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Investigation of the water purification efficiency of land treatment system by using microbial community as an index

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ABSTRACT

Artificial land treatment system was constructed to remove nutrient salt in the sewage by the physicochemical and biochemical mechanisms of plants, microorganisms and soil. The aims of this research were to investigate the relationship between the water purification efficiency using the land treatment system and the variation of microbial community, and to study the interactive relation between microbial community and flora by observing the flora succession. The results showed that the vegetation coverage facilitated the soil steadiness. After the fortieth day, the average moisture content of soil and capacity of cation exchange in the restoraion zone tended to steadiness due to the increase of vegetation coverage. Besides, the observed results indicated that the microbial community was affected by the flora succession. When composite plants became the dominant species, the Aeromonas family (*Aeromonas hydrophila/caviae, Aeromonas salmonicida*) and *Burkholderia cepacia* were the main species of bacteria in the two zones. However, when *Burkholderia cepacia* caused the phosphate-solubilizing so that removal rate of ortho-phosphate decreased. Summary all results displayed that microorganisms, plants and water quality have the interactive relation. Therefore, the study results of microbial community and flora succession could be used as an index for the water purification efficiency, which ensure that land treatment systems achieves optimal performance.

Keywords: Land treatment system; Second ecosystem; Microbial community; Nutrient salt

1. Introduction

The source of wastewater land treatment systems originated in wastewater irrigation, hundreds of years ago in Sweden and Germany. At the end of 18th century, this technology became prevalent in the United Kingdom, and introduced to the United States in the latter half of the 19th century. At that time, wastewater irrigation technology was primarily adopted as a convenient means to dispose of pollutants, but the processes were never examined scientifically. Strictly speaking, pre-20th century treatment technologies were not the true treatment technologies, as we understand them today; they were just the prototypes [1].

The twentieth century brought on a rapid economic progress, and with the development of high-density urbanization, the generation of household wastewater increased exponentially. A number of traditional sewage treatment technologies were already in full use, but their construction, operation and maintenance was very costly. With water shortages becoming increasingly urgent, a new and revolutionary technology in wastewater treatment had to be found. The solution turned out to be land treatment systems [2]. Despite their simplicity in design,

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they were capable of effective secondary and advanced tertiary level treatment. As a result, more and more countries began actively researching the land treatment system for wastewater, simultaneously promoting the concept of water recycling and reusing [3,4].

The objective of this study was to investigate the composition of the microbial community and examined the way they react to changes in water quality. After many years of continuous operation, the system had become unstable, and its water purification was no longer effective. The unstable part of the land treatment system was restored through soil and rock primed. When the system was restart, the relationships among the flora successions, the change of the microbial community, and the efficiency of water purification system were investigated to establish a reference of information background for improvements in the design and management of land treatment systems.

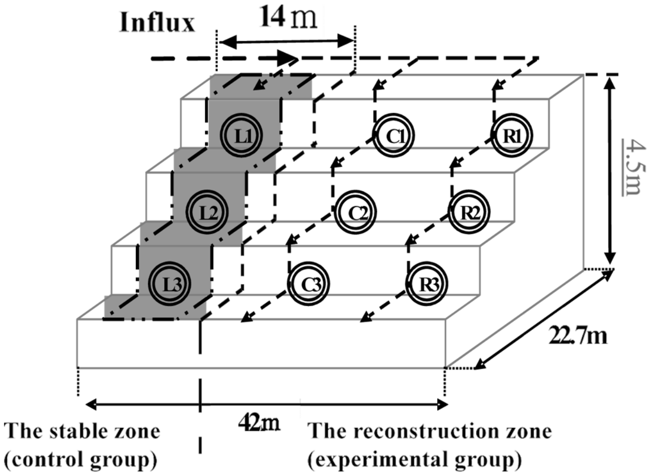

Fig. 1. The sampling sites of land treatment system.

