

Spatio-Temporal Patterns of Nitrogen and Phosphorus Stoichiometry in Cascade Ponds in an Agricultural Small Watershed and Their **Influencing Factors**

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Abstract

Ecological stoichiometry of nitrogen and phosphorus is an important indicator to characterize the nitrogen and phosphorus trophic status in aquatic ecosystems. The study of the spatio-temporal patterns of nitrogen and phosphorus stoichiometry is beneficial to the nitrogen and phosphorus pollution management in pond ecosystems. In this study, 18 groups (36 in total) of typical cascade ponds were selected as long-term observations to investigate the spatial distribution patterns of nitrogen and phosphorus component ratios (ratio of total nitrogen to phosphorus: TN:TP, ratio of dissolved nitrogen to phosphorus: TDN:TDP, ratio of particulate nitrogen to phosphorus: PN:PP) in water bodies in the tropical agricultural watershed of Jinjing. The results showed that the average values of TN:TP and TDN:TDP in the upstream ponds were 26.4 and 53.4, respectively, and were more than those in the downstream (22.95 and 48.1, respectively). In contrast, the PN:PP (13.78) in the upstream was significantly lower than that of the downstream (30.39). Furthermore, the factors of rainfall, agricultural land use and fish farming influenced the spatio-temporal variability of the N:P ratios. The ratios of TN:TP and TDN:TDP were higher in the wet season and lower in the dry season. Agricultural land use and fish farming reduced the ratios of the above three nitrogen and phosphorus components in cascade ponds in the study area. Our results show that strengthening agricultural land pollution control and aquaculture management could help to improve water quality of pond ecosystems in the study area.

Keywords

Agricultural Land Use, Fish Farming, Pond Ecosystems, Nitrogen and

Phosphorus Component, Nitrogen and Phosphorus Stoichiometry

1. Introduction

Water pollution management has been the focus of worldwide social attention in recent years. How to economically and efficiently reduce nitrogen and phosphorus loads in watersheds is the key to water body nitrogen and phosphorus pollution management. The emerging ecological stoichiometry provides a new perspective for the prevention and control of nitrogen and phosphorus pollution in water bodies. The nitrogen and phosphorus stoichiometry theory of water bodies suggests that selective control of N or P, which play a limiting role in the primary productivity of aquatic ecosystems, is an effective measure to mitigate eutrophication in water bodies [1] [2]. Ecological stoichiometry theory is derived from the classical Redfield ratio (a fixed 106:16:1 molar ratio of carbon, nitrogen and phosphorus in marine phytoplankton, *i.e.* a mass ratio of 41.1:7.2:1) and has since been advanced by several scholars [3] [4] [5]. Nitrogen and phosphorus nutrients are the material basis for the primary productivity of water bodies such as ponds and are key factors in causing cyanobacterial outbreaks in lakes [6] [7]. The ratio of nitrogen and phosphorus ratio s can express the nutrient structure characteristics of water bodies, and to a certain extent could be used to determine the limitation state of nitrogen and phosphorus nutrients [8] [9] [10]. The study of the spatial and temporal characteristics of the ecological stoichiometry of nitrogen and phosphorus in water bodies contributes to the understanding of eutrophication processes, competition of aquatic organisms and population succession in aquatic ecosystems [5] [8] [9].

Ponds and reservoirs are generally small surface depressions that hold still and shallow water [11] [12]. The global number of small ponds ($<0.01 \text{ km}^2$) is estimated to be $5.47 \times 108 - 3.2 \times 109$, with great uncertainty owing to failure to be detected by traditional satellite [13] [14]. Thus, the importance of ponds has been ignored in the ecosystems. In recent years, the ecological function of ponds for ecosystems and climate change has gain more and more attention [15] [16] [17] [18] [19]. Farming ponds serve as ecological functions of hydrological regulation, pollution mitigation, biodiversity conservation and socioeconomic benefits. Ponds and reservoirs are widely distributed across southern China due to their abundant precipitation and agricultural demand, such as fish farming and paddy planting [1] [2].

In particular, due to differences in elevation, there are many cascade ponds that are connected upstream and downstream in the hilly areas of southern China. Cascade pond system is a wetland system in which multiple ponds are connected by ditches, surface runoff or groundwater runoff, which has the ecological function of intercepting surface runoff and sediment, as well as effectively controlling non-point source pollution and effectively intercepting nitrogen and phosphorus nutrients in the watershed. The cascade ponds are a combination of multiple ponds, which are related upstream and downstream due to differences in altitude and topography. Cascade ponds are the important nodes at which materials from surface runoff are deposited and retained in the water columns. Previous studies have focused on the variability of nitrogen and phosphorus in pond waters and the affecting factors [15] [17] [19] [20] [21]. The spatialtemporal characteristics of the ecological stoichiometry of the nitrogen and phosphorus components are still limited. To explore the spatio-temporal patterns of nitrogen and phosphorus stoichiometry in pond ecosystems, especially cascade pond system, 18 groups (36 in total) of typical cascade ponds were selected in water bodies in the tropical agricultural watershed of Jinjing. We determined the concentrations of the contents of different nitrogen and phosphorus components, and investigate the spatial distribution patterns of nitrogen and phosphorus component ratios (ratio of total nitrogen to phosphorus: TN:TP, ratio of dissolved nitrogen to phosphorus: TDN:TDP, ratio of particulate nitrogen to phosphorus: PN:PP). Our results could help to both improve water quality of pond ecosystems in the study area.

