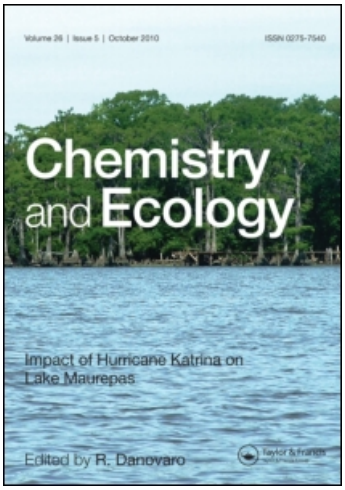

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Norio Tanaka[a]; A. K. Karunarathna[a]; K. B. S. N. Jindasa[a]
[a] Graduate School of Science and Engineering, Saitama University, Saitama, Japan

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Effect of coconut coir-pith supplement on nitrogen and phosphate removal in subsurface flow wetland microcosms

Norio Tanaka*, A.K. Karunarathna and K.B.S.N. Jindasa

Graduate School of Science and Engineering, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama 338-8570, Japan

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A series of sub surface flow wetland microcosms planted with and without *Phragmites australis* were established to remove nitrogen and phosphorus from synthetic wastewater at concentrations of 20–105 mg TN L^{-1} and 12–33 mg PO_4-P L^{-1}. Three planted microcosms were amended with coconut coir-pith aiming better performances for nitrogen and phosphorus removal. The coir-pith had a significant effect ($p < 0.05$) on N removal that was clearly demonstrated for high TN loading as urea, in which coir-pith amended microcosms showed most efficient TN removal (92–99%) followed by untreated planted microcosms (85–88%), and controls without plant (24–44%). Phosphorus removal was equally efficient (>99%) in planted and unplanted microcosms for first 120 days of experiment, then unplanted microcosms showed elevated levels of P in effluent waters that was higher than ($p < 0.05$) the planted microcosms. Although there was a small difference between two planted systems for P removal ($p > 0.05$), we conclude that the coir-pith supplement has no effect on P removal. The possible mechanisms for high N removal by coir-pith supplement supposed to be the enhancement of denitrification activity.

Keywords: coir-pith; constructed wetland; nitrogen; *Phragmites australis*; phosphorus

1. Introduction

Nitrogen and phosphorus are major nutrients in secondary and tertiary wastewaters that have to be reduced effectively to achieve water quality standards before discharging into natural watercourses and lakes [1]. Possible nitrogen (N) removal mechanisms in wetlands are microbiological process (ammonification, nitrification and denitrification), plant uptake and retention in wetland soil [2–4]. Among the N removal processes, denitrification is supposed to be the ultimate N removal process since the denitrification converts N in aqueous phase (NO_3-N) into gaseous N (N_2O and/or N_2) that are expelled into atmosphere. However, unlike the conventional biological denitrification processes in reactors, which involves controlling an anoxic condition, wetland denitrification occurs in anoxic zones in bed media. Wetland denitrifying microbes potentially use plant productivity, either from biomass (organic matter) or root release, as the source of energy and carbon to fuel denitrification [2]. However, root release may be insufficient and organic carbon

*Corresponding author. Email: n-tanaka@post.saitama-u.ac.jp

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limitations would prevail in newly constructed wetlands than older natural wetlands. Studies have been conducted in order to investigate the optimum carbon allocation for efficient denitrification in wetlands. In most cases, readily soluble carbon had been used to introduce external carbon and those wetlands showed significant increase in denitrification [5]. Conversely, incorporation of plant biomass in wetlands has also shown potential increase in denitrification [6]. However, except for soluble carbon sources and plant litter detritus, little is yet known about the effect of other carbon sources on N removal in wetlands.

Phosphorus (P) removal in wetlands is mainly occurred by geochemical process in which soluble P is adsorbed or precipitated by insoluble cations such as Fe, Al or Ca presence in the bed media [7]. However, all these mineral soils reduce the adsorptive capacity when the bed media saturated with P sorption. In such instances, minor processes like plant absorption and sedimentation of organic matter bound P should also be taken into account [8].

