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Plant Rhizodeposition and Rhizosphere Microflora: Their Relationship and Its Consequences in Wetlands

Bachelor Thesis

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Annotation: Plant and microbial relationships in the rhizosphere have been briefly reviewed. The research of tropical wetland ecosystem in northern Belize has been summarized. After that a synthesis of both parts results in the hypothesis of carbon, nitrogen and phosphorus flows between *Eleocharis cellulosa*, *Typha domingensis* and their rhizosphere.

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- bakalářská práce v nezkrácené podobě -

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Contents:

1. INTRODUCTION

Rhizosphere is a distinctive part of the soil, which is considered to be the most diverse and complicated environment. Due to plant carbon supply, various biogeochemical processes are triggered or suppresed in the rhizosphere. Thus, this little area in the root vicinity is also important in ecosystem-scale changes (e.g. plants in symbiosis with N_2 fixers are good pioneers during primary and secondary succesion). After the eutrophication of oligotrophic P-limited wetlands in northern Belize, *Typha domingensis* replaced sparse vegetation of *Eleocharis* spp. and cyanobacterial mats. How can *Typha domingensis* change its environment? And, why is *Eleocharis* spp. able to thrive on under such extreme conditions? Definitely, something happens in their rhizosphere. For this reason a review on recent knowledge about rhizosphere follows. Then a short summary of previous research in Belizian wetlands is added. By the synthesis of both parts the hypotheses on carbon (C), nitrogen (N) and phosphorus (P) flows between *Typha domingensis* and *Eleocharis cellulosa* plants and their rhizosphere are proposed.

Figure 1. Roots and rhizosphere of *Eleocharis cellulosa*.

2. REVIEW ON THE RHIZOSPHERE

2.1 The Rhizosphere

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

What makes rhizosphere a rhizosphere? First of all, it is necessary to mention its enormous heterogeneity caused by the gradients of various chemicals and environmental characteristics. The gradients occur both with the distance from the root to the soil and with the location along the root length (Richards 1987). Oxic conditions, pH, concentration of organic compounds and other characteristics vary even within short parts of a single root. Anyway, there is something that all rhizosphere types have in common: the increased amount of organic substances released by the root into its environment. This easily available C consequently triggers the microbial activity in the rhizosphere (Vančura 1988b).

According to recent findings an essential role of this easily available C in evolution of root and its rhizosphere is more obvious. There was higher concentration of CO_2 in the atmosphere at the time when plants started to colonize the terrestrial ecosystem (in the mid-Palaeozoik era between 480-360 million years ago, Kenrick and Crane 1997). Therefore, plants were able to photosynthetize more effectively than nowadays. The surpluses of assimilates were released by plant-parts and iniciated the concentration of microbial communities around them. Surprisingly, before real roots were formed, some of recently known rhizosphere interactions had evolved (e.g. arbuscular mycorrhizae, Brundrett 2002).

2.2 Roles of The Plant

Plants influence and change their environment in multiple ways (e.g. changes in water cycle or in concentration of gases in the atmosphere). For simplicity we focus only on the effects, which might influence soil plant-microbe relationships in a substantial manner (rhizodeposition, nutrient uptake and litter production). Plants provide the main source of C input to the soil, so they are crucial to trigger various microbial transformations present there. Thus, they influence a myriad of biogeochemical cycles (e.g. mineralization, N_2 fixation). Moreover, in the evolutionary perspective, plants with their rhizosphere microbial communities played a pivotal role in mineral weathering and soil formation (Lambers et al. 2009).

2.2.1 Rhizodeposition

The term rhizodeposition means the general release of organic substances by roots. It encompasses wide range of processes: root cap and boarder cell loss, death and lysis of root cells, flow of C to root-associated symbionts, gaseous losses, leakage of solutes from living cells (real root exudates) and insoluble polymer secretion from living cells (mucilage). The term exudation, however, is used in two different meanings: 1) as a synonym for rhizodeposition and 2) as one particular case of rhizodeposition – the passive release of organic C by living cells. This can be misleading; therefore, the term rhizodeposition is now prefered for the general phenomenon of C release. Consistent with that, the term exudation is predominantly used in its narrower sense in this study.

As reviewed by Jones et al. (2009), plants alocate about 40% of their net fixed C belowground. From this aproximately 50% (i.e. 19% of net fixed C) retains in root biomass, 33% (i.e. 12% of net fixed C) is respired back to the atmosphere by rhizosphere (mostly root and microbial) respiration, 12% (i.e. 5% of net fixed C) can be recovered as soil residues and a small amount of C could be lost in leaching and surface runoff. Litton and Giardina (2008) related the portion of C alocated belowground to the temperature. Indeed, there occurred a clear trend of proportionally higher C alocation belowground with higher temperature or, in a broader sense, with warmer climate.

The most intense rhizodeposition occurs in the growing parts of the root. Exudation has been observed in the zone of extension growth of cells about 300 mm behind the tip of both lateral and main roots (Vančura 1988a). Partly it is due to vesiculation when the cytoplasmatic membrane is being prolonged (Ovečka et al. 2005); partly because of the absence of any efficient transport

barrier as is the secondary cell wall, which forms later in cells of rhizodermis and endodermis (Franke and Schreiber 2007). Moreover, the quantity and composition of rhizodeposits change during plant ontogenesis. Vančura (1988a) described the exudation characteristics for germinating seeds, seedlings and and intact (not injured) roots of various age. Rhizodeposition is generally higher during the day and lower at night and the composition of rhizodeposits is also different (Melnitchouck et al. 2005). Rhizodeposition is influenced by complex interactions of many biotic and abiotic factors displayed in Table 1.

Table 1. 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	**Abiotic factors**
Plant species	Temperature
Developmental status	Moisture
Shoot herbivory	Humidity
Photosynthesis	Wind speed
Supply of C from shoot to root	Light intensity
Evapotranspiration	Elevated CO_2
Nutrient Deficiency	Pesticides
Root age	Available space
Root architecture	Atmospheric N deposition
Cytosolic concentration	Ozone
Membrane permeability	Physical disturbance
Membrane electrochemical potential	Fire
Release of microbial signals	Irrigation
Allelochemical release	Erosion
Mycorrhizae	Altitude
Nodulation	Latitude
	Rhizodeposition
Root herbivory	Compaction
Mycorrhizae	Soil type
Microbial community size	Soil pH
Microbial community structure	Salinity
Microbial community activity	Metal toxicity
Toxin production	Water availability
Root membrane permeabilisers	Organic matter
Release of root signal molecules	Cation and anion exchange
Quorum sensing	Drainage and aeration
Pathogens	Rooting depth
Biocontrol agents	Soil texture
Phytohormon production	Soil structure
Mesofauna	Redox potential
Soil biotic factors	**Soil abiotic factors**

2.2.1.1 Origin and Composition of Rhizodeposits

A terminology based on the origin of rhizodeposit and on the mechanism of its release (e.g. active and passive transport) was proposed by Rovira (1979 in Richards 1987). Despite numerous other nomenclatures occurred, this one has been used most extensively. Jones et al. (2009) in their review described the most important categories of rhizodeposition with respect to recent findings: 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 and roots are expected to exert little direct control over this diffusion. According to Jones et al. (2009), the critical factors, which influence the rate of C losses are the root-soil concetration gradient, permeability of plasmatic membrane and spatial location of the solutes in the root tissue. Not to mention the most important factor creating all the rhizodeposits prior to their release – the rate of photosynthesis.

Exudates are usually free sugars, amino acids and organic acid. Lambers et al. (2009) reported that this passive leakage of exudates represents less than 5 % of C daily fixed in the photosynthesis. Although the C flow to symbionts originated most probably as the passive exudation (Brundrett 2002), compared to the passive leakage C costs are much higher: from 4 to 20% of daily assimilated C (Tinker et al. 2004, Morgan et al. 2005).

