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Abstract. Consequences of sulfide toxicity on succession in wet calcareous dune slacks were investigated. Sulfide may exert an inhibitory effect on dune slack plants, but several pioneer species exhibit ROL (Radial Oxygen Loss) and thereby protect themselves against free sulfide. Under oxic conditions free sulfide will be oxiginated to harmless sulfate. However, successive species when not capable of ROL may be sensitive to free sulfide and cannot invade the area. Therefore, the occurrence of free sulfide may have a stabilizing effect on the pioneer vegetation.

Data on the vertical distribution of oxygen, redox and sulfide were collected in mesocosms with *Littorella uniflora* or *Carex nigra*, with and without microbial mats and compared to control mesocosms. Also, *in situ* data were collected in a dune slack on the Frisian Island of Texel.

In the mesocosms, free sulfide was detected only at night-time in *C. nigra* populated mesocosms and in unvegetated units, but not in *L. uniflora* vegetated mesocosms. In the field, sulfide and redox profiles showed distinct differences between the groundwater exfiltration and infiltration site of the dune slack. At the exfiltration site, sulfide was only occasionally found; in contrast, measurable amounts of free sulfide were regularly found at the infiltration site of the slack.

Since *Phragmites australis* dominates in the infiltration site of the slack, the results suggest that free sulfide accelerate the succession, rather than slowing it down by the exclusion of some plant species.

Introduction

Vegetation succession in wet calcareous dune slacks seldom is gradual. The vegetation development is rather discontinuous. This is most clearly demonstrated by the rapid shift from a low-productive, apparently stable stage with many endangered plant species to a more productive stage in which only a few common plant species dominate the vegetation (Lammerts et al. 1995). This is indicative of the presence of one or more positive feedback mechanisms (Wilson & Agnew 1992). Through these mechanisms a gradual succession, in which the vegetation slowly but constantly changes in time, turns into a successional shift, in which the early successional stage converts rapidly in later more productive stages, after a period in which the vegetation apparently did not change, which may last for decades..

Since groundwater of dune slacks has a relatively high sulfate content, up to 1.8 mM (Stuyfzand 1993), it is anticipated that sulfide, originating from sulfate reduction, can exert an inhibitory effect on vegetation development in dune slack ecosystems. The actual presence of sulfide in dune slacks was demonstrated earlier (Adema et al. 2002*)*. This paper reports on the relevance of sulfide in wet calcareous dune slack succession.

Microbial activity plays an important role in early stages of dune slack succession. Firstly, micro-organisms stabilize dune slacks by aggregation of sand (van den Ancker & Jungerius 1985; Forester & Nicolson 1981; de Winder 1990). Secondly, in particular cyanobacteria are capable of N_2 fixation (Joye & Pearl 1994; Stal 1995; Steppe et al. 1996) and therefore they increase the nutrient availability of the soil. These two activities accelerate dune slack succession rather than slow it down. However we also can hypothesize a positive feedback mechanism that arrest the succession.

Microbial mats often cover the soil surface in open pioneer vegetation. The combined metabolic activities of different functional groups of microbes result in steep environmental micro-gradients, particularly of oxygen

and sulfide (van Gemerden 1993). Due to the occurrence of sulfide only some characteristic dune slack pioneer species that can protect themselves against the toxic sulfide by releasing oxygen from their root system can survive. This results in open stable pioneer vegetation with a microbial mat that cannot be invaded by later species not adapted to anoxic soils containing free sulfide.

The microbial community consists not only of the oxygenic phototrophic cyanobacteria. At the oxic-anoxic interface, colourless sulfur bacteria, which oxidize sulfide, may proliferate. In deeper layers, devoid of oxygen, fermenting and denitrifying heterotrophic bacteria, in addition to sulfate-reducing bacteria, are of importance. Sulfate-reducing bacteria, colourless (and purple) sulfur bacteria all are involved in the production of sulfide (van Gemerden 1993), which is a highly toxic compound for many plants (Havill et al. 1985; Fürtig et al. 1996; Armstrong et al. 1996; Grootjans et al. 1997; Lamers et al. 1998).

Early, low-productive, states in dune slack succession contain many plant species that are adapted to root in anoxic soils (Schat 1984); typical species are *Littorella uniflora, Schoenus nigricans* and *Samolus valerandi*. These plants oxidize the rhizosphere by leaking oxygen from their roots, a phenomenon known as radial oxygen loss (ROL) (Armstrong 1975). The released oxygen enables micro-organisms, such as colourless sulfur bacteria, to oxidize the sulfide in the root zone and, consequently, free sulfide cannot harm these plants. Such a mechanism is not known for most of the common species in the more productive states, for example *Carex nigra* and *Calamagrostis epigejos*. Therefore, these species are more vulnerable with respect to sulfide toxicity. *C. epigejos* and *Juncus alpinoarticulatus* were affected by sulfide concentrations of 30-50 μM (Grootjans et al. 1997), while *Carex* species, tested in a mesotrophic wetland meadow vegetation, were even more sensitive and showed a response at sulfide concentrations less than 10 μM (Lamers et al. 1998). One of the least sensitive late successional species is *Phragmites australis,* showing toxic effects at sulfide concentrations just below 500 μM (Fürtig et al. 1996). Considering these observations, we can expect the occurrence of a positive feedback mechanism; the presence of sulfide in early, low productive, successional stages appears to be in favour of these stages because species from later, more productive, states cannot enter the area because of the presence of sulfide.