This study examined the possibility of coconut (*Cocos nucifera* L.) coir-pith (coir dust) to use as an organic matter supplement in subsurface flow (SSF) wetland microcosms. Coir-pith is known to be a by-product from coconut (*Cocos nucifera* L.) fiber processing industries in tropical coconut growing countries [9]. Emergent macrophyte *Phragmites australis* (Cav.) Trin.ex Steud., commonly found in natural and created wetlands [8,10], was selected as the plant component of this study. Series of SSF wetland microcosms were set up to investigate the effect of coir-pith supplement on wetland performances. The objectives of this study were to; (i) estimate the effect of coir-pith supplement on N removal, (ii) examine the possible changes in P removal due to coir-pith supplement.

2. Material and methods

2.1. *Microcosm wetlands preparation*

As illustrated in Figure 1, eight fiberglass tanks were modified to microcosm wetlands with sub-surface flow and placed in a green house.

Each tank was divided vertically into three apartments: inlet, middle stratum, and outlet, separated by two orifice baffles that made from (0.2 cm grid size) plastic mesh. Inlet and outlet regions were filled with 2 ± 1 cm size gravels in order to achieve high porosity and uniform flow. The weight and volume of the filled gravel and sand material were measured at the site and sub samples were dried in an oven at 120 °C to obtain the absolute dry weight. Coconut coir-pith bricks brought from a plant nursery store were wetted and dispersed into their natural size (<0.02 cm)

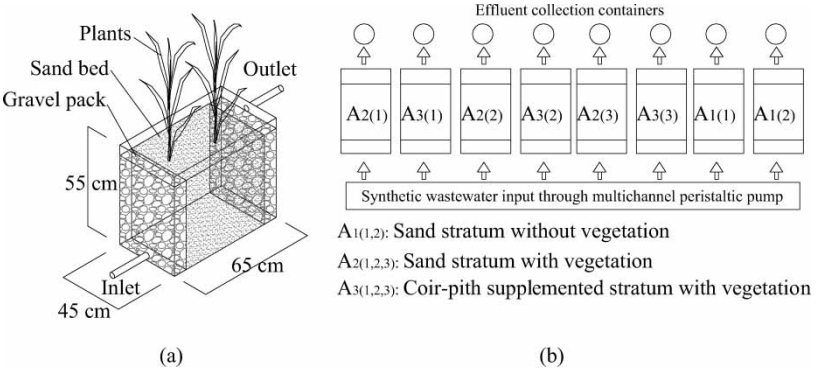

Figure 1. (a) Schematic diagram of a microcosm wetland and, (b) layout of the experiment. Microcosm A_2 and A_3 are planted with *P. australis*, and A_1 is unplanted.

and placed in an oven at 70 °C for 72 h to obtain dried coir-pith. The dried coir pith was mixed with sand in stratum region up to 2% of weight of sand in three microcosm wetlands.

P. australis plants that had been grown in a greenhouse during the previous year were uprooted, washed with tap water and debris and dead parts were removed. Six microcosm wetlands were planted with rhizomatous cuttings of about $313 \pm 7\ g\ m^{-2}$ (dry matter) in each tank. Then each microcosms wetland was filled up to field capacity with tap water and kept until shoot emergence in late April 2005. The nutrient addition began in early May 2005, at which time the plants were well established.

2.2. *System operation*

The experiment was separated into three phases (Phase 1 to 3) based on the wastewater loading rates and nutrient source. Synthetic wastewater that contains known amounts of NO_3-N, NH_4-N and PO_4-P were made mixing weighted amounts of fertilizer solution in tap waters. In addition, Urea ($CO(NH_2)_2$) was used as N source and Potassium Hydrogen Phosphate (K_2HPO_4) used as the P source of the Phase 3 of the study. Prepared synthetic wastewater was stored in a large container of 240 L, and replaced every three days by a new solution. The multi-channel peristaltic pump (Model 7553-70, Cole-Parmer) connecting the storage tank and each microcosm was operated 30 minutes period, 10 times a day at different pumping rates in different phases supplying a total of 7–8 l day per each microcosm wetland. The daily effluent collection was measured and discharged every evening, if the sampling was not done on particular day.

2.3. *Water quality monitoring*

Influent and effluent water samples were collected to 250 ml plastic bottles and immediately transfer to the analytical laboratory. The water qualities (pH, conductivity, Dissolved Oxygen, Total Dissolved Solids, Oxygen Reduction Potential, and Temperature) of samples were measured in situ by using a portable water quality analyzer (Model U-20XD, Horiba Ltd.). At the laboratory, samples were filtered (size 0.45 μm) and immediately analyzed for total nitrogen (TN), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), and phosphate phosphorus (PO_4-P). All the analytical methods were referenced by the Standard Methods [11].

