

# Rhizosphere microbial community of duckweed in pilot-scale wastewater treatment system

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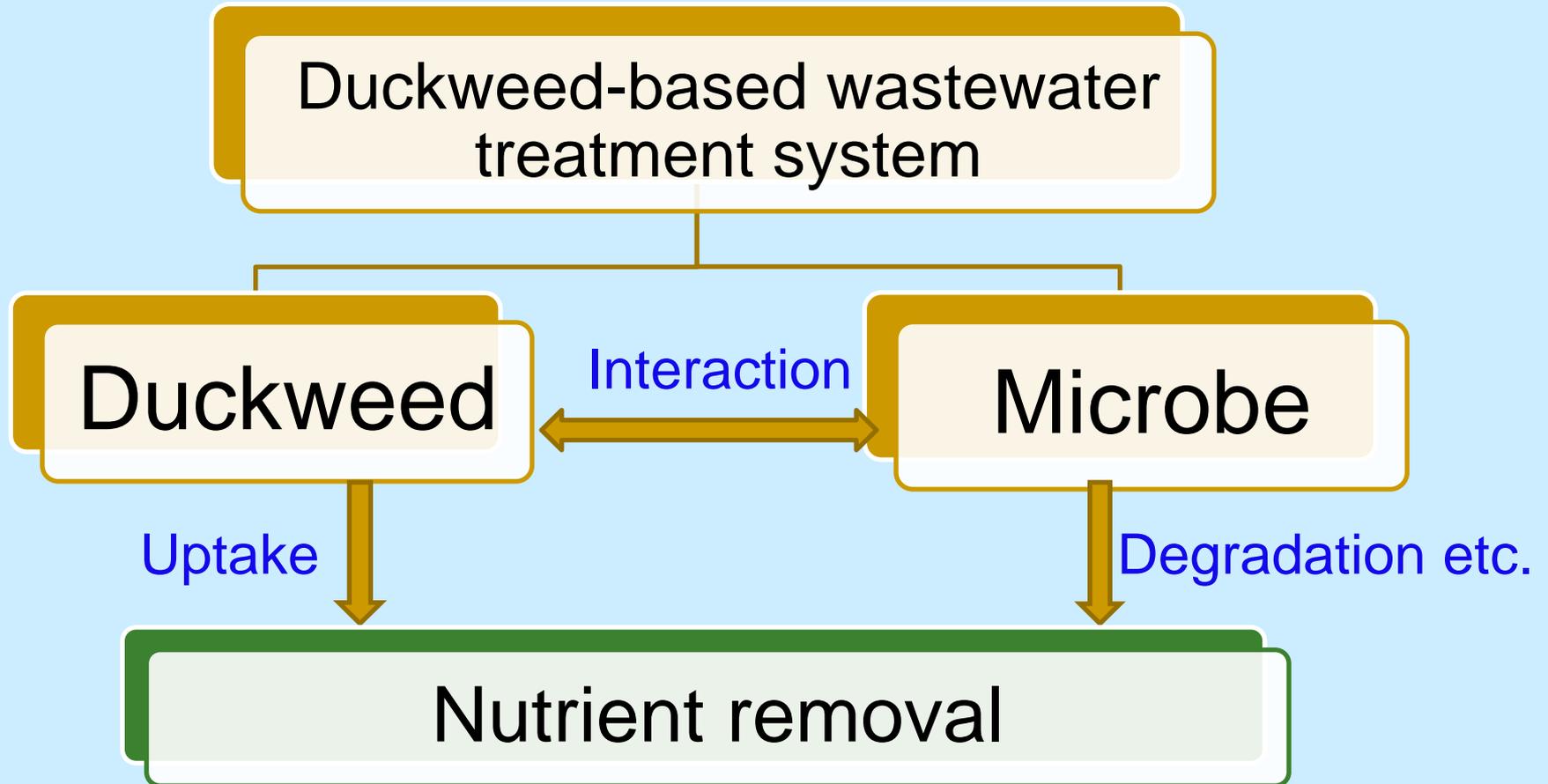
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# Outline

- Background
- Introduction of the experiment
- Results
- Conclusion

# Background



# The roles of microbes in duckweed wastewater treatment system

- COD Removal: degradation
- N Removal: nitrification, denitrification and assimilation
- P Removal: assimilation and accumulation
- Indirect roles: such as symbiosis by rhizobacterium and antagonism by pathogen

## **Relative to vulnerable microbes in wastewater, rhizosphere microbes are more**

- Stable
- abundant
- important in nutrient removal.

