Long-term nitrogen compound removal trends of a hybrid subsurface constructed wetland treating milking parlor wastewater throughout its 7 years of operation

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ABSTRACT

This study evaluated the nitrogen compound removal efficiency of a hybrid subsurface constructed wetland, which began treating milking parlor wastewater in Hokkaido, northern Japan, in 2006. The wetland's overall removal rates of total nitrogen (TN) and ammonium (NH_4^+-N) improved after the second year of operation, and its rate of organic nitrogen (Org-N) removal was stable at 90% efficiency. Only nitrate (NO_3^--N) levels were increased following the treatment. Despite increased NO_3^--N (maximum of 3 mg-N/L) levels, TN removal rates were only slightly affected. Removal rates of TN and Org-N were highest in the first vertical bed. NH_4^+-N removal rates were highest in the second vertical bed, presumably due to water recirculation and pH adjustment. Concentrations of NO_3^--N appeared when total carbon (TC) levels were low, which suggests that low TC prevented complete denitrification in the second vertical bed and the final horizontal bed. In practice, the beds removed more nitrogen than the amount theoretically removed by denitrification, as calculated by the amount of carbon removed from the system. This carbon-nitrogen imbalance may be due to other nitrogen transformation mechanisms, which require less carbon.

Key words | ammonium, cold climate, dairy farming, high concentration organic wastewater, nitrate, organic nitrogen, total nitrogen

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INTRODUCTION

The livestock industry is a major source of nitrogen pollution. In Japan, the livestock industry annually generates about 83 million tons of waste and 70 thousand million tons of nitrogen (MAFF 2009). Annual nitrogen waste was reduced from approximately 95 million tons in 1997 to about 83 million tons in 2013, using methods such as composting, converting to manure, treatment, and incineration. In spite of this achievement, the livestock industry faces pressure to reduce waste further. Roughly one-quarter of complaints against the livestock industry concern water pollution (MAFF 2010). Currently, the inorganic nitrogen level in waste effluent is limited to 700 mg-N/L (NH_4^+-N $\times$ 0.4 + NO_2^--N + NO_3^--N). This level might be reduced even further by regulators in 2016. However, stricter standards (total nitrogen (TN) limit of 120 mg-N/L, daily average of 60 mg-N/L) are only applied to facilities that release more than 50 m^3/day. Since many small to medium scale farmers release less than that amount, these wastewater discharges are not strictly regulated. Farmers should be aware that regulation of nitrogen pollution is likely to become more stringent in the future.

Where adequate space exists, constructed wetlands offer an alternative to conventional activated sludge treatment. A constructed wetland mimics a natural wetland to treat different kinds of wastewater, including domestic and industrial sewage, agricultural wastewater, and acid-mine drainage wastewater (IWA 2000). Constructed wetlands have several benefits; they require little to no energy input, have low operation and maintenance cost, and can be designed to resemble the natural environment so as to benefit the local ecosystem (IWA 2000). There are two types of constructed wetlands: one uses free surface flow and the other uses subsurface flow. Among subsurface flow constructed wetlands,

some flow vertically and others horizontally. In a vertical flow subsurface constructed wetland, water flows perpendicular to the bed; in a horizontal flow subsurface constructed wetland, water flows parallel to the bed. A hybrid subsurface constructed wetland (HSCW) uses both vertical and horizontal flows.

In a constructed wetland system, nitrogen undergoes many transformations before it is removed (IWA 2000; Vymazal 2007). Often some nitrogen compounds are not removed due to the complex route they must take. In a classic nitrogen removal pass-way for example, high organic nitrogen (Org-N) wastewater is first ammonified or mineralized. Next, ammonium (NH_4^+-N) is nitrified into nitrite (NO_2^--N), and then to nitrate (NO_3^--N). Finally, NO_3^--N is denitrified into nitrogen gas (N_2). In addition to this classical route, there are absorption, adsorption, assimilation, and routes recently discovered by researchers. These new routes are partial nitrification-denitrification, Anammox, and Canon. Engineers are attempting to incorporate these processes in their wastewater systems to remove nitrogen because they require less oxygen and carbon compared with classical route processes. All of these processes, including those of the classical route, are sensitive to environmental conditions (such as temperature, pH, dissolved organic and carbon sources) (Vymazal 2007) and system maturity. Hence, all nitrogen compounds and their removal rates must be carefully followed and quantified. However, there are very few examples of constructed wetlands that work with high organic wastewater. Furthermore, the monitoring periods for many of them are very short. Therefore, a thorough investigation of these systems, over a prolonged period, is crucial.