Secretion

To this category belong low and high-molecular weight compounds, which are expelled actively, in other words at the expense of energy (e.g. by active membrane transport or by exocytosis). Some of them have a signalling character, promote or inhibit the growth of microbes or other plants (allelopathy, Rudrappa et al. 2007). Others are exoenzymes, which cleave specific chemical bonds to gain more 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 phytosiderophores produced 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. Organic acids are known to both enhance the mobility of P and reduce 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 exudation, the secretion is much more controlled by the plant. Ueno and Ma (2009) reported on intensive secretion of phytosiderophores under certain temperature 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 soil nutrients.

However, the most of organic C is usually secreted in the form of mucilage. This mixture of polysaccharides, proteins and phospsholipids is released by exocytosis from the root cap cells to form a gelatinous protective layer around the root (Jones et al. 2009). Indeed, 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 the plant. Carboxylic groups of the mucilage protect meristem by complexing potentially toxic metals (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 attack (Sobolev et al. 2006). Furthermore, the gelatinous layer also reduces frictional resistance when the root tip is moving through the soil (Hawes et al. 2003). Remarkable is the water-intrinsic affinity of mucilage - its water content can be 100 000 times greater than its dry weight (McCully and Boyer 1997). This suggests an important role of mucigel in water supply 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)

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

Senescence-derived compounds

This category is contains chemicals released into 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 spontanoeus (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 (relatively continuous input of inorganic C) and dead roots (prevalently seasonal 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 2.

Table 2. 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 microorganisms to interact with the rhizodeposits and it is affected by the temporal and spatial distribution of organic compounds and microorganisms in the soil. It encompasses not only the direct bioavailability of released original compounds but also the bioavailability of these compounds after their hydrolyzation by exoenzymes (Marschner and Kalbitz 2003). In the bulk soil, bioavailability of dissolved organic matter (DOM), depends mainly on soil characteristics (size of pores, soil aggregates, sorption activity of the soil and also on drought, Marschner and Kalbitz 2003). The bioavailability of rhizodeposits also depends on the location of microbes in the rhizosphere. In the case of symbiotic relationships where the exudates are delivered directly to symbionts, we can expect their high bioavailability. Other rhizosphere inhabitants compete for the rhizodeposits and are forced to utilize some less biodegradable compounds.

Biological Degradability

The biodegradability itself also 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). It is expresses as a ratio of microbial biomass to total C utilized – so-called microbial assimilation efficiency or yield factor (Cheng and Gershenson 2007).

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

In comparison to bioavailability, biodegradability is controled by three 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) and 3) by external factors (e.g. seasonality in temperature, moisture, input of organic matter, etc.). The groups of factors, which influence the biodegradability of rhizodeposits, are shown in Figure 2.

By the release of particular well-available substances, plants can influence biogeochemical cycles. For instance, STRÖM et al. (2003) brought an interesting evidence that plant species (especially cotton grass, *Eriophorum scheuchzeri*), which release lot of acetate induce activity of methanogens associated on their roots.

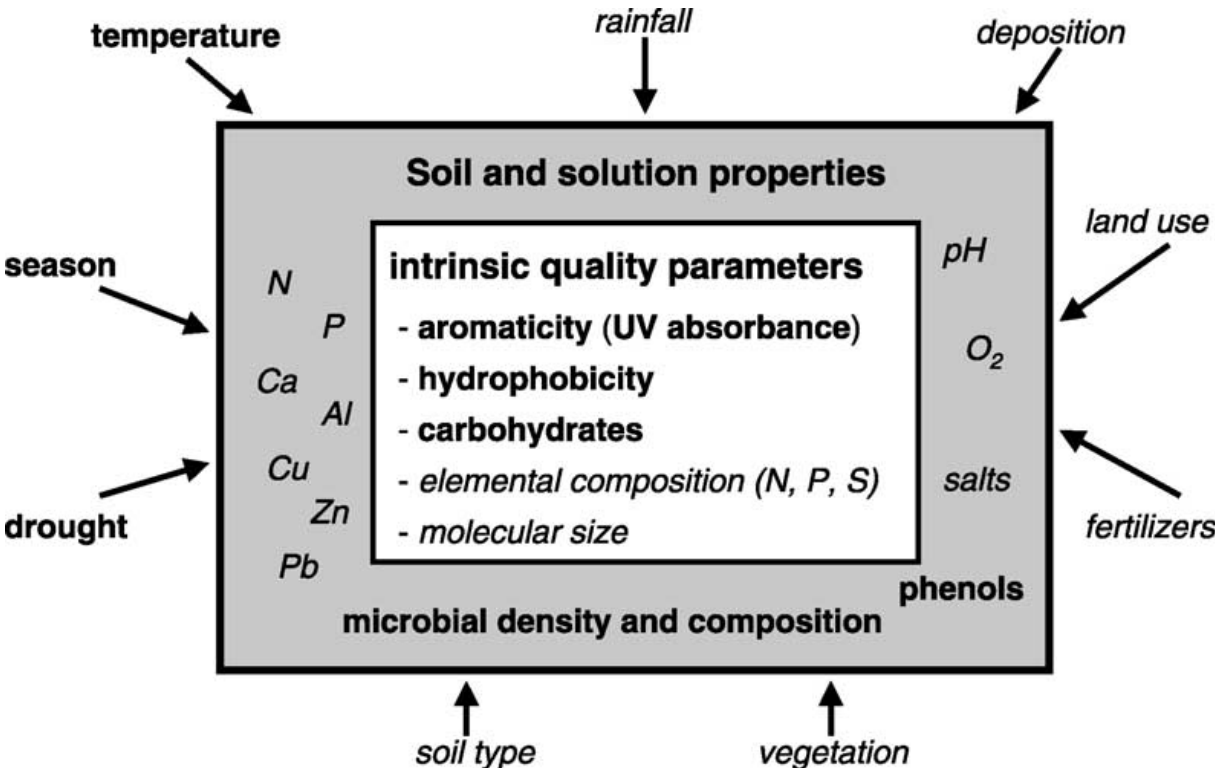

Figure 2. Summary of parameters, which have been identified as controlling factors for DOM biodegradability. Bold: verified in several studies; italic: with conflicting or circumstantial evidence in some studies or assumed factor. Modified from Marschner and Kalbitz (2003).

2.2.1.3 Other Biological Roles of Rhizodeposits

Besides being the substrate for microbiota, rhizodeposits maintain a variety of other functions in the rhizosphere. Their role in the nutrient uptake is summarized in chapter 2.2.2. The following 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 to these complex processes is limited (Hartmann et al. 2009). Most information has been obtained on the rhizobial nodule formation. Legume root produces flavonoids and betaines to communicate with *Rhizobia*. The signalling compound enters

the 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 root hair curling on the plant and cortical cell division, which becomes the initial part of the 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 plant secondary metabolits (e.g. salicylate) are known to trigger microbial biodegradation of xenobiotics in polluted soils (Singer et al. 2003).

Another interesting type of signalization are the tritrophic interactions. Grazing on plant root induces a production of volatile compounds by the root. These chemicals attract entomopathogenic nematodes, which prey on soil herbivorous larvae (Rassman and Turlings, 2008).

Negative Communication

Leaving aside other negative interactions like competition for resources, plants can, by secretion of particular compounds, inhibit the growth of microorganisms (Wu et al. 2009), other plant species (allelopathy, described on *Fragmites australis*, Rudrappa et al. 2007) and even the species itself (autotoxicity, Zhang et al. 2009). The inhibition of microbes operates directly (Singer et al. 2003) or indirectly by promotion of microbial and mycorrhizal symbionts, which produce pathogen-inhibiting compounds (Martin et al. 2001).

