Influence of substrates on nutrient removal performance of organic channel barriers in drainage ditches

Feng Liu a,b, Yi Wang a,b, Runlin Xiao a,b, Jinshui Wu a,b,s, Yong Li a,b, Shunan Zhang a,c, Di Wang a,b, Hongfang Li a,c, Liang Chen a,b

a Key Laboratory of Agro-ecological Processes in Subtropical Regions, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, PR China
b Changsha Research Station for Agricultural & Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, PR China
c Graduate University of Chinese Academy of Sciences, Beijing 100039, PR China

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S U M M A R Y
Pilot-scale field trials were performed to investigate the nutrient removal capacity of organic channel barriers (OCBs) with the objective of developing innovative technology for effectively reducing nutrient transport in simulated drainage ditches. The performance comparison of three OCBs, which were composed of rice straw (RS), pine sawdust (PD), and activated carbon with <1 mm quartz sand (AC), indicated that the RS OCBs produced the highest mean removal rate of 73% for ammonium-N (NH₄-N) and 96% for nitrate-N (NO₃-N). Performance of the RS OCBs with RS application rates at 0, 45, 75, and 120 kg dry weight showed the quantity of RS had a significant positive correlation with NH₄-N removal (r = 0.754, p < 0.01) and NO₃-N removal (r = 0.969, p < 0.01), but an insignificant negative correlation with phosphate-P (PO₄-P) removal (r = -0.492, p = 0.104). Release of carbon (C) from the RS OCBs primarily occurred during the first three weeks. Total C loss accounted for less than 5% of the initial C mass. These findings indicated that RS OCBs can be used to construct a low-cost treatment system that requires minimal maintenance and can be easily integrated into drainage ditches to reduce nutrient export from agricultural areas.

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1. Introduction

In agroecosystems, a large proportion of nutrients applied for crop production are transported to aquatic ecosystems due to low fertilizer utilization efficiency. This transport has become a key factor in nitrogen (N) and phosphorus (P) overloading in natural water bodies around the world (e.g., Chatterjee, 2009; Galloway et al., 2003). Diffuse agricultural pollution also results in more difficulties in preventing water pollution (e.g., Conley et al., 2009; Merriam et al., 2002; Royer et al., 2006). The first national Chinese pollution census of 2007 listed agriculture as the main source of N and P pollution revealing that agriculture is responsible for 57% and 67% of the total national discharges of N and P, respectively (National Pollution Source Survey Staff, 2010). The control of agricultural pollution will be urgent in Chinese rural areas in the coming years (Qiu, 2011). Drainage ditches comprise the primary water conduits in agricultural lands; they also serve as the dominant pathways for nutrient transport from agricultural lands to downstream water bodies (Kröger et al., 2007). The use of drainage ditches to control nutrient loss from agricultural catchments would constitute a significant advancement. However, natural ditches and streams exhibit short-term nutrient retention times and low nutrient removal capacities (e.g., Merriam et al., 2002; Moore et al., 2010).

Therefore, a number of field practices, such as aquatic plant vegetation, water-control structures, in-stream wetlands, and bioreactors, have been implemented in drainage ditches to tackle diffuse pollution from agriculture (e.g., Hunt et al., 1999; Leu et al., 1998; Liu et al., 2013; Needelman et al., 2007; Robertson and Merkley, 2009; Woli et al., 2010).

The cost of building and operating an agricultural wastewater treatment system is an important factor in the selection of a treatment technique (Ruane et al., 2011). Denitrification bioreactor designs, including barrier, wall, bed, layer, filter, and trench types, are selected in polluted agricultural areas because they are
low-cost options that require minimal maintenance and are effective strategies for nitrate-N (NO$_3^-$–N) removal (Schipper et al., 2010). In bioreactors, organic substances act as external carbon (C) sources and electron donors to stimulate heterotrophic denitrification, which causes permanent N removal (e.g., Schmidt and Clark, 2012; Woli et al., 2010). Solid organic substrates are convenient low-cost fillers for bioreactors. Wood media, which exhibit a high permeability and a high C-to-N (C:N) ratio, have been extensively used in field trials. Denitrification bioreactors filled with wood media are capable of providing consistent long-term NO$_3^-$–N removal without C replenishment. In situ investigations have indicated that wood media annually consumes less than 5% of the initial C mass within bioreactors (e.g., Robertson et al., 2000; Robertson and Merkley, 2009; Schipper and Vojvodic-Vukovic, 2001; Schmidt and Clark, 2012).

