Microbial transformations of nitrogen, sulfur, and iron dictate vegetation composition in wetlands: a review

Leon P. M. Lamers1, Josepha M. H. van Diggelen2, Huub J. M. Op den Camp3, Eric J. W. Visser4, Esther C. H. E. T. Lucassen1, Melanie A. Vile5, Mike S. M. Jetten4, Alfons J. P. Smolders1,5 and Jan G. M. Roelofs1

1 Department of Aquatic Ecology and Environmental Biology, Institute for Water and Wetland Research, Radboud University Nijmegen, Nijmegen, Netherlands
2 B-Ware Research Center, Nijmegen, Netherlands
3 Department of Microbiology, Institute for Water and Wetland Research, Radboud University Nijmegen, Nijmegen, Netherlands
4 Department of Experimental Plant Ecology, Institute for Water and Wetland Research, Radboud University Nijmegen, Nijmegen, Netherlands
5 Department of Biology, Villanova University, Villanova, PA, USA

The majority of studies on rhizospheric interactions focus on pathogens, mycorrhizal symbiosis, or carbon transformations. Although the biogeochemical transformations of N, S, and Fe have profound effects on vegetation, these effects have received far less attention. This review, meant for microbiologists, biogeochemists, and plant scientists, includes a call for interdisciplinary research by providing a number of challenging topics for future ecosystem research. Firstly, all three elements are plant nutrients, and microbial activity significantly changes their availability. Secondly, microbial oxidation with oxygen supplied by radial oxygen loss from roots in wetlands causes acidification, while reduction using alternative electron acceptors leads to generation of alkalinity, affecting pH in the rhizosphere, and hence plant composition. Thirdly, reduced species of all three elements may become phytotoxic. In addition, Fe cycling is tightly linked to that of S and P. As water level fluctuations are very common in wetlands, rapid changes in the availability of oxygen and alternative terminal electron acceptors will result in strong changes in the prevalent microbial redox reactions, with significant effects on plant growth. Depending on geological and hydrological settings, these interacting microbial transformations change the conditions and resource availability for plants, which are both strong drivers of vegetation development and composition by changing relative competitive strengths. Conversely, microbial composition is strongly driven by vegetation composition. Therefore, the combination of microbiological and plant ecological knowledge is essential to understand the biogeochemical and biological key factors driving heterogeneity and total (i.e., microorganisms and vegetation) community composition at different spatial and temporal scales.

Keywords: biodiversity, eutrophication, heterogeneity, nutrients, plant–microbe interactions, plants, rhizosphere, toxicity

INTRODUCTION

Plant ecologists have long been focusing mainly on above-ground processes, while a significant part of the driving mechanisms of ecosystem functioning is taking place below-ground (Waisel et al., 1996). This strongly pleads for studies including biogeochemical transformations in soils and the important interactions between their key players: microorganisms and plants. This paper will focus on the roles of microbial conversions of the elements nitrogen (N), sulfur (S), and iron (Fe), with respect to their major role in the functioning and succession of plant communities in wetlands. The rationale lies in the fact that all three elements can be converted to chemical species that can serve as nutrients, as terminal electron acceptors and electron donors, and can also be toxic. We hope in this review to show the fascinating biogeochemical interactions between plant communities and microbial communities, and encourage microbiologist and plant ecologists to further integrate their disciplines in the hidden half of the ecosystem (Figure 1). Wetland overall communities (i.e., the integration of micro- and macro-communities) provide excellent examples for this complex interaction playground, the rhizosphere. We define the rhizosphere as that part of the soil where plant roots significantly interact with soil biological and chemical processes, at the scale of 10−4 m or less to 10−1 m. One should realize that the root systems of plants can be very large, up to 112 km m−2 of soil (Robinson et al., 2003) and reaching a depth between 10−3 and 10 m (or even deeper for trees; Canadell et al., 1996). Therefore, a major part of the upper soil of all vegetated wetlands can be defined more or less as a potential rhizosphere. This urges the use of an integrated approach of microbiologists and plant ecologists, in order to understand wetland biogeochemistry, biodiversity, and resilience with respect to ecosystem changes. The purpose of this paper is to show how microbial activity related to the biogeochemical transformation of N, S, and Fe also has profound effects on the activity and competitive strength of plants in wetlands. In
This way, they shape the structure, succession, and biodiversity of vegetation.

HETEROGENEITY IN SOILS DRIVES BIODIVERSITY AND FUNCTIONING

Unvegetated wetland soils, such as deeper parts of lake sediments or sediments of turbid shallow lakes (with insufficient light for underwater plant photosynthesis) are relatively homogeneous, except for the sediment-water boundary layer that generally shows a steep redox profile related to the successive depletion of terminal electron acceptors (Wetzel, 2001). One of the interesting characteristics of vegetated soils, and particularly of wetland soils, is their heterogeneity in space and time (Paul and Clark, 1989; Conrad and Frenzel, 2002; Bodenier, 2011). Microbiological batch experiments, even those containing multiple organisms, hardly resemble these soils. One of the challenges in microbial ecology is therefore to include this heterogeneity in resources (nutrients, substrates, electron acceptors) and conditions (e.g., oxygenation level, pH) under experimentally controlled conditions. Even under apparently stable conditions, the outcome of competition for one or several nutrients may be chaotic (Huisman and Weissing, 1999), but heterogeneity is even more relevant, ecologically speaking, because it facilitates the formation of multiple niches and generates larger fluctuations in conditions over time. As such, heterogeneity strongly affects competition between species (or operational taxonomic units at around species level), and diversity will differ from that under homogeneous conditions. Competitive exclusion, occurring under homogeneous, stable conditions, can be prevented by spatial and temporal heterogeneity, both for microorganisms and for macroorganisms.