A common feature 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, which neighbouring plants suffer from. However, it is not easy to evaluate the importance of allelopathy, in many cases the exepriments were set up in the way, which did not correspond with the natural conditions (e.g. study on *Typha domingensis* allelopathy, Gallardo-Williams et al.2002). The persistance of allelochemical compounds in soils depends on soil characteristics (e.g. sorption, Tharayil et al. 2008). Interestingly, some allelochemical compounds are reported to persist in soils for 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) exude several autotoxic compounds (e.g. cinnamic acid). Crop rotation is then an efficient management to avoid gradual declines in crop yields (Lambers et al. 2009). Autotoxicity could be also involved in root growth regulation. In responce 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).

2.2.1.4 Research on Rhizodeposits

In spite of numerous experimental setbacks the rhizosphere has been widely studied; a fact that is supported by more than 11,000 of publications on this topic on the Web of Science database (number to April 2010)[1)]. This fact also reflected on the outcome of this review - the amount of studied literature is definitely limited by the time and human skills / abilities. For our purposes it is reasonable to discuss briefly the challenges of rhizosphere research and after that shortly comment on methods of biodegradability measurements.

Phillips et al. (2008) summarized a set of common challenges, which need to be overcome in all exudation methods: 1) to capture exuded C before it is assimilated by microbes, 2) to select a medium that does not affect root physiology and exudate recovery and 3) to distinguish exuded compounds from other soluble C compounds in the solution.

1) Roots growing under sterile conditions do not provide representative data on plant exudation. Generally, the results are underestimated because the flow of C from the root is not driven by the steep gradient caused by microbial uptake of exudates. The increace of organic C in the growth solution then slows down the exudation. Vančura (1988a) presented the comparison between exudation of sterile and inoculated plant roots. He also commented on the issue of sampling period choice. Frequent sampling intervals (e.g. every two days) also reflected on the root length shortening (about 20%) and on the root biomass decrease (about 11%; Vančura and Přikryl 1980 in Vančura 1988a). Thus, the percolating (non-static) systems of exudate trap solution should be prefered to the static ones in the culture-based experiments (Phillips 2008).

   When there is microflora present on the roots, the study is more relevant to the natural conditions. However, it is still imposible to measure the amount of exuded C qualitatively and quantitatively; as it 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).

2) When grown in solution without any mechanical support, root system changes its architecture (Lavelle and Spain 2001). Preferentially, some kinds of solid substrate should be 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 exudation 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 definitely is to sample root rhizodeposits *in situ*. For qualitative research, various microsuction cups were developed (Dessureault-Rompré et al. 2007). For microscale research of rhizodeposits, agar, specialized resins and filter papers were used (Gregory and Hinsinger 1999). Phillips et al. (2008) proposed a new method of quantitative exudate collection *in situ*. The rhizodeposits were collected in 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 good point of this experiment.

3) For the purposes of qualitative research, axenic laboratory experiments are acceptable. To measure C flux in natural systems a method of isotope labeling is suitable (Cheng and Gershenson 2007). To trace the fate of assimilated C, plants are placed into a labelled $^{13}CO_2$ (or $^{14}CO_2$) atmosphere. Then, the amounts of these heavy isotopes are measured in plant tissues, growing solution and microbes (Kaštovská and Šantrůčková 2007). However, as mentioned in point 1), on non-sterile roots, the rhizodeposits are immediately metabolized by micorbes. Thus, it is almost imposible to distinguish whether these ^{13}C compounds are real exudates or if they are the products of microbes.

Quite reasonably, there is a tendency to move the rhizosphere experiments to the field. With this fact, other issues occur: A) the laboratory methods (so far inaccurate with respect to these three 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).

2.2.2 Plant Nutrient Uptake

Plant ability to acquire nutrients is said to be restricted to few, mostly inorganic, chemical compounds. Recently, however, the evidence of root ability to uptake organic compounds has been reported (Sauheitl et al. 2009). 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), 2) organic nutrient (e.g. amino acid) capture from soil and 4) tranfer 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 boreal and arctic regions where N limitation prevales (Martinelli et al. 1999). It could be partly explained by the old age of tropical soils, partly by the temperature optimum for nitrogenase activity. This enzymatic activity reaches maximum at 26^{o}C, hence lower N_2 fixation occurres in colder areas (Houlton 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 to the system and at the same time the source of P (the maternal bedrock) is slowly getting exhausted. Thus, the shift in N: P availability of very old soils (reaching to almost) results in P limitation.

Plants have developed various strategies to sustain their life under nutrient limiting conditions, such as changes in root morphology and functions, secretion of chemically active compounds or various symbioses (Figure 3). An interesting strategy have been developed by carnivorous plants (Allison 2006). However, the wide-spread and also best-studied adaptations are symbiotical interactions with fungi 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). Plant invests relatively more to 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 limiting 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 (cluster roots) improve P uptake efficiency. So far, we do not know any specialized root structures for enhacement of N uptake, except for nodular symbiotic structures. The explanation is in much higher mobility of N nutrients than that of P compounds (Brady and Weil 2002). Overall, root morphological adaptations in symbiotic relationships are more (nodules and short ECM roots) or less (AM intracellular arbuscules) apparent but definitely the best-studied ones.

2.2.2.2 Nitrogen Uptake

Plants are able to acquire N in both inorganic (NH_4^+ and NO_3^-) and organic (amio acids) form (Sauheitl et al. 2009). Nevertheless, plants are not able to fix the ubiquitous N_2 from the atmosphere by themselves. That is why they evolved symbioses with procaryotic 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*, *Pleurozium schreberi*, Gentili et al.), 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 has been used as a biological rice-field fertilizer (Choudhury and Kennedy 2004).

In addition, associative N_2 fixing microbes in the rhizosphere (e.g. *Azotobacter*, *Rhizobium*) could be also important for plant N-budget. However, they do not supply N to the plant directly as do the symbionts, but the organic N must first be released to the soil via microbial turnover and only then (usually after an additional mineralization) can it be absorbed by the root.

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 aminopeptide bonds in soil organic matter and thus make N more accesible (Adamczyk et al. 2009). In fact, it might be a little contra-productive to uptake N at the expense of production of N-rich enzymes.

This can perhaps explain why proteases seem to be of much lower importance for the plant than phosphatases or phytases.