Rhizosphere microbes of duckweed from wastewater

### **In lab-scale**

- synthetic wastewater → limited
- shorter running period → unstable

The result can't show the really contribution of microbes in the system of practical application

## **In our previous pilot-scale study**

- Water hyacinth-based system performs better mainly owe to contributions of microbe
- By regulating and optimizing the functional microbe, it is possible the duckweed-based system achieve even more than Water hyacinth

## **It is very important to investigate**

- rhizosphere microbial community
- their contributions to nutrient removal
- regulating and optimizing functional microbes

**to improve the nutrient removal capacity**

# Introduction of the experiment

## ■ Duckweed pilot-scale reactor

Made by nickelclad, five basins in parallel. Each basin features an area of 12 m<sup>2</sup>(L × W × H = 24 m × 0.5 m × 0.6 m)

## ■ Operation

- Four different hydraulic detention times (HRT): 3d, 6d, 10d, 15d (corresponding inflow rate 2, 1, 0.6 and 0.4m<sup>3</sup>/d, respectively)
- Location: Beside the Dianchi lake in Kunming city, China
- Duckweed: local *Lemna minor*
- The coverage rate : 412 g/m<sup>2</sup> (about 150% )
- Harvest regime: 4 days
- Wastewater sampling: everyday



## ■ **Analysis of microbial communities**

- Roots collection: at the day 35<sup>th</sup> from four HRTs
- DNA extraction
- PCR amplification: 16S rDNA
- 454 Pyrosequencing
- Analysis of sequences

# Results

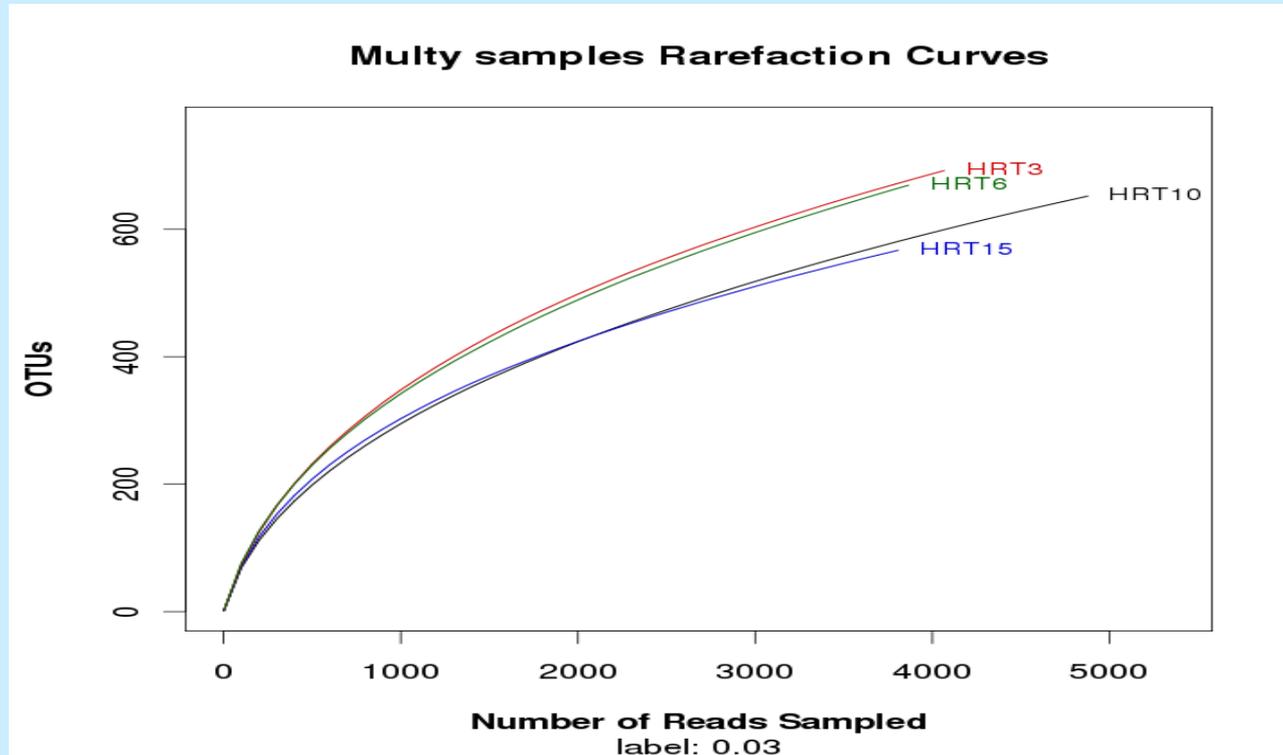
## ■ Amount of sequences from four samples

