

## Effect of external organic matter on nutrient removal and growth of *Phragmites australis* in a laboratory-scale subsurface-flow treatment wetland

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**Abstract** Coconut dust, which is used intensively in horticultural applications, was tested as an external organic additive in a series of laboratory-scale subsurface-flow constructed wetlands planted with *Phragmites australis*. The systems were fed with a mixture of  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, and SRP in tap water to simulate high nutrient loads. In the absence of plants, TN removal efficiency was 66%, and the efficiency increased to >80% in the microcosm wetlands. TN and  $\text{NO}_3^-$  removal efficiencies were marginally increased by coconut-dust treatment in comparison with sand-bed microcosms. Analysis by ANOVA showed that the TN removal from a coconut dust-supplemented sand-bed microcosm was significantly different from a sand-bed microcosm ( $0.0437 < p < 0.05$ ). All the systems showed an equal capacity to treat  $\text{NH}_4^+$  nitrogen under low influent concentration levels. Phosphorus removal efficiencies were >98% in all three systems, and a difference between planted and unplanted systems was not observed. Shoot height and shoot densities of *P. australis* grown in the coconut dust-supplemented medium were significantly higher than those grown in the sand-bed medium. The difference in *P. australis* growth in response to the coconut dust addition revealed that the added material has the potential to create favourable conditions for plant growth.

**Keywords** ANOVA model; coconut dust; denitrification; organic carbon; phosphorus sorption

### Introduction

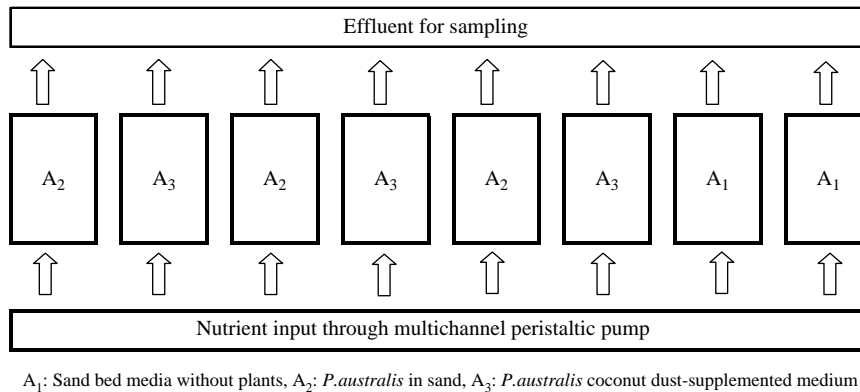
Constructed wetlands have been shown to have the capacity to treat secondary wastewater. The fundamental biogeochemical processes that enable constructed wetlands to treat wastewaters, and the roles of microbes and vegetation in the treatment process, have been widely studied (Kadlec and Knight, 1996). Secondary wastewaters are characterised by high quantities of nutrients that are readily available for biological uptake. Nitrogen and phosphorus are among the nutrients that have to be effectively reduced to achieve water quality standards appropriate for discharge into natural water bodies. Among the possible nitrogen removal mechanisms in wetlands are plant uptake and denitrification (Yang *et al.*, 2001; Hunt *et al.*, 2003). Denitrification is believed to be the dominant long-term mechanism, particularly when the nitrate-nitrogen loading rates are high (Lin *et al.*, 2002), although ammonia volatilisation, sedimentation and filtration of particle-bound nitrogen are some of the other mechanisms used in exclusive conditions (Davidsson and Stahl, 2000). Unlike the conventional biological denitrification processes in reactors, which involve maintaining a requisite anoxic condition, wetland denitrification occurs in anoxic zones in bed media. Wetland-denitrifying microbes potentially use plant productivity, either from biomass or root release, as the source of energy and carbon to fuel denitrification (Hunt *et al.*, 2003). Moreover, Davidsson and Stahl (2000) suggested that denitrification could be limited by insufficient oxygen for nitrification when the receiving waters