This paper outlines how the nitrogen compounds were transformed and removed in a HSCW, which has treated dairy farm wastewater for more than 7 years.

MATERIALS AND METHODS

The studied HSCW is located in the Town of Embetsu, Hokkaido, northern Japan (N44 °45.0′ latitude and E141 °48.4′ longitude). The town's average annual temperature is 6.45 °C and total annual precipitation is 1,112 mm. This HCSW treats milking parlor effluent, discharged from a free stall-milking parlor that holds about 120 dairy cows.

The HCSW is constructed of two vertical beds (VB) and one horizontal bed (HB). The areas of the VBs are 160 m² each and the HB is 336 m², as shown in Figure 1 and Table 1. The main vegetation is *Phragmites australis*. Feces,
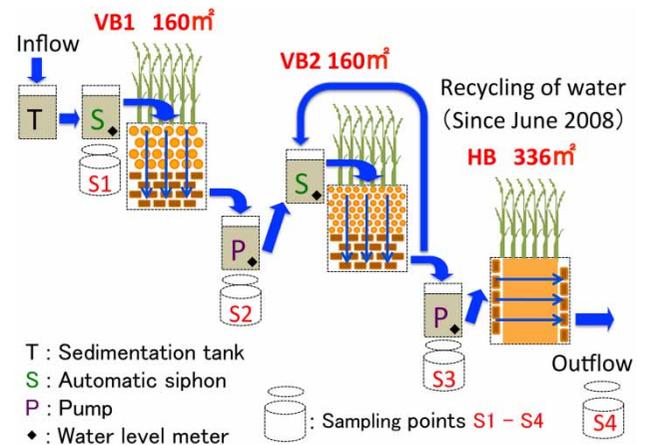
Figure 1 | Schematic diagram of the constructed wetland. HB and VB represent horizontal bed and vertical bed, respectively.

Table 1 | Bed sizing and media composition

Bed	Area (m²)	Average depth (m)	Volume (m³)	Bed material	Cover material
VB1	160	0.75	120	River gravel	Supersol
VB2	160	0.71	114	Clinker ash River gravel	Supersol
HB	336	0.72	242	Sand	ALC
Total	656	–	476	–	–

urine, non-shippable milk, and detergent are sent from the milking parlor, then mixed and stored in the sedimentation tank (underground storage tank). The stored sewage flows into the first vertical bed (VB1), and then into the second vertical bed (VB2). Part of the VB2 effluent is recycled back to the inlet of VB2 to increase removal efficiency (RE). This recycling is done by a recirculation pump equipped with a timer and it is activated every other hour. The remaining water from VB2 flows into the HB and then finally water is discharged into a nearby stream. Inlets of VB1 and VB2 are each equipped with a pump and a French based self-priming siphon (Molle *et al.* 2005) to supply water intermittently. The inlet of HB is also equipped with a pump. Details of the bed media are listed in Table 1. Porous recycled glass material called Supersol© is used on the surface beds of VB1 and VB2, and autoclaved aerated lightweight concrete (ALC) is used on the bed of HB. Both these materials insulate the beds to prevent water from freezing in winter (Kato *et al.* 2013). These cover materials and reinforced bypass tubes are used in all of the beds to prevent clogging, which is a problem for all constructed wetlands particularly during the startup period (Kato *et al.* 2013).

These improvements enabled this system to treat wastewater with high loads of organic pollutants, even in extreme cold conditions. VB1 and VB2 are partitioned into two long strips, so one of the sections can be in a dry phase to facilitate oxidation and drying of the organic layer. The sections are used alternately during the growing seasons.

Samples were collected on a monthly to bimonthly basis at the inlet of the system and the outlet of each bed. The samples were analyzed for TN, NH_4^+-N, NO_3^--N, NO_2^--N, and total carbon (TC). TN and TC were measured using an elemental analyzer (Elementar vario MAX; Elementar Analysensysteme GmbH, Hanau, Germany). Ammonium nitrogen was measured using a segmented-flow analysis system (QuAAtro; SEAL Analytical GmbH, Norderstedt, Germany). Org-N was calculated by subtracting the inorganic nitrogen (NH_4^+-N + NO_3^--N) from TN.