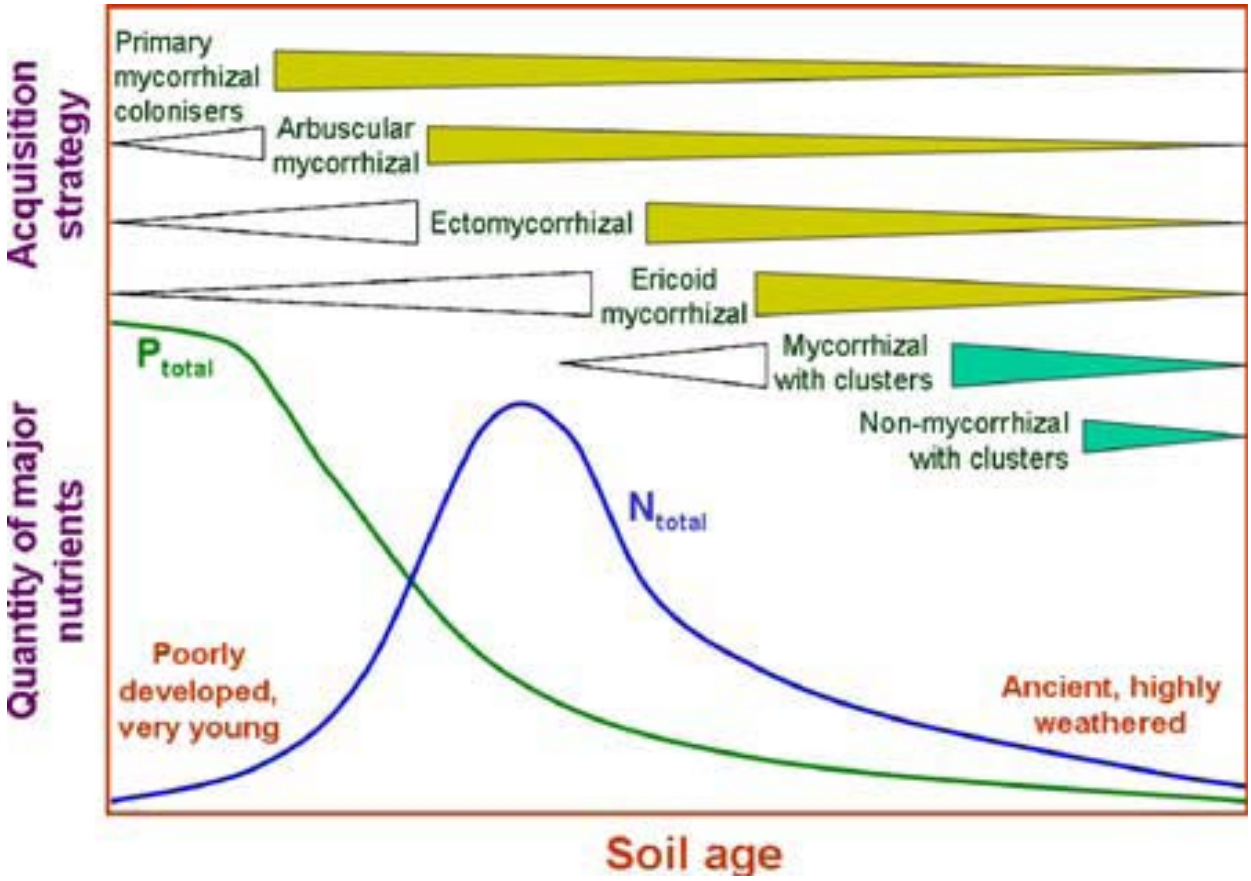

Figure 3. Changes in N and P soil contents as a function of soil age and plant P acquiring strategies. The soil age scales from 'poorly developed very young soils' (e.g. soils resulting from recent volcanic eruptions) to 'ancient weathered soils' (i.e. soils that have been above sea level and have not been rejuvenated by glaciation over several millions of years). 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}. Modified from 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 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 3). However, this non-renewable source can get 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 (Mitch and Gosselink 2000). P availability does, however, change substantially according to pH values (Brady and Weil 2002). It is known that in alkaline (basal) soils, P is trapped into insoluble complexes with Ca. As a result of that, calciphilous plants have the ability to secrete higher amounts of organic acids in order to mobilize this unavailable P (Jones 1998).

Particular forms of P compounds act differently under various environmental conditions. For our purposes it is useful to distinguish between low and high-turnover forms. Orthophosphate in the soil solution, its proportion reversibly attached to the soil particles (exchangeable P) and some P organic compounds undertake rapid turnover. 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. Also, occluded inorganic P (physically encapsuled by minerals) and some other organic P compounds are involved in the low turnover-rate processes. In a shorter time scale, most of the P is acquired from dead organic material and microbial biomass is one of the most important organic pools (Coyne 1999)

Exudation of Low Molecular-Weight Organic Acids

Common strategy to acquire more P is to secrete low molecular-weight organic acids (Jones 1998). Their carboxylic groups serve as complexing agents for metal cations present in the soil solution. Coupled with that, displacement of anions bound in soil matrix happens. Thus, it could be not only P but also Fe and other micronutrients, which are mobilized. On the other hand, not only nutrients but also toxic elements act similarly, particularly Al and Ni, which are toxic for plant growth (Ahonen-Jonnarth et al. 2000). Consistent with that, 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 / kept AM mycorrhiza to gain P as well as to avoid Ni toxicity, which would happen in case of increased organic acid release. Last but not least, organic acids are able to release P from its insoluble forms in minerals and, therefore, serve as important weathering agents in the process of pedogenesis (Jones 1998).

Cluster Roots

Although these specialized roots have 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. Often this secretion is not continuous but occurs in an exudative outburst (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" (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). Thus, 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 mono- and di-esters, Adams 1992). Phytases are efficient in phytate (= *myo*-inositol penta- and hexa-phosphates) hydrolysis. In addition, mycorrhizal fungi are known to secrete high amounts of phosphatases (Ahonen-Jonnarth et al. 2000), which is important for mycorrhizal plants.

As exoenzymes consist of C and N, N-limitation can also result in decrease of exoenzyme secretion. Hence, the activity of N_2 fixers can indirectly influence the production of exoenzymes (Houlton et al. 2008).

In addition to the strategies described above, Lambers et al. (2006) completed the list of P acquiring strategies with 1) hydraulic redistribution: this redistribution of water within roots (upwards, downwards and horizontally) helps to transport soluble P compounds from soil to the root,this is of particular importance in arid soils; 2) the secretion of phenolics and mucilage, an often neglected factor of plant P-acquisition; 3) 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 moved (translocated) from senescing tissues and reused or stored. Common percentage of N and P reused by plants is around 50% for each of them (Aerts 1996). However, this proportion fluctuates according to nutrient availability in the environment. For instance, in P-limited wetland plants can recycle about 80 % of their P content (Rejmánková and Snyder 2008). In contrast, the C content in the senescing tissues usually remains almost the same, as the 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 after rhizodeposition the second most considered C input to the soil. It is crucial to know how much is this organic matter available for soil microbiota and, therefore, for all other organisms. Litter bioavailability is primarily determined by its chemical composition. Logically, a nutrient-rich litter triggers the activity of microbial communities (Richards 1987). Chemical recalcitrance is known mostly for plant structural material (Lavelle and Spain 2001). Particularly, aromatic compounds (e.g. lignin, phenolics) can persist in the ecosystem for very long time and, thus, keep relatively big amounts of C and nutrients immobilized (e.g. an extreme example of more than 3000 years old undecomposed trunks of *Sequoiadendron giganteum* in the forest understory)[2]. In addition to chemically resistant compounds, some plants are known to produce biological conservants. For instance, polyuronic acid released by peat mosses inhibits microbial activity (Aerts et al. 1999) and, therefore, induces the accumulation of peat (partly decomposed plant material).

The rate of decomposition depends not only on the litter chemical composition but also on the microbial activity of decomposers, which are influenced by environmental conditions. Generally, the decomposition is faster under warm conditions than under cold, under wet conditions than under dry and under aerated conditions than in anoxia (Brady and Weil 2002). Wetlands are known to accumulate organic matter, because the decomposition is inhibited by lack of oxygen (Mitsch and Gosselink 2000).

2.3 Roles of The Rhizosphere Microflora

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

Generally, we distinguish positive and negative interactions, both direct and indirect. Furthermore, all these types have either loose (associative organisms) or tight (symbionts or parasites and pathogens) character. For the plant, the positive relationships represent mainly three 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. It is, however, much more difficult to evaluate the influences in the opposite direction (the effect of a plant on the microbes).

In a broader sense, there are some other positive and negative aspects in the rhizosphere. An example of a positive aspect is both plant and the microbial contribution to the development of environment favourable for their life. On the other hand, their competition for water and nutrients is a strong constraint. The line between negative and positive influences is usually very thin and intricate. According to Saikkonen et al. (1998), both symbiotic and parasitic fungi evolved from root endophytes.

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 few groups of nodular plants, these two phenomenons do not appear separately. Plants with nodular symbioses often maintain AM as well (Lambers 2009).