This paper focuses on the question of whether free sulfide does occur in wet calcareous dune slacks, and whether it can slow down vegetation succession if present. Special attention is given to differences in sulfide production during day and night. Plants, algae and oxygenic phototrophic cyanobacteria produce oxygen profusely during daytime. Since most sulfate-reducing bacteria are obligate anaerobic, even low oxygen concentrations may slow down the production of sulfide. As a result, one can expect higher sulfide concentrations during the night.

Material and Methods

Study area

The study site was situated in the 'Buiten Muy', a wet calcareous dune slack, considered as a so-called flow-through dune lake (Stuyfzand 1993; Stuyfzand & Moberts 1987), in which exfiltrating (upwelling) groundwater enters at the southeastern side ('exfiltration site'), while surface water leaves the slack at the northwestern side infiltrating in a dune ridge ('infiltration site') (Adema et al. 2002). The slack is situated on the Frisian island of Texel (53°07' N 5°47' E) and originates from a beach plain that was separated from the sea by a dune ridge between 1920 and 1925 (Westhoff & van Oosten 1991). The 'Buiten-Muy' forms part of the Nature reserve 'De Muy', which was established in 1908. The 'Buiten Muy' can be divided in a northeastern area that was sod-cut in 1994, a southwestern area that is yearly mown to conserve rare species such as *Anagallis tenella*, *Epipactis palustris* and *Ophioglossum vulgatum*, and an unmanaged central area in which the observations were made. Most of this area consists of tall stands of reed dominated by *P. australis* or *Carex riparia*, which both are representatives of late successional stages. A pioneer stage (*Samolo-Littorelletum*) with a well-developed microbial mat is still present in the not managed area, despite more than 70 yr of undisturbed succession.

Mesocosm experiment

Laboratory mesocosms were set up to simulate the two dune slack successional vegetation states, the low productive early state (typical representative: *Littorella uniflora*) and the high productive later state (typical representative: *Carex nigra*). Mesocosms mimicked the situation at the exfiltration site of a flow-through dune lake, i.e. a seepage zone where the groundwater flow reaches the soil surface.

A total of 48 mesocosms, glass containers with a surface area of 25 cm × 18 cm and a height of 22 cm (Fig. 1), were filled with 16 cm calcareous sand from the beach of Texel after a 4 cm layer of fine river gravel was added to the bottom of each container. Sideways light penetration through the glass was prevented by a PVC sheet. A water inlet was placed in the gravel layers to ensure equal distribution of the artificial

ground water; the outlets were placed at the soil surface level. The composition of the water was derived from groundwater of the Frisian Island of Schiermonnikoog (Stuyfzand et al. 1992), supplemented with ammonium or ammonium plus sulfate (Table 1). The oxygen-free water, stored under an overpressure of 0.1 bar nitrogen, was supplied to each mesocosm at a rate of 250 ml.day^{-1} using multi-channel peristaltic pumps (Masterflex, Cole Parmer, USA).

A thin layer of a microbial mat, harvested in the 'Buiten Muy', was spread out on 24 mesocosms. One third of these received *L. uniflora*, one third received *C. nigra* and one third was used as control, the other 24 mesocosms were set up likewise, but received an anti-algae sheet (0.03 mm perforated PE foil) instead of a microbial mat on top of the sand. Half of the replicates (four for each treatment) were supplied with water amended with ammonium and the other half with water amended with ammonium plus sulfate (Table 1). *L. uniflora* and *C. nigra* plants were planted as single shoots, six *L. uniflora* and four *C. nigra* per mesocosm. All plants originated from the Wadden Sea area and were pre-grown in a greenhouse. After the microbial mats and the plants were added, the mesocosms were left undisturbed in a climate chamber with a 12hr/12hr light/dark cycle, a fluorescent light intensity during the light period of 350 µE.m^{-2}, a temperature regime of 22 °C during light periods and 17 °C during dark periods and an air humidity of 50-60%.