In addition to wood media, other natural organic media, such as cornstalks, wheat, maize cobs, barley straw, and rice husks, are used as alternative C substances for bioreactors, as noted by Schipper et al. (2010). Small-scale laboratory experiments demonstrate that alternative C substrates provide higher NO$_3^-$–N removal capacities than wood media and can be regarded as efficient C sources that rapidly release bioavailable C for the denitrification bacteria (Greenan et al., 2006). However, bioreactors with alternative C substrates also require frequent C replenishment due to rapid C depletion during processing. For example, filter bioreactors filled with wheat straw exhibit a substantial C loss of 37.7% of the initial mass during a 140-days experimental period. The time limitation of the bioreactor's working life must also be considered (Saliling et al., 2007). Denitrification bioreactors show mean NO$_3^-$–N mass removal rates in the range of 0.62–12.7 g N m$^{-2}$ d$^{-1}$ (Schipper et al., 2010). However, the occurrence of N-limiting situations under field conditions frequently limits the N mass removal capacity of denitrification bioreactors, which is reflected by rapid N depletion in bioreactors (e.g., Elgood et al., 2010; Schmidt and Clark, 2012). Although filling bioreactors with alternative C substrates for enhanced NO$_3^-$–N removal has been successful, the suitability of rice straw (RS) as an organic substrate in bioreactors for NO$_3^-$–N removal remains unknown. In addition, the potential ability of bioreactors to remove dissolved ammonium-N (NH$_4^+$–N) and phosphate-P (PO$_4^{3-}$–P) has not been addressed (e.g., Park et al., 2009; Ruane et al., 2011).

In this study, a pilot-scale field trial was performed to investigate the nutrient removal capability of organic channel barriers (OCBs) constructed in simulated drainage ditches. Objectives of this study were to: (i) compare N removal rates for the RS OCB and OCBs filled with two alternate C substrates—pine sawdust (PD) and activated C with sand (AC), (ii) analyze the performance of the RS OCB at different RS application rates in terms of nutrient removal efficiency of the channels and effects of hydraulic retention time on NH$_4^+$–N removal, and (iii) evaluate the amount of C loss by the RS OCB.

2. Materials and methods

2.1. Study site

This study was conducted at the Changsha Research Station for Agricultural and Environmental Monitoring (CRSAEM), located in the Jinjing catchment in Hunan Province of the PR China. The catchment covers an area of 105 km$^2$ and has a typical subtropical climate. A small meteorological station (InteliMet Advantage, Dynamax Inc., USA) at the CRSAEM was used to monitor the maximum and minimum temperatures and the total rainfall, which were 32.1 °C, 10.9 °C, and 1,141 mm, respectively, during the study period of April 2012–August 2012. The monitoring data from 2010 to 2012 showed that the stream NH$_4^+$–N, NO$_3^-$–N, and PO$_4^{3-}$–P concentrations in the jinning catchment were in the range of 0.01–8.05, 0.12–4.46, and 0.01–0.55 mg L$^{-1}$, respectively (Li et al., 2014a,b). These data prompted us to select 5 mg L$^{-1}$ NH$_4^+$–N, 5 mg L$^{-1}$ NO$_3^-$–N, and 0.5 mg L$^{-1}$ PO$_4^{3-}$–P wastewater as influents for this study. In other way, mean base flow rate for a natural drainage ditch at the CRSAEM was 6.12 m$^3$ d$^{-1}$ in 2011. Therefore, this observed rate of approximate 6.0 m$^3$ d$^{-1}$ was used in the pilot-scale filed trial.