2. Material and method

2.1. The system designing and restoration

The experiment site of this research was built near the irrigation ditch of the farm in Yunlin of Taiwan. The wastewater source is mainly the secondary effluent water from the sewage treatment plant at the campus (National Yunlin University of Science and Technology, TUNTECH), secondarily is the rainwater. This system was designed ladder shape (length 42 m, width 22.7 m and hight 4.5) in order to increase hydraulic retention time. The wastewater was flowed from top to bottom along ladder. The wastewater was flowed through the experiment site in order to remove excessive nutrients in the wastewater by natural purge, then water was passed into the ditch for irrigating the farm. Since the land treatment system was constructed in 2000, the right side of the structure in the system has collapsed due to hurricane or the other natural causes etc. This damage has altered the movement of water along the slope of the ladder, and the flow direction and rate cannot be controlled to achieve the expected removal efficiencies for nutrition in the system. Therefore, the collapsed portion of the land treatment system was restored in 2006. The damage was restored by soil and rock, and retaining wall was used to avoid the collapse happened again. All plants above the collapsed portion were harvested and removed. After restoring, the flora was into the secondary succession in the right side of the system.

2.2. Establishment of sampling sites

As Fig. 1 shows that the land treatment system was differentiated for two parts after restoring. Respectively, the stable zone was on the left side of the figure, the restoration zone was on the right side of the figure. There are nine sampling sites (L1–3, C1–3, and R1–3) in the system (Fig. 1). The sampling sites (L1, L2, and L3) in the stable zone are the controls. The sampling sites (C1, C2, C3, R1, R2, and R3) in the restoration zone comprise the experimental group. In the experiment period, the soil and water at the sampling sites were collected and tested respectively for the composition of the microbial community and water quality. At the same time, the succession of dominant plants was observed.

2.3. Analyzing methods

The soil below surface depth 5–10 cm was sampled around the sampling sites. The soil sampled was analyzed for four basic characteristics, including temperature (depth 5 cm), pH value (USEPA Method 9045D, 2004), moisture content (ISO11465, 1993), cation exchange capacity(CEC) (USEPA Method 9081, 1990). A part soil was the extracted for identifying species of the microorganism, by French Bio-Merieux API identification kits (20E and 20NE). Water was withdrawn at the sampling sites and analyzed for two nutrients, nitrate nitrogen (NO_3^--N) (No. NIEA W419.50A) and orthophosphate (PO_4^{3-}) (No. NIEA W427.52B), using the Standard Methods from the Taiwan Environmental Protection Administration. In the experiment field, the diversity of plants was changed with the season changed. The scientific names of the plants within a 1 m radius around the sampling sites were recorded, and the zone covered by the different plants was then calculated through the following method established by the Taiwan Environmental Protection Agency [5].

3. Results and discussion

3.1. The basic soil properties

3.1.1. Soil temperature and pH value

During the experiment period, an average temperature in the range of 16.6– 25.7°C (Table 1), similar to that

Table 1
The average climate temperatures during the experiment period

Exp. days	Relative month	Average temp. (°C)
10	Middle of October	25.7
20	End of October	25.1
30	Beginning of November	22.6
40	Middle of November	22.8
50	End of November	22.8
60	Beginning of December	20.3
70	Middle of December	17.9
80	End of December	16.6
90	Beginning of January	17.0
100	Middle of January	18.2

of subtropical winter conditions. As shown in Fig. 2, during the initial 20-day period, the average temperature of soil had a significant change in the system as the weather began cooling. Detailed observations of the differences in soil temperature indicated that the average soil temperature in the restored zone was 22.1°C while the stable zone averaged less than 2°C. This indicated that good plant life coverage in the stable zone had helped to reduce the average soil temperature. However, during the winter season, too low a soil temperature could cause the activity of microorganisms to decrease, which could indirectly affect the efficiency of water purification processes. As seen in Fig. 3, the average pH value of soil changed very little (approx. 6.8–7.3),. The result indicated that the soil system had excellent buffer ability to allow the pH value remain in the middle range, and to provide the opportune condition for nutrient removal.

