- Ovečka M, Lang I, Baluška F, Ismail A, Illeš P, Lichtscheidl IK (2005) Endocytosis and vesicul trafficking during tip growth of root hairs. Protoplasma 226, 39-54
- Pagano AM, Titus JE (2007) Submersed macrophyte growth at low pH: carbon source influences response to dissolved inorganic carbon enrichment. Freshwater Biology 52, 2412-2420
- Personeni E, Lüscher A, Loiseau P (2005) Rhizosphere activity, grass species and N availability effects on soil C and N cycles. Soil Biology & Biochemistry 37, 819-827
- Personeni E, Loiseau P (2005) Species strategy and N fluxes in grassland soil. a question of root litter quality or rhizosphere aktivity? European Journal of Agronomy 22, 217-229
- Phillips RP, Erlitz Y, Bier R, Bernhardt ES (2008) New approach for capturing soluble root exudates in forest soils. Functional Ecology 22, 990-999
- Pivničková B, Šantrůčková H, Rejmánková E, Snyder JM (2010) Heterotrophic microbial activities and nutritional status of microbial communities in tropical marsh sediments of different salinities: the effect of phosphorus additionand plant species. Plant and Soil, 336 (1-2), 49-63
- Rai AN, Söderbäck E, Bergman B (2000) Cyanobacterium-plant symbioses. New Phytologist 147, 449-481
- Rashid MH, Asaeda T, Uddin MN (2010) Litter-mediated allelopathic effects od kudzu (Pueraria montana) on Bidens pilosa and Lolium perenne and its persistence in soil. Weed Biology and Management 10, 48-56
- Rassman S, Turlings TCJ (2008) First insight into specifity of belowground tritrophic interactions. Oikos 117, 362-369
- Rasmussen HN (2002) Recent developments in the study of orchid mycorrhiza. Plant and Soil 244, 149-163
- Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289, 1920-1921
- Rejmánková E, Sirová D (2007) Wetland macrophyte decomposition under different nutrient conditions: relationship between decomposition rate, enzyme activities and microbial biomass. Soil Biology & Biochemistry 39, 526-538
- Rejmánková E, Sirová D, Carlson E (2011) Pattern of activities of root phosphomonoesterase and phosphodiesterasein wetland plants as a fiction of macrophyte species and ambient phosphorus regime. New Phytologist, 190 (4), 968-976
- Rejmánková E, Snyder JM (2008) Emergent macrophytes in phosphorus limited marshes: do phosphorus usage strategies change after nutriet addition? Plant and Soil 313, 141-153
- Richards BN (1987) The Microbiology of Terrestrial Ecosystems. Longman Scientific & Technical, Longman Group
- Rout ME, Callaway RM (2012) Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that ´everything is *not* everywhere´. Annals of Botany 110, 213-222
- Rudrappa T, Bonsall J, Gallagher JL, Seliskar DM, Bais HP (2007) Root-secreted allelochemical in the noxious weed *Phragmites australis* deploys a reactive oxygen species response and microtubule assembly disruption to execute rhizotoxicity. Journal of Chemical Ecology 33, 1898-1918

- Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root secreted malic acid recruits beneficial soil bakteria. Plant Physiology 148, 1547-1556
- Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Annual Review of Ecology and Systematics 29, 319-349
- Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigma. Ecology 85 (3), 591-602
- Semchenko M, Zobel K, Heinemeyer A, Hutchings MJ (2008) Foraging for space and avoidanceof physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. New Phytologist 179, 1162-1170
- Singer AC, Crowley DE, Thompson IP (2003) Secondary plant metabolites in phytoremediation and biotranformation. Trends in Biotechnology 21, 123-130
- Sobolev VS, Potter TL, Horn BW (2006) Prenylated stilbenes from peanut root mucilage. Phytochemical Analysis 17, 312-322
- Squartini a (2001) Functional ecology of the rhizobium-legume symbiosis. In: Pinton R., Varanini Z. and Nannipieri P. (eds), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 297-326
- Ström L, Ekberg A, Mastepanov M, Christensen TR (2003) The effect of vascular plants on carbon turnover and methane emissions from a tundra wetlands. Global Change Biology 9, 1185-1192
- Ström L, Tagesson T, Mastepanov M, Christensen TR (2012) Presence of *Eriophorum scheuchzeri* enhances substrate availability and methane emission in Arctic wetland. Soil Biology & Biochemistry 45, 61-70
- Tharayil N, Bhowmik PC, Xing B (2008) Bioavailability of allelochemicals as affected by companion compounds in soil matrices. Journal of Agricultural and Food Chemistry 56, 3706-3713
- Tinker PB, Durall DM, Jones MD (1994) Carbon use efficiency in mycorrhizas: theory and sample calculations. New Phytologist 128, 115-122
- Ueno O (2001) Environmental regulation of C_3 and C_4 differentiation in the amphibious sedge *Eleocharis vivipara*. Plant Physiology 127, 1524-1532
- Ueno O, Ma JF (2009) Secretion time of phytosiderophore differs in two perrenial grasses and is controlled by temperature. Plant and Soil 323, 335-341
- Uren NC (2001) Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R., Varanini Z. and Nannipieri P. (eds), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 19-40
- Vančura V (1988a) Plant metabolites in soil. In: Vančura V. and Kunc F. (eds), Soil Microbial Associations. Akademia, Praha, pp. 57-144
- Vančura V (1988b) Microorganisms, their mutual relations and functions in the rhizosphere. In: Vančura V and Kunc F (eds), Soil Microbial Associations. Akademia/Praha, pp. 191-280
- Vessey JK, Pawlowski K, Bergman B (2005) Root-based N_2-fixing symbioses: Legumes, actinorhizal plants, *Parasponia* sp. and cycads. Plant and Soil 274, 51-78
- Vralstad T (2004) Are ericoid and mycorrhizal fungi part of a common quild? New Phytologist 164, 7-10
- Weston LA, Duke SO (2003) Weed and Crop Allelopathy. Critical Review in Plant Sciences 22, 367-389
- White SD, Deegan BM, Ganf GG (2007) The influence of water level fluctuations on the potential for convective flow in the emergent macrophytes *Typha domingensis* and *Phragmites australis*. Aquatic Botany 86, 369-376
- Winkel A, Borum J (2009) Use of sediment CO_2 by submersed rooted plants. Annals of Botany 103, 1015-1023

- Yang JX, Liu Y, Ye ZH (2012) Root-induced changes of pH, Eh, Fe(II) and fractions of Pb and Zn in rhizosphere soils of four wetland plants with different radial oxygen losses. Pedosphere 22 (4), 518-527
- Zhang Y, Min G, Shi K, Zhou YH, Yu JQ (2009) Effects of aqueous root extracts and hydrophobic root exudates of cucumber (*Cucumis sativus* L.) on nuclei DNA content and expression of cell cycle-related genes in cucumber radicles. Plant and Soil 327, 455-463

Internet references

[1)] http://apps.webofknowledge.com/WOS

[2)] http://en.wikipedia.org/wiki/Sequoiadendron_giganteum

[3)] http://kingfish.coastal.edu/biology/sgilman/778PrimProd.htm

[4)] http://www.ramsar.org

4. APPENDIX

NÁLEŽITOSTI PRO PREZENTACI PUBLIKACE VE STAGU

Bibliografické údaje:

Kubešová J, Rejmánková E, Šantrůček J, Šantrůčková H (2012) Effect of P enrichment on rhizodeposit quantity and bioavailability: a comparison of two macrophyte species.

Stav publikace v procesu vedoucím k publikaci:

Publikace bude odeslána do vědeckého časopisu během dvou týdnů.

Prohlášení studenta o podílu na publikaci:

Na publikaci jsem se podílela přibližně z 60 %. Prováděla jsem odběr exudátů a sklizeň rostlin v terénu. V laboratoři jsem měřila mikrobiální respiraci a koncentraci buněk v exudátech. Pomáhala jsem při mezokosmovém experimentu se stabilním izotopem ^{13}C. Publikaci jsem napsala.