2.2. Set-up of the simulated drainage ditch system and the OCBs

The simulated drainage ditch system was constructed at the CRSAEM in 2011, which had three primary components: water supply unit, 20 ditch cells, and water distribution equipment. Through a polypropylene pipe (110 mm in diameter), the main water supply unit of a 100 m$^3$ tank can obtain adequate clean water with TN < 0.5 mg L$^{-1}$ and TP < 0.1 mg L$^{-1}$ from a reservoir surrounded by a hill. Artificial wastewater in the study was prepared in the 100 m$^3$ tank by adding the appropriate quantity of chemicals. Each of the 20 ditch cells were constructed with equivalent dimensions: 16 × 2.0 × 0.35 m (length × width × depth). Cells were placed in a paddy field after removal of topsoil (depths from 0 to 0.1 m). Water distribution equipment included the stainless-steel flumes with dimensions of 2.0 × 0.1 × 0.1 m and a set of polyvinyl chloride (PVC) connection pipes. A flume was placed horizontally in the inlet of each ditch cell with a platform that was 0.4 m higher than the bottom level of the ditch to evenly distribute influents (Fig. 1). A set of PVC pipes was used to deliver water from the tank to the flumes. A tap and a water meter with a measuring precision of 0.1 L were installed in front of each flume to regulate and monitor flow flux. Two pipes were placed in the baffle wall at the end of each ditch cell; they were used as outlets to maintain a 0.1 m water depth in each ditch cell during the study period. In addition, effluents from the simulated drainage ditch were collected and delivered to an adjacent wetland with an area of approximately 0.2 ha to prevent the release of artificial wastewater into the natural aquatic environment.

A schematic of the OCBs in the ditch cells is shown in Fig. 1. OCBs were placed in the back of the ditch cells and a 3-m distance from the outlets was maintained. Stainless steel frame was used to construct OCBs with a width of 2 m and a thickness slightly less than 0.1 m to ensure that all OCBs were submerged. Lengths of the OCBs were not equivalent and were dependent on the quantity of C substrates in the study OCBs.

2.3. Experimental materials

Air-dried RS was collected from a paddy field near the simulated drainage ditches. RS samples had a total moisture content of 5.3%, a total organic C content of 396 g kg$^{-1}$, and a C:N of 31.3. PD was purchased from a local sawmill, and the activated C was purchased from Longkai Instrument Company (Changsha, China). Artificial wastewater with 5 mg L$^{-1}$ NH$_4^+$–N, NO$_3^-$–N, and PO$_4^{3-}$–P were prepared by ammonium hydrogen carbonate (NH$_4$HCO$_3$, 95% purity), potassium nitrate (KNO$_3$, 96% purity), and potassium dihydrogen phosphate (KH$_2$PO$_4$, 95% purity), respectively, which were purchased from Changsha Minle Chemical Companies (Changsha, China).

2.4. OCB experiments to evaluate nutrient removal in the simulated drainage ditches

Two tests were performed to study nutrient removal by the OCBs. Test 1 compared NH$_4^+$–N removal and NO$_3^-$–N removal of the OCBs filled with three types of C substrates: RS, PD, and
activated C with <1 mm quartz sand (AC). Based on results of test 1, RS was selected as the preferable C substrate for constructing the OCBs. Test 2 primarily investigated performance of the OCBs with four RS application rates in removal of NH$_4^+$–N, NO$_3^–$–N, and PO$_4^{3–}$–P.

### 2.4.1. Test 1: A comparison of N removal for the OCBs filled with different C substrates

Three types of OCBs, which were filled with RS, PD, or AC, were constructed in triplicate in the simulated ditch cells. These OCBs contained 75 kg RS, 450 kg PD, or 500 kg AC (activated carbon and sand in a 1:4 weight ratio), which covered approximately 25%, 25%, and 10%, respectively, of the ditch bottom area. Based on density ratio and volume-weight of the materials in OCB, porosity of OCBs with RS, PD, and AC were estimated to be 62.7%, 36.5%, and 11.7%, respectively. Three of the 20 ditch cells with no OCB were used as the control (CK). Clean water was imported into ditch cells for two weeks until ditch outlets achieved a steady effluent flow velocity. Nutrient removal from ditch cells was examined as follows: Artificial wastewater that contained 5 mg L$^{-1}$ NH$_4^+$–N was continuously delivered to ditch cells for seven days at a hydraulic retention time of 0.5 days, which corresponded to a hydraulic loading rate of 6.0 m$^3$ d$^{-1}$. Water samples were collected from ditch outlets on Days 1, 3, 5, and 7. Clean water was subsequently imported into the ditch cells for seven days. Artificial wastewater that contained 5 mg L$^{-1}$ NO$_3$–N was continuously fed to the ditch cells for another seven days at the same hydraulic retention time of 0.5 days; effluents were also sampled on Days 1, 3, 5, and 7. All water samples were either analyzed on the sampling days or stored at 4 °C until analysis.