2.3.1.1 Mycorrhizae

Several types of mycorrhizae are distingiushed: AM, 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 20 %

of the daily fixed C (Morgan et al. 2005). In return fungi provide more efficient uptake of water and nutrients to the plant.

Fungal hyphae increase the exchangeable surface with soil (mycorrhizosphere), reach further and grow faster than roots. In addition, hyphae can access the water and nutrients present in much smaller soil pores (Coleman and Crossley 1996). Fungi release exoenzymes to decompose complex organic substances and they are also able to absorb some organic compounds, which would, otherwise, remained unavailable for the plant. A second important ability of mycorrhizal fungi is the protection against plant pathogens. Besides competition with pathogens for space and resources, mycorrhizal fungi 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 mycorrhiza easily shifts to parasitism (Lambers et al. 2009).

Arbuscular Mycorrhiza (AM)

The oldest known evidence of root symbiotic structure is AM-like structure in "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). Consisten with this, all AM fungi belong to the 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 and saline) habitats and have evolved other strategies of obtaining P (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, 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 than is in AM. All of them come from woody gymnosperms and dicotyledons (except for one monocotyledonous species). ECM is substantially younger than AM; its origins are dated about 100 million years back. At that time, the initial development of flowering plant occurred as well(Brundrett 2002). In addition, ECM supplies the plant not only

with P and micronutrients (e.g. K, Mg and Ca) but also considerably contributes to 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 plants. 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 witht Basidiomycota (Rasmussen 2002). All orchids are fully dependent on their fungal symbionts because their tiny seeds would not germinate succesfully without the support of fungi (Johnson and Gehring 2007). However, it is not always advantagaeous for the fungi; some orchid species lack chlorophyl and thrive on assimilates moved by the fungus from other assimilating 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 origins have been estimated about 55 million years back. In that period, CO_2 concentration 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 during the primary succesion (Lambers et al. 2009).

2.3.2 Pathogens and Parasites

Morgan (2005) pointed out that if the 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 a complete rid of other microbiota. Plant – pathogen interactions are, in general, host specific and influenced by exudates (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 microbiota, which, consequently supress plant pathogens (chapter 2.3).

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

2.3.3 Associative Microorganisms

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

Positive Interactions

Assiciative N_2-fixers indirectly (via microbial turnover) improve plant N-budget. Other microbes release plant-growth promoting compounds, mostly phytohormones (e.g. IAA, Benizri et al. 2001, Brimecombe et al. 2001). Some microbes secrete substances to enhance the development of plant symbionts (e.g. MHB, mycorrhization helper bacteria, Johnson and Gehring 2007) or to eliminate plant pathogens. 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 and 3) volatile substances (e.g. ammonia, cyanide), which are believed to serve as a biocontrol. Further, the pathogens are controlled by competition for 4) nutrients and 5) for ecological niche. Next by 6) parasitism on pathogens and finally 7) by plant resistance to deseases induced by other microbes (ISR, induced systematic resistance). However, the mechanisms 2), 3) and 4) do not supress only pathogens but might have a negative eefect on the plant.

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

Negative Interactions

Besides those already meantioned, 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 formation of mycorrhizae and by counteracting the effect of N_2-fixers in the rhizosphere (Brimecombe et al. 2001). Similar to the tighter negative interactions, DRB are as well host-specific (Nehl et al. 1996).

Although there are positive effects of bacteriophage (microbial-growth control) and entomopathogenic (elimination of root grazers) Nematodes, it is necessary to mention that the majority of studied soil Nematodes belongs to phytopathogens (Richards 1987).

2.4 The Specifics of Wetland Ecosystem

As defined by Cowardin et al. (1978): 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. For wetlands hydric soils and hydrophytes (plants adapted to the life under wet conditions) are typical. This chapter aims to bring a quick overview of the main constraints for biota present in this ecosystem and how do the inhabitants deal with them.

First of all, the diffusion of atmospheric gases in flooded conditions is much lower. When O_2, is spent, aerobic metabolism is replaced by other metabolic pathways. NO_3^-, Mn_4^+, Fe_3^+, SO_4^{2-} and CO_2 or acetate serve, according to their redox potential, as electron acceptors for microbial catabolism (Mitch and Gosselink). Owing to this fact, the mineralization (decomposition) of organic matter is slower and the undecomposed material accumulates in wetlands (Lavelle and Spain 2001b).

Also wetland plants 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, *Taxodium* knee roots and buttresses, willow adventive roots) or by formation of aerial plant tissue - the aerenchyma. An aerenchyma can be aerated more efficiently by the effect of pressurized ventilation - an enhanced flow of air through the individual plant or even through the whole ramete (Armstrong et al. 1992). This pressurized air flow is based on different pressures and temperatures above young and older leaves, the mechanism is well-described for instance on yellow waterlily (Dacey 1981).

2) Plants tolerating anoxia by metabolic adaptations such as diversification of end products of glycolysis to avoid the accumulation of toxic metabolites or synthesis of antioxidants and enzymes to minimalize the post-anoxic stress after the water level drops down again (Mitch and Gosselink 2000). In addition the life under anoxia is often maintained by carbohydrate reserves stored in storage organs (e.g. rhizomes; Lavelle and Spain 2001).

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

Because of toxic Fe^{2+} and Mn^{2+}, which are mobilized under lower redox potential, wetland plants become more Fe^{2+} and Mn^{2} tolerant (Armstrong et al. 1992).

3. THE STORY OF *Eleocharis* AND *Typha*

3.1. Study Site and Research History

Our study sites are oligotrophic marshes scattered in the lowlands of northern Belize, Yucatan peninsula, Central America. This region is characterized by tropical wet-dry climate in Koeppen´s classification. The bedrock is formed by 2-3 km thick uplifted marine platform of limestone. Marsh hydrology is closely linked to groundwater and thanks to occasional intrusions of seawater the water conductivities vary from 0.2 to 7 μS within the marshes. Water level fluctuates mostly according to precipitation but despite the regime of wet and dry seasons, marshes seldom dry out completely. The amount of bioavailable P is very low, although there are prevalently anoxic conditions and P-rich bedrock (insoluble apatite). Detailed description of the study site was provided by Rejmánková et al. (2008)

In their pristine stage these marshes are dominated by few species of emergent macrophytes (*Eleocharis cellulosa* and *E. interstincta* and their hybrides) and by enormously diverse cyanobacterial mats (Rejmánková et al. 2004). A typical story of eutrophication has been happening since the second part of past century. At that time, the intensification of agriculture coupled with the increase of acreage of sugar cane plantages have multipled the nutrient input to the environment. Nutrients, particularly P, flushed with agriculture runoff entered, the marshes and in course of the time have caused a complete change in the vegetation cover (Rejmánková et al. 2008).

