



Nitrogen removal from landfill leachate in constructed wetlands with reed and willow: Redox potential in the root zone

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ABSTRACT

This study investigated the effects of reed and willow on bioremediation of landfill leachate in comparison with an unplanted control by measuring redox potential levels in the rhizosphere of microcosm systems in a greenhouse. Plants had a significant influence on redox potential relative to the plant-less system. Redox potential in the reed rhizosphere was anoxic (mean -102 ± 85 mV), but it was the least negative, being significantly higher than in the willow (mean -286 ± 118 mV), which had the lowest Eh. Redox potential fluctuated significantly in the willow rhizosphere during daylight hours, with large decreases in the morning. Levels of NH_4^+ decreased significantly in the first day of the experiment and remained at similar low levels in all three variants for the next four weeks of the experiment. Following this removal of ammonia significant peaks in NO_2^- occurred in the control and reed tanks on the 1st day, and again on 14th day in the control tank up to 13 mg/dm^3 . In the willow tank there was also one significant peak of NO_2^- in the first week, but only up to 0.5 mg/dm^3 . Significant accumulation, within 21 days of NO_3^- in all variants was observed, but in tanks with reed and willow the concentration of NO_3^- remained significantly lower ($<4 \text{ mg/dm}^3$) than in the unplanted tank ($\sim 35 \text{ mg/dm}^3$). Final levels of total-nitrogen, nitrate and chemical oxygen demand were considerably lower in the reed and willow tank than in the unplanted tank.

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1. Introduction

Treatment of LL normally uses high-tech solutions (e.g. activated sludge followed by reverse osmosis, ultrafiltration or ozonation) that present high operation and maintenance costs and, therefore, are not sustainable at many landfill sites, especially in rural areas. The use of ecological systems such as constructed wetlands (CW) has been seen as an economical and technically sustainable solution for LL treatment making it safe to discharge into the environment (Christensen et al., 1992; Schnoor et al., 1995).

Plants commonly used in constructed wetlands include: cattail (*Typha latifolia* L.), reed (*Phragmites australis* Trin ex Steudel), rush (*Juncus effusus* L.), yellow flag (*Iris pseudacorus* L.), and mannagrass (*Glyceria maxima*). As well as these typical natural wetlands plant species, willow (*Salix* sp.) may be used in constructed wetlands with high efficiency. Constructed wetlands (CW) with reed (Wojciechowska and Obarska-Pempkowiak, 2008; Wojciechowska et al., 2009) as well with willow (Białowiec et al., 2007; Randerson,

2006) have been shown to be effective in the removal of high levels of ammonia and total nitrogen (TN) from LL. A review of the potential for the use of willow filter beds and Short Rotation Plantations to treat LL by Duggan (2005) concluded that several studies showed success of willow filter beds in treating LL and that treatment improved with the number of the willows.

Oxygen released from roots creates aerobic conditions in the otherwise anaerobic rhizosphere, which induces growth of both heterotrophic and autotrophic aerobic bacteria (nitrifiers) and the aerobic breakdown of organic material (Brix, 1997). Enough oxygen is available in the root zone for respiration and release into the rhizosphere for the formation of an oxidative protective film around the root surface. Enhanced nitrification by microorganisms in the oxygenated rhizosphere is an important process in the treatment of LL with high levels of NH_4 . Removal of ammonia through nitrification is advantageous not only because it helps in the removal of N loads, but also because the end N specie (NO_3^-) is much less toxic and more bioavailable to plants than NH_4^+ (Jones et al., 2006). Plant uptake also plays an important role in the enhancement of N removal, especially in treatment wetlands containing fast-growing plants such as willows (Randerson, 2006). In addition, plants can facilitate removal of N from wetlands by microbial activity. Organic

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compounds, such as sugars, alcohols and acids, that are in the LL or released by plants into the rhizosphere can help in nitrate removal by acting as a carbon source for denitrifying bacteria (Brix, 1997). Aerenchyma also plays a role in the removal of nitrogen (N) from wetlands by releasing N_2 and N_2O produced by anaerobic denitrification of NO_3^- into the atmosphere (Reddy et al., 1989).