After five months of growth, oxygen, sulfide and redox data were collected using stainless steel needle electrodes with a sensing tip of < 0.1 mm (Microscale Measurements, The Netherlands; van Gemerden et al. 1989) and custom-made nA- and mV-meters (Electronic Workshop, University of Groningen). Profiles were recorded during the light periods applying depth increments of 0.5 mm over the 0-10 mm depth range, 1 mm over the 10-25 mm depth range and 10 mm over the
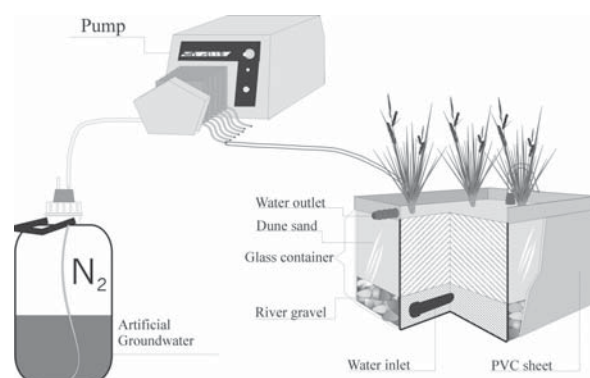
Table 1. Artificial groundwater composition as used in the mesocosm experiment. [*]marks the concentrations in the high sulfate treatment.

Cations	mM	Anions	mM
Ca^{2+}	2.63	SO_4^{2-}	1.31/3.11[*]
Na^+	3.96 /7.56[*]	Cl^-	3.98
K^+	0.10	HCO_3^-	3.00
Mg^{2+}	0.33	$H_2PO_4^-$	1.0 e-3
NH_4^+	0.60		
$Fe(II)^{2+}$	0.01	**Dissolved gas**	**mM**
Mn^{2+}	8.4 e-3		
Zn^{2+}	2.5 e-3	CO_2	1.50
Cu^{2+}	4.2 e-4		
Mo^{4+}	4.2 e-4		
B^{3+}	1.7 e-2		

Fig. 1. Experimental set-up for the mesocosm experiment in the climate room. Each mesocosm was supplied by a peristaltic pump with anoxic artificial groundwater to the bottom of the container.

50-120 mm depth range, by using a micromanipulator. Nocturnal continuous recordings were made at fixed depths of 2 mm, 20 mm and 50 mm using a multi-channel datalogger (Campbell Scientific CR10X) set to measure every 2.5 sec and store the average every minute.

Above- and below-ground biomass of the plant species were measured at the end of the experiment after nine months. At the same time numbers of sulfide-producing bacteria – being the summation of sulfate-reducing bacteria, S^0-reducing bacteria and thiosulfate- or sulfur-disproportionating bacteria – and colourless sulfur bacteria were estimated with the most probable number (MPN) technique (Visscher et al. 1992; Schaub & van Gemerden 1996; Heijs et al. 1999). From all mesocosms with low sulfate and plants a soil core of 2 cm diameter and 6 cm length was harvested. The cores were sliced in three sections, 0-1 cm, 1-3 cm and 3-6 cm depth. Calculations were performed using the MPN computer program of Clarke & Owens (1983).

Field measurements

In spring 2000, data on redox and sulfide were collected in the 'Buiten Muy' with a computer-controlled automatic device designed for the *in situ* measurements of multiple soil profiles in a 50 cm by 50 cm grid using the same type of stainless steel needle electrodes as in the experiment (Workshop, University of Groningen; Schaub & Van Gemerden 1996). After each profile the electrodes changed position in the grid to prevent repeated measurements in the same needle holes. Depth increments were 0.5 mm (0 to 30 mm depth) and 1 mm (30 to 64 mm depth). Measurements were carried out on both sides of the dune slack (sites of exfiltration and infiltration) to retrieve information about differences. Three grids at the exfiltration and three grids at the infiltration side are continuously profiled for 24 hr. In

time periods of 3 hr, the data in each profile at the same depth position is averaged ($n = 16$)

Oxygen production measurements were carried out to estimate differences in the activity of the microbial mats on both sides of the dune slack. A Clark-type oxygen electrode (Strathkelvin 1302 Microcathode oxygen electrode) was used. This electrode was placed in a measuring chamber consisting of a small plexiglass canister (16 cm height and 7 cm diameter) closed at one end and placed on a aluminium base ring of 5 cm height. A rubber ring sealed the connection. Two cm of the aluminium ring was placed in the soil to prevent leaking of oxygen through the soil. A temperature- (NTC resistor) and light- (LDR-resistor) sensor were used to correct for possible effects of temperature and light on the oxygen production. Since the Clark-type oxygen electrode measures the partial pressure of oxygen, it is very sensitive for pressure changes. To prevent pressure changes, we placed a capillary tube in the top of the canister.