### 2.4.2. Test 2: The performance of OCB nutrient removal for different RS application rates

After Test 1, ditch soils in the 0- to 0.1-m layer were removed, and a layer of sandy loam soil from an adjacent paddy field was added to each ditch to a depth of 0.1 m. The soil characteristics were listed as follows: a sandy loam texture (66.9% of sand, 20.7% of silt, and 12.4% of clay), with total C content of 8.2 g kg$^{-1}$, total N of 0.75 g kg$^{-1}$, total P of 0.36 g kg$^{-1}$, cation exchange capacity (CEC) of 4.38 cmol kg$^{-1}$, and a pH value of 6.02 (measured at soil to water ratio of 1:1 w/v). The practice of ditch soil replacement was used to eliminate the differences in nutrient adsorption capacities of ditch soils. The OCBs with RS application rates of 0, 45, 75, and 120 kg dry weight were constructed in ditch cells in triplicate; they covered 0, 15%, 25%, and 40%, respectively, of the ditch bottom area. RS was evenly distributed in the OCBs. Three ditch cells with no RS OCB were used as the CK.

The experimental procedure for Test 2 was divided into five stages. First, the OCBs were loaded with clean water for two weeks (days 1–14) to achieve a steady effluent flow velocity. Second, artificial wastewater containing 5 mg L$^{-1}$ NH$_4^+$–N was fed to ditch cells at a constant hydraulic retention time of 0.5 days for seven days (days 15–21). Third, after loaded with clean water for one week (days 22–28), artificial wastewater containing 5 mg L$^{-1}$ NH$_4^+$–N was fed to ditch cells for another seven days (days 29–35) at a hydraulic retention time of 0.33 days, which was equivalent to a hydraulic loading rate of 9.0 m$^3$ d$^{-1}$. Effects of the two hydraulic retention times on NH$_4^+$–N removal rates were compared. Fourth, the ditch cells were fed with clean water for another week (days 36–42). Last, the synthetic wastewater with 5 mg L$^{-1}$ NH$_4^+$–N, 5 mg L$^{-1}$ NO$_3$–N, and 1 mg L$^{-1}$ PO$_4^{3–}$–P was simultaneously supplied to ditch cells for seven days (days 43–49). Water effluent samples were collected on Days 15, 17, 19, 21, 29, 31, 33, 35, 43, 45, 47, and 49 as artificial wastewater being loaded into ditch cells. Occasional influent and effluent samples were collected to determine water quality parameters, including the pH, dissolved organic C (DOC), temperature, and bacterial populations during the entire experimental period of 49 days.

### 2.5. Analytical methods

After water samples had been filtered through a 0.45-μm membrane, the quantities of NH$_4^+$–N, NO$_3$–N, and PO$_4^{3–}$–P were determined using an automated flow-injection system (FIA-star 5000 analyzer, Foss Tecator, Höganäs, Sweden). To determine the total N and total P, unfiltered water samples were digested with a K$_2$S$_2$O$_8$–NaOH solution, and transformed NO$_3$–N and PO$_4^{3–}$–P were also determined by the flow-injection analyzer. DOC was measured with a total organic C analyzer (TOC-VWVP, Shimadzu, Japan). Bacterial populations (colony-forming units, CFUs) in water samples were evaluated according to the following procedures: Water samples were serially diluted with a sterile 0.85% NaCl solution, and 100 μl of each dilution was added to the plates with nutrient agar (CM0003, Oxoid Ltd, England), which were incubated at 30 °C for 72 h. After the sample was grown into a <1 mm powder (a fine powder), the total C and total N were measured with an elemental analyzer (Vario Max CN, Elemental Co., Germany).

### 2.6. Data analysis

Nutrient removal rate (NRR, %) was calculated using the following equation

$$NRR = \left( \frac{C_i - C_e}{C_i} \right) \times 100$$

where $C_i$ and $C_e$ are the influent nutrient concentration and the effluent nutrient concentration, respectively.