The combined nitrification–denitrification processes are considered to be the most common method for nitrogen removal in CWs (Kadlec et al., 2000; Vymazal and Kropfelova, 2008).

Determining the characteristics of oxygen release in the rhizosphere of different plant species and its impact on the treatment of LL in CW is important for a better understanding of the oxidising or reducing capabilities of the root zone. Measuring redox potential (Eh) may be used for this purpose, since oxidised aerobic environments generally have high Eh, while low Eh is linked to reduced anaerobic environments (Faulwetter et al., 2009). The protective oxidised layer around roots in the rhizosphere can create a gradient of Eh from ~ 500 mV at the root surface to ~ -250 mV 1–20 mm away from the root surface (Wießner et al., 2002).

In this paper we focused on the influence of plants on removal of N from LL by oxygen release, and control by plants of the subsurface aerobic/anoxic/anaerobic conditions.

2. Materials and methods

2.1. Experimental design

Experiments were carried out in a greenhouse with mean temperature 22.5°C ($SD = 7.67^\circ\text{C}$), which fluctuated with outside temperature and weather. The source of LL was the non-hazardous

landfill site (old section) at Lamby Way, Cardiff. Phytotoxicological tests, conducted according to Białowiec and Randerson, 2010, indicated the need for dilution of LL to 20% concentration to avoid toxic effects on plants.

Three glass tanks ($80 \times 5 \times 55$ cm; $L \times W \times H$) were used (Fig. 1). Tank A contained the common reed *P. australis* (Cav.) Trin. ex Steud, tank B the willow *Salix viminalis* \times *burjatica* (var. Ashton Stott), and tank C was a control tank with no plants. The tanks were filled to a depth of 33 cm with LECA 0.5–2 cm in size with a layer of gravel placed over the top to prevent floating. To enable Eh measurements and LL sampling within the root zone each tank had two perforated plastic inspection tubes (2 cm diameter) inserted between the LECA. Water pumps (Mini Aqua One, 300F-LV, 10 L/h, 2.5W) placed 2–3 cm below the surface of the LECA were used to circulate the LL solution in each tank.

Once the tanks were set up, on 15.06.2009 six *P. australis* rhizomes (5–10 cm long) with shoots were planted in tank A (two on each side of the inspection tubes), and six pre-cultivated (3 weeks old) willow cuttings were planted in tank B (two on each side of the inspection tubes). Tanks were then filled to the height of the LECA with the 20% (v/v) LL solution in tap water (A: 5.5L, B: 5.4L, C 5.2L) and the sides of each tank were covered in aluminium foil to prevent algal growth by excluding light. After 36 days of initial plant growth and microbial colonisation in the system, the first portion of diluted LL was replaced by new LL solution, and the experiment was run for a period of 144 days. Tanks were subject to continuous recirculation as in a horizontal flow CW.

On the 67th day of the experiment supplementary lighting between the hours of 05:00 and 21:00 was implemented to slow the senescence of the willow leaves.