In soils with plants (almost all soils in the world), heterogeneity increases as a result of rhizospheric effects (Marschner, 1995) and three-dimensional gradients (changes in concentrations with distance) at the micron-millimeter scale can be very steep. Different consortia of microbial species are present inside roots, on the root surface, and occur at different distances from roots, all of which are related to root exudate concentrations, oxygen availability, nutrient availability, and pH (Bruno et al., 2000; Bodenier, 2003; Bodenier et al., 2006). Plant roots even influence soil temperature, with significant effects on microbial activity (Boone et al., 1998). Rhizospheric interactions between microbes and plants have been studied to a large extent with respect to pathogens, mycorrhizal symbiosis, and carbon transformations (e.g., Callaway, 1995; Smith and Smith, 1997; Jones, 1998; Hanson et al., 2000; Whipp, 2001; Gilbert, 2002; Read and Perez-Moreno, 2003).

As a result of heterogeneity, the concentrations of terminal electron acceptors, nutrients, and toxins can differ greatly at even the micro-scale (Bodenier et al., 2006). In redox reactions, the oxidation of organic and inorganic compounds (electron donors) to generate energy has to be coupled to the reduction of electron acceptors, e.g., O_2, NO_3^-, Fe^{3+}, SO_4^{2-}. As a result of differences in energy generation, textbooks show a sequential “preference” for the use of electron acceptors, starting with O_2. This implies that microorganisms able to use a particular electron acceptor are expected to outcompete organisms using alternative, less favorable electron acceptors. However, small-scale differences in electron acceptor availability in heterogeneous soils explain the co-existence of different microbial groups, without outcompeting one another due to thermodynamic competitive advantage. In addition, the rapid transformation of reduced products by microbial re-oxidation, loss to the atmosphere (e.g., N_2), or chemical sequestration (e.g., FeS_3) may lead to favorable thermodynamic conditions that are inaccessible to those in closed batch systems.

Changes in plant species composition will strongly affect microbial communities in the rhizosphere. Especially in wetlands, where soils are periodically or constantly flooded or waterlogged, radial oxygen loss (ROL) from plant roots has an extreme impact on rhizospheric microbial communities, locally converting conditions in the wetland soil to those normally found in oxygenated upland soil. Using microelectrodes, Frenzel et al. (1992) showed that rice plants were well able to oxygenate the soil. Oxygen is transported through aerenchyma in the stolons and roots by diffusion or mass flow, and the extent and type of aerenchyma formation differs to a large extent among species (Jackson and Armstrong, 1999; Visser et al., 2000a,b; Voesen et al., 2006). As a consequence, the spatial pattern of ROL, also strongly varies among wetland species, due to differences in lignin and/or suberin deposits in the exodermis of roots. These deposits act as a barrier to prevent loss of O_2 in the upper soil layer and ensure O_2 supply to the active root tips (Armstrong, 1978; Laan et al., 1989; Colmer, 2003), and thereby prevent root anaerobiosis (Lambers, 1976; Vartapetian and Jackson, 1997; Jackson and Armstrong, 1999). This means that steep changes in oxygen availability, and hence in microbial communities, will prevail in the rhizosphere, in contrast to anaerobic bulk soil, depending on plant species composition. For this reason, microbial communities in wetland soils may be more diverse than in upland soils. The physiological ability to oxidize rhizospheres differs among plant species, which may not only facilitate conspecifics but also heterospecifics (Callaway, 1995). Next to this spatial heterogeneity, there will also be a strong temporal heterogeneity in the rhizosphere due to changes in temperature, plant presence, plant activity, and water table. Particularly in riparian wetlands, where water tables fluctuate frequently, both resource...
availability and conditions can vary widely, providing challenges and opportunities for microbes.

**NUTRIENT CYCLING AND UPTAKE IN THE RHIZOSPHERE**

For both heterotrophic and autotrophic organisms including plants, sufficient nutrient acquisition is essential to transform the carbon acquired into growth and reproduction and to safeguard the fitness of their populations. The rates of transformation of dead organic material into simple organic compounds and minerals, carried out by fauna and microorganisms, strongly determine the growth rates of plants. At high nutrient availability, fast-growing and highly competitive species will generally dominate at the expense of slow-growing species and monopolize light and space. As the quality of the dead organic matter strongly differs between plant species, decomposition (carbon mineralization) and the mineralization of the macronutrients N, P, potassium (K), and S, and of micronutrients including Fe and manganese (Mn), will depend on the species composition. Higher nutrient concentrations in dead material may stimulate mineralization rates, in this way providing a positive feed-back (Hobbie, 1992; Aerts and Chapin, 2000). This will, however, depend on the nature of nutrient limitation of the decomposition rate, which can be limited either by N, P or both. Although the C:N ratio of organic matter has traditionally been used as a quantitative indication of (potential) decomposition rates, the C:P ratio may be a better indicator for locations that show a high availability of N, e.g., as a result of high atmospheric N input. However, studies on the effect of organic matter quality on decomposition and nutrient mineralization rates still show much inconsistency (Kalbitz et al., 2000). Note that rates of vegetation growth and microbial decomposition do not necessarily have to be limited by the same element. Plants are able to actively forage for nutrients, leading to selective root placement at spots enriched with nutrients (Hutchings and De Kroof, 1994; Hodge, 2004). In this way, not only nutrient availability and its heterogeneity, but also traits and plasticity of plants with respect to root foraging strategies will drive below-ground competition, vegetation diversity, and biomass production (De Kroof et al., 2012). This means that there is a complex interaction between plant species composition and microbial communities including fungi, bacteria, and archaea. For technical reasons related to root sampling, many interesting characteristics of this hidden, but very important aspect of plant communities (Waisel et al., 1986) are yet to be discovered. New techniques including soil scanning in large-scale rhizotrons (Figure 2) enable an underground view on plant communities, which can be coupled to, e.g., fluorescence in situ hybridization (FISH) techniques visualizing the locations of different microbial groups or species, microarrays to rapidly check for known sequences, polymerase chain reaction (PCR) based community analyses and metagenomics (full community genomics), to gain insight into rhizospheric microbe-plant communities and interactions. Next generation DNA sequencing technologies can be expected to make rhizosphere community analyses much faster, cheaper, and easier, enabling for instance multiple comparisons between different plant species in one vegetation type, and between conspecifics under different environmental conditions.