In August 2001, 15 oligotrophic marshes dominated by cyanobacteria and sparse *Eleocharis* spp., were chosen. Three salinity levels (low, medium and high) were each represented by five marshes. At each marsh four 10 x 10 m plots were designed to study the ecosystem response to nutrient addition. One plot remained as a control and next three plots were enriched by nutrients: N, P and both N and P. The plots were enriched repeatedly (August 2001, August 2002, August 2005 and a half dose in September 2006) with doses corresponding to 20 g of N and 10 g of P per m^2 per year. As a source of N NH_4NO_3 served and for the P source triple super phosphate was used. In March 2003 one individual of *Typha domingensis* was planted in the middle of each plot. These *Typha* plants thrived in P- and NP-plots and often covered the whole plot within few years. Moreover, they would expand further to adjacent oligotrophic areas, therefore, a *Typha*-clearing activity at the plot borders has been maintained. On the contrary, there were not observed any differences in the spread of *Typha domingensis* between control and N-enriched plots, in both

treatments *Typha domingensis* disappeared after short time. In January 2005, the P-plots in six marshes were split into two parts, one dominated by *Typha domingensis*, second dominated by *Eleocharis* spp. Annual aboveground biomass estimates and N, P contents in plant tissues have been gathered since 2001. Since 2003 till 2007 the monitoring of *Typha domingensis* spread was conducted (Rejmánková et al. 2008). Aboveground and belowground *Eleocharis* spp. and *Typha domingensis* biomass and its decomposition were measured (Rejmánková and Houdková 2006, Rejmánková and Sirová 2007). Microbial sediment activities (respiration, mineralization, nitrification and N_2 fixation) are reported in Pivničková et al. (2010, in press). CBM and sediment phosphatase activity, (Rejmánková and Sirová 2007) and CBM profiles of oxygen and nutrients (Sirová et al. 2006) were described. More recently, since 2007, nitrogenase activity in the rhizosphere (Šantrůčková et al. 2010, in press) and root exudation (unpublished data) has been studied. Well-established long-term experimental plots and low macrophyte diversity of the system help then to interpret the results more trustworthy.

3.2 Rhizodeposition and Its Bioavailability

At the studied plots average plant biomass ranges from 150 to 250 g m^{-2} for *Eleocharis* spp. and from 450 to 820 g m^{-2} for *Typha domingensis*. Generally, plant biomass was higher in low-salinity marshes and lower in high-salinity ones (Rejmánková and Sirová 2006). Our study of C partitioning (Šantrůček et al., unpublished data) showed that within first five days after ^{13}C assimilation *Eleocharis cellulosa* lost much higher portion of fixed ^{13}C in favour of microbial biomass in the growing solution (about 40% when grown in P-limited and almost 80% when grown in P-enriched treatment) than *Typha domingensis* (about only 20% in both P-enriched and P-limited treatments). However, we do not know, which proportion of ^{13}C released to the solution was lost by microbial respiration. The rhizodeposit biodegradability (measured as a respiration of inoculated rhizodeposits) was higher in *Typha domingensis* rhizodeposits (Kubešová, unpublished data). This fact suggests an explanation for the lower percentage of ^{13}C in microbial biomass of *Typha domingensis* growth solution: the exuded ^{13}C might have been breathed out.

Cheng and Gerhenson (2007) pointed out the fact that nitrogenase activity can also signifficantly decrease the microbial assimilation (and, conversely, increase microbial respiration) of the inoculated medium. This is hence the N_2 fixation is an ATP-consuming process (usually 16 molecules of ATP are consumed to produce 2 molecules of NH_3, Mitch and Gosselink 2000). Considering this fact, the higher respiration measured in *Typha domingensis* rhizodeposits might

have been caused by a high proportion of N_2-fixing bacteria activity. N_2 fixation, however, was not measured.

Typha domingensis rhizodeposit biodegradability might have been even higher than was measured, as *Typha domingensis* mucilage was partly eliminated by filtration of rhizodeposits before the measurement.

3.3 N_2 Fixation

N_2 fixation significantly increased with P-enrichment both in the rhizosphere and in the sediment (Černá et al. 2009, Šantrůčková et al. 2010, in press). Expressed on dry weight, nitrogenase activity was higher in the rhizosphere then in the sediment. However, hence the roots occupy only little part of soil volume, the total N_2 fixation, expressed per m^{-2}, was higher in the sediment (Šantrůčková et al. 2010, in press).

Consistent with the proposed explanations of higher microbial respiration (or biodegradability) of *Typha domingensis* rhizodeposits, N_2 fixation in *Typha domingensis* rhizosphere was fourth times higher than in the rhizosphere of *Eleocharis cellulosa* (Šantrůčková et al. 2010, in press).

3.4 Enzyme Activity

In the decomposing *Eleocharis* spp. material, phosphatases were the most active enzymes when compared to amylopeptidase, arylsulfatase and β-glucosidase (Rejmánková and Sirová 2006). Phosphatases were also the most studied exoenzymes in the rhizosphere.

Expressed on dry weight basis, phosphatase activity was much higher on plant roots than in the sediment (Rejmánková and Macek 2008). Phosphatase activity was negatively correlated with P content in plant tissue and with the soluble inorganic P in the intersticial water (Rejmánková and Snyder 2008). Phosphatase activity on roots of *Eleocharis cellulosa* was consistently lower in P-enriched plots. On the other hand, *Typha domingensis* P-enriched and P-limited plots did not differ in this characteristic. As *Eleocharis cellulosa* disposes with more strategies of P acquisition than *Typha domingensis* (not only phosphatase activity and the ability to reabsorb P from senescing tissues but also organic acid production from dauciform roots); it might eliminate the most disadvantageous one. In the case of P-enriched but N-limited environments, the phosphatase production would be probably the most costly one and, therefore, decreased. Consistent with this hypothesis, in another experiment with *Eleocharis cellulosa*, the phosphatase activity was the

highest on *Eleocharis cellulosa* roots from N-enriched but P-limited system (Kubešová and Rejmánková, unpublished data).

3.5 Nutrient Content and Resorption

Rejmánková and Snyder (2008) presented the N and P tissue contents and the nutrient resorption from them. They found that P content in plant biomass increased five times for *Eleocharis cellulosa* and two times for *Typha domingensis*. However, the increase of biomass differed between plants and, thus, also differed the total nutrient storage. Whilst *Eleocharis cellulosa* increased its biomass five times, *Typha domingensis* was capable to multiply it 250 times. As a result, nutrient storage was much higher in *Typha domingensis* than in *Eleocharis cellulosa* biomass. N content in plant tissues of both species increased a little, but consistently, with P addition. Rejmánková and Snyder (2008) explained it by higher metabolical activity after the P-deficience was eliminated.

Under P-deficiency, both *Typha domingensis* and *Eleocharis cellulosa* reabsorbed about 80% of P present in their tissues. After two years of P-enrichment, *Eleocharis cellulosa* started to reabsorb less N and P and, therefore, the C/N and C/P ratios in its litter decreased. This low C/N and C/P ratios were reported to improve litter decomposition (Rejmánková and Houdková 2006). On the other hand, *Typha domingensis* did not seem to lower its nutrient resorption, as its growth probably had not reached its carrying capacity.

4. CONCLUSIONS

Finally, the information from rhizosphere review is combined with findings of research in Belizean wetlands. Hypotheses on the interactions between plant nutrient budgets, rhizodeposition and rhizosphere microflora are drawn up.

The hypothetized flows in the systems of different P status are viewed in Figures 4, 5 and 6. These schemes try to display the changes in plant rhizodeposition and nutrient uptake strategies and the responces of microbial loop. The intent is not to describe quantitatively the C, N and P fluxes but rather to sketch the potential processes, which are important in plant-soil ecology. Indeed, there is quite a difference between the systems of *Eleocharis cellulosa* grown under P-limited and P-enriched conditions. Contrarily, there is only one scheme for *Typha domingensis*, as it almost does not grow in oligotrophic marshes naturally.

Eleocharis cellulosa under P Limitation

Under P-limitation (Figure 4), *Eleocharis cellulosa* produces less biomass and less photosynthetize. The assimilated C is partly respired back via shoot, root and rhizosphere respiration. Another part of fixed C is incorporated into plant biomass and a substantial part is also released as some rhizodeposition (chapters 2.2.1.1 and 3.2). Part of the rhizodeposits is secreted in the form of extracellular phosphatases (chapters 2.2.2.3 and 3.4) and part of the rhizodeposits is utilized by rhizosphere microflora (chapter 2.2.1.2 and 3.2). In addition, when P-limited, *Eleocharis cellulosa* forms dauciform roots and produces substantial amounts of organic acids (chapter 2.2.2.3).