Number	Sequences	OTUs	Phyla
HRT3	4067	692	21
HRT6	3865	669	19
HRT10	4877	652	18
HRT15	3807	567	18
Total	16616		

All sequences are affiliated with 23 phyla, 1453 OTUs (label: 0.03)

High level of microbial diversity

## ■ Rarefaction curve



- About 4000 reads per sample is enough to really reveal the microbial communities

## ■ Coverage, abundance and diversity

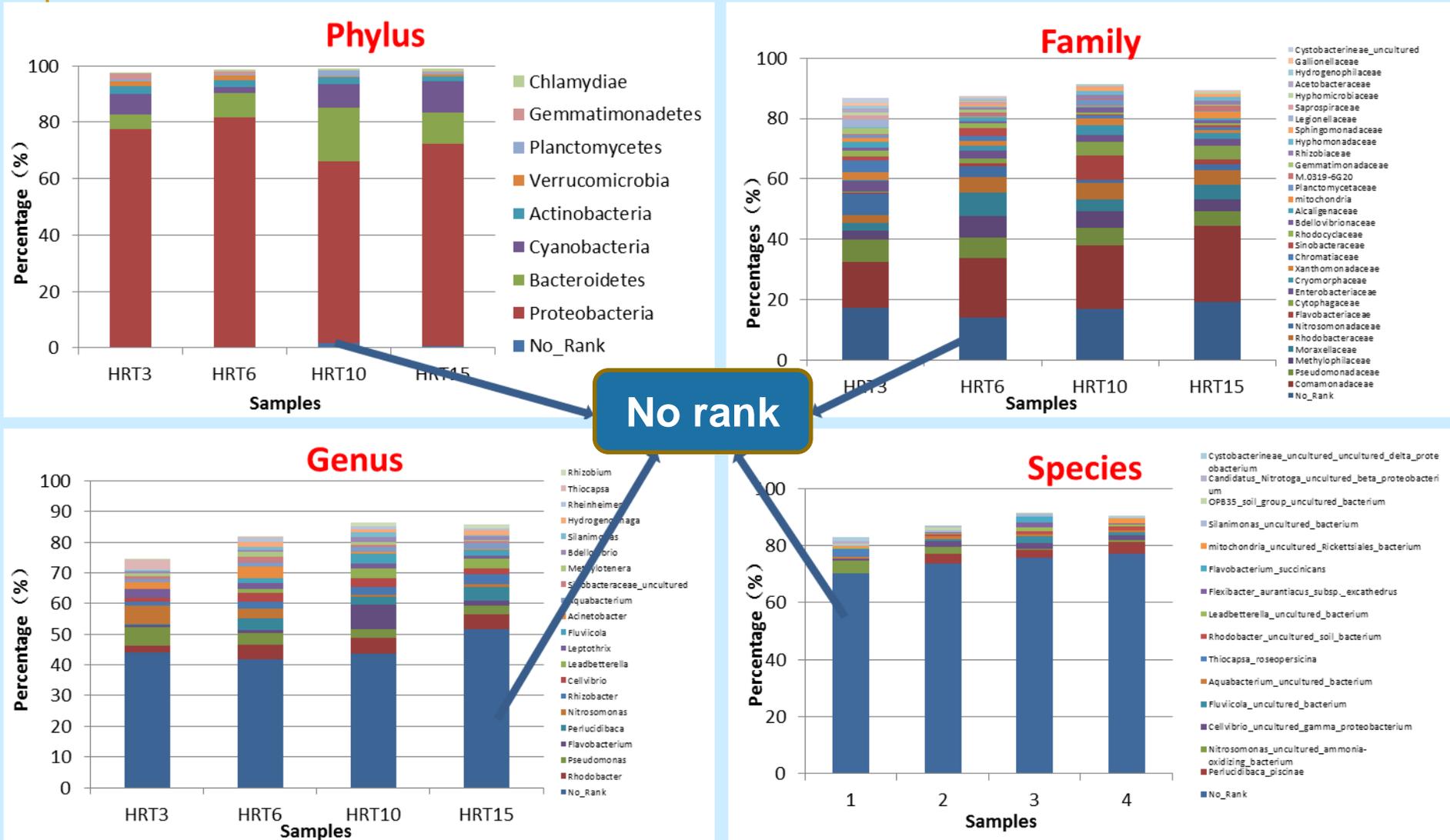
Index	Coverage	Ace	Chao	Shannon	Simpson
HRT3	0.939	1362	1112	5.50	0.0101
HRT6	0.935	1424	1158	5.56	0.0075
HRT10	0.925	1448	1068	5.26	0.0118
HRT15	0.921	1158	939	5.28	0.0131