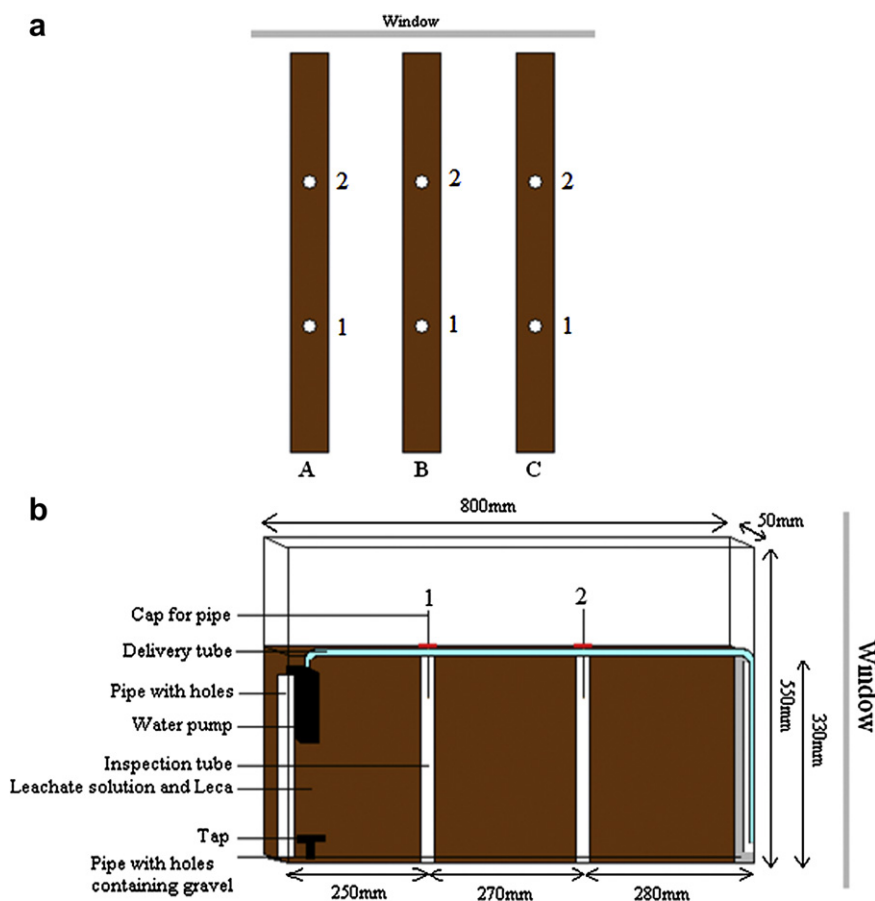


Fig. 1. (a) – Tanks: A – reed, B – willow and C – control (left to right) in the greenhouse, (b) construction and dimensions of tanks, with LL recirculation system, and sampling tubes.

2.2. Sampling and measurements

A multi-parameter analyser (Eijkelpamp 18.26) was used to take daily Eh measurements of the LL solution in each tank. A single redox probe was inserted in turn into each sampling tube to a depth of 12 cm, and left to equilibrate. Readings were taken from 20.07.09 to 24.01.10, and were started between 11:45 and 14:00 on week days and 07:00 and 09:00 on weekends. A thermometer attached to the wall of the greenhouse was used to record the ambient temperature at the time the readings were started. To measure any fluctuation in Eh during the day on three occasions (20.11.09, 27.11.09 and 10.12.09) Eh readings were taken hourly between the hours of 8:00 and 18:00 using the methods described above.

Once the Eh readings were complete the water levels of each tank were checked. Tap water was used to maintain water levels to the height of the LECA.

Samples of LL were taken once a week for the first four weeks and on days 137 and 144. Three 20–25 ml samples of LL solution were taken from each tank, one from each inspection tube and one from the tap. A 60 ml syringe and a piece of 5 mm tubing were used to withdraw samples from the middle level of each inspection tube. To remove any contamination on the apparatus the syringe, rubber tube and sample container were initially rinsed with LL solution from the sampling point. Samples from taps were poured directly into the sample container after it was rinsed. Samples of the 100% LL and 20% (v/v) LL solution were also taken on the first day of the experiment. The samples were analysed for levels of NH_4^+ , Kjeldahl nitrogen, nitrite, nitrate, and COD using the standard International Organisation for Standardisation (ISO) methods (ISO 7150-1:1984, ISO 5664:1984, ISO 5663:1984, ISO 6777:1984, ISO 7890-3:1988 and ISO 6060:1989).

Results were statistically verified. Differences between mean values of measured parameters were assessed using ANOVA at the significance level of $p < 0.05$. Post-hoc (*a posteriori*) Tukey's test was used to determine differences between means of specific variants. Correlation coefficients between Eh and greenhouse temperature were calculated.

3. Results and discussion

3.1. Redox potential

In all tanks Eh oscillated between days and over time (Fig. 2), but overall mean values were significantly different (ANOVA test: $F = 184.02$; $p < 0.01$). The least negative Eh was in the reed tank with a mean of -102 mV ($\pm \text{SD } 85$) (Tukey's test $p < 0.001$: against both willow, and control). The willow tank had the lowest Eh with a mean of -286 mV ($\pm \text{SD } 118$) (Tukey's test $p < 0.001$: against both

reed, and control). The mean value in the control tank was -158 mV ($\pm \text{SD } 67$).