contain high ammonium and organic nitrogen concentrations. Studies have also been conducted to investigate the optimum carbon allocation for efficient denitrification. In most cases, readily soluble carbon sources such as glucose (Davidsson and Stahl, 2000; Hunt *et al.*, 2003) and fructose (Lin *et al.*, 2002) have been used to introduce external carbon. Phosphorus removal, on the other hand, is mainly governed by an abiotic geochemical process in which soluble phosphorus is adsorbed to or precipitated by insoluble anions such as Fe, Al or Ca present in the bed medium (McGechan and Lewis, 2002). Materials used in bed construction have shown a wide range of absorbance capacities. When wetlands are constructed with particular attention to phosphorus removal, high capacity materials such as Lockport dolomite, Queenston shale, Fonthill sand (Pant *et al.*, 2001), bauxite, limestone, zeolite and fly ash (Drizo *et al.*, 1999) are used. However, all these materials lose adsorptive capacity when the material used in the bed medium is saturated. In such cases, plant absorption should also be taken into account in the later stages (Drizo *et al.*, 2000). This reveals that plants play an important role even in the phosphorus-removal process. The common reed, *Phragmites australis* (Cav.) Trin.ex Steud., is a widespread, highly productive, semi-aquatic plant that has been studied widely for its usefulness in aquatic ecosystems. The nutrient removal capacity of *P. australis* has been studied in detail, and this species is widely used in large-scale constructed wetland systems (Drizo *et al.*, 2000; Fraser *et al.*, 2004).

Coconut dust, a by-product from coconut processing industries in tropical regions, is widely used for horticultural purposes due to its constructive characteristics. Some of the interesting properties of coconut dust are its high water absorption capacity, decay resistance (lignin 425–475 g kg<sup>-1</sup>, cellulose 304–324 g kg<sup>-1</sup>), light weight and absence of phytotoxicity. With carbon/nitrogen ratios of 75/186 (trace amounts of water-soluble mineral nitrogen) and trace amounts of other essential plant nutrients, the nutritional value of coconut dust is negligible (Abad *et al.*, 2001). The use of coconut dust as a soil additive in agriculture is wide; coconut dust is used as a peat substitute for containerised ornamental plants (Abad *et al.*, 2001) and in field crop production. However, the use of coconut dust in constructed wetlands as a peat substitute or as an organic carbon supplement has not been studied widely. In this study, we hypothesised that coconut dust added to bed media could change the overall performance of constructed wetlands. The objectives were to study the performance of *P. australis* in a coconut dust-supplemented sand-bed microcosm and to investigate the changes in nutrient removal mechanisms.

### Material and methods

Eight laboratory-scale subsurface-flow constructed wetland tanks were set up in a greenhouse. Each tank had a volume of 160 L and was 65 cm long, 45 cm wide and 55 cm high. It was separated into three compartments, inlet, substratum and outlet zones, by two orifice baffles. The inlet and outlet compartments were filled with gravel 0.5–2.0 cm in size in order to produce a uniform flow distribution. The substratum was filled with washed river sand to a depth of 50 cm. The volume of the material in each tank was 145 ± 3 L with a pore space of approximately 30 L. Commercially available dry coconut dust (Irisohyama Inc.) was uniformly mixed with the substratum sand medium in three tanks. The sand/coconut dust ratio by weight was approximately 100:1. Three wetland system treatments were installed: sand beds without vegetation (A<sub>1</sub>), sand beds with vegetation (A<sub>2</sub>) and mixed coconut dust–sand beds with vegetation (A<sub>3</sub>), as shown in Figure 1. Rhizomes of *P. australis* were collected from a microcosm previously grown in a greenhouse and washed with tap water to remove debris and dead parts. Dead above-ground shoots were cut off 10 cm from the base. Then the colony was divided into individual rhizomes (15 ± 5 cm length). Each microcosm was planted with rhizomatous



**Figure 1** Schematic diagram of experimental tank setup

cuttings up to  $333 \text{ g m}^{-2}$ . At planting, a few samples of rhizomatous cuttings were dried to a constant weight in an oven at  $80^\circ\text{C}$  in order to estimate the dry weight.

The influent for all treatment tanks was a synthetic nutrient solution prepared by mixing tap water with two types of commercial liquid fertiliser (Shin-highspeedAX and MinerapuB, Sumitomo Chemical Co.) containing 7% (w/w)  $\text{NO}_3^-$ -N and 3% (w/w) phosphoric acid. The final solution contained  $10 \text{ mg L}^{-1}$  Ortho-P,  $10 \text{ mg L}^{-1}$  TN, and trace amounts of essential plant nutrients. A 200-L synthetic nutrient solution container was connected to each treatment tank via a multichannel peristaltic pump (Model 7553-70, Cole-Palmer). The pump was set to operate four hours a day at a constant flow rate of  $1.5 \text{ L h}^{-1}$ . With this arrangement,  $6 \text{ L day}^{-1}$  of nutrient solution passed through each tank with a residence time of five days. In order to avoid depletion of nutrients, the nutrient solution was replaced every four days.