Concurrently, data were downloaded from the pressure sensor (water level meter) with a data logger (DL/N70; Sensor TechnikSirnach (STS) AG, Sirnach, Switzerland, or S&DL Mini; Oyo Corp., Tokyo, Japan) placed inside each siphon pit and pump pit (Figure 1). The data logger recorded changes in water level at a 10-min interval. The system's daily inflow (unit of m^3/day) was calculated by multiplying the base area of the siphon tank by the changes in water level of the pit or tank and the number of times the siphon was activated. The daily flow was calculated by adding all water volumes that entered the pit per day.

Rainfall data were obtained using a tipping-bucket type rain gauge installed on site or from the Japan Meteorological Agency's local station (AMeDAS Embetsu Station).

The annual input loads were calculated by multiplying the concentrations of the samples by the total volume of the sampling periods. One sampling period was defined as the time from the sampling date to the day prior to the next sampling date. The total annual load was calculated by adding all the loads throughout the year. The average daily loads were calculated by dividing the annual load by total number of days, and the average annual concentration was calculated by dividing the total annual load by the total annual volume. Performance of the system was evaluated based on concentration and loads. The two different methods of evaluation were used. These methods are purification efficiency (PE; based on concentrations) and removal efficiency (RE; based on loads). The equations to calculate these rates are as follows:

$$PE = \frac{(C_{in} - C_{out})}{C_{in}} \times 100 \%$$ (1)

$$RE = \frac{(M_{in} - M_{out})}{M_{in}} \times 100 \%$$ (2)

where C_{in} is the influent concentration, C_{out} is the effluent concentration, M_{in} is the influent load, and M_{out} is the effluent load.