No relationship between Eh and greenhouse temperature was found for any of the tanks over the whole sampling period, and this could be due to many factors. Although, it has previously been found that there is a significant relationship between Eh and temperature, it is also known that the presence and species of plants can counteract the effects of temperature on Eh (Allen et al., 2002). However, no relationship between Eh and temperature was found in the unplanted control tank suggesting that other factors were affecting the Eh, such as microbial activity and evapotranspiration/evaporation (Dušek et al., 2008).

The temperature in the greenhouse was prevented from falling below 14°C , which may have affected any relationship between the temperature and Eh, as light intensity also plays a role in the relationship between these two factors and this may have been negated by the artificial heating. Values of Eh indicated that reed tends to raise Eh above typical (Randerson, 2006) anoxic levels by releasing either more oxygen or less organic exudates than willow. These exudates, which make up 5–25% of photosynthetically fixed carbon, assist denitrification and the degradation of toxic organic chemicals (Brix, 1997; Schnoor et al., 1995). Willow maintained Eh at anoxic levels but close to anaerobic, which could promote more efficient denitrification, and anaerobic decomposition of organic compounds. No direct comparisons have been made between reed and willow, as to their capabilities for oxygen release into the root zone, but it has been found that young (5 weeks old) willow *Salix viminalis* L. is able to release $0.18 [\text{gO}_2 \text{ m}^{-3} \text{ h}^{-1} \text{ plant}^{-1}]$ which corresponds to about 200 $[\text{gO}_2 \text{ m}^{-3} \text{ h}^{-1} \text{ kg}_{\text{dw}}^{-1}]$ of oxygen released in relation to root mass (Randerson et al., submitted for publication). It is apparent that oxygen released is sufficient to cover its consumption by respiration, decomposition of organic compounds, and nitrification. The strongly anoxic conditions in the rhizosphere of willow, probably caused by overloading of organic exudates, promotes more efficient denitrification than in the case of reed. Willow promotes more efficient nitrogen removal than in the case of reed (Section 3.2).

As well as the influence of plant species, the release of oxygen into the rhizosphere is related to some extent to photosynthesis, light intensity, stomatal aperture, and temperature (Stein and Hook, 2005). The amount of oxygen in the rhizosphere fluctuates over diurnal periods and varies between seasons. There is higher oxygen release in periods of illumination and even in periods of relatively low light intensity the amount of oxygen released into the rhizosphere can meet the respiratory oxygen demand of the roots and micro-organisms in the rhizosphere (Sheppard and Lloyd, 2002).

In the reed and control tanks Eh readings taken between the hours of 08:00 and 18:00 on the three separate occasions were constant over the day ($p > 0.05$) with only one exception, and

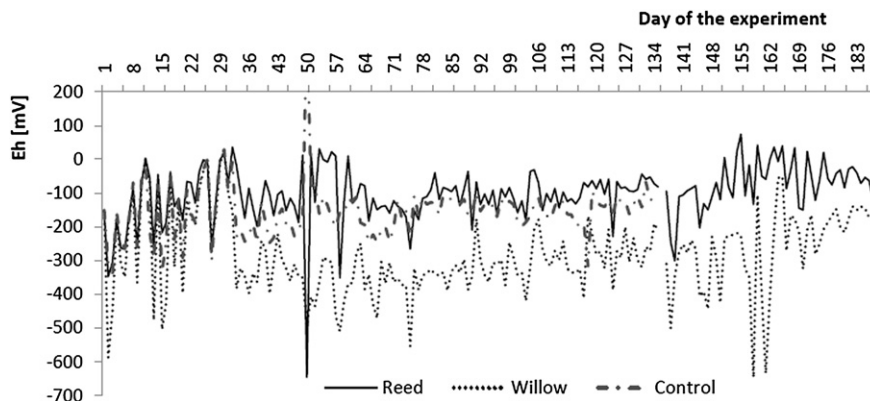


Fig. 2. Changes of Eh over time of whole experiment in reed, willow, and control tanks.

similar to each other, and between dates of measurement (Fig. 3). The lowest values (as low as -500 mV) were observed for willow, especially in the morning (8:00 to 10:00) tending significantly ($p < 0.05$) to increase towards 12:00 (Fig. 3). The duration of that tendency differs between dates of measurement.