Based on several previous studies (Drizo *et al.*, 2000; Lin *et al.*, 2002), nutrient analysis started after the first growing season (July 2004 to March 2005). From the 40th week, wastewater samples were taken from the influent nutrient solution container and effluent points of each tank, respectively, at 14-day intervals. During the sampling, additional *in situ* measurements of pH, conductivity (EC), dissolved oxygen (DO), turbidity, temperature (T), total dissolved solids (TDS) and oxygen reduction potential (ORP) were carried out using a portable water quality analyser (Model U-20XD, Horiba Ltd.). Water samples were immediately brought to the laboratory and kept at  $4^\circ\text{C}$  until analysis, which occurred within 48 h of sampling. The samples were analysed for the following parameters: total nitrogen (TN), ammonium nitrogen ( $\text{NH}_4^+$ -N), nitrate nitrogen ( $\text{NO}_3^-$ -N) and soluble reactive phosphorus (SRP, i.e.  $\text{H}_3\text{PO}_4 + \text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-} + \text{PO}_4^{3-}$ ). Soluble reactive phosphorus was determined using a spectrophotometer (Shimadzu UV mini 1240) according to the standard ammonium molybdate method. All other chemical analytical methods were performed according to the standard methods in the Examination of Water and Wastewater (APHA, AWWA, and WEF, 1998). Every month, (1) the live/dead status, (2) number of living shoots, (3) height of all living shoots, (4) diameter of all living shoots (average of three measurements 5 cm above ground level) and (5) number of dead and live leaves in each shoot were recorded for each tank microcosm. Two automatic temperature recorders (TR-71U, TECPEL Co., Ltd) were set 5 cm below the media surface in two randomly selected A<sub>2</sub> and A<sub>3</sub> tanks. Another TR-72U (TECPEL Co., Ltd) automatic temperature and humidity recorder was set inside the greenhouse to monitor the microenvironment conditions. The photosynthetic radiation inside and immediately outside the greenhouse was measured monthly in order to estimate radiation losses inside. All statistical tests were performed using SigmaStat statistical software (Systat Software Inc.). In cases of nutrient

removal, statistical significance was defined as  $p < 0.01$ ; the significance level of  $p < 0.05$  was selected for the plant-growth parameter analysis. A one-way ANOVA was used to determine the significance of vegetation and media type on nutrient removal. Tukey's LSD was applied to test for significance between treatment means.

## Results and discussion

A number of baseline measurements were conducted during the experiment. Air temperature, relative humidity and photosynthetically active radiation (PAR) in the greenhouse were compared with available meteorological data around the experimental site. The average daytime temperature during the summer inside the greenhouse was 4 °C higher than that of the immediate outside, while relative humidity was not significantly different from that immediately outside. The PAR on an average summer day was  $732.9 \pm 321.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  inside the greenhouse, which was 29% less than that immediately outside. The measured values of photosynthetic activity of *P. australis* leaves during the summer in the field and in the greenhouse were  $17.54 \pm 3.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $12.41 \pm 4.65 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively.