RESULTS AND DISCUSSION

Influent and effluent concentrations and loads

Figure 2 shows influent and effluent concentrations and loads for TN, NH_4^+-N, NO_3^--N, and Org-N. TN concentrations and loads were the highest in Year 1. This is likely due to the large amount of unshippable milk disposed into the constructed wetland in that year. Influent concentrations and loads fluctuated, but the effluent concentrations and loads remained stable. The PE and RE of TN improved during the first 4 years of operation and remained stable thereafter. Since PE and RE of Org-N were stable around 90% during all operational years, enhanced PE and RE of TN are due to improved NH_4^+-N removal. The NH_4^+-N removal rate was high in Year 1 and then decreased significantly in Year 2. However, the rate increased steadily after Year 2. This pattern occurred because NH_4^+-N was removed via adsorption to bed media during Year 1. After Year 2, the growth of plants and their root systems facilitated NH_4^+-N removal by bacterial colonies in the rhizomes. Also, increased soil biota and other macro-organisms, such as earthworms, presumably contributed to nutrient recycling. However, simple reduction of NH_4^+-N (transformation to NO_3^--N through nitrification) did not contribute to TN reduction. This indicates that NH_4^+-N was further transformed into N_2 gas by the denitrification process or through other mechanisms. Other mechanisms may include assimilation by biomass, plant uptake and ammonia volatilization. However, removal by these mechanisms should have been minimal compared with the overall nitrogen removal (IWA 2000). The influent concentrations and loads of NO_3^--N were very low and they increased significantly in the effluent. Even with the increased NO_3^--N (maximum of 3 mg-N/L), PE and RE of TN were only slightly affected. The effect was minimal because the average TN concentration of the effluent was 20.17 ± 10.69 mg-N/L and the average load was 118.24 ± 54.83 g-N/day. Thus, even the highest NO_3^--N concentration was responsible for just 15% of TN concentration.
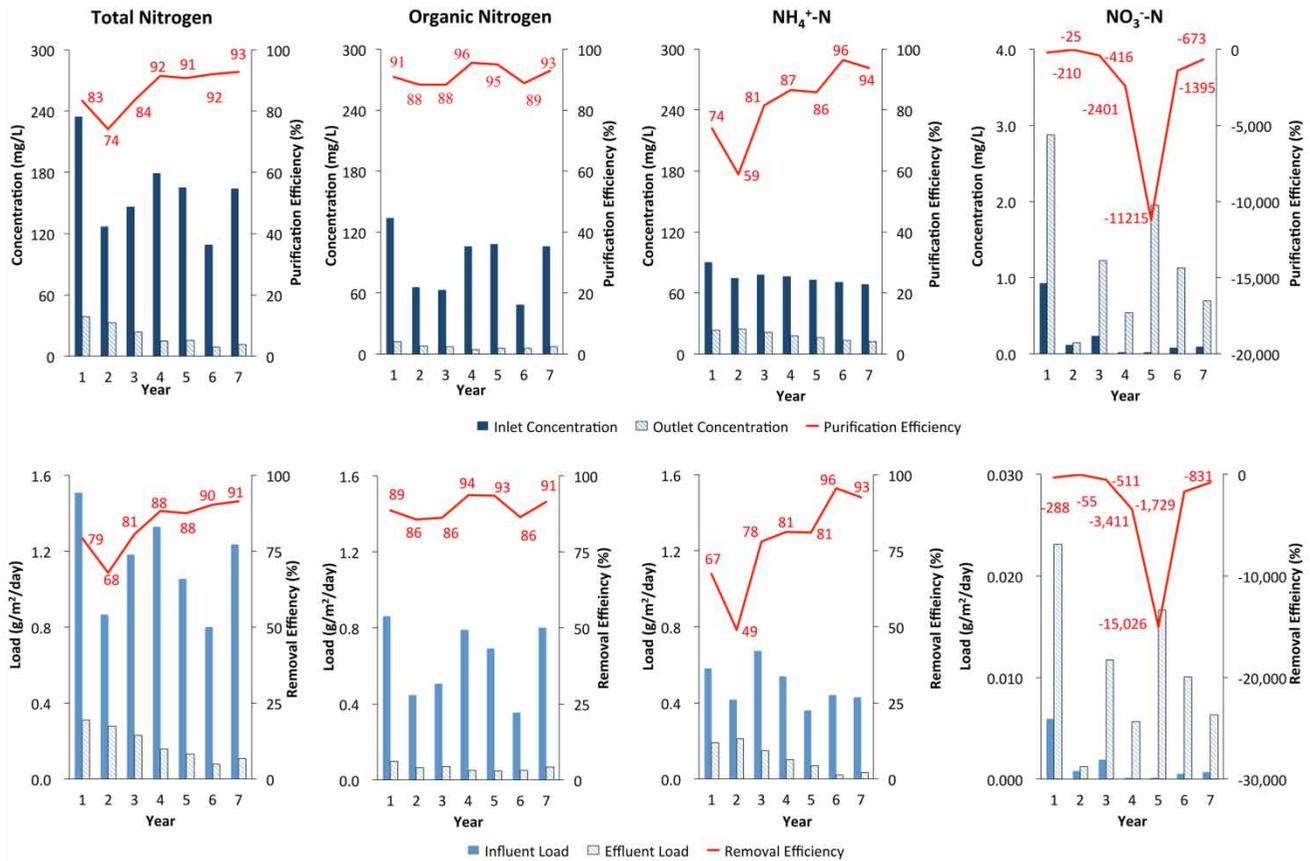
Figure 2 | Inflow and outflow concentrations, loads and removal efficiencies for nitrogen pollutants.

Composition of wastewater in the system

Figure 3 shows nitrogen composition, based on the initial load at each sampling point. For the initial inflow, NH_4^+-N and Org-N concentrations were about 43 and 56% of the initial TN, and NO_3^--N concentration was almost zero. In
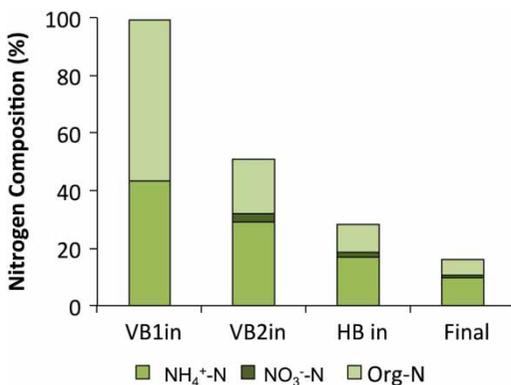

Figure 3 | Average composition of nitrogen compounds in the wastewater based on the initial TN load (initial TN = 100%).

the effluent, Org-N and NH_4^+-N concentrations were approximately 6 and 10% of the original TN load, respectively. Nitrate concentration drastically increased after passing through VB1, and then decreased gradually to about 1% of the initial TN concentration.

Generally, Org-N is removed by ammonification, nitrification, and denitrification. However, the Org-N load decreased drastically without an increase in NH_4^+-N load in VB1. There should have been two removal mechanisms. The first should have been the classical route, which involves adsorption, ammonification, nitrification, and denitrification. The increase in NH_4^+-N load could not be observed because of the spontaneous reaction or the adsorption. The second mechanism should have been physical filtering of particulate Org-N.