Oxygen concentration was measured every 2 seconds in a 5 minute period using a datalogger (Campbell Scientific CR10 X). On each measuring place this was done five times. From these time series we calculated the oxygen production rate by the derivative of a saturation curve (Michaelis-Menten equation) for $t_{(0)}$, assuming that the rate of oxygen production did not change within the 5 minutes period.
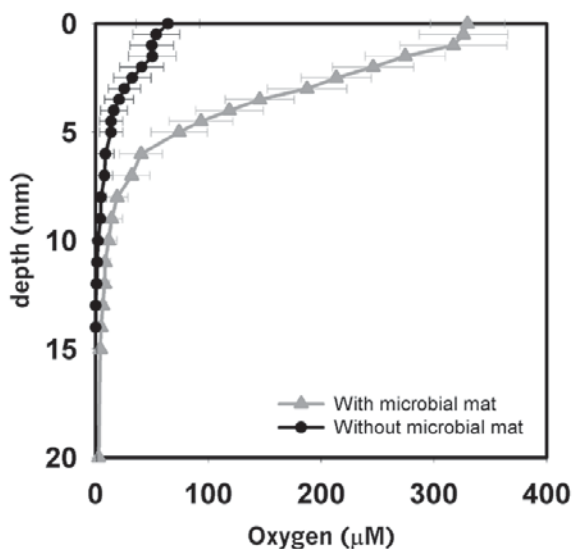

Fig. 2. Average profiles with standard error of oxygen in the top 20 mm of the soil as measured in the unvegetated mesocosms in the climate chamber experiment during the light period. Symbols mark measuring points and error bars represent the standard error ($n = 8$).

Results

Mesocosm experiment

Plant biomass of both species did not show significant differences between the treatments without (–) and with (+) a microbial mat:

L. uniflora: – mat 94 g.m^{-2} (SE 18 g.m^{-2}); + mat 114 g.m^{-2} (22 g.m^{-2}):
Carex nigra: – mat 1131 g.m^{-2} (69 g.m^{-2}); + mat 1093 g.m^{-2} (65 g.m^{-2}).
It appeared that the biomass of *Carex nigra* showed a negative correlation with the amount of sulfide found but this was not significant.

Profiles

The presence of mats resulted in supersaturated oxygen concentrations in the top layers (Fig. 2) as a result of the activities of oxygenic phototrophic organisms. During the daytime, oxygen penetrated somewhat deeper in mesocosms with microbial mats, compared to those without.

Oxygen and redox profiles recorded in the light period show clear differences between the mesocosms with *L. uniflora* and the other treatments (Fig. 3); in the former oxygen was detectable up to 80 mm depth, whereas in the other treatments oxygen became undetectable below 10 mm. This was also reflected in the redox profiles: in the mesocosms with *L. uniflora,* redox potentials were + 200 to + 300 mV and did not decrease in the 0-80 mm layer, while in the other mesocosms the values decreased from the surface downwards and were lower, albeit nowhere becoming negative. The level of sulfide in all mesocosms was below the detection limit in the light period. Effects of elevated sulfate concentration in the artificial groundwater were not observed.

Continuous measurements

The nocturnal continuous measurements of all treatments showed striking differences between light and dark periods, a typical example of oxygen, sulfide and redox fluctuations recorded in a mesocosm with *C. nigra* and a microbial mat is shown in Fig. 4. The oxygen content at the 2 mm depth horizon increased during daytime and even became saturated at the end of the light period. The same trend, though at much lower concentrations, was found at 20 mm depth, while no oxygen was detected at 50 mm depth. A steep decline in the oxygen content was observed after the light had been switched off; at the 2 mm deep horizon the oxygen content dropped from over 200 µM to about 50 µM within three hours. The same trend was observed at a depth of 20 mm.

Sulfide concentrations were below the detection limit at the 2-mm and the 50-mm depth horizons, whereas at 20 mm depth a build-up was observed during the dark periods and a rapid decrease after the light had been switched on. It appears that the plant/microbe community became more active in the course of the observation period since the build-up of sulfide increased, and the same is true for oxygen. Similar patterns were observed in the mesocosms that were treated identically: measurable amounts of sulfide were found in mesocosms with *C. nigra* (maximum during day 2: without mat 8.1 μM, sd 2.8; *n* = 3; with mat 8.9 μM; sd 6.5; *n* = 7) and in mesocosms without vegetation (maximum during day 2: without mat 8.2 μM, sd 2.7; *n* = 3; with mat 4.6 μM, sd 3.6; *n* = 6). Sulfide concentrations in mesocosms with *L. tÁ]flora* were below the detection limit regardless of the presence of a microbial mat ($n = 8$). MPN estimations, carried out towards the end of the experiment indeed showed high numbers of sulfide-producing bacteria in the top layers of mesocosms with *C. nigra* and microbial mats, while much lower numbers were found in mesocosms with *L. uniflora* and microbial mat, and even lower numbers were found in all other treatments (Fig. 5). Colourless sulfur bacteria were present in very low numbers in all treatments.