There could be many reasons for such fluctuations in Eh. Dušek et al. (2008) found fluctuations in Eh over diurnal cycles in wetlands containing *P. australis* with Eh decreasing in light and increasing in dark periods. The decrease in Eh during light hours was attributed to the ability of plants to transport oxygen and to the release of plant-root exudates into the rhizosphere, which served as a substrate for heterotrophic microorganisms resulting in increased microbial activity and utilisation of oxygen, thus decreasing Eh. Wießner et al. (2005) also found fluctuations in Eh in the rhizosphere of *Juncus effusus*, however the Eh increased during the light and decreased during the dark due to increased amounts of oxygen released by the plants into the rhizosphere during periods of light.

It appears that species differences affect the fluctuations of Eh in the rhizosphere during diurnal cycles, as there was a marked difference in the fluctuations between the reed and willow tanks, with the reed tanks having only slightly more variation in Eh than the control tank (Fig. 3).

Williams et al. (in press) found that diurnal cycling of oxygen levels in willow rhizospheres were affected by weather and by day/night changes.

The low values of Eh in the willow tank in the morning could be attributed to the release of photosynthetic exudates into the rhizosphere after the sun rose, and the subsequent increase in Eh could be attributed to the later release of oxygen into the rhizosphere. Dušek et al. (2008) also found that the fluctuations in Eh were most pronounced in the upper 20 cm of the wetland. In this study Eh was measured at a depth of 12 cm and it may be that fluctuations of Eh differed at different depths in the tanks.

Table 1

Pollutant concentrations (column 2–3) in 100% (v/v) LL and 20% (v/v) LL at the start of the experiment, and (columns 4–7) in tanks A (Reed), B (Willow) and C (Control) at 137 and 144 days (mean values \pm SD).

Parameter	%LL	Conc mg/dm ³	Day	Concentration (mg/dm ³)		
				Reed	Willow	Control
NH ₄	100	800.8	137	1.4 \pm 0.10	1.5 \pm 0.06	1.3 \pm 0.06
	20	155.6	144	1.6 \pm 0.39	1.3 \pm 0.14	–
NO ₂	100	0.14	137	0.0 \pm 0.02	0.1 \pm 0.01	0.1 \pm 0.00
	20	0.03	144	0.1 \pm 0.02	0.1 \pm 0.02	–
NO ₃	100	5.7	137	1.0 \pm 0.18	0.9 \pm 0.07	13.2 \pm 5.86
	20	1.1	144	0.8 \pm 0.17	0.6 \pm 0.00	–
Kieldahl-N	100	952	137	37.3 \pm 0.81	36.9 \pm 2.14	37.3 \pm 1.62
	20	192	144	55.1 \pm 20.26	39.7 \pm 2.91	–
Organic-N	100	151.2	137	35.9 \pm 0.90	35.4 \pm 2.19	36.0 \pm 1.65
	20	36.4	144	53.5 \pm 20.62	38.3 \pm 3.01	–
Total-N	100	957.84	137	38.3 \pm 0.65	37.8 \pm 2.07	50.6 \pm 7.03
	20	193.13	144	55.9 \pm 20.08	40.3 \pm 2.90	–
COD	100	1219	137	49.3 \pm 17.21	86.0 \pm 6.08	101.3 \pm 46.11
	20	253	144	90.3 \pm 22.12	96.3 \pm 18.01	–

3.2. Landfill leachate treatment

The 20% (v/v) LL used in the tanks was characterised by high levels of NH₄⁺ and COD (Table 1, Fig. 4). NH₄⁺ in all tanks and sampling points fell significantly ($p < 0.05$) from about 155 mg/dm³ during the first day of operation and remained below 2 mg/dm³ until the end of the experiment (Table 1, Fig. 4). NH₄⁺ was removed successfully with higher than 95% efficiency in all tanks, with the willow tank being the most efficient, having significantly ($p < 0.05$) lowest levels after the first week. This fall corresponded to significant ($p < 0.05$) peaks in the NO₂⁻ concentration in the reed and control tanks (Fig. 4). NO₂⁻ in the willow tank remained significantly ($p < 0.05$) lower than in reed and control tanks (Fig. 4).