The three elements that we focus on here, N, S, and Fe, all are essential nutrients for the metabolism and growth of both plants and microorganisms. These elements are predominantly taken up by plants from the soil via the roots, as only few plant species may take up high amounts of nutrients via the leaves. An exception are aquatic macrophytes that take up nutrients from the surface water, especially when soil concentrations are low or when the plants lack roots. On a quantitative base, N is the most important macronutrient (Marschner, 1995; Larcher, 2003), essential for the synthesis of amino acids, amines, and peptides (including proteins). As the availability and uptake of this element is a major factor limiting biomass production in many ecosystems (Vitousek and Howarth, 1991; Larcher, 2003), its availability and mobility are important drivers of species composition and biodiversity. Enhanced N availability changes biodiversity and heterogeneity, both above-ground and below-ground. Due to selective root placement, spots enriched in N contain more roots. For plants including Fabaceae (formerly called Leguminosae), the symbiotic relation with rhizobial strains of a variety of genera of Proteobacteria including *Rhizobium*, is of vital importance for atmospheric dinitrogen (N$_2$) fixation in soils that are low in N (Dakora and Phillips, 2002). The symbionts are attracted by the exudation of phenolics and aldonic acids from roots. For non-Fabaceous plants including the tree and shrub genera *Alnus*, *Casuarina*, *Ceanothus*, and *Myrica*, atmospheric N$_2$ is supplied by *Frankia* Actinobacteria. The cyanobacterial symbiont *Anabaeena* provides atmospheric N to waterferns (*Azolla* spp.). The yearly amounts of nitrogen provided by these symbionts can be as high as 100 kg N ha$^{-1}$ or more, which is similar to the highest anthropogenic airborne N fluxes reported. As a result, these plant species have a strong competitive advantage over other species under N limitation, especially under riparian conditions with coupled nitrification (during dry episodes) and denitrification (of generated nitrate, during wet episodes). Note that N limitation will also occur when P levels are high and P is no longer limiting biomass production, which explains the dominance of the *Azolla–Anabaena* complex and free-living N-fixing aquatic cyanobacteria under high P loads (Lamers et al., 2002b). In addition to the above-mentioned “usual suspects,” a large phylogenetic variety of...
free-living and symbiotic microorganisms including other Proteobacteria, Archaea, and Verrucomicrobia is now known to be able to fix atmospheric dinitrogen gas (Kneip et al., 2007; Khadem et al., 2010; Reed et al., 2011).

Although less frequently, low availability of S may also hamper plant growth (Dijkshoorn and van Wijk, 1967; Marschner, 1995). Like N, S is a vital constituent for protein structuring. While N and S are macronutrients, Fe is considered a micronutrient (average N:S:Fe in plant shoots is 500:15:1 on a molar basis; Marschner, 1995). The availability of Fe is essential for photosynthesis, as it is a cofactor for chlorophyll synthesis. During anaerobic soil conditions in wetlands, Fe is mainly present as Fe$^{3+}$ as a result of microbial Fe reduction, and its mobility and availability are relatively high. This may even lead to Fe toxicity (see below). During aerobic conditions, Fe is oxidized to Fe$^{3+}$ and a large part precipitates as hydroxides and oxyhydroxides, and Fe acquisition becomes hampered. To acquire Fe from Fe(III) compounds, plants have developed different mechanisms through evolution that facilitate uptake (Jones, 1998; Dakora and Phillips, 2002; Hell and Stephan, 2003). Graminaceous plants exude metal chelating amino acids called phytosiderophores, which are also important for the uptake of other micronutrient metals such as Mn, zinc (Zn), and copper (Cu). Non-graminaceous monocots, dicots, and bacteria release protons in the rhizosphere, or exude reducing and chelating compounds such as phenolics (Marschner, 1995; Schmidt, 1999). Soil Fe is, however, also strongly linked to phosphorus (P) availability, as both elements form a variety of chemical complexes, making P less available. Therefore, mechanisms to increase Fe availability may also be regarded as P mobilizing mechanisms (Bolan, 1991), next to the infection with mycorrhizal fungi.

Plants and microbes may compete for N, S, and P, depending on their relative availabilities (Hobbie, 1992), and competition models should therefore include this competition, yet few studies have. Especially at low nutrient levels, microbial uptake may significantly hamper nutrient uptake by plants, and hence reduce growth rates. As a result, they may even be able to increase the competitive advantage of slow-growing plant species and influence biodiversity. Not only competition at short-term, but also release rates from the microbial pool should be accounted for. As an example, selective root placement may be much slower than microbial colonization of a nutrient-rich patch, but subsequent release of N and P from the microbial pool can be effectively intercepted by these roots (Hodge et al., 2000).