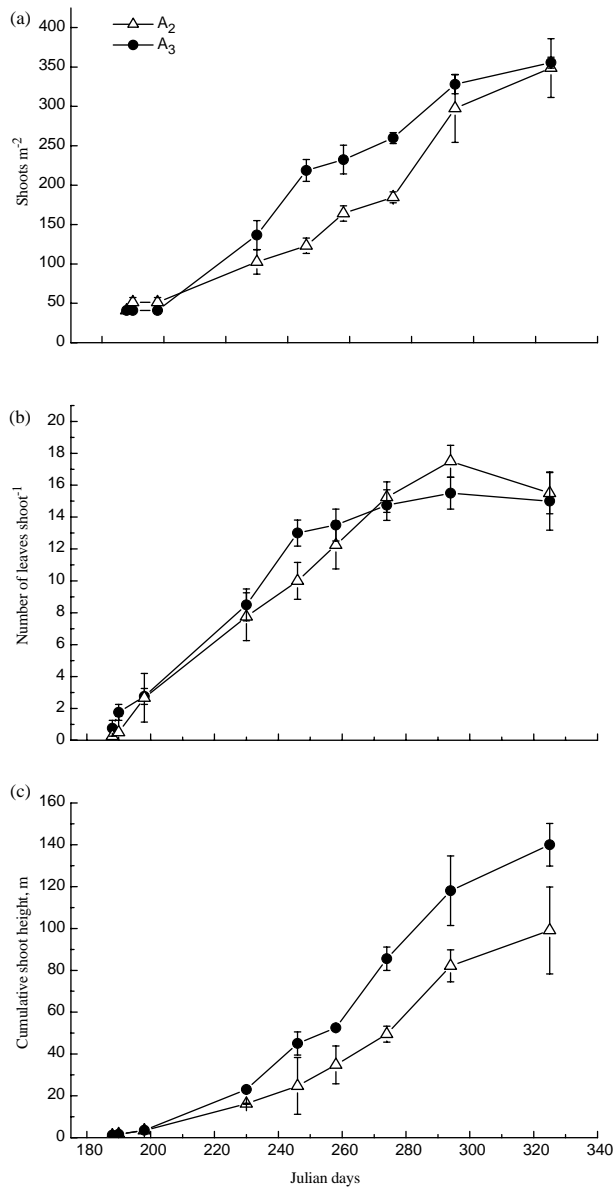
### Growth of *P. australis*

The plant growth data were collected from May 2004 until senescence in late October 2004. Average measurements over time of shoot density, number of leaves per shoot (obtained by combining and averaging data from three replicates in each treatment) and cumulative shoot height of all shoots in each tank are shown in Figure 2. The average shoot heights were compared between two microcosms. *P. australis* grown in the sand-bed medium ( $A_2$ ) reached a maximum shoot height of  $1.25 \pm 0.28$  m at the beginning of senescence while that in the coconut dust-mixed medium microcosm ( $A_3$ ) reached a maximum height of  $1.47 \pm 0.29$  m. The average shoot diameters of microcosms  $A_2$  and  $A_3$  were small with average values of  $2.40 \pm 0.25$  mm and  $2.92 \pm 0.25$  mm, respectively, and the change in diameter over time was not statistically significant. The numbers of shoots produced in the two treatment microcosms during the middle part of the growing season (days 230–294) were significantly different ( $0.0382 < p < 0.05$ , Figure 2a). However, when the shoot densities of  $A_2$  and  $A_3$  microcosms reached the maximum values of  $348 \pm 37$  shoots  $\text{m}^{-2}$  and  $355 \pm 7$  shoots  $\text{m}^{-2}$ , respectively, the difference was not statistically significant. As shown in Figure 2b, the number of leaves produced by the two microcosms was not considerably different between treatments. These results imply that the initial shoot growth of *P. australis* in microcosm  $A_3$  was slightly better than that in microcosm  $A_2$  at any given time.

### Nutrient removal efficiency

Table 1 illustrates the qualities of influent and effluent waters during the study period. The system operated with an influent concentration of  $7.37 \pm 0.13$  mg  $\text{NO}_3^- \text{-NL}^{-1}$  and  $3.31 \pm 0.29$  mg  $\text{NH}_4^+ \text{-NL}^{-1}$  ( $10.83 \pm 1.08$  mg TN  $\text{L}^{-1}$ ), which was equal to the surface loading rate of  $151.13 \pm 2.74$  mg  $\text{NO}_3^- \text{-N m}^{-2} \text{ day}^{-1}$  and  $67.89 \pm 6.02$  mg  $\text{NH}_4^+ \text{-N m}^{-2} \text{ day}^{-1}$  ( $222.10 \pm 22.16$  mg TN  $\text{m}^{-2} \text{ day}^{-1}$ ). The total effluent nitrogen concentrations in the  $A_1$ ,  $A_2$  and  $A_3$  systems were reduced by 66, 81 and 89%, respectively.