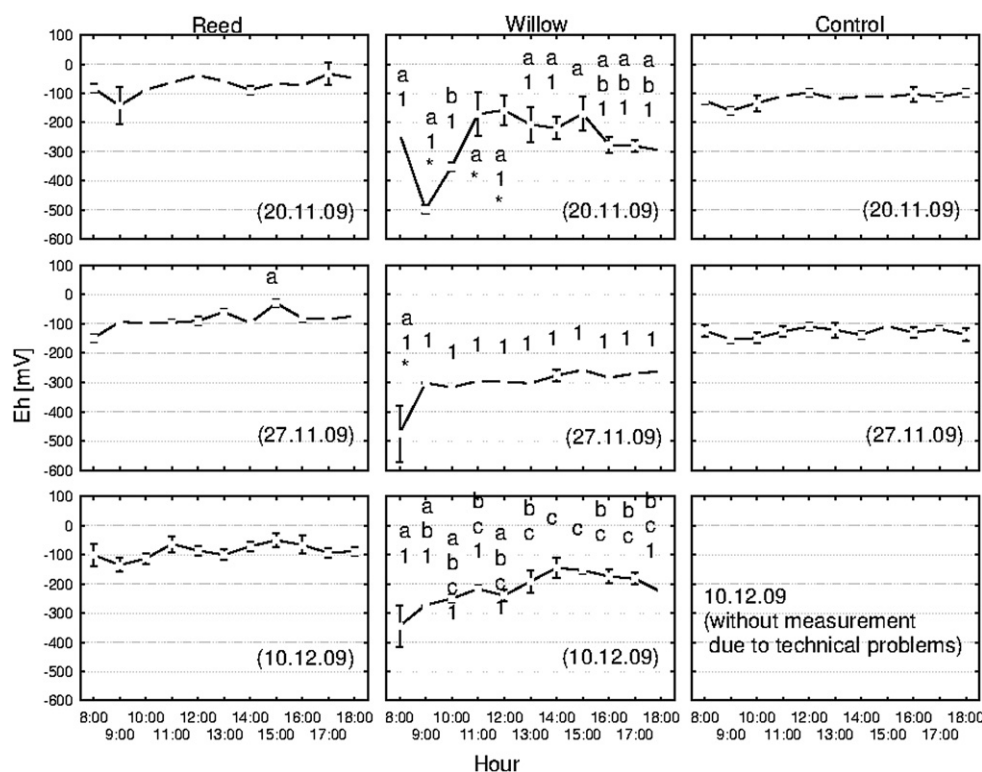


Fig. 3. Changes of Eh during a day between 08:00 and 18:00 in tanks with reed, willow, and control, on: (20.11.09 – sunny day), (27.11.09 – over cast 8:00–11:00, sunny 11:00–14:00, overcast 14:00 onwards), (10.12.09 – sunny day). Statistical significance ($p < 0.05$) of Eh differences between times of day is shown by letters; between tanks for individual time points is shown by asterisks.