**BELOW-GROUND CARBON TURNOVER**

The die-off of plant rhizomes, roots and above-ground litter, and their shredding by macro-, meio-, and microfauna strongly determines the availability of organic substrates for fermentation and the availability of organic electron donors (e.g., lactate, acetate, succinate, propionate) for a variety of microbial anaerobic and aerobic redox processes. The decomposability of dead plant material greatly varies between species, not only due to varying N and P concentrations and pH, but also as a result of varying concentrations of recalcitrant compounds such as lignin, and of potentially toxic secondary metabolites including other phenolic compounds and alkaloids. Consequently, plant species composition of a vegetation type has a great impact on decomposition rates and nutrient cycling (Hobbie, 1992). Decomposition rates of graminoid species such as grasses (Gramineae) and sedges and sedges of Cyperaceae are, for instance, much faster than those of peat mosses (Sphagnum), which contain high concentrations of polyphenolics and pectin-like polysaccharides (Verhoeven and Toth, 1995; Hajek et al., 2011). Even though net primary production of Sphagnum can, for instance, not compete with the highest production rates of vascular plants, low decomposition rates may still lead to fast peat accumulation. Long term accumulation rates may, however, differ from present-day rates depending on changing balances between production and decay rates (Clymo et al., 1998; Wieder et al., 2009). Next to species effects on dead organic matter (OM), microbial breakdown can also vary within one particular species. High atmospheric deposition rates of N may, for instance, decrease the concentration of polyphenols in Sphagnum peat and concomitantly increase decomposition rates, with consequences for net C sequestration (Bragazza and Freeman, 2007). The actual decomposition rates are regulated by the interaction between the nutrient (N, P) concentrations in the OM, recalcitrant or toxic compounds, and soil conditions. Peat accumulation (i.e., sequestration of atmospheric C) depends on the precarious balance between production and decomposition, and high production rates are generally coupled to higher decomposition rates as a result of organic matter quality.

Phenolic compounds have been shown to block the decomposition in peatlands to a large extent by inhibiting the activity of various crucial microbial enzymes in carbon transformation (Freeman et al., 2001). Oxygen intrusion during low water tables activates phenol oxidase activity, lowering phenol concentrations, and thereby stimulating overall decomposition. In addition, oxygen acts as the most favorable electron acceptor, stimulating decomposition rates by a factor of two or more. ROL from roots can be expected to have the same impact. A spectacular example is the large-scale rhizosphere oxidation by the vascular peatland plant species Astelia pumila and Donatia fascicularis, that may appear miniscule in stature above-ground, but possess extensive root systems that strongly stimulate decomposition rates to a depth of more than 1 m (Fritz et al., 2011). As a result of low nutrient levels in the pristine Patagonian peatlands where these species occur, carbon is preferentially allocated to root production. The strong soil oxygenation also nullifies methane emission by the inhibition of methanogenic communities and/or the stimulation of methanotrophic communities. In oligotrophic lakes, small-leaved (so-called isoeetid) species with relatively large root systems have a similar effect on decomposition rates (Smolders et al., 2002). The growth rates of facultatively aerobic, heterotrophic bacteria including Pseudomonas chlororaphis are significantly enhanced under flooded conditions by ROL of emergent macrophytes such as Glyceria maxima and Oryza sativa (Bodier et al., 1997, 1998), demonstrating the strong effect root systems may have on microbial composition and processes.

**MICROBIAL SOIL ACIDIFICATION AFFECTS PLANTS**

The cycling of N, Fe, and S in the soil is not only determined to a large extent by microbial activity, but also by uptake, release, and transformations of species of these elements due to root activity.
Plants can change the pH of the rhizosphere by the exudation of protons, organic acids, HCO$_3^-$, and OH$^-$ (Dakora and Phillips, 2002). By the excretion of root exudates, acids, and oxygen, plants control microbial resource availability, concentrations of oxygen as a potential toxin and electron acceptor, and pH in the rhizosphere. As a result of ROL or temporary water table draw-down (e.g., in riparian wetlands), pH can be strongly lowered due to microbial and chemical oxidation of N, S, Fe, generating acid (oxygen literally means acid generator). This may even lower the availability and uptake of redox-sensitive metals (e.g., Fe, Mn) or increase the availability and uptake of pH-sensitive metals that are not redox-sensitive (e.g., aluminum, Al). The pH change by the oxidation of ammonium and nitrite by nitrifying proteobacteria (e.g., the genera Nitrosomonas, Nitrospira, Nitrobacter, Nitrospina) or crenarchaeae, reduced S including sulfides to S and SO$_4^{2-}$ by S oxidizing proteobacteria (e.g., Beggiatoa, Thiobacillus), and ferrous iron to ferric iron (e.g., Gallionella ferruginea) not only influences microbial communities, but also plant communities. Most plant species are sensitive to low soil pH values (pH <5), not only by direct effects on membrane physiology, but also indirectly, by Al toxicity and by the impairment of the uptake of potassium (K), calcium (Ca), and magnesium (Mg; Marschner, 1995). Although Al is one of the most common metals in soils, its toxicity is completely dependent on its mobility, which is strongly regulated by pH. The pH in the rhizosphere may be much lower than that of the bulk soil. The regulation of pH is a combined effect of ROL activity, rhizospheric oxygen consumption, proton loss by roots (e.g., for NH$_4^+$ uptake), availability of electron donors like N, Fe, and S, and the acid neutralizing capacity (ANC) of the soil. Depending on the pH range (from 8 down to 3), ANC is provided by bicarbonate and carbonate, exchange of base cations such as Ca and Mg at soil cation exchange sites, and the dissolution of Fe and Al-oxides and hydroxides and silicates. Where bulk soil will be buffered under anaerobic conditions by the microbial reduction of N, manganese (Mn), Fe, and S (all generating alkalinity, i.e., consuming H$^+$), local oxidation by ROL will reverse this process. As such, high ROL activity can mimic the lowering of the water table in wetlands at a very local, but crucial scale. Under flooded or waterlogged conditions, most bulk soils are buffered around pH 6–6.5 by the CO$_2$ - HCO$_3^-$ buffering mechanism. As microbial decomposition (generating inorganic carbon) is generally coupled to microbial mineralization of P and N, microbially derived HCO$_3^-$ concentrations can even be used as a proxy for nutrient availability in freshwater and marine soils (Van der Heide et al., 2010).