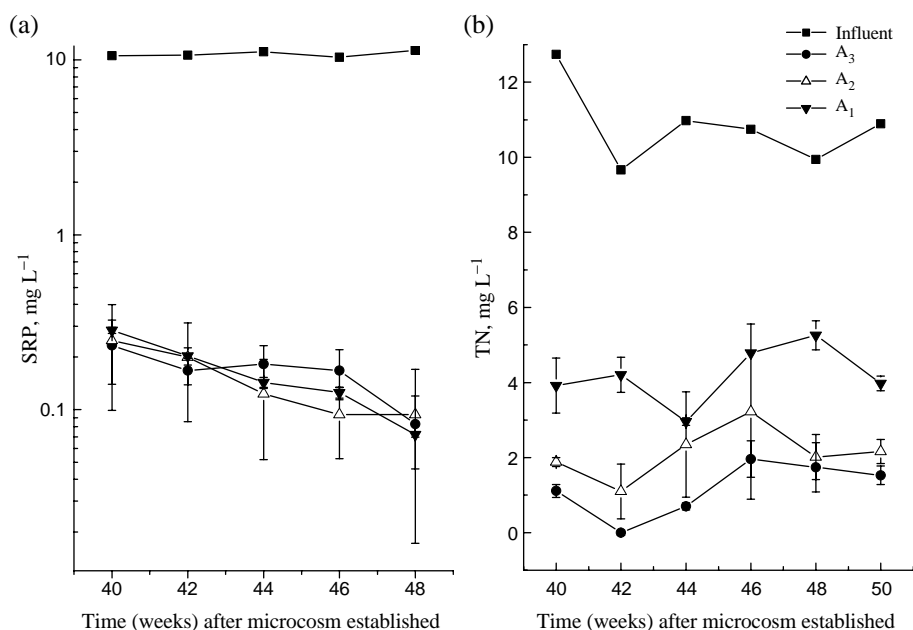
A comparison of the amount of TN removed by the three treatments across sampling dates is shown in Figure 3. To test the effect of coconut dust supplementation on TN removal, one-way ANOVA was performed. Each treatment had three replicates, and the test was performed on absolute concentration values. Results of the ANOVA showed a significant difference between treatments  $A_3$  and  $A_2$  ( $0.0437 < p < 0.05$ ), but the average variation over time was large in both systems, resulting in a failure to achieve the



**Figure 2** Changes in growth of *P. australis*: (a) shoot density; (b) number of leaves per shoot; (c) cumulative shoot height in sand bed medium (A<sub>2</sub>) and coconut dust-supplemented sand bed medium (A<sub>3</sub>)

**Table 1** Quality of influent and effluent waters ( $n = 6$ ,  $\pm$  = standard deviation)

Parameter	Influent	Effluent of microcosm wetland		
		A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>
pH	6.85 ± 0.3	7.47 ± 0	7.36 ± 0.05	7.33 ± 0.09
EC, mS cm <sup>-1</sup>	78.1 ± 0.8	46.8 ± 0.2	57.5 ± 1.4	62.2 ± 6.2
Turbidity, NTU	74 ± 1	25 ± 3	16 ± 6	19 ± 12
DO, mg L <sup>-1</sup>	5.6 ± 0.6	5.9 ± 0.1	5.2 ± 0.3	5.2 ± 0.4
T, °C	20.1 ± 0.4	23.1 ± 0.1	23.0 ± 0.2	23.1 ± 0.1
TDS, g L <sup>-1</sup>	0.50 ± 0.0	0.30 ± 0	0.37 ± 0.01	0.40 ± 0.04
ORP, mV	160 ± 8	132 ± 8	117 ± 30	118 ± 18



**Figure 3** Comparison of changes in SRP and TN concentrations in influent and effluent waters during the study period

0.01 level of significance. The total nitrogen removal efficiency of the unplanted tank system remained lower (61%) and was significantly smaller ( $0.002 < p < 0.01$ ) than those of planted systems.

The reason for the lower TN concentrations in planted systems might be that plant uptake was more significant than the TN removal mechanism in the unplanted system, although various processes involved in TN reduction, denitrification and nitrogen assimilation by biota could be the dominant process in this system because the system runs without organic nitrogen input (Lin *et al.*, 2002). The removal of  $\text{NO}_3^-$ -N followed a trend toward higher values in planted systems (91 and 95%) and lower efficiency in unplanted systems (38%). The one-way ANOVA test demonstrated a significant difference between planted and unplanted systems ( $1.3 \times 10^{-5} < p < 0.01$ ) for  $\text{NO}_3^-$ -N removal, but the difference between A<sub>2</sub> and A<sub>3</sub> was not significant. However, the average removal rate in the A<sub>3</sub> system was slightly higher than that in the A<sub>2</sub> system (Table 2). In general, nitrate nitrogen is removed by absorption by plants, use in microbial cell synthesis and denitrification. Development of denitrifying microbes is inhibited in the absence of carbon sources, which could be the reason for the low efficiency of unplanted systems (Yang *et al.*, 2001). Excretion of small amounts of organic substances into bed media by plants would not be adequate for a complete denitrification process, consequently creating a higher effluent nitrogen concentration in the A<sub>2</sub> than that in the A<sub>3</sub> microcosm.