➤ **Coverage:** about 93%, most of the microbes can be covered

➤ **Abundance** (ace and chao): HRT15 shows the lowest abundance because of deficiency of nutrient

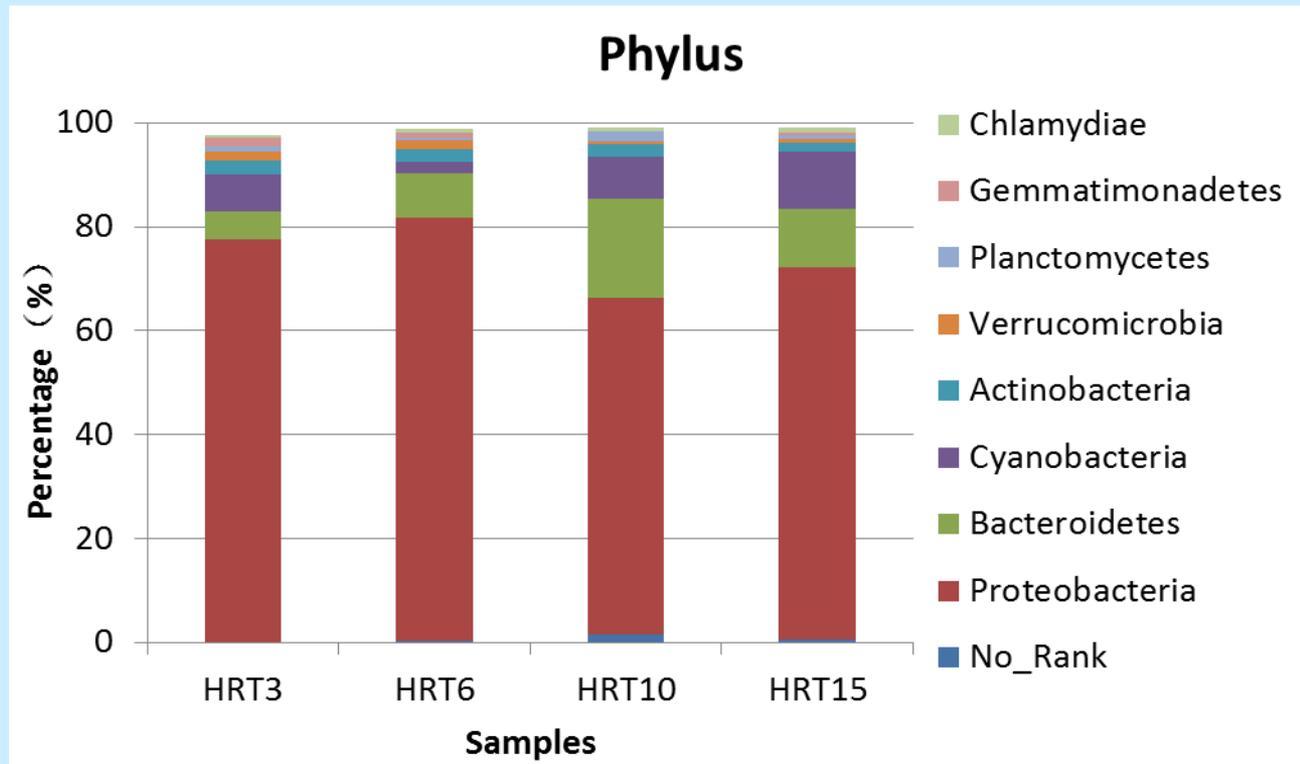
➤ **Diversity** (shannon and simpson): HRT6 shows the highest abundance, diversity is related to the concentration of nutrient, both higher (shorter HRT) and lower concentration (longer HRT) can decrease microbial diversity

# Dominant microflora (abundance $\geq 1\%$ )



The smaller the taxonomic unit is, the higher the percentage of No rank became  
 The taxonomic status of most reads can't be identified in species or genus level

## ■ Phylum



- *Proteobacteria* (60-80%) is the most abundant phylum
- Other dominant phyla were *Bacteroidetes*, *Cyanobacteria*, *Actinobacteria*, *Verrucomicrobia* and so on.

## Comparing with Matsuzawa's study (2010)

Similar in microbial community composition , but different significantly in percentage of phyla.