The redox potentials (Fig. 4), being strongly influenced by oxygen and sulfide, declined during the night and showed a rise during the day.

Field measurements

At the the inland side of the 'Buiten Muy' where groundwater exfiltrates, sulfide concentrations were almost all below 10 μM, except for one measurement at 6 mm depth just below the microbial mat (Fig. 6, top panel). In contrast, at the the seaward side where the surface water infiltrates the soil, free sulfide (up to 30 μM) was invariably found in the deeper layers (Fig. 6, lower panel).

Redox data collected at the exfiltration site showed marginal differences in the 0-60 mm depth layer analysed. During an 18-hr period (12 a.m. to 6 a.m. the next day) the redox potential gradually changed from + 30 mV during the day to –100 mV at the end of the night with only marginal depth differences (data not shown). No relation was found to the one-time observed somewhat higher sulfide concentration mentioned above, probably indicating that the latter was restricted to a very small area. Redox potentials at the infiltration site tended to become somewhat lower in deeper layers, presumably due to the presence of low sulfide concentrations in these layers. The fact that redox potentials at the exfiltration site invariably were lower than at the infiltration site, despite the observed distribution of sulfide at both sites, may indicate that redox couples other than sulfide were involved.

Oxygen production

The mean oxygen production rate at the inland side of the slack in the 'Buiten Muy' was 1.16 μmol.cm^{-2}.sec^{-1} (sd = 0.11; n = 2) and at the seaward side it was 0.42 μmol.cm^{-2}.sec^{-1} (sd = 0.26; n = 3). These differences are significant (P = 0.024, *T*-test). No significant correlation was found between the oxygen production rate and temperature or light intensity.
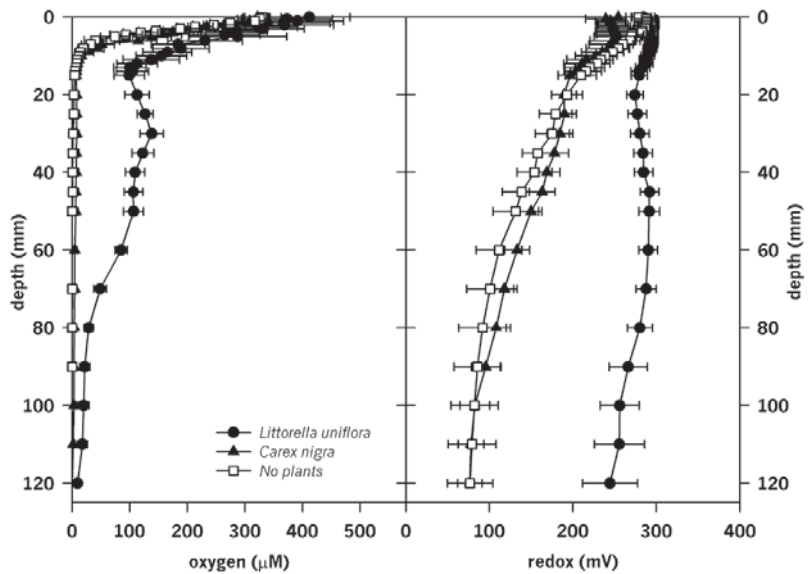