Rhizosphere microflora is able to decompose soil organic matter by production of exoenzymes (chapter 2.2.2.3). This makes the nutrients available not only for the microbiota but also for plant. In addtition, microbes enrich the system with N via N_2 fixation (chapters 2.2.2.2 and 3.3). Microbial N and P are available for the plant after they are release from microbial cells during microbial turnover. When *Eleocharis cellulosa* is limited by nutrients, it performs high ability to reabsorb them from its senescing tissues (chapter 3.5). Thus, its litter is N- and P- impoverished. Both plant and microbial community secrete signalling compounds to communicate to each other (chapters 2.2.1.3 and 2.3.3).

Eleocharis cellulosa under P Enrichment

Eleocharis cellulosa in P-enriched plot (Figure 5) is not P limited, thus, it can produce more biomass and assimilate more C. The assimilated C probably undertakes the same fates like in the P-limited plant but in different rates. For example, the nutrient resorption is observed to increase in first two years after P-addition but later, when the P is still well-available in the environment, *Eleocharis cellulosa* does not reabsorb so much. Consequently its litter is of higher quality (chapter 3.5).

The rhizosphere microbiota is not limited by P and its activity is triggered. Therefore, the N_2 fixation is also higher. Hence *Eleocharis cellulosa* does not need to use its C for P acquisition, the dauciform root formation (and organic acid secretion) is lower. In addition, the production of phosphatases decreases (chapter 3.4) as the plant does not need to expense N and C for enzyme synthesis and can invest them into the growth.

Typha domingensis under P-Enrichment

The C, N and P flows in *Typha domingensis* would be probably different in comparison to *Eleocharis cellulosa* (Figure 6). First of all, *Typha domingensis* is much robust plant, which assimilates more C. More of that C is respired back to the atmosphere and incorporated into plant tissues. A substantial portion is also allocated belowground (chapter 3.2). *Typha domingensis* also well reabsorbs the nutrients (chapter 3.5).

In the rhizosphere, the microbial activity is high. Also the N_2 fixation is higher compared to the both cases of *Eleocharis cellulosa* (3.3). The N released by microbial turnover can be taken up by the plant and after that used also for exoenzyme synthesis. As *Typha domingensis* does not maintain any other of known strategies of P acquisition (chapter 3.1), the phosphatases might be of high importance for the plant. However, there is a probability that, similar to its relatives *Typha angustifolia* (Tang et al. 2001) and *Typha latifolia* (Ray and Inouye 2006); *Typha domingensis* might be involved in AM symbiosis. Furthermore, *Typha domingensis* is reported to secrete allelochemicals (chapter 2.2.1.3) which might decrease the fitness of other plant species in the surroundings.

Future Goals

To achieve better understanding to rhizodeposit role in P acquisition, the examination *Eleocharis cellulosa* dauciform roots and *Typha domingensis* mycorrhiza should be done. Also, the nitrogenase activity during the experiments of rhizodeposits biodegradability should be measured. To evaluate real contribution of associative N_2 fixers to the plant nutrient budget a challenging labeling by $^{15}N_2$ might be conducted.

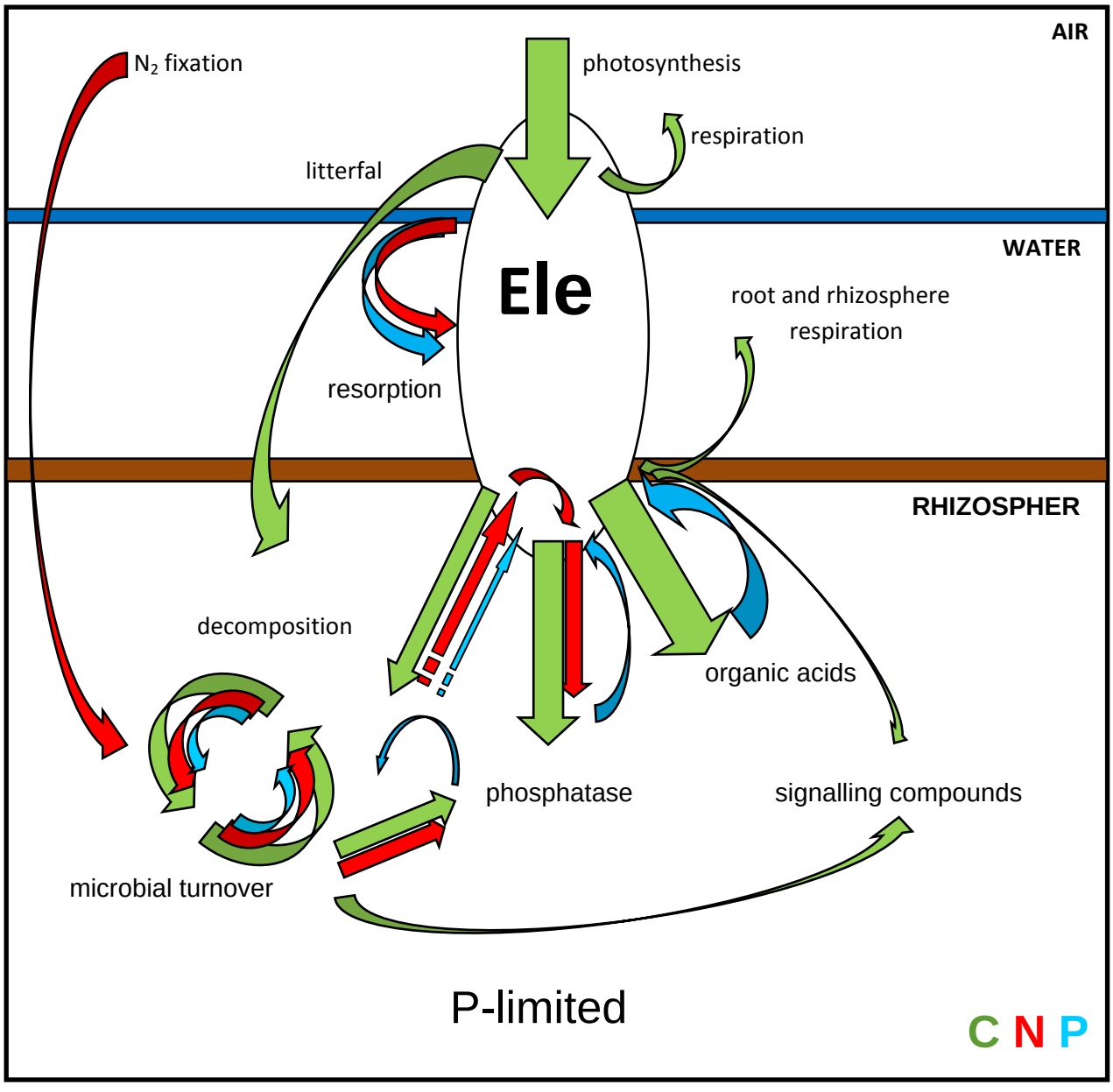

Figure 4. A scheme of hypothetized C, N, P flows in the system of *Eleocharis cellulosa* (Ele) and its rhizosphere under P-limited conditions. Green arrows – C; red arrows – N; blue arrows – P.