2.4. *Estimation of nutrient assimilation by plants and soil nutrient content*

At the end of experiment (day 233), all the plants were removed from each tank and measured the wet and oven dried weight of plant organs that were separated into leaves, shoots, rhizomes, buds and roots. The Cation Exchange Capacity (CEC) of the sand that was used in the experiment was determined by ammonium replacement method.

2.5. *Experimental design and statistical analysis*

According to the types of stratum media and conditions with or without vegetation, three treatment wetland systems were installed; sand stratum without vegetation (A_1) and with vegetation (A_2) and coconut coir-pith supplemented stratum with vegetation (A_3) as shown in Figure 1 (b). In order to study the difference between three systems for particular nutrient removal for a particular day, absolute values of effluent nutrients were compared by using a one-way ANOVA model. Tukey's least significant difference (LSD) was applied to test for significance between treatment means. All the statistical analysis was performed by using SigmaStat 3.11 [12] software.

3. Results and discussion

3.1. *Effect of coir-pith supplement on N removal*

As shown in Table 1, during the Phase 1 of the study TN removal efficiencies of all vegetated tanks were more than that of unvegetated sand bed systems (A_1). Although the influent TN concentration increased about double in the Phase 2, TN removal efficiencies of A_3 microcosms remained high compared to A_2 microcosms. The small amounts of NO_3-N and NH_4-N which were presented in the influent waters (Table 1) may be derived by decomposition of Urea in the influent storage container.

Table 1. Average levels of nutrient, water quality parameters and removal efficiencies of effluent of microcosms over three phases of study.