Fig. 3. Average profiles of oxygen concentrations and redox-potential for each species in the mesocosms with microbial mats as measured in the climate chamber experiment during the light period. Symbols mark measuring points and error bars represent the standard error (n = 8).
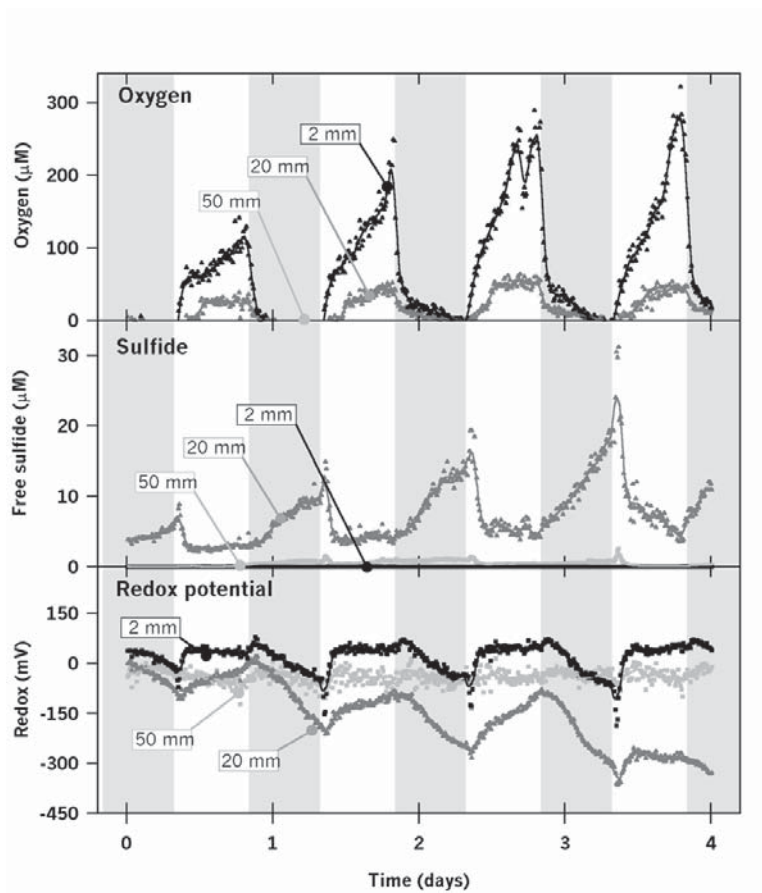
Fig. 4. Diurnal cycles in oxygen concentration, sulfide concentration and redox potential in a mesocosm with *Carex nigra* and a microbial mat. The parameters were measured at three depths: 2 mm, 20 mm and 50 mm. Light bands represent the light period, dark bands the dark period.
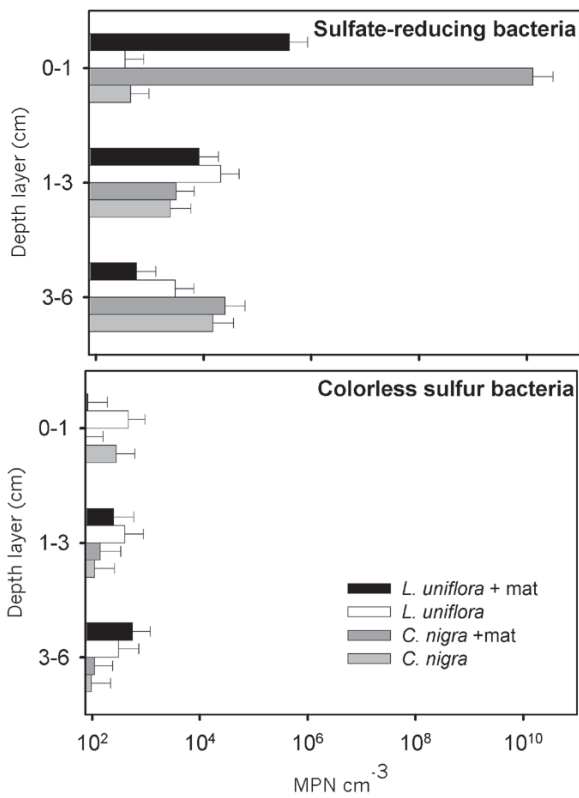

Fig. 5. Most probable number (MPN) of sulfate-reducing bacteria and colourless sulfur bacteria at three depth layers in the mesocosms with *Littorella uniflora* and *Carex nigra* both with and without a microbial mat.
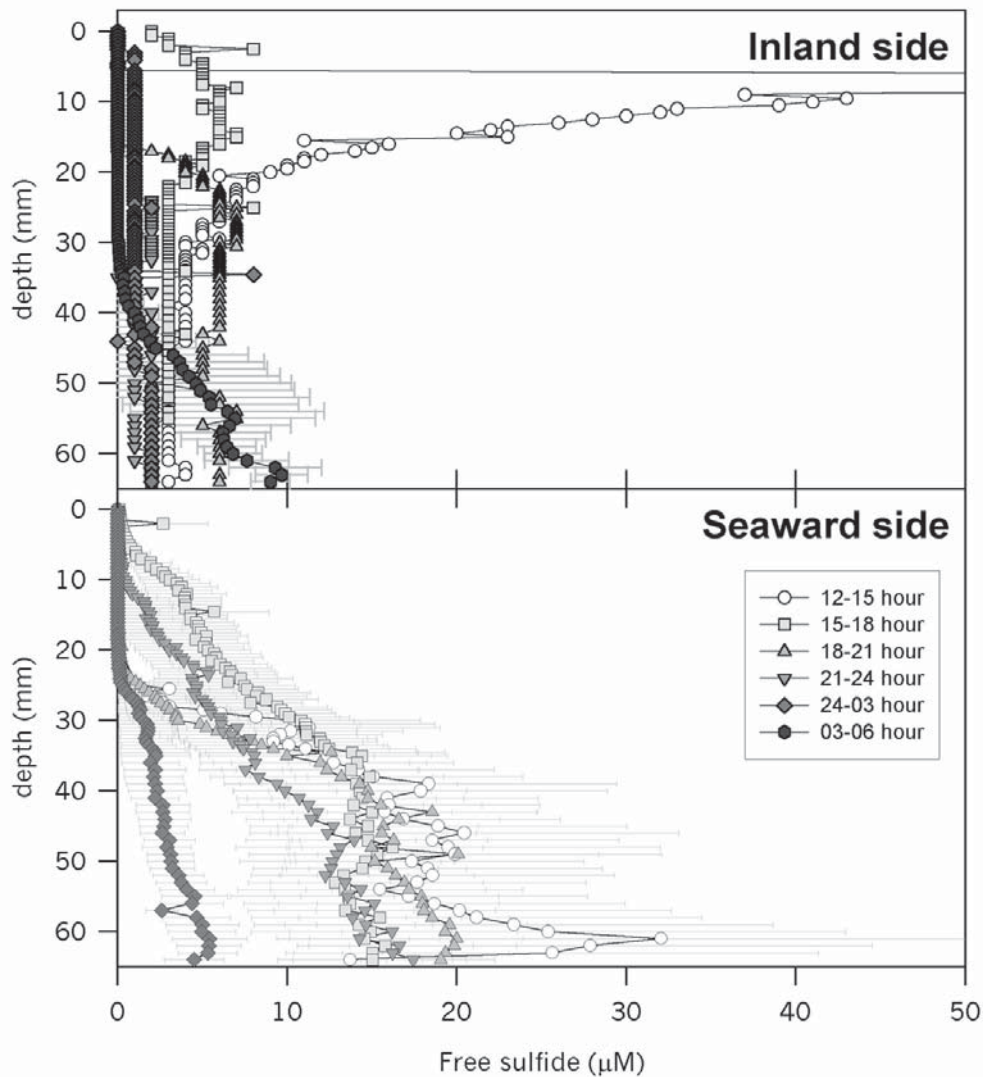
Fig. 6. Vertical sulfide profiles as measured in the 'Buiten Muy' on Texel. The top panel shows the sulfide profiles at the exfiltration site, and the bottom shows the results at the infiltration site. Depth of measuring is marked as symbols and error bars represent the standard error.