The reasons might be

- **Difference of duckweed species**

*Lemna minor*; *Spirodela polyrrhiza*

- **Research method**

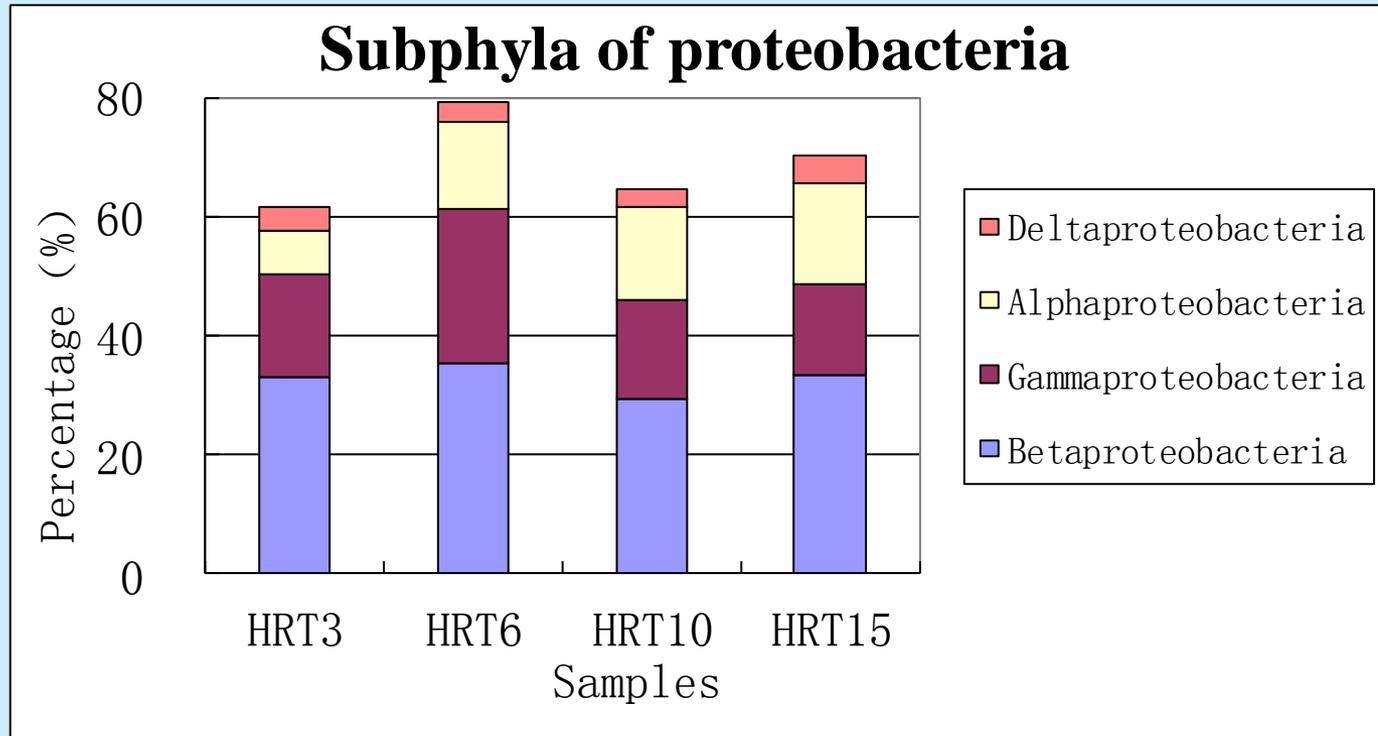
454 Pyrosequencing; Clone library  
(112 clones)

- **Growing environment**

Pilot-scale in field; Lab-scale

Phyla	Percentage	
	This study	Matsuzawa
Alphaproteobacteria	14.5	14
Betaproteobacteria	32.5	45
Gammaproteobacteria	20.8	2
Deltaproteobacteria	4.2	2
Bacteroidetes	11.5	11
Verrucomicrobia	1.1	2
Planctomycetes	1.1	2
Cyanobacteria	7.1	22