Item	Units	Influent	Effluent A_1	Removal A_1 (%)	Effluent A_2	Removal A_2 (%)	Effluent A_3	Removal A_3 (%)
					Phase 1			
TN	mg L^{-1}	19.5±3.5	10.8±5.6	19–29	1.9 ± 0.8	88–90	1.2 ± 0.7	92–94
	mg m^{-2} d^{-1}	548 ± 30	427 ± 51	78	477 ± 23	87	496 ± 29	91
NO_3-N	mg L^{-1}	6.8 ± 4.0	9.1 ± 1.0	> 0–15	0.6 ± 0.2	91–93	0.4 ± 0.1	94–95
NH_4-N	mg L^{-1}	16.2 ± 2.5	<0.1	>99	<0.1	>99	<0.1	>99
PO_4-P	mg L^{-1}	11.8 ± 0.4	<0.1	>98	<0.1	>98	0.1 ± 0.1	>98
	mg m^{-2} d^{-1}	283 ± 11	279 ± 12	98	281 ± 11	99	278 ± 12	98
pH	–	6.8 ± 0.1	7.4 ± 0.1	–	7.0 ± 0.5	–	7.3 ± 0.1	–
Conductivity	mS sec^{-1}	48 ± 1.2	45 ± 1.2	–	51 ± 2.0	–	60 ± 3.0	–
DO	mg L^{-1}	6.0 ± 0.1	6.1 ± 0.4	–	5.1 ± 0.1	–	5.0 ± 0.1	–
TDS	mg L^{-1}	315 ± 7	295 ± 7	–	310 ± 17	–	383 ± 15	–
ORP	mV	174 ± 8.5	144 ± 43.6	–	139 ± 5.8	–	98 ± 3.2	–
					Phase 2			
TN	mg L^{-1}	43.2 ± 10.4	32.7 ± 21.7	−24–29	4.9 ± 3.7	78–89	2.8 ± 1.4	96–98
	mg m^{-2} d^{-1}	1212 ± 65	223 ± 213	18	978 ± 100	81	1094 ± 58	90
NO_3-N	mg L^{-1}	15.3 ± 7.4	7.7 ± 5.6	41–49	0.5 ± 0.4	96–97	0.3 ± 0.1	98–99
NH_4-N	mg L^{-1}	32.4 ± 9.2	4.5 ± 6.0	75–86	0.2 ± 0.3	>99	<0.1	>99
PO_4-P	mg L^{-1}	20.4 ± 5.7	11.7 ± 3.2	42–43	1.7 ± 0.3	91–92	1 ± 1.2	91–95
	mg m^{-2} d^{-1}	522 ± 116	453 ± 103	87	461 ± 106	88	461 ± 117	88
pH	–	6.8 ± 0.4	7.5 ± 0	–	7.4 ± 0	–	7.3 ± 0.1	–
Conductivity	mS sec^{-1}	78 ± 18	47 ± 0	–	58 ± 1	–	62 ± 6	–
DO	mg L^{-1}	5.9 ± 0.3	5.9 ± 0.1	–	5.4 ± 0.1	–	5.0 ± 0.3	–
TDS	mg L^{-1}	510 ± 122	298 ± 4	–	370 ± 10	–	397 ± 38	–
ORP	mV	170 ± 24	132 ± 8	–	117 ± 44	–	112 ± 13	–
					Phase 3			
TN	mg L^{-1}	105.1 ± 13.9	59.5 ± 15.6	24–44	33.4 ± 9.6	85–88	7.8 ± 6.5	92–99
	mg m^{-2} d^{-1}	3031 ± 199	1147 ± 330	38	2062 ± 310	68	2660 ± 262	88
NO_3-N	mg L^{-1}	3.4 ± 1.5	12.9 ± 8.8	<0	16.8 ± 8.1	<0	3.4 ± 1.5	0–2
NH_4-N	mg L^{-1}	3.3 ± 0.9	20.5 ± 2.9	<0	10.3 ± 5.6	<0	2.1 ± 2.2	<0–36
PO_4-P	mg L^{-1}	33.2 ± 6.2	24.1 ± 2.7	27–32	9.0 ± 2.7	70–73	7.3 ± 2.0	76–79
	mg m^{-2} d^{-1}	813 ± 157	407 ± 230	50	652 ± 133	80	673 ± 74	83
pH	–	6.7 ± 0.2	7.1 ± 0.1	–	7.0 ± 0.2	–	6.0 ± 1.7	–
Conductivity	mS sec^{-1}	65 ± 3	71 ± 1	–	79 ± 0	–	76 ± 2	–
DO	mg L^{-1}	5.7 ± 0.7	6.7 ± 0.2	–	7.0 ± 0.1	–	5.5 ± 0.3	–
TDS	mg L^{-1}	420 ± 17	455 ± 7	–	505 ± 7	–	487 ± 15	–
ORP	mV	179 ± 12	158 ± 1	–	155 ± 4	–	122 ± 10	–

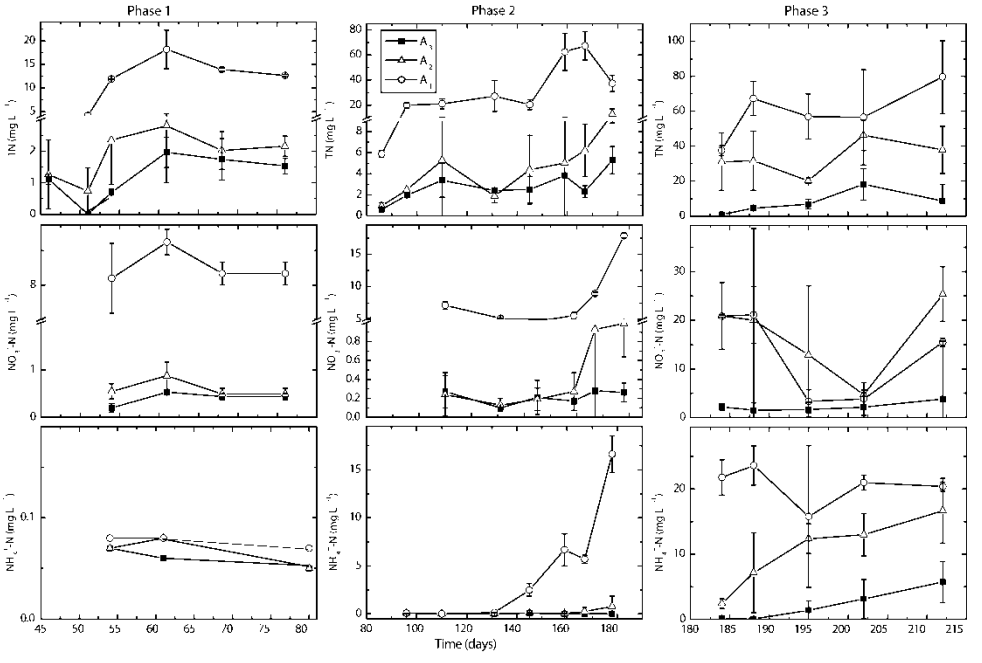

Figure 2. The mean TN, NO_3-N, and NH_4-N of effluents of three microcosm wetlands; A_1, A_2 and A_3 over three phase of the study. Vertical bars represent the standard deviation.