Discussion

Oxygen penetration

Needle electrodes are powerful tools for the *in situ* establishment of soil parameters; however, data collected in this way are spot measurements. Soil with rooted plants by definition is heterogeneous and care has to be exercised when interpreting individual oxygen and redox profiles since differences (observed as 'spikes' in the profile) could be due to the vicinity of roots. The depth profiles of oxygen and redox (Fig. 2) do not show such spikes because these are averages of profiles re-

corded in each of the eight replicate mesocosms.

Enhanced oxygen penetration was observed only in mesocosms with *L. uniflora*. The presence of a microbial mat, generating high concentrations of oxygen in the superficial soil layers, will have resulted in some downward transport, but it is clear that the presence of oxygen in the deeper layers is the result of ROL (radial oxygen loss). This oxygen could have oxidized sulfide. Since sulfide was not detected during day time in either the *L. uniflora* mesocosm or the mesocosms lacking plants that generate ROL, this absence cannot be attributed to sulfide oxidation. It appears that the production of sulfide by sulfate reduction during the light periods was a

process of marginal importance, and probably the same holds for the dark periods in mesocosms with *L. uniflora*. Similar results were found for submerged populations of *L. uniflora* growing in sediment with low organic content (Holmer et al. 1997). This explains why raised sulfate concentrations had no effect.

Sulfide production during the dark period

The time series taken in the mesocosms by electrodes at fixed depths of 2 mm, 20 mm, and 50 mm illustrated the dynamics of both oxygen and sulfide production and consumption. In a mesocosm with *C. nigra* and a microbial mat it was observed that oxygen concentrations at the 2 mm depth horizon increased in the early hours of the light period, indicating that oxygen was produced in, or near, that depth by oxygenic phototrophs harboured by the microbial mat since it was not observed in microcosms without microbial mat (data not shown). The electrodes, positioned at 20 mm depth, recorded less pronounced and also slower oxygen increases, suggesting that this was due to downward transport; *in situ* production appears unlikely at that depth. On average, the oxygen concentration at 20 mm during the light period was somewhat higher than observed during profiling in the mesocosms (Fig. 3, left panel). The oxygen concentration at 50 mm was below the limit of detection, which is consistent with the data shown in Fig. 3.

The rapid decline of the oxygen concentrations at 2 mm depth after the light had been switched off, followed by a slower decline after 1-2 hr, indicate that cessation of oxygen production was more important than aerobic respiration by both phototrophic and heterotrophic organisms. In the latter process, mat organisms appear to have played a dominant role since the decline was less rapid at 20 mm depth.

In the dark periods a gradual build up of sulfide was observed at the 20 mm depth horizon, which continued in the first hour of the light period, but was rapidly consumed as soon as ample oxygen became available. These observations may be taken as proof that colourless sulfur bacteria were present and active, despite the fact that MPN counts were low, at least at the end of the experiment. However, during the dark periods oxygen was short in supply both at the 2 mm and at the 20 mm depth horizon while sulfide concentrations progressively increased. This means that the roots of *C. nigra* must have been exposed to sulfide, but possibly not at toxic levels as growth of *C. nigra* showed only a slightly negative response to the occurrence of sulfide.