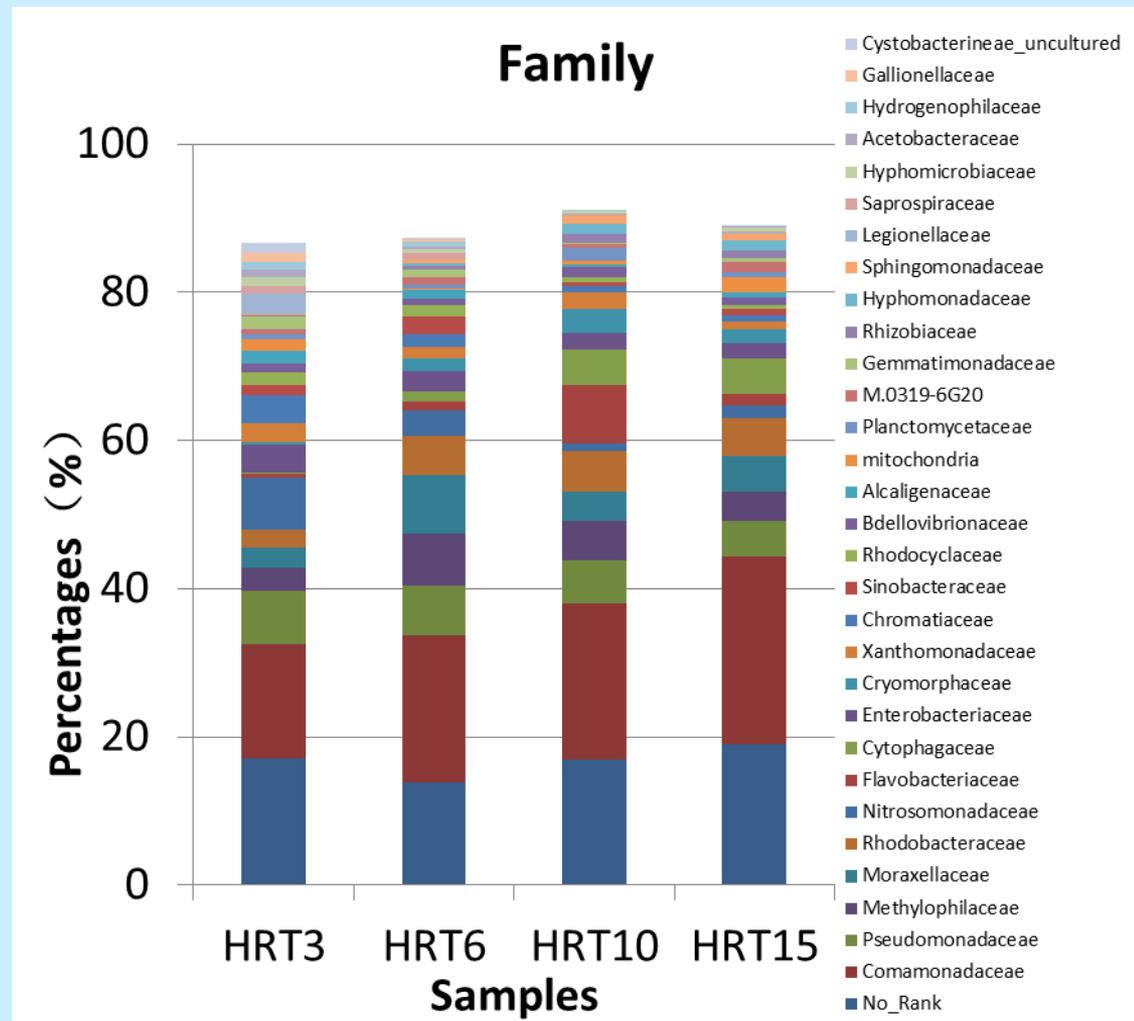
## Percentage of subphyla of *proteobacteria*



- Significantly difference in different HRT samples

## ■ Family

- *Comamonadaceae* (about 20%) is the most abundant phylum
- with the HRT is increasing, the percentage of *Comamonadaceae* increased
- *Comamonadaceae* is the most common functional bacterium in wastewater treatment system



## ■ Genus

HRT(d)	Effluent (mg/L)		Duckweed removal (g)		Microbe removal (g)		Duckweed removal rate (%)		Microbe removal rate (%)	
	ammonia	P	ammonia	P	ammonia	P	ammonia	P	ammonia	P
3	7.99	1.34	246.78	59.23	239.57	22.05	50.74	72.87	49.26	27.13
6	3.81	0.77	230.09	47.34	150.47	13.16	60.46	78.24	39.54	21.76
10	1.06	0.36	199.28	32.29	106.01	13.28	65.28	70.85	34.72	29.15
15	0.24	0.15	173.73	23.47	51.41	10.37	77.16	69.35	22.84	30.65

### *Pseudomonas*

**(HRT3: 6.17%, HRT6: 3.91%, HRT10: 2.92%, HRT15: 2.86%)**

- Some strains are heterotrophic nitrifying bacterium, can remove COD and nitrogen simultaneously.
- The abundance is positive correlated with the gross of N removal by microbes.