3.1.2. *Soil moisture*

The average moisture content of soil in the stable zone was 32.6–38.8% greater than that of in the restoration zone (Fig. 4). The results indicate that there was a very close relationship between the moisture content of soil and the coverage of surface plants. When restoring engineering was finished, all plants in the restoration zone were removed. Therefore, the flora coverage of the restoration zone was much lower than the stable zone. The plants coverage on the soil surface could reduce water evaporation caused by wind and sun. During the entire construction period, the irrigated water system had to be shut down, leading to a gradual loss of moisture in the soil. When the first samples were taken after water was restored on day 10, the average moisture content in the soil had dropped by approximately 7% in both the control and experimental group. After the system was restarted, the coverage of plants and moisture content of the soil in the stable zone had noticeably outgrown that of in the restoration zone. After 20 days, the moisture content of the soil had stabilized, and appeared to follow the accelerated increase in the flora coverage. However, after 40 days, the increase rate had sharply fallen, indicating that when the all plants was removed during reconstruction period, the soil moisture in the restoration zone had been negatively affected by various climactic factors. The mois-

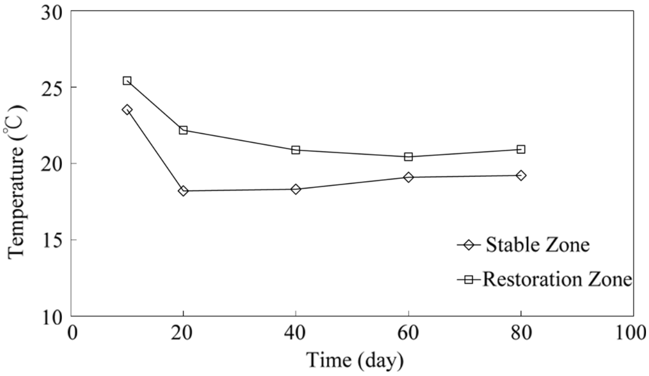

Fig. 2. The temperature of the soil in the land treatment system.

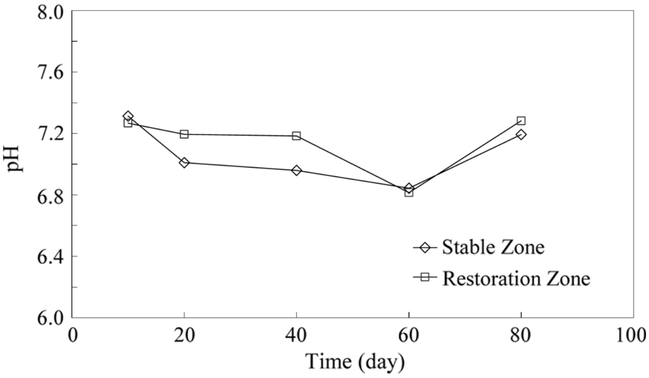

Fig. 3. The pH value of the soil in the land treatment system.

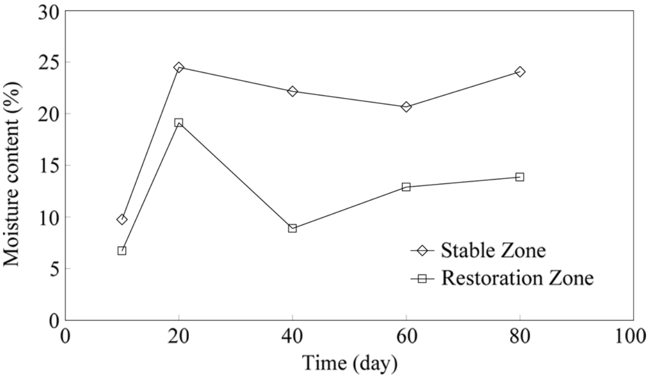

Fig. 4. The moisture content of the soil in the land treatment system.

ture content of the experimental group was found to be relatively unstable compared to that of the control group.

3.1.3. Cation exchange capacity (CEC)

As shown in Fig. 5, cation exchange capacity in the stable and restoration zone followed more different in development. As time passed, in the stable zone, the ability of the soil to exchange cations had gradually reached the saturation point, whereupon its cation exchanging capacity began to fall. The moisture in the soil seemed to have affected the viscosity of soil particles, which then affected water penetration into the soil. Low moisture content caused soil particles to pack closely together, resulting in an increase in molecular attraction leading to an increase in adhesion. The soil then began to aggregate as water became unable to penetrate into the soil. In fact, water penetrated into the soil more easily when the moisture content was higher, and cation exchange between water and soil increased. This explains the reduction in cation-exchange capacity within the stable zone.

