Seasonal variation in phosphorus removal processes within reed beds – mass balance investigations

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Abstract The phosphorus (P) removal processes in two pairs of High and Low Loaded reed beds were investigated during five periods within a 27-month study. The uptake/release of P was measured in seven mass balance compartments. With the exception of the first year of operation, the reed beds consistently removed over 96% of the influent P load, with total phosphorus (TP) concentrations being reduced from 0.5 mg/L to generally less than 0.005 mg/L across the range of loading rates and seasons studied. During the first year, uptake by *Phragmites australis* accounted for greater than 75% of P removed, and was equally distributed between above and below-ground biomass. During the second and third years, three seasonal stages were identified in the uptake and cycling of P by *P. australis*. A period of rapid above-ground growth and uptake occurred during spring fuelled partly by P reserves accumulated in rhizomes during the previous year. During summer, uptake by above-ground biomass was governed by the influent P loading rate, while the amount of P held in below-ground biomass remained relatively stable. During autumn and winter, P appeared to be translocated from senescent shoots to reserves in the rhizomes. Approximately 85% of the below-ground biomass P occurred in the top 20 cm of the substrate. Gravel fixation increased in importance from 12% in the first year to approximately 30% of P removed in the second year, with a highly significant correlation between the influent P loading rate and P fixed by the gravel. The weakly-bound P fraction from a sequential extraction was the dominant form of P fixed by the gravel. HCl extracts were inappropriate for the examination of sorption processes as they dissolved large amounts of mineral P from within the basaltic gravel. The bottom 30 cm of the substrate became the most important site for gravel fixation during the second year. Incorporation of P into the detritus/microbiota/other compartment increased after the first year to become one of the most important P removal processes, probably consisting mainly of leaf litter and slowly accreted organic sediments.

Keywords Mass balance; phosphorus removal; reed bed

Introduction

In Australia, subsurface horizontal flow wetlands (reed beds) are increasingly being used as an inexpensive, low-tech means of removing nutrients from a range of agricultural wastewaters, including runoff from plant nurseries. In order to avoid the eutrophication of waterways, the reduction of phosphorus (P) loads in runoff is often a high priority. Researchers have identified a number of broad mechanisms by which P removal from wastewater is achieved in reed beds. These are via: (a) fixation onto the substrate (Arias et al., 2001; Pant et al., 2001); (b) plant uptake (Breen, 1990; Tanner, 1996); (c) sediment accretion (Nguyen, 2000); and (d) microbial immobilisation (Mitsch et al., 1995). Fixation of P onto the substrate is often a major removal pathway in a young reed bed. However, once P sorption sites become saturated, substrate fixation will cease and P may be remobilised into solution (Horne and Goldman, 1994). Consequently, the sustainable removal of P from applied wastewater generally relies on sediment accretion and the harvesting of above-ground plant biomass. In order to identify the relative importance of the various P removal pathways, a mass balance approach can be used similar to that developed by Breen (1990).
In this study a number of P compartments were monitored in four reed beds treating nursery runoff over a 27-month period. Phosphorus balances were conducted during five sequential sampling periods, by conceptually partitioning the reed beds into the following four measured P compartments:

1. influent loading;
2. effluent loading;
3. change in plant material (above-ground and below-ground); and
4. change in substrate-bound (loosely-adsorbed, Fe/Al-bound, Ca-bound and Residual-P).

A fifth, unmeasured, compartment (detritus/microbiota/other) was assumed to have accumulated the difference between Compartment 1 and Compartments 2–4. The aim of the study was to determine the relative importance of each of these compartments in the removal of P from reed beds receiving simulated nursery runoff at two different loading rates across a number of different seasonal periods.