It cannot be deduced from the data whether the production of sulfide was restricted to the dark periods,

as the low sulfide concentration (< 5 μM) observed during the light periods may have resulted from upward diffusion from deeper layers, but the impact of oxygen on the activities of sulfate reducing bacteria is not the same for all species (Widdel 1988). The reduction of sulfate has been reported for oxidized marine sediments (Jørgensen 1977), as well as for sediments actually containing oxygen (Visscher et al. 1992).

Sulfide production and the hydrological regime

Since the pioneer vegetation is zonated towards the exfiltration site of the 'Buiten Muy', the occurrence of free sulfide would be expected at this site if sulfide toxicity act as positive feedback mechanism that slows down the succession. However the *in situ* data collected at the 'Buiten Muy' showed that sulfide concentrations, with one exception, were below the detection limit at the exfiltration site. Although higher, or at least equal sulfide, production would be expected at the inland side compared to the seaward side since the conditions for sulfide production are better at the exfiltration site. The supply of anoxic, reduced, and sulfate-rich groundwater will maintain anoxic conditions. Moreover, the microbial mat is more developed at the inland side. Since the organic matter content in young calcareous dune slacks is low (Berendse et al. 1998), the presence of organic compounds originating from the microbial mat could be necessary for the production of sulfide by sulfate-reducing bacteria.

So the question to be answered is why the sulfide concentrations at the exfiltration site were lower compared to the infiltration site. It appears that the explanation for these phenomena lies in the presence of compounds, such as reduced iron (Fe^{2+}) that quickly and effectively bind sulfide. Ion-specific sulfide electrodes do not detect bound sulfide (such as FeS and FeS_2). Any sulfide produced in the mat will have resulted in the chemical reduction of ferric iron to ferrous iron, part of which will have precipitated as FeS.

Ecological consequences

On a cross-section, the vegetation in the undisturbed part of the 'Buiten Muy' can be divided into three vegetation types. From the inland to the seaward side we find subsequently a *Caricetum ripariae* stand, a *Samolo-Littorelletum* stand, and a *Scirpo-Phragmitetum* stand.

The occurrence of free sulfide may partly explain this vegetation zonation. On the seaward side of the dune slack, where sulfide was found, the vegetation is dominated by *P. australis*. This species is relative tolerant to sulfide; Chamber et al. (1988) found that the uptake of ammonium by *P. australis* declined signifi-

cantly at 375 µM sulfide, while a smaller, not significant, decline was observed at 195 µM. *P. australis* is therefore not harmed by the sulfide concentrations observed in the field. Indirectly, it can even take advantage of the free sulfide; in the presence of free sulfide the pool of phosphate that can be assimilated will increase due to the liberation of phosphate from the Fe-bound and possibly also the Ca-bound phosphate pool (Sperber 1958; Patrick 1974; Caraco et al. 1989; Stal et al. 1996; Beltman et al. 2000; Heijs et al. 2001).

C. riparia is probably restricted to the exfiltration site of the dune slack because of the sulfide sensitivity of *Carex* species (Lamers 1998). At this site we did not measure free sulfide; moreover, the higher part, where *C. riparia* in particular dominates, will not be waterlogged in the growing period of the plant species. The effects of free sulfide in the *Samolo-Littorelletum* stands are still uncertain. This pioneer vegetation in the central part of the slack is almost permanently waterlogged during wet years. Most of the soil is without vegetation and covered with a well-developed microbial mat. In the laboratory we found under similar hydrological conditions occasionally > 30 µM of free sulfide in *C. nigra* stands during the night. However, *L. uniflora* shows ROL; the low density of this species in the 'Buiten Muy' may only aerate their own rhizosphere, therefore, free sulfide may expected to occur further away from the plants. The establishment of *C. riparia* could be limited in the central area of the slack if indeed similar concentrations as found under experimental conditions would be produced – Lamers et al. (1998) showed a negative response of the growth of sedges at sulfide concentrations < 10 µM. Although the organic matter content in the pioneer stand is very low, it is unlikely that sulfate reduction is limited due to a lack of organic matter, as sufficient organic matter is present in the microbial mat (van Gemerden 1993).

In conclusion, the occurrence of free sulfide may explain most of the vegetation gradient in the 'Buiten Muy'. More conclusive results would be obtained during early spring during the night. This is the period with strong groundwater discharge from surrounding areas, and when temperatures are high enough to sustain sulfate reduction. The question if sulfide toxicity can slow down the succession in dune slacks remains, therefore, unresolved. It is likely, however, that sulfide can accelerate vegetation succession at the infiltration side of the slack, due to enhanced phosphate availability at low sulfide concentrations.

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