The mass removal rates of TN were calculated by measuring the outflow volume and concentrations, and are presented in Table 1. During the Phase 1 and 2 of the study, the levels of NO_3-N in effluents of A_2 and A_3 microcosms remained well below the influent concentrations, but unplanted microcosm showed high concentrations of NO_3–N in all phases of the study (Figure 2). During Phase 3 of the study, the mean NO_3-N levels in effluent waters of A_2 and A_1 were higher than that in the influents ($p < 0.05$) but the A_3 microcosms had comparatively low levels of NO_3-N in effluents (Table 1).

During the high TN loading stage at Phase 3, more than 30% of TN in effluent waters of A_1 microcosms occurred as NH_4-N (Figure 2), similarly A_2 microcosm also showed comparatively high levels of NH_4-N in effluents.

A portion of the TN levels (in Phase 1 to 2) determined in the effluents of planted and unplanted microcosms was neither NH_4-N nor NO_x-N, but possibly organic residual nitrogen. The organic residual N portion in effluents of A_1, A_2 and A_3 microcosms were; 19%, 48% and 33% of TN detected. Huett et al. [13] reported that the amount of residual dissolved organic fraction in effluents of planted bed increased >50% when the influent TN concentration was 1.35 to 18 mg L^{-1}. Thus, it is reasonable to assume that the unplanted systems accommodate comparatively low microbial activities, consequently, produce fewer amounts of dissolved organic N.

During the Phase 1 and 2 of the study, NH_4-N had been removed efficiently in all microcosms until 120 days of experiment. A portion of applied NH_4-N might remain in the sand bed of microcosms since the sand used in this study was capable of adsorbing considerable amount of NH_4-N by cation exchange (40–60 mg kg^{-1}). However, total amounts of NH_4-N applied to the system for 120 days (23.9 g) exceeded the amount that potentially retained in the bed media by cation exchange (9.6 ± 0.1 g of NH_4-N). Therefore, substantial amount of loaded NH_4-N could have been converted into NO_3-N through nitrification yet in unplanted microcosms. Although the unplanted microcosms were not supplied with organic matter or microbial inoculations, it is reasonable to expect a reasonable development of microbial community since we observed algal

formations. However, the potential of unplanted systems to reduce NO_3-N is lower ($p < 0.05$) than that of planted systems. The presence of plant potentially enhances the key nitrogen removal processes by uptaking NO_3–N and NH_4-N [4], augmentation of oxygen transport [1], and root release of organic matter that enhances the denitrification [3]. The estimated biomass assimilation of N and retention in bed substrate has not revealed a significant difference between planted microcosms. If the other minor nitrogen processes (NH_4-N stripping, microbial assimilation, burial through litter falling) occurred in wetlands is considered similar and negligible in both vegetated systems, the possible reason for higher TN removal in coir-pith supplemented microcosms is the enhancement of microbial activities [14]. Since the net removal of N from the system is exclusively through denitrification, it is assumed that the major difference between two planted microcosms occurred by different denitrification potentials. Accordingly, A_1, A_2 and A_3 microcosms showed 38%, 49% and 61% of overall N mass losses through denitrification and other minor processes throughout the period of this study, hence, A_3 microcosms had treated about 12% of additional N masses compared to other planted systems.

Coir-pith is amorphous fine dust like organic matter that likely to have favorable anaerobic microsites for denitrifies [15]. This hypothetical estimation of denitrification amounts are comparable with the data reported by Matheson [16] in which harvested or un-harvested plant-inhabited microcosms were able to denitrify 61–63% of added ^{15}N-NO_3, and the rest was plant assimilated (24–26%) and reduced to ammonia (11–15%).