Methods

Site and study description

Phosphorus mass balances were compiled for four identical reed beds located at the New South Wales Centre for Tropical Horticulture, Alstonville in the sub-tropical zone of the Australian east coast. Each reed bed was 4 m long by 1 m wide with a 0.5 m water depth, and contained a 10 mm basaltic gravel substrate into which Phragmites australis was planted in April 1999 (Headley et al., 2001). This paper presents monitoring from this date through to July 2001. The reed beds received a nursery runoff solution characterised by relatively low concentrations of nitrogen (10 mg/L total nitrogen as nitrate) and P (0.5 mg/L total phosphorus (TP) as soluble reactive P (SRP)) and virtually no organic matter or suspended solids. The four reed beds were grouped into two pairs and operated at different hydraulic loading rates (HLRs). For the first 11 months (Period 1) all beds were operated at the same HLR. One pair of beds (Low Loaded Beds) was then maintained at a relatively constant HLR throughout the study, while the HLR on the other pair of beds (High Loaded Beds) was periodically increased. For the final period (Period 5), both pairs of beds were operated at similar HLRs (Table 1). Measurement of the P content of the plant biomass and the gravel-bound compartments took place at the same time that changes were made to the HLR. The amount of P that had been taken up or released by these compartments during each period was then calculated. Influent and effluent P loadings for each period were calculated from weekly samples. The balance between the influent compartment and the sum of the other measured compartments was assumed to consist of the detritus, microbiota and sediment that had accumulated during each period, and was collectively termed the “detritus/microbiota/other” compartment.

Table 1 Dates and seasons covered by the sequential study periods, and the mean HLRs and corresponding Hydraulic Residence Times – HRT (italicised in parentheses) of the High and Low Loaded Beds. Units: HLR = mm/day; HRT = days

<table>
<thead>
<tr>
<th>Study period</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start Date</td>
<td>22nd Apr-99</td>
<td>1st Apr-00</td>
<td>9th Sep-00</td>
<td>23rd Jan-01</td>
<td>21st Mar-01</td>
</tr>
<tr>
<td>Finish date</td>
<td>1st Apr-00</td>
<td>9th Sep-00</td>
<td>23rd Jan-01</td>
<td>21st Mar-01</td>
<td>22nd Jun-01</td>
</tr>
<tr>
<td>Seasons covered</td>
<td>Entire year</td>
<td>Autumn–winter</td>
<td>Spring–summer</td>
<td>Summer–autumn</td>
<td>Autumn–winter</td>
</tr>
<tr>
<td>HLR High Beds</td>
<td>48 (5.4)</td>
<td>63 (4.0)</td>
<td>106 (2.6)</td>
<td>176 (1.5)</td>
<td>108 (2.2)</td>
</tr>
<tr>
<td>(HRT) Low Beds</td>
<td>49 (5.3)</td>
<td>50 (4.9)</td>
<td>52 (4.9)</td>
<td>53 (4.6)</td>
<td>101 (2.8)</td>
</tr>
</tbody>
</table>
Influent and effluent monitoring

Water samples were collected weekly from the inlet and outlet of each bed and analysed for TP. Flow volumes were measured using flow meters and used to calculate the mean weekly influent and effluent TP loading rates for each bed. The pH of the effluent was measured in-situ.

Above-ground biomass “shoots”

At the start and end of each sample period (Table 1) a non-destructive method of biomass estimation was used which related shoot height to dry weight. Initially, the height and oven dry weight (70°C) of a range of shoots was measured. After a log conversion of the data, regression analysis was used to derive Eq. (1), which describes the relationship between individual shoot height (ht) and dry weight.

\[
\ln \text{Dry weight} = 20.639 - 16.206(\ln \text{ht}) + 3.799(\ln \text{ht})^2 - 0.2618(\ln \text{ht})^3 \quad (r^2 = 0.94)
\]  

100 mm wide quadrats that traversed the width of the bed were established within the inlet, middle and outlet sections of each bed. At the start and end of each sampling period, the number of shoots was counted within each quadrat, by grouping the shoots according to height in classes 1–500 mm, 501–700 mm, 701–900 mm […] progressing in increments of 200 mm thereafter. In addition to the non-destructive estimates, complete harvests of all of the standing shoots were conducted in July 2000 and July 2001 (Southern Hemisphere Winter). Standing shoots within each section were cut at the gravel surface, oven dried at 70°C and weighed. At the time of the non-destructive biomass estimations and the winter harvests, representative shoot sub-samples were collected for TP analysis.

Below-ground biomass and gravel sampling

Roots and rhizomes were collectively sampled as below-ground biomass at the start and end of each sampling period using a 155 mm diameter steel corer. Cores were sunk in the inlet, middle and outlet sections of each bed to correspond with above ground sampling. The below-ground biomass and gravel were excavated from the in-situ core by hand at two depths (0–20 cm and 20–50 cm). The below-ground biomass was manually separated from the gravel. All material was washed to remove loosely attached nutrients, sediment and organic films. Gravel samples were air dried, while biomass was oven dried at 70°C and weighed. Representative sub-samples of the below-ground biomass were ground for TP analysis.