To compare nutrient mass removal rates (NMRs, g m$^{-2}$ d$^{-1}$) between OCBs with different quantities of RS and CK, the NMR was calculated as the difference between the nutrient concentration of the influents and the nutrient concentration of the effluents multiplied by the hydraulic loading rate (HLR, m$^3$ d$^{-1}$) and divided by the surface area of the entire ditch cell ($A$, m$^2$). The equation for the NMR is expressed as
\[
\text{NMR} = \frac{(C_i - C_e) \times \text{HLR}}{A}
\]

where \(C_i\) and \(C_e\) represent the same variables as in Eq. (1) and \(A\) is equal to 32 m².

Based on the DOC concentration in effluents over time during the experimental period, the total C loss from the OCBs was calculated by using the method as described in a published paper by Schmidt and Clark (2012).

Statistical significance of a univariate analysis of variance (ANOVA) was calculated with Duncan’s test in SPSS 13.0 for Windows, which was set at an alpha level of 0.05 (two-tailed, \(p < 0.05\)).

3. Results

3.1. N removal capacity of OCBs with three different C substrates

Nutrient removal rates of \(\text{NH}_4^+\text{--N}\) by the OCBs with RS, PD, AC, and CK exhibited the following ascending order: CK < PD < AC < RS (Fig. 2a). The three C substrates stimulated \(\text{NH}_4^+\text{--N}\) removal. However, the RS OCB resulted in greater \(\text{NH}_4^+\text{--N}\) removal, with an mean removal rate of 73%, which differed significantly from the mean removal rates of the remaining two C substrates (\(p < 0.05\)). The CK exhibited the lowest \(\text{NH}_4^+\text{--N}\) removal capacity, with a mean removal rate of 25%. The OCBs exhibited removal performances for \(\text{NO}_3^--\text{N}\) in the channels in the following order: RS > PD > AC > CK. The CK achieved a \(\text{NO}_3^--\text{N}\) removal rate of approximately 5%, whereas the AC OCB achieved a slightly higher \(\text{NO}_3^--\text{N}\) removal rate than the CK. The RS and PD OCBs achieved significantly higher \(\text{NO}_3^--\text{N}\) removal rates than the AC OCB and the CK (\(p < 0.01\)) (Fig. 2b). The RS OCB had the best performance in terms of N removal compared with the OCBs with the remaining two C substrates. After the background nutrient removal in the CK was subtracted from the RS OCB, the RS OCB yielded an enhanced mean removal rate of 58% for \(\text{NH}_4^+\text{--N}\) and 92% for \(\text{NO}_3^--\text{N}\).

3.2. Nutrient removal performance of the RS OCBs at different RS application rates

The OCBs that contained RS quantities of 45, 75, and 120 kg and the CK showed varied \(\text{NH}_4^+\text{--N}\) removal capacities. Changes in effluent \(\text{NH}_4^+\text{--N}\) concentrations and the removal rates in the channels with OCBs were distinct when the hydraulic retention time was adjusted from 0.5 days to 0.33 days. Given a retention time of 0.5 days, the RS OCB with 45 kg of RS produced only slightly more \(\text{NH}_4^+\text{--N}\) removal than the CK, whereas the RS OCBs with 75 and 120 kg of RS exhibited similar \(\text{NH}_4^+\text{--N}\) removal. The lowest mean \(\text{NH}_4^+\text{--N}\) removal (0.17 g N m⁻² d⁻¹) and the highest mean \(\text{NH}_4^+\text{--N}\) removal (0.55 g N m⁻² d⁻¹) were observed for the CK and the RS OCB with 120 kg of RS, respectively (Fig. 3a). For the hydraulic retention time of 0.33 days, the difference in the \(\text{NH}_4^+\text{--N}\) removal between the RS OCB and the CK was significant (\(p < 0.05\)), and the difference between the RS OCBs with 75 and 120 kg of RS was also significant (\(p < 0.05\)). However, there was little difference in the \(\text{NH}_4^+\text{--N}\) removal rates for the RS OCBs with 45 and 75 kg of RS (Fig. 3b). The RS OCB with 120 kg of RS produced the highest

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**Fig. 2.** Comparison of the (a) \(\text{NH}_4^+\text{--N}\) and (b) \(\text{NO}_3^--\text{N}\) removal rates of the OCBs with the three test substrates and the CK. The error bars represent the standard deviations (\(n = 3\)), and the different letters in the graph indicate significant differences (\(p < 0.05\)).