### *Nitrosomonas*

**(HRT3: 6.12%, HRT6: 3.13%, HRT10: 1.16%, HRT15:0.49%)**

- *Nitrosomonas* is an important player in converting ammonia to nitrite
- The abundance is also positive correlated with the gross of N removal by microbes

## ***Rhizobacterium***

**(HRT3: 1.48%, HRT6: 2.38%, HRT10: 2.79%, HRT15: 3.13%)**

- Has the ability to fix nitrogen for plant growth
- The abundance is negative correlated with the ammonia concentration in wastewater, shows *Rhizobacteria* can multiply and fix more nitrogen for duckweed growth in low ammonia conditions
- Other study also showed (Zuberer, 1982) about 15-20% came from nitrogen fixation in total nitrogen needed for duckweed growth

## ***Rhodobacter***

**(HRT3: 2.11 %, HRT6: 4.76%, HRT10: 5.13%, HRT15: 4.68%)**

- The most-studied photosynthetic organism
- Has various metabolic pathways and ability to degrade pollutants

## ***Flavobacterium***

**(HRT3:0.54 %, HRT6: 1.14%, HRT10: 7.87%, HRT15: 1.58%)**

- Mainly found in soil, water and rhizosphere environments.
- Plays an important role in degradation of organic matter

## ***Perlucidibaca***

(HRT3:0.15 %, HRT6:3.73%, HRT10:2.60%, HRT15:4.33%)

- A new genus, first discovered and named from freshwater in 2008
- *Perlucidibaca\_piscinae*, was isolated from wastewater treatment system by Hao (2012) , showed excellent nitrification ability in the conditions of low temperature (10°C)
- First finds *Perlucidibaca* is the dominant microflora in the rhizosphere

## ***Cellvibrio***

(HRT3:1.08%, HRT6:2.90%, HRT10:2.83%, HRT15:1.97%)

- A group of bacteria with ability of cellulose degradation

## ***Leadbetterella***

(HRT3: 0.05%, HRT6: 1.17%, HRT10: 3.10%, HRT15: 3.10%)

- A new genus was first discovered and named from freshwater in 2008
- The abundance was positive correlated with the HRT(or negative with nutrient concentration)

## ***Leptothrix***

**(HRT3: 2.73%, HRT6: 1.91%, HRT10: 1.50%, HRT15: 1.00%)**

- Easy to thrive in sewage treatment plant and lead to block pipes and equipment
- The abundance is negative correlated with the HRT(or positive with nutrient concentration)

## ***Aquabacterium***

**(HRT3:0.96 %, HRT6: 1.24%, HRT10: 1.46%, HRT15: 1.84%)**

- The abundance is positive correlated with the HRT(or negative with nutrient concentration)

## ***Acinetobacter***

**(HRT3: 2.19%, HRT6: 3.75%, HRT10: 0.88%, HRT15: 0.34%)**

- Some strains have the ability of heterotrophic nitrification or denitrifying phosphorus removal
- May be related to the removal of COD, N and P

### **Thiocapsa:**

**(HRT3:3.05 %, HRT6: 0.13%, HRT10: 0%, HRT15: 0%)**

- *The abundance decrease sharply with the increasing of HRT, The abundance in HRT3 was 3.05%, but nothing could be detected in HRT10 and HRT15*

### **Legionella:**

**(HRT3:2.61%, HRT6: 0.026%, HRT10: 0.021%, HRT15: 0.026%)**

- *The abundance in HRT3 is about one hundred times than that in others*

### **OM27\_clade:**

**(HRT3:0.59%, HRT6: 0%, HRT10: 0%, HRT15:0%)**

- *First discovered and named from seawater in 2006, no any other detailed information can be found out*
- *Only found in HRT3*

### **Chitinimonas**

**(HRT3: 0%, HRT6: 0%, HRT10: 0%, HRT15: 0.60%)**

- *A group of bacteria with ability of chitin degradation*
- *Only found in HRT15*

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# Conclusion

- Revealed a high level of microbial diversity and a significantly difference of microbial community in the rhizosphere of duckweed under different HRTs.
- The abundance of N removal bacteria was positive correlated with the ammonia concentration and the gross of N removal in wastewater
- The abundance of N-fixing bacteria was negative correlated with the ammonia concentration in wastewater

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Thank you

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