Gravel sequential phosphorus extraction

Four inorganic P fractions were sequentially extracted from the gravel sub-samples using a method similar to that developed for soil analysis by Hedley et al. (1982). 75 g samples of gravel were sequentially extracted with:

Step 1. 50 mL of 0.5 M NaHCO₃ to remove weakly-bound P, thought to consist of P adsorbed onto surfaces of more crystalline P compounds, sesquioxides, or carbonates;
Step 2. 50 mL of 0.1 M NaOH to remove Fe and Al-bound P, thought to have a lower plant availability and to be associated with amorphous and some crystalline Al and Fe phosphates;
Step 3. 100 mL of 1 M HCl to remove Ca and Mg-bound P; and
Step 4. 25 mL of hot concentrated HCl to remove Residual-P.
Steps 1, 2 and 3 were extracted for 16 hours at room temperature. For Step 4, the gravel was extracted for 20 minutes in 25 mL of concentrated HCl at 80°C, before the addition of a further 10 mL of concentrated HCl and being left to stand for one hour at room temperature. Following each step, the gravel was washed sequentially with 25 mL of 1 M KCl and distilled water to remove any re-adsorbed P. These KCl and distilled water washes were added to the supernatant solutions from each step and analysed for inorganic P. Sample blanks, consisting of raw gravel that had not been used in the reed beds, were run through the sequential extraction with each batch of samples.

**Chemical analyses**

All influent and effluent samples were analysed for TP in accordance with the Standard Methods for the Examination of Water and Wastewater (1995) using the ascorbic acid/ammonium molybdate technique by Flow Injection Analysis on a Lachat QuickChem 8000 Automated Ion Analyser after autoclave digestion with persulphate. Plant and sediment samples were digested for TP using a semi-micro Kjeldahl technique, with sulphuric acid and a selenium catalyst. The digested samples were then analysed for SRP using the ascorbic acid/ammonium molybdate method. Aliquots from the gravel sequential extractions were analysed for SRP using the method of Murphy and Riley (1962).

**Results and discussion**

With the exception of the first year of operation, the reed beds consistently removed over 96% of the influent P load, with TP concentrations being reduced from 0.5 mg/L to generally less than 0.005 mg/L, over the range of loading rates and seasons studied. An adaptation period was apparent during the first year of operation (Period 1) when the influent P loads were lowest, with TP reductions of 91% and 84% for the High and Low Loaded Beds.
respectively. The mean daily phosphorus mass uptake or release rates for each compartment during the sequential periods are presented in Figure 1. Negative values represent a release of P during that period. The gravel-bound P compartment consists of the sum of the weakly-bound and Fe/Al-bound P fractions.

In the first year of operation (Period 1), while both sets of beds were receiving similar influent loading rates, plant uptake was the dominant removal process, accounting for greater than 75% of the P removed. Incorporation into the above-ground biomass compartment accounted for 36% and 63% of the P removed in the High and Low Loaded Beds respectively, while below-ground biomass uptake was responsible for 39% and 36% respectively. This dominance of plant uptake in the removal of P is to be expected in newly planted reed beds, as both the above-ground and below-ground components of the reeds rapidly expand during the first year of growth. The importance of the below-ground biomass compartment decreased substantially following Period 1, accounting for less than 10% of the P removed during Periods 2, 3 and 4 in both sets of beds. Collectively, these periods made up the second year of growth, during which time the net change in the amount of P contained in below-ground biomass was relatively small. This suggests that, following rapid growth in the first year of establishment, the amount of below-ground biomass P may remain somewhat constant throughout much of the year. A substantial decrease of 0.0101 g/m²/day was observed in the P content of the below-ground biomass of the Low Loaded Beds during Period 4 (late summer of 2001), possibly due to reed mortality. This decrease in below-ground biomass P was due to a decrease in the amount of biomass, rather than the concentration of P in the biomass, and corresponded with a decrease in the above-ground biomass compartment for that period. The highest rate of increase in the below-ground biomass compartment occurred in Period 5 (autumn/winter 2001) for both the High and Low Loaded Beds (0.0207 and 0.0120 gP/m²/day respectively). This was due to both an increase in the amount of below-ground biomass and an increase in the P concentration within that biomass. *P. australis* often exhibits a seasonal growth pattern where reserve material is accumulated in the rhizomes and roots during autumn to be exploited for new shoot growth in the following spring (Fiala, 1978; Hocking, 1989a).