**NITROGEN: A MOBILE NUTRIENT THAT MAY BECOME TOXIC**

Either the availability of electron donors or the availability of electron acceptors determines the nature and rates of microbial redox reactions in wetland soils. In the rhizosphere of plants showing high rates of ROL, oxidation of N, S, and Fe takes place (Bodelier, 2003). This implies that there will be a strong gradient in microbial communities at the micro-scale, from close to the roots toward the bulk soil. Ammonium (NH$_4^+$) produced in the bulk soil is oxidized to nitrate/nitrite (NO$_3^-$/NO$_2^-$) by N oxidizing communities, including Crenarchaeota (Leininger et al., 2006; Jetten, 2008). Such local redox processes create a variety of N species, which have effect on plant performance. Although plants can use oxidized inorganic N (NO$_3^-$), reduced inorganic N (NH$_3^+$), and organic N (amino acids) as N source, plants adapted to soils with low pH and low redox potential prefer NH$_4^+$ (Marschner, 1995). For plants adapted to soils with higher pH, it has been shown that NH$_4^+$ can be toxic to a variety of plant species, mainly by leading to nutrient imbalances (K, Mg deficiency) or internal acidification in roots. Aquatic macrophytes prefer either NH$_4^+$ or NO$_3^-$, depending on the pH of the surface water where the species mainly occurs (Roelofs et al., 1984). Most rooting wetland plants are evolutionary adapted to NH$_4^+$ uptake, although NH$_4^+$ may also be taken up after rhizospheric oxidation to NO$_3^-$.

In riparian wetlands, however, are known to face problems at a high NH$_4^+$: NO$_3^-$ ratio in acidified soil during lower water tables in the growing season (Lucassen et al., 2003; Van den Berg et al., 2005). Increased anthropogenic NH$_3$ inputs, as a result of ammonia (NH$_3$) emissions in regions with intensive cattle breeding, have for this reason led to the decrease of sensitive species and loss of biodiversity (Stevens et al., 2011). In riparian wetlands with a low soil ANC, acidification due to high N and S inputs increases the NH$_4^+$: NO$_3^-$ ratio due to a decrease of the activity of N oxidizers. In soils that have been acidic for longer time, nitrification rates may be higher due to the development of acid-resistant N reducers (De Boer and Kowalchu, 2001). In soils with a high pH (>8.5), a significant proportion of NH$_4^+$ (ammonium) is present as ammonia (NH$_3$; pk$_\text{a}$ = 9.25), which is highly toxic to plant roots (Schenk and Wehrmann, 1979). Similarly, high pH values of surface water, e.g., as a result of aquatic photosynthesis (pH >10), can lead to NH$_3$ toxicity to submerged vegetation and fauna in N loaded aquatic systems (Thurston et al., 1981). The actual toxicity of N depends on its accumulation in plant tissues and becomes higher under P-limited conditions due to extra accumulation. By the production of N-rich free amino acids, plants are able to detoxify excess internal N, but only to a certain level (Tomassen et al., 2003).

It has been shown that increased availability of NO$_3^-$ /NO$_2^-$ by increased nitrification rates in the rhizosphere may raise denitrification in the anaerobic bulk soil the root zone with sufficient oxygen supply (Reddy et al., 1989; Arth et al., 1998). In addition, higher rates of anaerobic ammonium oxidation (anammox) or dissimilatory nitrate reduction to ammonium (DNRA) can be expected to increase at the oxic/anoxic interface (Jetten et al., 1998; Burgin and Hamilton, 2007; Hu et al., 2011). The uptake of NH$_4^+$ or NO$_3^-$ by plant roots may, however, lower denitrification rates in freshwater and marine wetlands by reducing the N pool (Bodelier et al., 1996; Welsh et al., 2000). For vegetation, the rates of these N conversions are important, as most of the gaseous reaction products (N$_2$ and N$_2$O) leave the soil compartment and are no longer available (except through microbial N$_2$ refixation). N$_2$ production by N reduction is strongly reduced by lowering of pH from 7 to 4, but the (net) N$_2$:O$_2$ production ratio shows an exponential increase in this transect as a result of the inhibition of N$_2$O reduction at low pH (Van den Heuvel et al., 2011). The heterogeneity with respect to N$_2$O emission rates seems to be explained by differences in soil structure affecting gas transport rather than by microbial composition (Van den Heuvel et al., 2009), and probably also by vegetation composition. As such, N biogeochemistry is far more complex and variable than that of P, which is only released to the atmosphere in very small quantities as phosphate (Wetzel, 2001).
Although Fe is an essential micronutrient, high levels of Fe may cause deficiency in vegetation (Smolders and Roelofs, 1993), but also microbial Fe reduction for thermodynamical reasons (hence N limitation) through the community level (competition between species under N limitation) to the ecosystem level (N exchange with the surroundings, including the atmosphere). In addition, there is high variability at the temporal scale, mostly coupled to seasonal change and water table fluctuations (Neubauer et al., 2005).