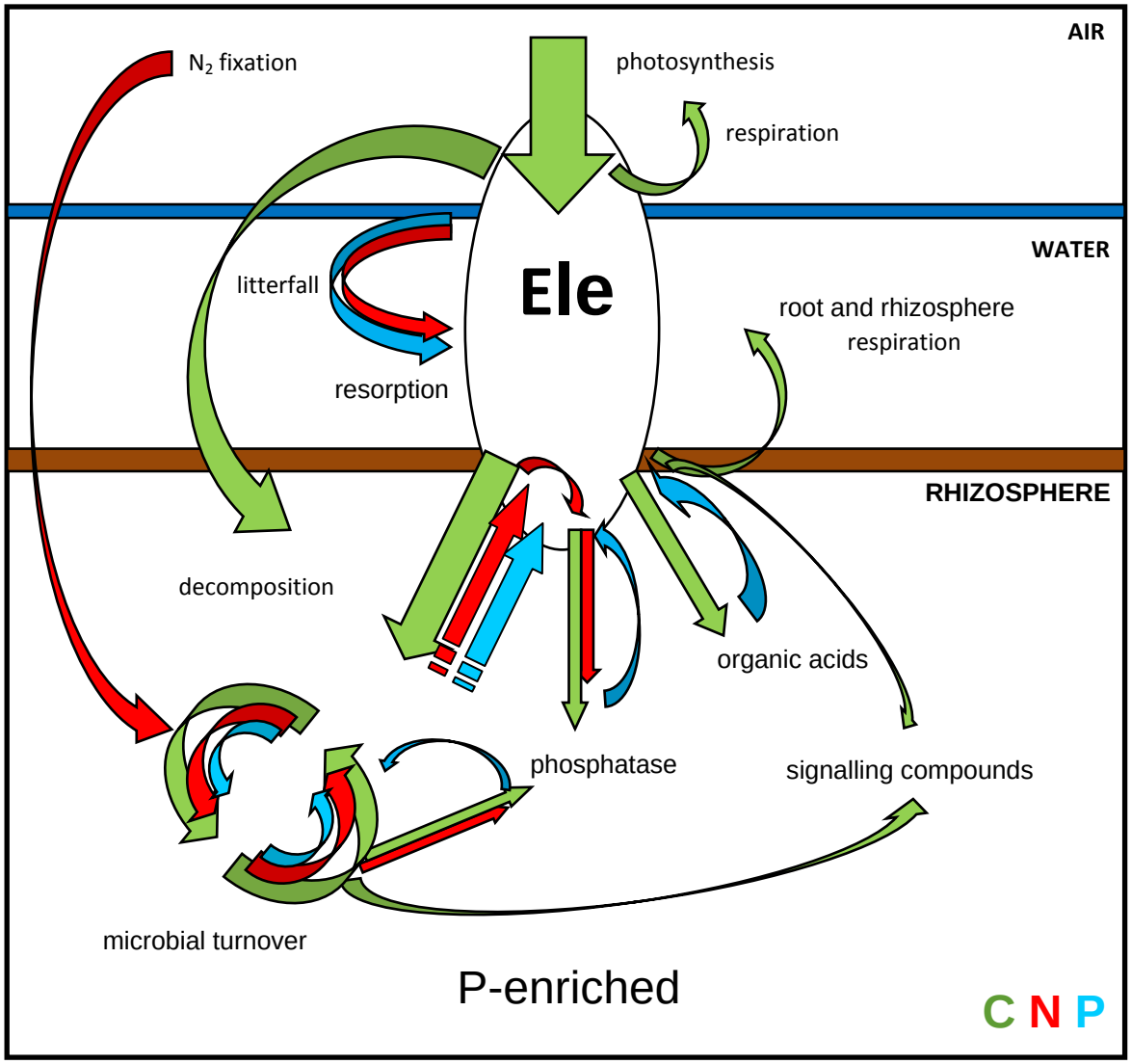

Figure 5. A scheme of hypothetized C, N, P flows in the system of *Eleocharis cellulosa* (Ele) and its rhizosphere under P-enriched conditions. Green arrows – C; red arrows – N; blue arrows – P.

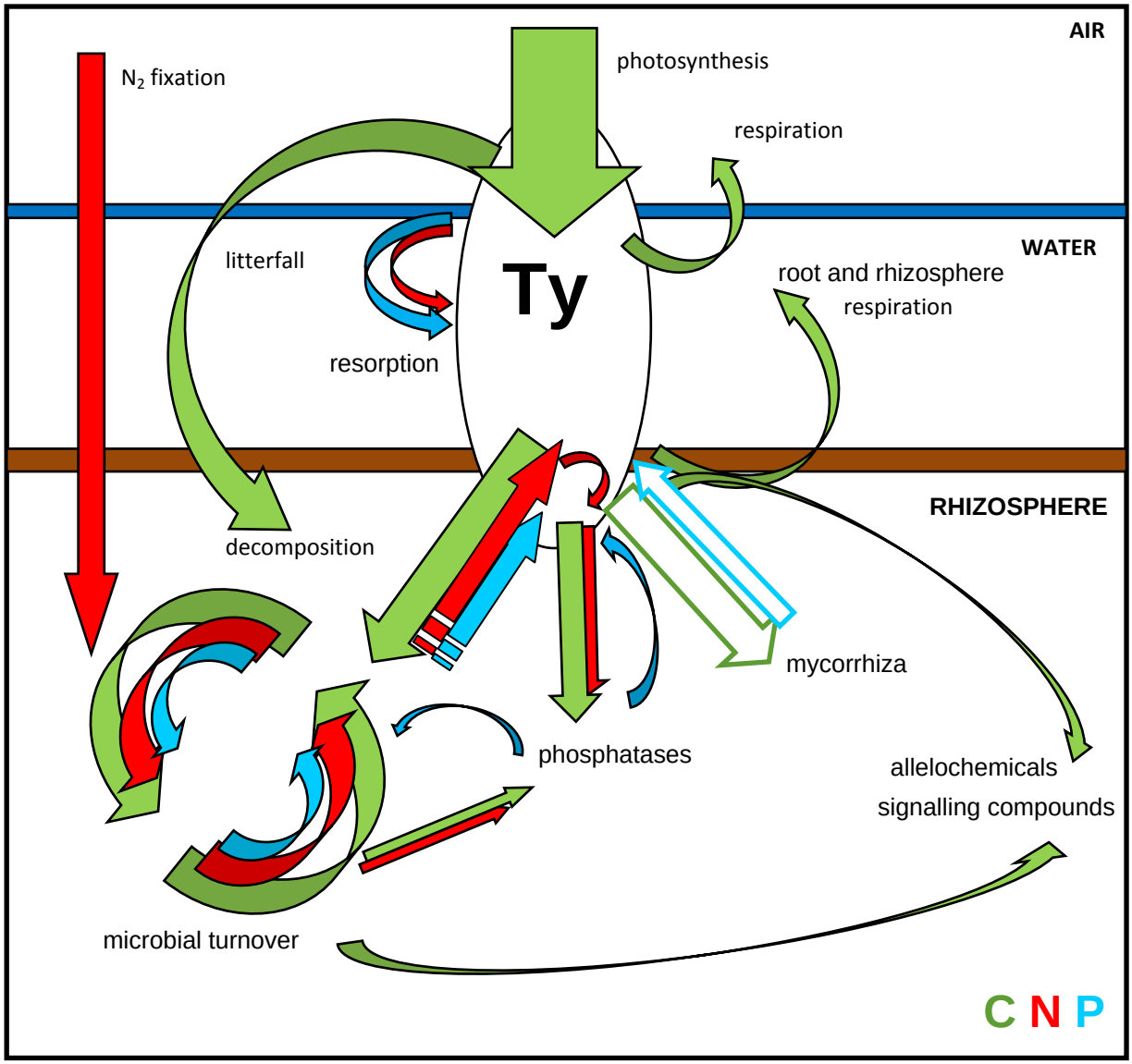

Figure 6. A scheme of hypothetized C, N, P flows in the system of *Typha domingensis* (Ty) and its rhizosphere under P-enriched conditions. Green arrows – C; red arrows – N; blue arrows – P; blunk arrows – hypothetized mycorrhizal symbiosis.

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