Figure 4 shows nitrogen removal performed by each bed. More than half the removal of TN and Org-N was done by VB1. Since solid waste accumulation mainly occurred in VB1 (organic layer of about 25 cm in depth) compared with VB2 (organic layer of about 5 cm), it is likely that removal of particulate nitrogen, including Org-N, occurred in VB1.
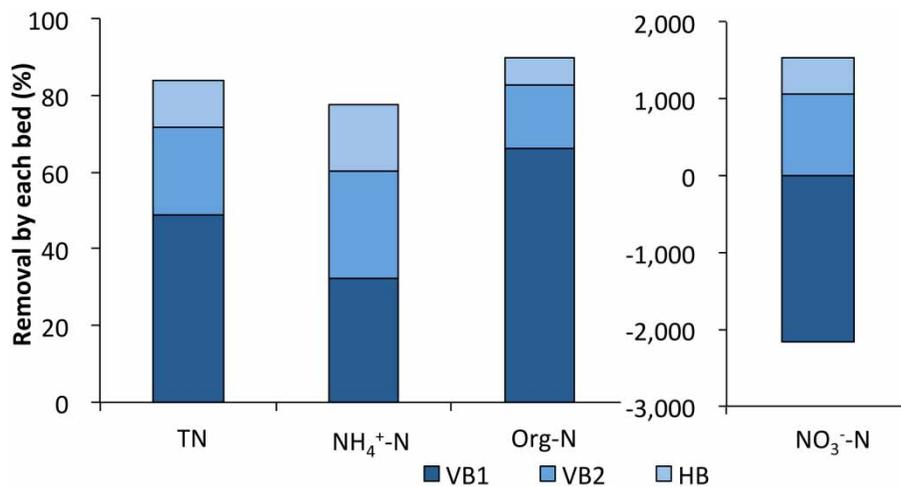
Figure 4 │ Contribution of each bed for removal of different nitrogen pollutants.

Therefore it is likely that the highest removal of TN occurred in VB1 due to the physical removal of particulate Org-N. Ammonium removal was the same in VB1 and VB2. The high removal rate in VB2 was probably due to recirculation of the effluent and neutralization of pH from clinker ash. In many studies, NH_4^+-N removal efficiencies were improved by recirculation (Brix & Arias 2005; Ayaz *et al.* 2012). Since denitrifying and nitrifying bacteria favor a neutral pH, it is possible the clinker ash neutralized the wastewater and helped the system to maintain high removal rate. Unlike TN, NH_4^+-N, and Org-N, NO_3^--N concentrations increased rapidly in VB1 and were removed by the following beds.

Relationship between nitrogen and carbon

Figure 5 shows the concentrations of NH_4^+-N and NO_3^--N in VB2in and HBin, plotted against corresponding TC concentrations. Concentrations of NH_4^+-N and TC were positively correlated ($R^2 = 0.76$). In contrast, NO_3^--N concentrations had a completely different relationship. NO_3^--N concentrations were low at high TC concentrations but started to appear at low TC. This may have occurred because the denitrifying bacteria did not have enough carbon to denitrify all NH_4^+-N. Therefore, NH_4^+-N was able to be nitrified to NO_3^--N, but was not denitrified and NO_3^--N was released. A similar trend was observed in HB, but with a lower NO_3^--N peak. If further denitrification is required in the future, one approach could be the addition of external carbon. However, current TN concentrations in the effluent are far below the limit so further reduction may not be necessary.