High inputs of NO$_3^-$ in wetland soils are known to inhibit microbial Fe reduction for thermodynamical reasons (hence N reduces outcompete Fe reducers). This may not only lead to Fe deficiency in vegetation (Smolders and Roelofs, 1993), but also to reduced mobilization and availability of P in the rhizosphere (Lucassen et al., 2004). A significant part of soil P is sequestered by ferric (Fe$^{3+}$) iron phosphate compounds and is preferentially taken up by plants. The reduction of ferric (Fe$^{3+}$) iron phosphate compounds (Richardson, 1985; Scheffer and Schachtschabel, 2002). For the vegetable involved, increased N inputs may have contrasting effects, depending on the actual nutrient limiting the rate of biomass production. For N-limited systems, biomass production will increase and plant biodiversity will generally decrease by out-competition of slow-growing species. For P-limited systems, productivity may become lower and biodiversity become increased as a result of lower Fe reduction rates, leading to lower P availability. The latter is the reason why P-loaded lakes have even been treated with NO$_3^-$ in order to change the eutrophic state, dominated by algae, to a less eutrophic state in which underwater vegetation receives more light (Ripl, 2010).

**TOXICITY OF S AND Fe, AND THEIR INTERACTIONS WITH P AVAILABILITY**

Fe reduction by bacteria, including sulfate reducing bacteria (e.g., *Geobacter sulfurreducens*), and archaea converts ferric compounds into ferrous iron (Lovley and Phillips, 1988; Coleman et al., 1993; Lovley et al., 2004; Weiss et al., 2004), which is much more mobile and is preferentially taken up by plants. The reduction of ferric iron, produced as a result of ROL, may suppress other anaerobic processes including methanogenesis (Frenzel et al., 1999). Although Fe is an essential micronutrient, high levels of Fe may become phytotoxic. This toxicity may even become visible above-ground as necrotic spots on the leaves of wetland plants (Lucassen et al., 2000). The oxidation of Fe by ROL may detoxify Fe due to the microbial or chemical precipitation of Fe hydroxides in the soil (Armstrong, 1978; Bienfait, 1989). However, excess Fe in combination with relatively low ROL can generate problems for plants due to Fe plaque formation on the root surface or by Fe deposition in the root apoplast (Taylor et al., 1984; Smolders and Roelofs, 1996; Emerson et al., 1999). These incrustations include Fe oxides (e.g., ferrihydrite), Fe oxyhydroxides (e.g., goethite), Fe carbonates (e.g., siderite), and Fe phosphates (e.g., vivianite), microbially derived organic-metal complexes, bacterial biofilms, and co-deposition of other metals such as manganese, copper, zinc, and lead (Batty et al., 2006; Hansel et al., 2001). Root physiology can be negatively affected by this plaque that acts as a physical barrier and interferes with P metabolism (Snowden and Wheeler, 1995). An interesting aspect of Fe hydroxide precipitation on microbes is the fact that they act as nanowires shuttling electrons in microbial communities (Lovley, 2006; Rabaey et al., 2007), and also electrically connect them to plant roots.

The activity of sulfate reducing prokaryotes (SRP) is also of high relevance to vegetation development. Dissimilatory sulfate reducers include both Bacteria (e.g., *Desulfovibrio*, *Desulfobacter*) and Archaea (e.g., *Archaeoglobus*) producing sulfide (H$_2$S). Whereas oxidized states of S (SO$_4^{2-}$, S) are hardly toxic to plants, H$_2$S is highly toxic by inhibiting the activity of cytochrome c oxidase in mitochondria, leading to a subsequent blocking of energy production in roots, and by inhibiting other metal containing enzymes (Koch et al., 1990; Raven and Scrimgeour, 1997). The rhizospheric oxidation of S (Figure 3) by microbial activity of S oxidizers including *Beggiaota* detoxifies H$_2$S, and ROL activity of plants will therefore moderate the sensitivity of plants to sulfide. All three pH-dependent species of sulfide (H$_2$S, HS$^-$, and S$^{2-}$, pK$_1$ = 7.2; pK$_2$ = 13.7) seem to be equally toxic to plants, but for most anoxic soils the gaseous H$_2$S will be prevailing due to HCO$_3^-$ - CO$_2$ buffering around pH 6–6.5. For marine wetlands receiving sufficient organic matter to keep electron donor concentrations high, H$_2$S production affects the growth of marine and brackish water macrophytes in the millimolar range. The phytotoxin is known to hamper the establishment and growth of the two main mangrove genera *Avicennia* and *Rhizophora* (McKee et al., 1988) and of seagrasses including *Thalassia testudinum* and *Zostera marina* (Frederiksen et al., 2006; Koch et al., 2007; Holmer et al., 2009). Fluctuations in ROL of seagrass plants lead to low H$_2$S concentrations during daytime and increased concentrations during nighttime. At the end of the growing season decreasing ROL, higher microbial O$_2$ consumption and H$_2$S toxicity in concert induce the die-off of vegetation in brackish wetlands (Azzoni et al., 2001).