Throughout the study the majority of the below-ground biomass was restricted to the top 20 cm of substrate, accounting for approximately 85% of the below-ground biomass P. This seems to be a common trait of macrophytes grown in subsurface horizontal-flow treatment wetlands (Adcock and Ganf, 1994; Rogers *et al*., 1990), possibly due to a reduced need to seek out nutrients in the eutrophic conditions.

The rate of P uptake into the above-ground biomass compartment of the High Loaded Beds increased from 0.0086 g/m²/day in Period 1, to 0.0123 g/m²/day in Period 2, and to 0.0281 g/m²/day in Period 3, in response to the increasing influent loading rate (represented by the total column height in Figure 1). Conversely, in the Low Loaded Beds uptake of P into the above-ground biomass decreased to only 10% of the P removed in Period 2 following the rapid growth of the first year (Period 1). Although Period 2 included the winter months of 2000, P uptake by the shoots in the Low Loaded Beds never regained the rate observed in Period 1. The reeds in the Low Loaded Beds displayed signs of nutrient deficiency during most of the study, suggesting that they were unable to compete with other removal processes (such as gravel fixation) for the limited supply of P in the influent. The loss of P from the above-ground biomass in the Low Loaded Beds (0.0027 g/m²/day) observed in Period 4 (late summer/autumn 2001) was echoed in the High Loaded Beds (0.0207 g/m²/day) in Period 5 (autumn/winter 2001). This reflected a general decrease in the standing crop of shoots and a possible translocation of nutrients from above-ground to below-ground plant parts as the reeds finished flowering and became senescent. This is characteristic of the seasonal growth pattern of *P. australis* in which nutrients accumulated
in shoots during rapid Summer growth are redistributed from senescent shoots to rhizome reserves in Autumn and Winter (Fiala, 1978; Hocking, 1989b). Consequently, removal of P in constructed wetlands may be enhanced by harvesting above-ground biomass in late summer, before P is translocated from shoots to rhizomes. This will not only capture the maximum amount of P in above-ground biomass, but may also increase uptake by below-ground biomass in order to supplement rhizome reserves to be used for shoot growth in the following spring.

To calculate the amount of P bound by the gravel only the weakly-adsorbed and Fe/Al-bound P fractions from the sequential extraction were used. The Ca and Mg bound-P (Step 3: 1 M HCl) and the Residual-P (Step 4: hot conc. HCl) fractions yielded unreliable results, with supernatant P concentrations being very high for both the samples and the sample blanks. In many instances, raw gravel that had not been in the reed beds (sample blanks) yielded more P than gravel taken from the beds. This indicated that both of the acid extractants dissolved relatively large amounts of native P from within the structure of the basaltic gravel, thus rendering these extractants of little use when examining P processes on the gravel surface. Nevertheless, the data indicate that the formation of insoluble Ca compounds (such as hydroxyapatite) and incorporation into the more permanently removed Residual-P compounds did not play a major role in the fixation of P by the gravel. The pH of water within the reed beds remained slightly acidic throughout the study, with a mean pH of 6.2, which would favour formation of Fe and Al compounds over Ca products (Iyamuremye et al., 1996).

Fixation of P by the gravel in Period 1 accounted for 9% and 12% of the P removed from the High and Low Loaded Beds respectively. This increased in Period 2 to 33% (High Loaded Beds) and 31% (Low Loaded Beds) of the P removed, and remained an important removal pathway throughout the rest of the study. The highest rate of P fixation in the High Loaded Beds occurred in Period 4 (0.0305 g/m²/day) and Period 5 (0.0236 g/m²/day). These were also the periods where the influent loading rates were greatest in these beds. A similar trend occurred in the Low Loaded Beds, with the highest fixation rates occurring in Period 5 in response to an increase in the influent loading rate. Analysis of the combined data from the High and Low Loaded Beds found a highly significant correlation between the gravel fixation rate and influent loading rate ($r = 0.891; n = 12; p < 0.005$).