The amount of theoretical carbon used for denitrification was calculated using the assumption that all NH_4^+-N was denitrified, and none was lost due to ammonia volatilization, plant assimilation or biomass uptake. Under this very simplified assumption, every gram of NO_3^--N consumed
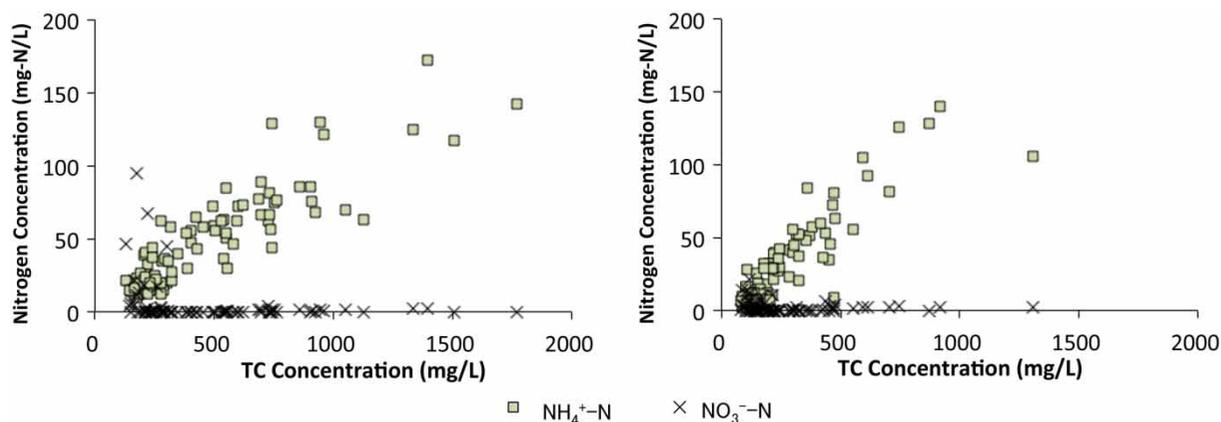

Figure 5 │ Nitrogen versus TC concentration for VB2in (left) and HBin (right).

1.07 g of carbon for denitrification (Vymazal 2007). There-fore, the theoretical carbon consumption could be calculated as follows:

$$Carbon_{removed,\ denitrification} = 1.07(NH_4^+\text{-}N_{removed}$$
$$+ NO_3^-\text{-}N_{removed}) \qquad (3)$$

where $Carbon_{removed,\ denitrification}$ represents the mass of carbon used if all the NO_3^--N (and transformed NH_4^+-N) were denitrified into N_2. NH_4^+-$N_{removed}$ is the total amount of NH_4^+-N removed from that bed, and NO_3^--N.

When $Carbon_{removed,\ denitrification}$ was compared with the actual amount of TC removed from the same bed, there were several instances where $Carbon_{removed,\ denitrification}$ exceeded the actual amount of TC removal. This indicates more NH_4^+-N was removed than projected by the theoretical model. Therefore in at least some instances, it is assumed that TN was reduced due to other inorganic nitrogen removal mechanisms, which require less carbon. In other studies, there are cases where Anammox bacteria were possibly involved in treating wastewater from the dairy industry or at least the theoretical potential of Anammox was recognized (Tao *et al.* 2012; Waki *et al.* 2013).

Both partial nitrification-denitrification and Anammox require nitrite (NO_2^-) (van Dongen *et al.* 2001). Many samples had undetectable levels of NO_2^--N but some had more than 1 mg-N/L. Concentrations of NO_2^--N exceeding 1 mg-N/L (high as 6.1 mg-N/L) were observed at all sites except in the outflow. It is commonly understood that the Anammox process requires the ratio of NH_4^+-N:NO_2^--N to be 1/1.32, but this system never reached that ratio. However, NO_2^--N readily decomposes so this compound may have decomposed within the bed and during transportation of the samples.

CONCLUSIONS

A thorough analysis of the removal of nitrogen compounds indicates that the PEs and REs of TN improved during the first 4 years and remained stable thereafter. The system's PE and RE of Org-N were stable at around 90% and NH_4^+-N improved from 40% PE and 50% RE in Year 2 to about 90% in Year 6. This improvement was likely due to the growth of reed and their roots, flourishing of nitrifying and denitrifying bacteria in the rhizomes, development of soil biota, and ecosystem features like earthworms that enhance recycling of nutrients and stabilize infiltration

rates. The removal rates of TN and Org-N were highest in VB1 and gradually decreased throughout the system. This is likely due to the physical filtration by VB, which was effective in removing particulate Org-N. Implementation of water recycling in VB2 most likely contributed to an increase in overall removal of NH_4^+-N. The TC concentration was positively, but moderately, correlated with NH_4^+-N concentration. On the other hand NO_3^--N was not observed at high TC concentrations, but started to appear at low TC concentrations. This indicates there were occasions when insufficient carbon was available to completely denitrify all inorganic nitrogen. Finally, there were cases where more NH_4^+-N was being removed relative to the expected amount of removed TC from denitrification. Therefore, other mechanisms may have played a role in ammonia removal, such as partial nitrification-denitrification and Anammox.

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