Although S concentrations are generally lower in freshwater wetlands, high input rates due to volcanic activity or other geological conditions, and as a result of S pollution of the atmosphere, groundwater, and surface water have raised SO$_4^{2-}$ concentrations at a global scale. In anaerobic freshwater wetland soils, SO$_4^{2-}$ reduction may therefore become a dominant process next to Fe reduction (Lamers et al., 1998), while its rate can be determined by the availability of either electron donors or SO$_4^{2-}$ (Lamers et al., 2002a). A recent review showed that up to 50% of the anaerobic decomposition in freshwater wetlands can be attributed to SO$_4^{2-}$ reduction (Pester et al., 2012). Sulfide concentrations in the range of 10–100 µmol L$^{-1}$ are known to be toxic to the roots of freshwater plants (Tanaka et al., 1968; Armstrong et al., 1996; Smolders and Roelofs, 1996; Lamers et al., 1998; Geurts et al., 2009). Like for marine plants, H$_2$S toxicity to roots can be prevented by the activity of sulfur bacteria using O$_2$ from the roots (Joshi and Hollis, 1977). In addition, volatile S compounds including H$_2$S and, after its methylation, dimethylsulfide, and methanethiol are released to the atmosphere (Lomans et al., 2002). As the thermodynamical characteristics of Fe reduction and S reduction do not differ very much, these conversions take place in the same soils, and Fe$^{3+}$/precipitation occurs (Lamers et al., 2002a). By this sequestration the concentration of both potential phytotoxins is reduced (Van der Welle et al., 2006), and plants may even become Fe deficient (Smolders and Roelofs, 1993).

Already in the 1950s of the former century, the prominent role of S in aquatic nutrient biogeochemistry was described (Ohle,
FIGURE 3 | Radial oxygen loss (ROL) of Juncus acutiflorus is shown by the oxidation of added H₂S in an anaerobic rhizotron with living plants and natural organic wetland soil. This was confirmed by the use of oxygen microelectrodes. Yellow-whitish haloes of S₀ and S precipitates have been formed by microbial S oxidation (Lamers and Janssen, unpublished results).

1954). As SO₄²⁻ will serve as a terminal electron acceptor, it will speed up overall anaerobic decomposition. Increased inputs of SO₄²⁻ may additionally mobilize Fe bound P in the soil, due to its reduction to H₂S competing for Fe binding sites (Sperber, 1958; Caraco et al., 1989; Roden and Edmonds, 1997; Lamers et al., 1998; Smolders et al., 2006) or mobilization of P from polyphosphates by sulfur bacteria (Golterman, 2001). Whether or not sulfate-induced eutrophication occurs, depends on the levels of P in the sediment and its type of binding (Lamers et al., 2002a; Geurts et al., 2008; Loeb et al., 2008).

The large spatial and temporal heterogeneity in O₂ supply (as determined by the balance between local ROL and local oxygen consumption) enables the coupling of oxidation and reduction of the same element (S, Fe, N) at a small-scale in the rhizosphere. In this way, consortia of microorganisms using the same element can coexist in wetland soils, but the directions of the transformations are governed by plant physiology and soil characteristics.

In addition to the use of organic electron donors, the reduction of one of the three elements can be coupled to the oxidation of the other (chemolithotrophy). Sulfide, both dissolved and as FeSₓ deposits in the soil can be used as an electron donor by proteobacteria such as *Thiobacillus denitrificans* coupled to the use of NO₃⁻ (Haaijer et al., 2006, 2007; Burgin and Hamilton, 2008). Even in peat soils rich in organic compounds, NO₃⁻ pulses are still able to stimulate to chemolithotrophic denitrification while organo-trophic denitrification rates were very high (Lamers, unpublished results). For plants, this means that the detoxification of sulfide extends beyond the direct influence of ROL, and that S-rich situations may show lower N-availability. At the landscape scale, N pollution of the catchment will mobilize S from subsoil FeSₓ deposits, and the resulting S pollution of groundwater will lead to P pollution in wetlands where this polluted water is discharged. In this way, there is a microbial "S bridge" between N and P at this hydrological scale (Smolders et al., 2010). In wetland soils, partial H₂S oxidation to S₀ and pyrite (FeS₂) can also be coupled to Fe reduction (Thamdrup et al., 1994), with consequences for the availability and toxicity of both elements to plants, and for the availability of P.

GLOBAL CHANGES OF N, S, AND Fe BIOGEOCHEMISTRY

As a result of anthropogenic forcing, the input of all three elements, N, S, and P, into wetlands has strongly changed at a global scale (Schlesinger, 1997; Vitousek et al., 1997). As a result of urbanization, industrialization, and intensive farming, surface waters of many wetlands have become polluted by N and P (Carpenter et al., 1998). In contrast to P, increased N inputs (NOₓ and NHₓ) are also the result of atmospheric deposition, which has strongly increased in industrialized regions and in regions with intensive farming (Vitousek et al., 1997; Bobbink et al., 1998). Whereas S deposition rates in Europe have been reduced by 80–90% as a result of legislation, they are strongly increasing in other parts of the world. In fast-developing regions such as Asia (particularly China), SO₂ emissions may soon equal the combined emissions of North America and Europe as a result of the large-scale use of coal as a fuel (Shah et al., 2000). In addition to N and S pollution, many wetlands have been drained for land use change (Lamers et al., in press), which has not only resulted in the loss of wetlands, but also
in significant changes in the hydrochemistry of wetlands that have not suffered from complete dehydration. Lowering of groundwater tables, also due to the extraction of drinking water and industrial process water, has decreased the influence of groundwater in wetlands to a large extent. As a result, the discharge of anaerobic, Fe-rich groundwater has decreased. This means that

**FIGURE 4** Microbe–plant interactions in the biogeochemistry of N, S, and Fe in wetland soils. Losses of volatile N and S compounds to the atmosphere also occur through plant tissues, which has not been depicted here. See text for further explanation. DNRA, dissimilatory nitrate reduction to ammonium; SR, sulfate reduction; FeR, iron reduction; NOX, N oxidation to NOX, SOX, S oxidation; FeOX, Fe oxidation; DN, denitrification; NFix, N fixation; Anammox, anaerobic ammonium oxidation; ROL, radial oxygen loss (by roots).