3.2. Observation of the flora and the microbial community

3.2.1. The succession of dominant plants

Fig. 6 illustrates the total coverage of plants in the stable and restoration zone during the experiment period. The average flora coverage of the stable zone was higher than the restoration zone, but in the two zones, the flora coverage was increased with growth of dominant plants. Table 2 shows the succession of dominant plants over regular time intervals. The stable zone included three plants: *Bidens pilosa* and *Mikania micrantha* of Compositae family, *Mikania micrantha*, and *Brachiaria mutica* of Poaceae family. Initially, *Brachiaria mutica* was the dominant species, followed by *Mikania micrantha*, but after the fortieth day, *Mikania micrantha Brachiaria mutica* was gradually replaced by *Bidens pilosa* that become the dominant specie in the stable zone. Onsite records showed that *Bidens pilosa* began growing rapidly and blooming in a wide range after the fortieth day. According to past studies [6], most plants species became the dominant species during their blooming phase, occupying most of the zone within the system. However, *Mikania micrantha* survived mainly by clambering above *Brachiaria mutica*, its ability to compete for sun's energy diminished followed a decrease in the population of *Brachiaria mutica*.

As shown in Table 2, when construction engineering had been completed in the restoration zone, *Kyllinga brevifolia* of the Cyperaceae family was observed in sampling sites C. It started to spread from its original clump, gradually expanding in a bell-shaped distribution pattern, to become the dominant plant species of the restoration zone during the initial period. After the appearance of *Kyllinga brevifolia*, the much taller *Ageratum houstonianum* became the dominant species gradually. Under the explosive reproductive growth of *Ageratum houstonianum, Kyllinga brevifolia*, which grew much closer to the surface, was in a much weaker position to compete for the available energy of the sun. After the sixtieth day, *Kyllinga brevifolia* was completely replaced by *Ageratum houstonianum*.

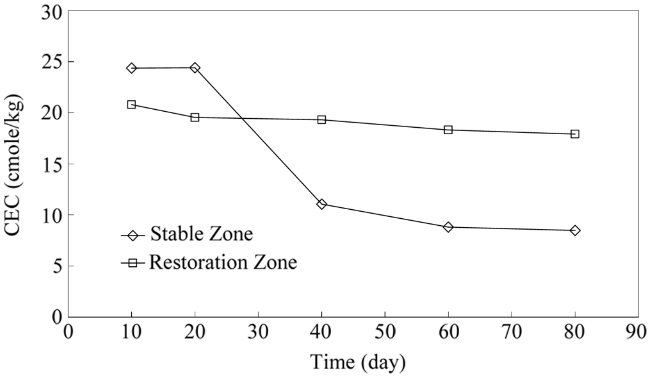

Fig. 5. The cation exchange capacity of the soil in the land treatment system.

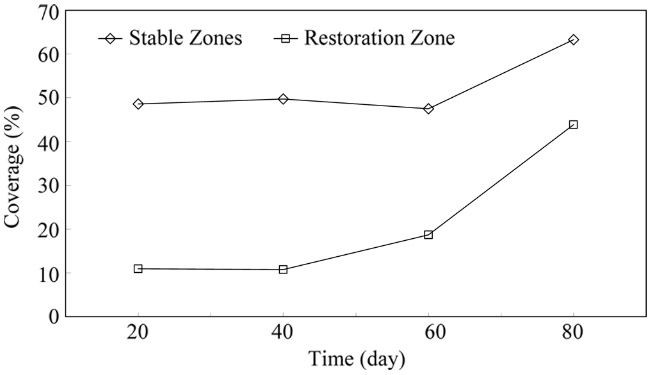

Fig. 6. The coverage of the dominant plants in the land treatment system.