The weakly-bound P fraction was the dominant form of gravel-bound P throughout the study, although the amount of Fe/Al-associated P increased with time. The weakly-bound P fraction would consist mainly of adsorbed P that is plant available and can be easily remobilised if changes occur in the pH, redox or equilibrium P conditions (Grobbelaar and House, 1995; Hedley et al., 1982; Hillbricht-Iłowkska et al., 1995). While weakly-bound P may not represent a permanently removed pool, once the P is held at the gravel surface by adsorption it may become more permanently bound through inner-sphere complexation and precipitation reactions (Sparks, 1995), thus progressing into the Fe/Al-associated P fraction.

The majority of gravel-bound P occurred in the bottom 30 cm of the reed beds. This may have been due to scavenging of P from the substrate by the roots of the $P. australis$, since the majority of below-ground biomass occurred within the upper 20cm of the reed beds. Considering the relatively low P loading rates observed in this study, the reeds may have been forced to strip weakly-bound P from the gravel.

Incorporation of P into the detritus/microbiota/other compartment was low during Period 1. This can be explained by the fact that a large proportion of the P in this compartment would be derived from decaying plant and microbiological material, and sediment accretion. The formation and deposition of this material would be slow during the first year of operation, while the plant and microbial communities are establishing (Kadlec and Knight, 1996). Following the first year of operation, incorporation of P into the
detritus/microbiota/other compartment grew to become a major P removal process, accounting for greater than 30% of the P removed in both sets of beds throughout the rest of the study. Phosphorus accumulation rates in this compartment peaked in Period 4 for both the High (0.0679 g/m²/day) and Low Loaded Beds (0.0316 g/m²/day), and remained high in Period 5. The magnitude of this compartment was estimated by taking the difference between the influent loading rate and the sum of all other measured compartments, and therefore includes any cumulative errors from the measurement of the other compartments. However, it is unlikely that error alone would account for all of the P accumulated in this compartment. A substantial component of the detritus/microbiota/other compartment would be undecomposed litter from senescent reeds accumulated at the gravel surface. Accumulation of this material would be particularly rapid during autumn and winter when the reeds are becoming senescent, corresponding with Periods 4 and 5. A portion of this sloughing leaf material would also be lost from the relatively small and exposed reed beds by wind dispersal. While some of this deposited leaf litter may eventually become mineralised, thus releasing P back into the water column, much of it will break down into a stable residual, providing a sustainable P removal pathway (Kadlec and Knight, 1996).

Conclusions
With the exception of the first year, the reed beds consistently removed over 96% of the influent P load, with TP concentrations being reduced from 0.5 mg/L to generally less than 0.005 mg/L, over the range of loading rates and seasons studied. An adaptation period was apparent during the first year of operation (Period 1), with TP reductions of 91% and 84% for the High and Low Loaded Beds respectively. Plant uptake accounted for greater than 75% of the P removed during the first year, and was equally distributed between above and below-ground biomass. During the second year, uptake of P by below-ground biomass declined, accounting for only 10% of the P removed. The majority of below-ground biomass occurred in the top 20 cm of the substrate. Three seasonal stages were identified in the uptake and cycling of P by P. australis during the second and third years of growth. A period of rapid above-ground growth and uptake occurred during spring fuelled partly by P reserves accumulated in rhizomes during the previous year. During summer, uptake by above-ground biomass was governed by the influent P loading rate, while the amount of P held in below-ground biomass remained relatively stable. During autumn and winter P appeared to be translocated from senescent shoots to rhizome reserves. It is therefore recommended that above-ground biomass be harvested at the end of summer to prevent P from being redistributed to rhizomes. Gravel fixation accounted for less than 12% of the P removed in the first year of operation, but increased in importance during the second year, accounting for approximately 30% of P removed. A highly significant correlation existed between the influent P loading rate and P fixation by the gravel. The weakly-bound P fraction was the dominant form of P bound by the gravel. The bottom 30 cm of the substrate became the major site for gravel fixation after the first year, possibly due to stripping of weakly-bound P by the abundant reed roots in the top 20 cm of the reed bed. The two HCl extracts used in the sequential extraction yielded unreliable results due to dissolution of large amounts of P from the internal structure of the basaltic gravel. Accumulation of P in the detritus/microbiota/other compartment increased after the first year to become one of the most important P removal processes, probably consisting mainly of leaf litter and slowly accreted organic sediments.

References