**FIGURE 5** Four filters created by microbial activities related to N, S, and Fe biogeochemistry influence the occurrence and functioning of wetland plant species, and their interspecific competition. A positive influence on the parameter mentioned on the filter frame is denoted by +, a negative by −.
the role of Fe in regulating vegetation composition, P availability, and sulfide detoxification in wetlands has been changed (Lamers et al., in press). In combination with increased inputs of N, S, and P, large-scale changes of wetland communities (microbial communities and their vegetation) have resulted, and ecosystem services such as C sequestration, flood protection, water storage, and biodiversity have been altered. The resilience of ecosystems to global change depends to a large extent on the responses microbial communities and their positive and negative feedbacks with respect to these changes. For this reason, changes in microbial composition and activity should be incorporated in ecosystem models (Treseder et al., 2011).

**GENERAL CONCLUSION**

The biogeochemical cycling of N, S, and Fe shows strong interactions in microbe–plant communities at various levels, from rhizosphere to landscape (Figure 4). The composition and activity of microbial communities dictate vegetation composition to a large extent by their strong effects on four important drivers (below-ground environmental filters) of the growth, composition, and biodiversity of wetland vegetation, namely root oxygen availability, phytotoxicity, pH, and nutrient availability (Figure 5). Microbe–plant interactions provide challenging and complex new topics for highly interesting ecological research carried out by a consortium of specialists. In addition to correlative research, experimental research in the field and lab is essential to determine causal relationships. The fast development of new and much cheaper molecular techniques opens up a whole new world of rhizosphere research, in which basic and applied ecological issues on plant–microbe communities (Table 1) can be addressed that were impossible or much more difficult in the recent past. In addition, new students should be challenged and trained to address ecological topics in this multi-disciplinary way. Especially on a rapidly changing globe, it is vital that both microbiologists and plant ecologists meet in the hidden half of plant communities to better understand ecosystem changes at multiple levels.

### Table 1 | Examples of fundamental and applied ecological challenges regarding plant–microbe communities in wetlands (and other ecosystem types).

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<tr>
<th>LANDSCAPE/ECOSYSTEM</th>
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<tr>
<td>Interrelations among geohydrology, microbial communities, and vegetation development</td>
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<td>Biogeochemical controls of carbon dynamics (primary production–decomposition feedbacks)</td>
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<td>Impacts of anthropogenic hydrological changes (e.g., change from discharge of Fe-rich groundwater to recharge) on the interactions between microbial communities and vegetation</td>
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<td>Pollution effects (e.g., N and S pollution groundwater, P pollution surface water, increased airborne N and S) on plant–microbe interactions</td>
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<td>Climate change (e.g., changes in precipitation and temperature patterns), effects on landscape biogeochemistry</td>
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<td>Microbial ecology in conservation biology</td>
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<td>Microbial ecology in restoration ecology</td>
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<th>ECOSYSTEM</th>
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<tr>
<td>Role of microorganisms in vegetation succession and development</td>
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<tr>
<td>Role of vegetation in the succession and dynamics of microbial communities</td>
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<tr>
<td>Role of soil heterogeneity (e.g., nutrients, organic substrates, toxins, temperature, electron acceptors) in the functioning of plant–microbe communities, including the competition among microorganisms</td>
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<tr>
<td>Biodiversity in plant–microbe communities: niche differentiation, disturbance, fluctuation, neutral theory</td>
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<td>Biodiversity of plant–microbe communities and ecosystem functioning and resilience</td>
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<td>Microbial consortia in three-dimensional rhizosphere gradients</td>
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<td>Rhizosphere community dynamics (e.g., diurnal, seasonal)</td>
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<tr>
<td>Plant host specificity of microbial communities and symbionts (e.g., comparison of species, same species under different environmental conditions)</td>
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<td>Competition among plants based on their microbial communities (e.g., rhizosphere community as a plant trait)</td>
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<td>Competition among microbes as a result of plant community changes</td>
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<td>Competition for nutrients between microbes and plants, community effects</td>
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<tr>
<td>Dispersal of microbes and plants</td>
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<tr>
<td>Climate change effects (temperature, rainfall, CO₂) on plant–microbe interactions</td>
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<td>Optimization of water purification in constructed wetlands based on the manipulation of soil–plant–microbe interactions</td>
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<th>ECOPHYSIOLOGY</th>
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<tr>
<td>Microbial controls on nutrient and water uptake by plants (including costs and benefits of mycorrhiza)</td>
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<td>Gas (e.g., CO₂, CH₄, N₂, N₂O) exchange in the plant–microbe continuum; effects of methane oxidizing symbionts on photosynthesis (e.g., Sphagnum mosses)</td>
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<td>Selective root placement and microbial communities</td>
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<td>Dynamics in anaerobiosis and microbe–plant interactions</td>
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<td>Microbial manipulation of phytotoxic compounds (including agricultural crops, e.g., H₂S oxidation by symbiotic S oxidizers)</td>
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<td>Generation of electricity in constructed wetlands</td>
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*In addition to correlative research, experimental research in the field and lab is essential to determine causal relationships. The fast development of powerful and less expensive molecular techniques will strongly facilitate comparative and experimental community studies.*
REFERENCES


Lamers et al.