**Fig. 3.** \(\text{NH}_4^+\text{--N}\) mass removal rates of OCBs with different quantities of RS and the CK at hydraulic retention times of 0.5 day (a) and 0.33 day (b). CK, 45 kg, 75 kg and 120 kg represent the OCBs filled with 0, 45, 75, and 120 kg RS, respectively (hereinafter the same in Figs. 4 and 5).
mean NH₄–N mass removal of 0.72 g N m⁻² d⁻¹, which was also higher than the mass removal of 0.55 g N m⁻² d⁻¹ at a retention time of 0.5 days.

Table 1 lists the nutrient removal rates when NH₄–N, NO₃–N, and PO₄–P were simultaneously fed to the ditches. The RS OCBs had significantly higher NH₄–N removal rates than the CK (p < 0.05). Mean NH₄–N mass removals of the RS OCBs ranged from 0.47 to 0.77 mg N m⁻² d⁻¹; they were higher than the 0.25 g N m⁻² d⁻¹ value of the CK. Mean NO₃–N mass removal rates of 0.57, 0.65, and 0.85 g N m⁻² d⁻¹ were observed for the RS OCBs with 45, 75, and 120 kg of RS, respectively. The highest mean NO₃–N mass removal rate of 0.85 g N m⁻² d⁻¹ for the RS OCB with 120 kg of RS was almost six times higher than the lowest mean mass removal rate of 0.14 g N m⁻² d⁻¹ for the CK. The RS OCBs also achieved more PO₄–P mass removal than the CK; however, the RS OCB with 120 kg of RS yielded less PO₄–P mass removal than the RS OCBs with 45 and 75 kg of RS.

Correlation analysis showed the quantity of RS in the RS OCBs were positively correlated with the NH₄–N and NO₃–N removal rates (r < 0.01). In addition, the correlation of the NO₃–N removal rate with the quantity of RS (r = 0.969) was higher than the correlation of the NH₄–N removal rate with the amount of RS (r = 0.754). Conversely, the amounts of RS showed a negative correlation with the PO₄–P removal rate (r = -0.492); however, this relationship was not significant (r = 0.104).

3.3. Effluent dynamics of dissolved organic C and the bacterial population

Release of C from the RS OCBs that were installed in simulated ditches was evaluated using the observed effluent DOC concentrations (Fig. 4). During the 49-day study period, influent DOC concentrations ranged from 4.63 to 6.50 mg L⁻¹. The two highest effluent DOC concentrations were observed in the OCBs with 120 kg of RS during the first few weeks. The two highest effluent DOC concentrations of 56.3 ± 4.87 and 62.7 ± 18.2 mg L⁻¹ (n = 3) were observed on Days 7 and 10, respectively. After these peaks, the release of C from the RS OCB sharply declined to a constant rate, which was reflected by the < 8 mg L⁻¹ effluent DOC concentrations after day 25. The total C mass loss from the RS OCB for 49 days was estimated at 4385 g, with 90% occurring during the first three weeks. A dark brown color was observed in the effluents from the RS OCB during the first three weeks. However, the C mass loss from the RS OCB was relatively small, which accounted for approximately 5% of the initial RS C mass. Effluent DOC concentrations for the CK remained relatively low; they ranged from 5.31 to 9.7 mg L⁻¹.

The bacterial population dynamics in the influents and effluents of the CK and the RS OCB with 120 kg of RS were similar to the DOC during the study period (Fig. 5). A higher amount of effluent bacteria was detected for the RS OCB than the CK during the initial observation days; these values climbed to a peak value of 6.6 × 10⁶ CFUs ml⁻¹ on Day 10 and eventually decreased toward the CK level.

4. Discussion

4.1. Alternative C substrate and denitrification in OCB

The C source serves an important role in supporting energy and electron donors during the heterotrophic denitrification process. Although fragmented wood media in the denitrification bioreactor have been frequently proposed as a feasible C source for the removal of NO₃–N, alternative C media may provide more labile C to obtain a higher NO₃–N removal rate compared with wood media (e.g., Gibert et al., 2008; Greenan et al., 2006). In this study, the C substrates of RS, PD, and AC were used to construct OCBs in the simulated drainage ditches. The OCBs, which were submerged
underwater, were assumed to act as the reported denitrification bioreactors with high NO$_3$–N removal capability. Results from this study demonstrated that the RS OCBs resulted in removal rates higher than 90% for NO$_3$–N, which indicated that RS OCBs were capable of serving as efficient denitrification bioreactors.