It has demonstrated that the mineral soils or gravel used as substrate in constructed wetlands can frequently limit denitrification due to low levels of organic matter [17], the limitation is more common in newly built constructed wetlands than in older natural wetlands [18]. Hence, incorporation of coir-pith in substrate would increase the organic matter content in such instances.

3.2. *Effect of coir-pith supplement on P removal*

PO_4-P removal efficiencies were >98% in all microcosms wetlands from the start to end of Phase 1 and was not different between treatments.

Although the three microcosm systems were equally effective at the start of Phase 2, unplanted microcosms showed a reduced efficiency ($p < 0.05$) for PO_4-P removal at the end of Phase 2 (Figure 3). Though the A_3 microcosms showed slightly better removal efficiency than the

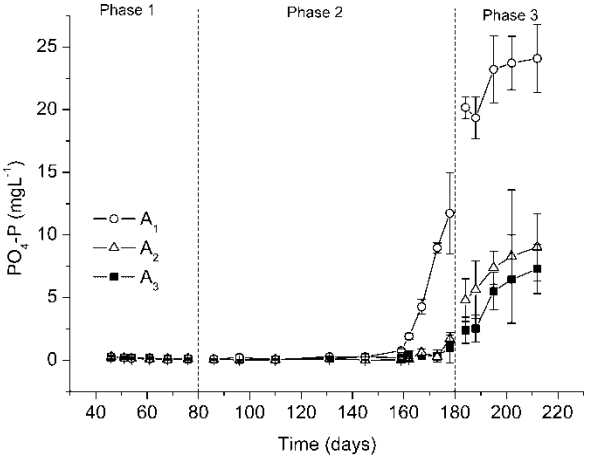

Figure 3. The mean PO_4-P of effluents of the microcosm A_1, A_2 and A_3 wetlands over time for entire study period. Vertical bars represent the standard deviation.

microcosm A_2, the difference was not significant ($p > 0.05$). The area-based mass removal rates of PO_4-P were calculated and presented in Table 1.

The saturation of sand bed media with adsorbed phosphorus is revealed by the gradual decrease of the removal capacity in all wetland microcosms. However, the planted systems showed higher phosphorus removal capacity, even at the end of the Phase 3 (>73%). Principally, the higher removal rates of P in planted tanks occurred by the increase of uptake by the plant community. However, the difference between two planted systems for P removal remained the same and this indicated that the effect of coir-pith on P removal process is not significant.

3.3. *Nutrient assimilation by plants and retention in soil*

Although the coir pith supplement showed marginally higher values of phenological characteristics in few growth stages, the overall difference was not significant between two microcosms [19]. Thus, *P. australis* perform equally or slightly better in coir pith amended microcosms [19].

The plant assimilation of N and P were estimated based on last (day 233) biomass samples that were collected during the senescence, onset of winter. The Nitrogen contents of plant organs were adapted from the records by Hocking [20]. Based on the estimations, *P. australis* grown in A_2 and A_3 microcosms have potentially assimilated 80.4 g N m^{-2} and 70.0 g N m^{-2}, respectively.

The analysis of bed substrate material for N retention at the end of experiment (day 233) showed that the three microcosms wetlands A_1, A_2, and A_3 had retained; 32.8 ± 0.1 g N m^{-2}, 33.5 ± 0.8 g N m^{-2}, and 34.7 ± 0.9 g N m^{-2}, respectively.

4. Conclusions

In this study, coir-pith demonstrated a significant potential to enhance nitrogen removal. The planted systems supplemented with coir-pith showed significantly low effluent N levels with a range of nitrogen loading from two different N sources. Although the difference was not significantly revealed under low N loading, there was a marked difference under high N loading. The unplanted sand bed SSF wetland showed only trivial potential to remove N in all tested N applications. The potential effect of coir-pith on N removal enhancement would be the supply of substrate organic carbon to fuel denitrification and creation of anaerobic microsites to accommodate denitrification bacteria. The addition of coir-pith into SSF wetland microcosms did not show effect on the P removal process. However, the vegetation showed a significant effect on P removal in comparison with unplanted microcosms. Hence, the phosphorus saturation of SSF bed media could be extended by maintaining the plant growth. This study revealed the potential use of coir-pith in SSF wetlands to enhance denitrification.

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