3.2.2. The soil microbial community

Tables 3 and 4 show what occurred in the restoration zone after soil bacteria had been purified, analyzed, and identified. Until day 40, only 10 types of microorganism had existed, but by day 40–80, the number had grown to 15.

Table 3 shows the change of microbial species in the stable zone. The composition of the microbial community in the stable zone during the initial forty days period were represented mainly by *Aeromonas hydrophila/caviae, Aeromonas salmonicida* spp. *Salmonicida*, *Enterobacter cloacae*, and *Enterobacter sakazaki*i. Within day 40–80, they were represented by *Aeromonas salmonicida* spp. *Salmonicida*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*. Among all the microorganisms, *Aeromonas* species had

Table 2
The succession of dominant plants in the land treatment system

Zone	Sampling group L	Sampling group C	Sampling group R
Exp. days			
20	1. *Brachiaria mutica* 2. *Mikania micrantha* 3. *Bidnes pilosa*	1. *Kyllinga brevifolia* 2. *Brachiaria mutica*	1. *Kyllinga brevifolia*
40	1. *Brachiaria mutica* 2. *Mikania micrantha* 3. *Bidnes pilosa*	1. *Kyllinga brevifolia* 2. *Brachiaria mutica* 3. *Ageratum houstonianum*	1. *Kyllinga brevifolia* 2. *Ageratum houstonianum*
60	1. *Bidnes pilosa* 2. *Brachiaria mutica* 3. *Mikania micrantha*	1. *Ageratum houstonianum* 2. *Kyllinga brevifolia*	1. *Ageratum houstonianum* 2. *Kyllinga brevifolia*
80	1. *Bidnes pilosa* 2. *Brachiaria mutica* 3. *Mikania micrantha*	1. *Ageratum houstonianum* 2. *Kyllinga brevifolia*	1. *Ageratum houstonianum* 2. *Kyllinga brevifolia*

[a] In this table, the order from top to the bottom is the dominant order

[b] Compositae: *Ageratum houstonianum, Bidnes pilosa, Mikania micrantha*
Poaceae: *Brachiaria mutica*
Cyperaceae: *Kyllinga brevifolia*

Table 3
The change of microbial structure in the stable zone

Microbial species	Time (day)					Frequency
	10	20	40	60	80	
Aeromonas hydrophila/caviae	■	■	■			3
Aeromonas salmonicida ssp. *salmonicida*		■	■	■	■	4
Aeromonas hydrophila/caviae/sobria					■	1
Brevundimonas vesicularis					■	1
Burkholderia cepacia			■	■	■	3
Enterobacter cloacae		■	■			2
Enterobacter sakazakii		■	■			2
Non-fermenter spp.					■	1
Pseudomonas aeruginosa			■	■		2
Pantoea spp.			■	■	■	3
Pseudomonas luteola			■			1
Pasteurella pneumotropica / Mannheimia			■			1
Sphingomonas paucimobili			■	■		2
Stenotrophomonas maltophilia				■	■	2
Vibrio alginolyticus					■	1
Sum species	1	4	10	6	8	
New detected species	1	3	6	1	4	
Accumulative detected species	1	4	10	11	15	

Note: ■ indicates that the microbial species was detected at this time.

been sighted most frequently, especially after day 20 *Aeromonas salmonicida* spp. *Salmonicida* was found continuously. This demonstrated that *Aeromonas salmonicida* spp. *Salmonicida* was a strong and well-adapted microorganism, perfectly suited for the stable zone.

Table 4 shows that microorganisms in the restoration zone during the initial 40-day period were mainly represented by *Brevundimonas vesicularis, Enterobacter cloacae,* and *Sphingomonas paucimobilis*. within day 40–80, they were represented by *Aeromonas hydrophila/caviae*, *Burk-*

Table 4
The change of microbial structure in the restoration zone

Microbial species	Time (day)					Frequency
	10	20	40	60	80	
Aeromonas hydrophila/caviae			■	■	■	3
Brevundimonas vesicularis		■	■			2
Burkholderia cepacia				■	■	2
Enterobacter cloacae	■	■	■			3
Pantoea spp.					■	1
Stenotrophomonas maltophilia				■	■	2
Sphingomonas paucimobilis			■			1
Serratia liquefaciens					■	1
Sum species	1	2	4	3	5	
New detected species	1	1	2	2	2	
Accumulative detected species	1	2	4	6	8	

Note: ■ indicates that the microbial species was detected at this time.

holderia cepacia, and *Stenotrophomonas maltophilia*. Before 40 days, there were only four species of microorganism in existence. By day 40–80, the number had increased to eight species. This result indicated that microorganism species was more abundant as plant species increased in the restoration zone. *Aeromonas* species was sighted most frequently that the similar result in the stable zone.