**Table 2** Influent and effluent nutrient concentrations ( $n = 6$ ,  $\pm$  = standard deviation)

Parameter	Influent	Effluent of microcosm wetland		
		A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>
TN (mg L <sup>-1</sup> )	10.83 ± 1.08	5.19 ± 0.79	2.06 ± 0.80	1.17 ± 0.73
$\text{NO}_3^-$ -N (mg L <sup>-1</sup> )	7.37 ± 0.13	4.59 ± 0.57	0.64 ± 0.21	0.39 ± 0.17
$\text{NH}_4^+$ -N (mg L <sup>-1</sup> )	3.31 ± 0.29	< n.d.*	< n.d.	< n.d.
SRP (mg L <sup>-1</sup> )	10.87 ± 0.45	0.21 ± 0.08	0.19 ± 0.07	0.19 ± 0.05

As shown in Table 2, the effluent  $\text{NH}_4^+$ -N concentrations of all three systems were below the detectable limit throughout the study period, which could be due to the low concentrations of  $\text{NH}_4^+$ -N in influent waters. Successful removal of  $\text{NH}_4^+$ -N from planted systems was explained by the high affinity of *P. australis* for  $\text{NH}_4^+$ -N uptake (Tylova-Munzarova et al., 2005). However, the level of  $\text{NH}_4^+$ -N supplied seems to be inadequate owing to further reduction of  $\text{NO}_3^-$ -N in planted systems compared with the reduction in unplanted systems. Nitrification can occur only in oxic conditions (Kadlec and Knight, 1996). The highly porous (21%) bed medium had greater potential for oxygen transport to deeper layers, leaving space for a complete  $\text{NH}_4^+$ -N reduction in the unplanted system. Thus, the transport of oxygen from atmosphere to the bed medium through the plant was not elucidated by the results. A considerable amount of TN in effluent waters of planted systems is in the form of neither  $\text{NO}_3^-$ -N nor  $\text{NH}_4^+$ -N. The remaining nitrogen might be in the form of organic nitrogenous compounds because nitrite nitrogen was not detected in effluent samples. According to the effluent concentrations of nitrogen components, the reduction by the planted systems was two times that by the unplanted system. However, previous studies reported that the reduction of nitrogen components by planted systems could be 10 or more times that by unplanted systems (Drizo et al., 2000; Fraser et al., 2004). The reason for the low efficiency might be the low influent concentration in this study.

A comparison of SRP removal by the three treatments and across sampling dates is shown in Table 2 and Figure 3. All treatment systems showed a higher percentage removal of SRP (>98%) during the test period. SRP removal efficiency was marginally increased in planted systems compared with the unplanted system (Table 1). However, analysis by a one-way ANOVA model showed that there was no difference among any of the treatments for SRP removal. The high phosphorus removal efficiency values obtained established the fact that phosphorus removal is a geochemical process rather than one of biological assimilation.

## Conclusions

This study demonstrated that the nitrogen removal efficiency and plant productivity of subsurface flow treatment wetlands could vary with the addition of external organic carbon. The addition of coconut dust as an organic carbon to a sand-bed medium improved nitrogen removal in planted microcosms. The total nitrogen and nitrate nitrogen removal efficiencies were marginally higher in coconut dust-supplemented microcosms than in the unplanted control, possibly due to higher denitrification activity. However, planted and unplanted systems were equally effective in ammonium nitrogen removal under the low loading rate tested in this study. The characteristics of SRP removal showed that all systems were capable of achieving higher removal efficiencies during the early establishment period. The observed difference in *P. australis* growth in response to the addition of coconut dust revealed that the material has the potential to create favourable conditions for plant stand development. The addition of coconut dust to sand-bed medium microcosms would be a constructive measure for nitrogen removal enhancement by increasing the productivity of the microcosm.

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