Concentrations of bioavailable C, oxygen (O$_2$), and NO$_3$–N are the dominant factors in the control of the heterotrophic denitrification rate (Seitzinger et al., 2006). Compared with the total solid fraction of organic C, the amount of DOC representing the bioavailable C source was closely related to heterotrophic denitrification rate (Rivett et al., 2008). This study revealed decreasing NO$_3$–N removal by OCBs in the following order: RS > PD > AC > CK (Fig. 2). Enhanced NO$_3$–N removal by RS indicated this substrate supplied more labile C to stimulate the microbially mediated denitrification process than the remaining two C substrates of PD and AC, during the study period. A similar result was observed that fresh wheat straw in up-flow laboratory reactors was capable of supplying adequate soluble C fraction to engage high NO$_3$–N removal during the initial weeks of the operation (Soares and Abeliovich, 1998). Maize and wheat straw OCBs were also suggested to provide more labile C sources than wood media, which resulted in higher NO$_3$–N removal rates (Cameron and Schipper, 2010). Additional C substrates resulted in NO$_3$–N removal in the following ascending order: wood chips < wood chips and oil < cardboard < cornstalks, which revealed that the cornstalks had a greater ability to remove NO$_3$–N than the wood media (Greenan et al., 2006). These findings indicated alternative agricultural organic wastes, such as RS, cornstalks, maize, and wheat straw, can serve as more efficient C substrates for heterotrophic bacteria than wood media because they achieved higher denitrification rates and more efficient NO$_3$–N removal.

The RS OCBs with different amounts of RS yielded NO$_3$–N mass removal rates within the range of 0.51–0.89 g N m$^{-2}$ d$^{-1}$ (Table 1). Our observed data are comparable to the data from previous field investigations. For example, a similar mean NO$_3$–N mass removal rate of 0.7 g N m$^{-2}$ d$^{-1}$ was observed for a reactive subsurface barrier filled with fine wood particles (0.73 m$^2$) in an agricultural area of southwestern Ontario, in which the mean influent NO$_3$–N concentration was 11.5 mg L$^{-1}$ during a 20-month period (van Driel et al., 2006). The performance of a reactor (40 m$^3$) that was entrenched in the bottom of an agricultural drainage ditch in southern Ontario also exhibited a temperature-dependent NO$_3$–N mass removal rate that ranged from 0.26 g N m$^{-2}$ d$^{-1}$ at 3°C to 5.28 g N m$^{-2}$ d$^{-1}$ at 14°C (Robertson and Merkley, 2009). In another study, a stream-bed denitrifying bioreactor was observed to yield high and low monthly mean NO$_3$–N mass removal rates that ranged from 0.50 to 1.25 g N m$^{-2}$ d$^{-1}$ in the spring and the fall and that ranged from 0.28 to 0.38 g N m$^{-2}$ d$^{-1}$ during the cold months from December to March (Elgood et al., 2010). Based on studies of NO$_3$–N mass removal via denitrification bioreactors, simulated ditches that contained the manmade RS OCBs produced higher N mass removal than constructed wetlands (e.g., Hernandez and Mitsch, 2007; Mitsch et al., 2005).

Field investigations showed that nitrate limitation consistently caused low NO$_3$–N mass removal by denitrifying bioreactors (e.g., Elgood et al., 2010; Robertson et al., 2000; Robertson and Merkley, 2009; Schmidt and Clark, 2012). The RS OCB with 120 kg of RS continuously removed more than 95% of the influent NO$_3$–N. It also maintained an effluent NO$_3$–N concentration of less than 0.5 mg L$^{-1}$, which indicated a limited NO$_3$–N concentration of 5 mg L$^{-1}$ in the influent. Therefore, a higher influent NO$_3$–N concentration is required to evaluate the threshold for the NO$_3$–N mass removal rate for the RS OCBs. Meanwhile, the rapid N depletion observed in this study implied that the RS OCB with 120 kg was very strong for the almost completely removal of current influent NO$_3$–N.