3.2.3. Influence of flora succession on the soil microbial community

By comparing the number of the microbial species at different time intervals as shown in Tables 3 and 4, it was found that the number of the microbial species peaked right at the time of dominant plant succession. This plant succession process occurred on day 40 for the stable zone and the restoration zone. The number of the microbial species that had increased was ten and four for stable and restoration zone respectively. This result showed that the microbial structure of the soil had experienced a huge transformation during the flora succession period.

After day 60, we made a further comparison of microbial community relative to changes of the dominance plants in both zones. It showed that when the dominant plants was *Compositae, Burkholderia cepacia* and *Stenotrophomonas maltophilia* were common species of the microbial structure in the both zone. This result indicated that the microbial community and the dominance plants had link of a certain degree. The research confirmed, when microorganisms and multiple plants occupied the same limited space, they interacted with each other as a means to improve their chance of survival [7,8].

Further analysis of soil properties indicated that a number of factors had altered following the changes in the two zones. Moisture content and the exchange of cations fluctuated widely during the initial 40-day period of the experiment, and only gradually began to stabilize after day 40. The environment of the soil changed with a change of the flora succession, but when the flora coverage growth had stabilized, the changes in soil environment decreased. Even though soil properties had undergone the changes which did not affect the diversification of the microbial structure simultaneously. Based on the results generated thus far, it can be said that the microbial community and the flora succession were closely related, and the influence of plant on microorganisms within the plant rhizosphere far exceeded that around the soil [9]. Thus, the interaction between plants and microorganisms was the important factor most likely to have affected the efficiency of water purification in land treatment systems.

3.3. Influence of the microbial community on water purification

3.3.1. Removal of orthophosphate

Fig. 7 shows the efficiency of ortho-phosphate removal within the two zones. As indicated, those results were poor during the initial 20 days of the experiment, and various explanations have been proposed. For example, it was speculated that when the system took on wastewater at the beginning, basic soil properties had not yet stabilized and the microbial structure had not yet fully formed; therefore, the system's mechanisms for nutrient removal could not be implemented effectively. Between day 60–80 however, the flora coverage had reached the general level in the two zones; soil properties and the composition of the microbial community had become stable. This result indicated that nutrient removal mechanism had become effective. Therefore, the removal rate of ortho-phosphate gradually moved from drastic change to stable state.

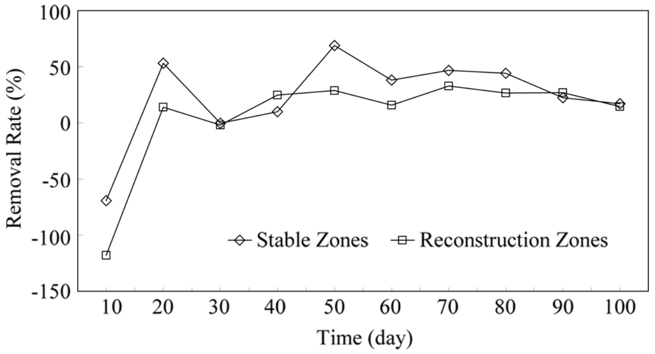

Fig. 7. The ortho-phosphate removal rate in the land treatment system.