Abstrakt v původním znění:

This study compares rhizodeposition and rhizosphere microbial activity of two macrophyte species growing in tropical marshes of northern Belize. *Eleocharis* spp. is adapted to oligotrophic P limited conditions, while *Typha domingensis* Pers. is a strong competitor, which spreads over eutrophicated areas. We studied rhizodeposits (exudates s.l.) of both species under P-limited and P enriched conditions. Rhizodeposits were collected in the field for two days, after that DOC (dissolved organic carbon), DON (dissolved organic nitrogen), mineral N, pH and cell concentrations were measured. Biodegradability of rhizodeposits was tested by mineralization experiment (microbial respiration, N mineralization and phosphatase activity). To estimate relative DOC fluxes from root to rhizosphere and to microbial cells, ^{13}C partitioning was examined in mesocosm experiment. *Eleocharis* spp. released relatively (per g of assimilated ^{13}C) and quantitatively (per root dry weight and root surface area) more DOC and DON than *T. domingensis S*quare estimates of net and gross rhizodeposition fluxes in monospecific macrophyte stands were based on ^{13}C partitioning ratios and were higher in *Eleocharis* spp. stands under both P enrichment and P limitation. The two species responded to P enrichment differently: *Eleocharis* spp. enhanced while *T. domingensis* decreased the relative rhizodeposition. Consequently, *Eleocharis* spp. rhizodeposits were more mineralized than *T. domingensis* rhizodeposits. The effect of plant species was generally higher than the effect of P enrichment. Marly clay sediment seemed less favourable for both plants and rhizosphere microorganisms than peaty clay sediment. Our findings helped us to interpret the processes present in the rhizosphere in the context of previous studies about sediment conditions and plant ecology. The possible impact of species composition change on the biogeochemistry of eutrophised areas is outlined.

Abstrakt přeložený do češtiny:

Vliv eutrofizace fosforem na množství a biologickou dostupnost exudátů: porovnání dvou mokřadních druhů rostlin. (název práce)

Práce porovnává dva druhy mokřadních rostlin rostoucí v tropických mokřadech severního Belize. *Eleocharis* spp. je přizpůsobena k životu v oligotrofních podmínkách limitovaných fosforem, zatímco *Typha domingensis* Pers. je silným kompetitorem schopným zarůstat eutrofizovaná území. Studovali jsme exudáty obou druhů v oligotrofních a fosforem eutrofizovaných podmínkách. Odběr exudátů přímo v mokřadech trval dva dny, poté byly v roztoku exudátů stanoveny koncentrace DOC (rozpuštěného organického uhlíku), DON (rozpuštěného organického dusíku) a minerálních forem dusíku, také koncentrace buněk a hodnota pH. Biologická rozložitelnost exudátů byla zjišťována následným mineralizačním experimentem v laboratoři. Jeho součástí bylo určení rychlosti respirace, mineralizace N a fosfatázové aktivity. Pulsní značení stabilním izotopem ^{13}C posloužilo k stanovení relativních toků asimilovaného C do podzemních částí rostlin, do exudátů a míry zabudování exudátů do mikrobiální biomasy v rhizosféře. *Eleocharis* spp. exudovala relativně (vyjádřeno na množství asimilovaného C) i kvantitativně (vyjádřeno na hmotnost a povrch kořene) více DOC než *T. domingensis* Rostliny odpověděly na přídavek fosforu odlišně: *Eleocharis* spp. zvýšila relativní rychlost exudace, zatímco *T. domingensis* ji snížila. Plošné odhady hrubého a čistého toku uhlíku ve formě exudátů do sedimentu byly opět vyšší pro *Eleocharis* spp. než pro *T. domingensis* při obou hladinách fosforu. Rozdílné životní strategie rostlin se kromě odlišného upravení exudace při eutrofizaci projevily také v rozdílné biologické dostupnosti exudátů. Mineralizace C a N probíhala rychleji v exudátech *Eleocharis* spp. než u *T. domingensis*. Vliv rostliny byl obecně významnější než vliv přídavku fosforu. Jílový sintr (vápencový sediment) byl méně příznivý pro růst rostlin a mikroorganismů než organický jíl. Výsledky této práce nám pomohly propojit dosavadní poznatky o vlastnostech sedimentu a rostlin a zasadit je do kontextu ekosystému. Možné důsledky změn druhového zastoupení makrofyt na biogeochemické procesy v eutrofizovaných mokřadech jsou nastíněny.