### 4.2. NH$_4$–N and PO$_4$–P removals in RS OCBs

The NH$_4$–N removal performance of RS OCBs was influenced by the amount of RS and the hydraulic retention time (Fig. 3). The RS OCBs with a large quantity of RS supplied a more stable buffering capacity for NH$_4$–N removal, even at the shorter hydraulic retention time, which was reflected by the changes in NH$_4$–N removal by the RS OCBs with 45 and 75 kg of RS at the hydraulic retention times of 0.5 and 0.33 days. For the longer hydraulic retention time of 0.5 days, the CK produced higher NH$_4$–N removal rates, which may be attributed to ditch-soil adsorption. The differences in NH$_4$–N removal between the RS OCBs and the CK indicated that the RS OCBs exhibited a stable NH$_4$–N removal capacity during the study period. Ammonia (NH$_3$) volatilization may contribute minimally to NH$_4$–N removal because effluents from the RS OCBs exhibited neutral pH values in the range of 6.1–7.4 (e.g., Leu et al., 1998; Hunt et al., 2002).

RS is predominantly composed of cellulose, hemicellulose, and lignin, which supply an abundance of functional groups, such as –OH and carbonate CO$_3$–OH. These functional groups facilitated the removal of cations, such as Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ (Rocha et al., 2009). Due to its negatively charged functional groups, RS was not favorable for PO$_4$–P adsorption, which resulted in low P removal capacity for the RS OCBs and a negative relationship between the quantity of RS and the amount of PO$_4$–P removal. A similar result was observed for the woodchip filters of an on-farm treatment system for soilled dairy water, which achieved a poor PO$_4$–P removal rate of 31% (Ruane et al., 2011).

### 4.3. C loss and bacterial population in RS OCBs

The effluent DOC concentrations increased from 7.4 to 62.7 mg L$^{-1}$ within the first two weeks and then rapidly declined to a stable value of less than 10 mg L$^{-1}$ (Fig. 4), which indicated substantial C loss from the RS OCBs. A similar phenomenon of significant C loss was also detected during the start-up period in the denitrification wall or filter (e.g., Healy et al., 2006; Schmidt and Clark, 2012). Using a mass-balance method, the accumulated amount of DOC can be used to estimate the longevity of the C media in a bioreactor (e.g., Robertson et al., 2000; Robertson and Merkley, 2009). The accumulated C loss from the RS OCB at 120 kg was approximately 4400 g, accounting for less than 5% of the initial C mass. The amount of C loss during the first two weeks comprised 70% of the total quantity of C loss for the entire experimental period. If the study RS OCB with 120 kg of RS was continuously operated for one year, the amount of C loss was estimated to be approximately 10% of the initial C mass. Despite this finding, the RS OCBs had a greater C loss than the field bioreactors with wood media, with less than 3% of the initial C mass (e.g., Robertson et al., 2000; Robertson and Merkley, 2009). Therefore, as indicated for the alternative C substrates of maize, cornstalks, and wheat straw (e.g., Cameron and Schipper, 2010; Salling et al., 2007), RS in the OCBs was attributed to higher NO$_3$–N removal rates compared with PD of wood media; however, a more frequent replenishment would be required when RS was used in the OCBs.

In our study, the highest effluent bacterial population was observed in the RS OCB with 120 kg RS during the first two weeks, which exhibited a similar variational trend for the DOC (Figs. 4 and 5). This finding indicated that sufficient quantities of DOC were present during the initial period, which resulted in rapid microbial growth. A similar result was also reported in a denitrification bioreactor experiment with fresh wheat straw during a start-up period performed by Soares and Abeliovich (1998). Although a large reduction was observed in the bacterial population during the last stage, the RS OCB contained an effluent bacterial
population that was ten times greater than the effluent bacterial population of the CK. This phenomenon suggested a continuous release of bioavailable C from the RS OCB, which was beneficial to the growth and reproduction of the heterotrophic denitrifying bacteria in the OCB. As a result, the denitrifying bacteria engaged in consistent NO₃⁻ bacteria in the OCB. As a result, the denitrifying bacteria engaged to the growth and reproduction of the heterotrophic denitrifying population that was ten times greater than the effluent bacterial population. Furthermore, the RS OCB had lower PO₄³⁻ and C loss of less than 10% of the initial C mass over one year (especially in rural China), is an ideal and effective material for investigations. Moreover, the optimization of the OCB size for efficiently treating the agricultural runoff under field conditions is needed for further investigations.

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