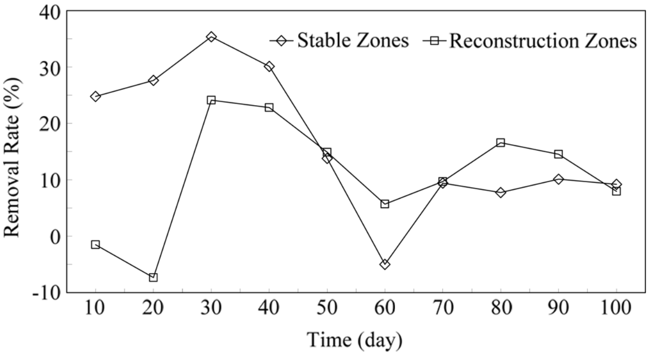

Fig. 8. The nitrate nitrogen removal rate in the land treatment system.

Between days 60–80, the concentration of ortho-phosphate from the incoming wastewater was approximately 0.32 mg/L, while ortho-phosphate removal rate of the system was very poor (18.7%). The research showed that when plants entered their blooming phase, phosphorus absorption increased [10]. Therefore, during those periods when dominant plants such as *Bidens pilosa* and *Ageratum houstonianum* were blooming, the demand for phosphorus was significantly higher than during their other growth stages. Under low concentration level conditions, the system was unable to provide sufficient phosphorus for the plants to grow.

At the same time, *Burkholderia cepacia* was discovered in the stable zone and restoration zone. The research showed that *Burkholderia cepacia* had the capacity to convert insoluble calcium phosphate into water-soluble phosphate compounds [11]. Biologically available phosphorus in nature was easily combined with cations (calcium, iron, aluminum, magnesium, etc.) in soil, to form insoluble phosphorus compounds (such as calcium phosphate, iron phosphate, aluminum phosphate, and magnesium phosphate). Those compounds were difficult for plants to absorb [12]. The most plant roots contain phosphate-solubilizing bacteria, which can transform biologically unavailable phosphorus into useful orthophosphate that plants can easily absorb [13–15]. It makes sense that the microbial composition of soil could indirectly affect the removal efficiency of ortho-phosphate from the system. Under low concentration level conditions, when wastewater phosphorus content was insufficient for plants to grow, phosphate-solubilizing bacteria in soil was prompted to increase ortho-phosphate within the system. Therefore, the ortho-phosphate removal rates of the system was significantly lower in this research.

3.3.2. Removal of nitrate nitrogen

Fig. 8 shows efficiency rate of nitrate nitrogen removal in the two zones. During day 30–40 in the experiment, this rate indicated a significant downward trend. This result was speculated that the species of bacteria in the soil had altered compositionally when succession of the dominant plants happened. That may have led to an interruption in the reaction to soil denitrification, with a corresponding loss in the ability to remove nitrate nitrogen. These results suggested that the change of bacteria composition in the soil was more obvious, the nitrate nitrogen removal rate of the system was affected significantly.

According to previous studies [16,17], when denitrified phosphorous bacteria are in an anaerobic environment, they make use of nitrate as electron acceptor, converting nitrate into nitrogen gas, with a release of phosphorus in the ensuing reaction. After day 60 of the experiment, under stable soil conditions, the nitrate nitrogen removal rate had gradually increased in the two zones. During this period, a large amount of *Aeromonas* (*Aeromonas hydrophila/caviae/sobria/salmonicida*) played the role of releasing the phosphorus and denitrification. In addition to converting phosphorus into orthophosphate that plants could use, it had also converted nitrate into nitrogen releasing it into the air, thus achieving the purpose of removing nitrate nitrogen from the wastewater.

4. Conclusions

When conditions of soil environment are stable, a succession of the dominant plants leads to the change of microbial composition in the soil, thereby affecting the performance of water purification systems.The existence of the special dominant plant was associated with the particular the microbial species. The presence of phosphate-solubilizing bacteria in soil indirectly affects efficiency of phosphate removal in the land treatment system. When *Compositae* family is the dominant plants, and *Burkholderia cepacia* exists in the soil, variations in phosphate concentration in inflow wastewater are the primary factor affecting the efficiency of phosphate removal in the system. In the future, about management of similar land treatment systems, there is a need to screening for the key dominant plant, as well as a need for regular testing of the microbial community, in order to ensure that land treatment systems achieves optimal water treatment performance.

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