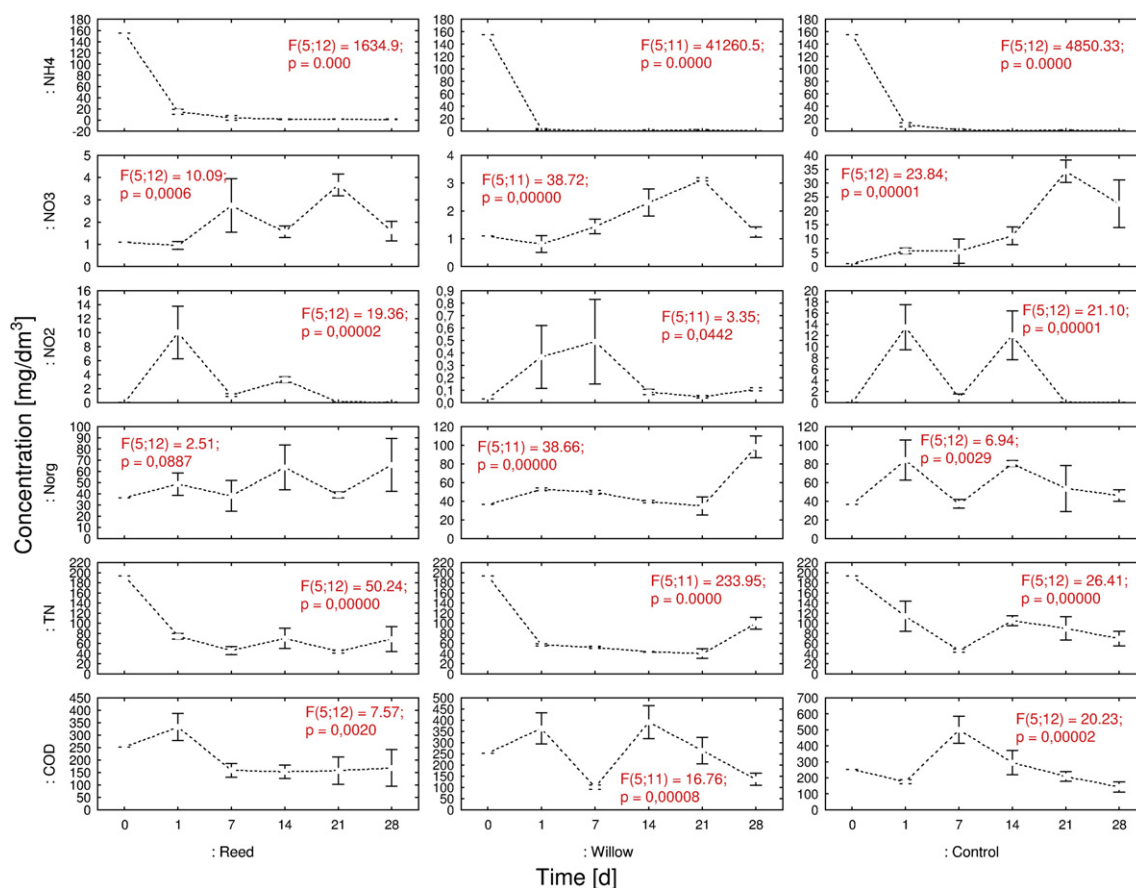


Fig. 4. Changes in concentration of N–NH₄, N–NO₂, N–NO₃, organic-N total-N, and COD, in samples of LL (A-reed, B-willow, C-control). ANOVA *F* statistics and *p* values have been given for each variant of the experiment and measured compound.

The highest concentration of NO₃[−] was observed in the third week of sampling, particularly for the control tank, which had much higher levels of NO₃[−] (mean value 34.3 mg/dm³), than the planted tanks (Tukey's test *p* < 0.001: against both reed, and willow). Furthermore, this peak in NO₃[−] corresponded with a fall in the concentration of NO₂[−] in the control tank (Fig. 4).

Interestingly, the decline in NH₄⁺ in the willow tank was not followed by a rise in NO₂[−] or NO₃[−], which would be expected if nitrification had taken place. It may be that full or partial nitrification and full denitrification occurred simultaneously and quickly during the first week of the experiment. Plant uptake could also explain the removal of the NH₄⁺ in the willow tank. Alker et al. (2003) found a combination of plant uptake and nitrification in LL treatment wetlands containing willow resulted in an almost complete removal of NH₄⁺, with nearly half of the N removed stored in the wetland system, principally in plant tissues as organic N.

In the reed tank there was a significant (*p* < 0.05) peak in NO₂[−] suggesting that nitrification occurred, but there was no rise in concentration of NO₃[−] which would be expected with the removal of NO₂[−] through nitrification (Randerson, 2006). It could be that nitrification of NO₂[−] to NO₃[−] and subsequent denitrification of the NO₃[−] took place, but because of the relatively long time period between sampling the changes in the concentration of the NO₃[−] were not detected. Organic carbon is one of the primary regulators of denitrification of NO₃[−] (Schipper et al., 1993) and both the reeds and the LL itself may have provided an organic carbon source for the denitrification process. Another possible reason for the lack of NO₃[−] in the reed tank could be plant uptake, as it has previously been found that in rhizospheres with low N availability competition

between plant uptake and microbial use of NO₃[−] can limit denitrification (Fromin et al., 2005). A further potential pathway for NO₂[−] removal, which does not involve denitrification to NO₃[−], is direct denitrification of NO₂[−] to N₂O and N₂ (Faulwetter et al., 2009). However, this only occurs under conditions of oxygen stress (Stevens et al., 1998), which is compatible with the low Eh in the reed tank during the initial experiment.

In the control tank the decline in NH₄⁺ with the subsequent peak and decline in NO₂[−] and later peak in NO₃[−] suggests that nitrification of NH₄⁺ and NO₂[−] took place. Lekang and Kleppe (2000) established a similar NH₄⁺ removal process in an unplanted system containing LECA with almost 100% of NH₄⁺ removed, which is akin to this study. The slight, but significant (*p* < 0.05) decline in NO₃[−] after week three suggests that some denitrification took place in the control tank. However, levels remained significantly higher than in planted tanks (Tukey's test *p* < 0.001: against both reed, and willow), which could have been due to a lack of organic carbon needed for further denitrification to take place (Schipper et al., 1993) and the absence of plant uptake.

The presence of reeds and willows in the tanks in this study clearly affects the removal of NH₄⁺ with utilisation of different pathways or different rates of nitrification and denitrification.

The amounts of TN fell significantly (*p* < 0.05) over the sampling period (Table 1, Fig. 4). Levels of organic N in each tank fluctuated over the sampling period (Fig. 4), but were at about the same levels on days 137 and 144 as the 20% (v/v) LL at the start of the experiment (Table 1). Levels of COD also fluctuated during the first four weeks, but generally showed a significant (*p* < 0.05) decline in levels (Table 1, Fig. 4).

Even though the final level of NH_4^+ did not differ between the three tanks, the final concentration of TN and NO_3^- did. Final NO_3^- concentration in the control tank was more than 13 times higher than that in the reed or willow tanks, while TN was 1.3 times higher than in tanks with plants. This shows that the presence of plants in the system enhanced the removal of TN and NO_3^- in LL, which has also been found in previous studies (De Foe, 2007; Morgan et al., 2008).

The presence of the reeds and willows also appears to have had a positive effect on the COD levels, which were much lower in the reed (50%) and willow (14%) tanks than the control tank in the final LL sample. Previous research has also found that the presence of plants can significantly affect COD removal compared to unplanted controls (Allen et al., 2002).

4. Conclusions

Significant fluctuations of Eh during daylight hours occurred only in the willow rhizosphere containing LL. Reed tends to maintain typical anoxic conditions, but willow regulates Eh close to anaerobic. Control tank Eh was significantly higher than in willow, but lower than in the reed tank. These different patterns of redox reflected differences in pathways of nitrogen removal between variants.

Ammonia nitrogen was removed efficiently in all variants. In tanks with plants NO_3^- peaked up to 4 mg/dm^3 , but with the control tank within 3 weeks of experiment significantly higher accumulation of NO_3^- up to 35 mg/dm^3 , was observed. In reed and control tanks significant peaks of NO_2^- (up to 13 mg/dm^3) appeared, whereas in willow NO_2^- remained very low. Reeds and willows positively affect the treatment of LL as there are lower TN, NO_3^- , and COD concentrations in tanks containing these plants after 4.5 months than in an unplanted control. This is probably achieved through plant uptake and the effects of enhanced levels of oxygen and organic carbon in the rhizosphere on nitrification and denitrification.

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