2. Materials and Methods

2.1. Study Area

The headwater watershed of Jinjing (105 km²) was selected as the study area in a hilly subtropical region of the Hunan Province, China. The annual precipitation in the study area ranges from 1200 to 1500 mm, and the elevation ranges from 56 m to 434.8 m. The land use in the study area is dominated by three major types, namely forest, paddy fields, and tea plantations [22] [23], which account for 58.5%, 31.6%, and 4.3% of the total area, respectively. 5.6% of the remaining areas are waters and residential ones. In areas like this, agricultural products and aquaculture are generally the main sources of income for farmers.

2.2. Selection of Sampling Locations for Cascade Ponds

The high-resolution imagery (December 2016, 0.5 m resolution) was used to extract a spatial distribution map of ponds in the study area Using ArcGIS software. There were a total of 2012 small water bodies of ponds in the study area, with a distribution density of 19 ponds·km⁻². Eighteen representative groups of cascade ponds were selected, that was, one pond in each group upstream and downstream, for a total of 36 ponds. The GPS coordinates of the pond sampler and land use types around the ponds were recorded, and the spatial distribution of the sample sites was shown in **Figure 1**. The results of the previous study showed that the TN of the upstream ponds was lower than that of the downstream, the TP content of the upstream and downstream ponds was close, and the surrounding landscape types and land use practices were important factors influencing the nitrogen and phosphorus contents of the cascade ponds [24]. This paper continued to investigate the spatial and temporal distribution of the



Figure 1. The spatial distribution of pond samples in the subtropical hilly watershed of Jinjing, China.

ratios of the three different nitrogen and phosphorus components in the 36 selected in the study area.

2.3. Water Sample Collection and Analysis

The water samples were collected below the surface of 30 - 50 cm from June 2017 to May 2018. All water samples were filtered through 0.45 μ m membrane filters to remove non-dissolved solid particulate matter within 24 h after sampling and stored at -20° C until analysis. Water samples were transported to the laboratory where various N and P fractions, such as total nitrogen (TN), total phosphorus (TP), dissolved total nitrogen (TDN), dissolved total phosphorus (TDP), were determined. The alkaline potassium persulfate digestion-UV spectrophotometric method was used to measure the contents of N fractions, and sulfuric/perchloric acid digestion-spectrophotometric method for ones of P fractions. The contents of particulate nitrogen (PN), and particulate phosphorus (PP), were calculated indirectly, *i.e.* the differences between the total nitrogen and phosphorus contents and the dissolved nitrogen and phosphorus contents.

3. Results

3.1. Statistical Description of the Ratios N and P Fractions

The annual mean values of TN:TP, TDN:TDP and PN:PP in the water bodies of the cascade ponds in the study area had a large variability (Table 1). The annual mean values of TN:TP in the upstream ponds ranged from 13.4 to 55.5, with a mean value of 26.3, while the annual mean values of TN:TP in the downstream ponds ranged from 15.6 to 47.1, with a mean value of 23.2. The average values of TDN:TDP in the upstream ponds ranged from 14.6 to 127.85, with a mean value of 53.24. The values of PN:PP in upstream and downstream varied from 9.92 to 40.39 and 8.46 to 87.72, with mean values of 13.78 and 30.39, respectively. Overall, TN:TP and TDN:TDP in the upstream ponds were higher than those in the downstream ponds. In contrast, the average values of PN:PP in the upstream was smaller than that in the downstream ponds. The trends in the ratios of the three nitrogen and phosphorus components may be related to the differences in pollutant inputs between the upstream and downstream ponds. This resulted in a situation where the nutrient status of the water bodies in the downstream ponds were more limited by phosphorus than that of the upstream. This explains why the nitrogen to phosphorus ratios were higher in the downstream than in the upstream.

3.2. Monthly Variation and Spatial Distribution Characteristics of N:P Ratios in Cascade Ponds

From June 2017 to May 2018, the ratios of nitrogen and phosphorus components had a large fluctuation over time in the upstream and downstream ponds in the study area. The monthly mean values of TN:TP ratios in the upstream and downstream ponds fluctuated between 17.7 and 46.8 (**Figure 2**), with high values occurring in spring and summer and low values mainly in winter. Compared to the TN:TP ratio, the variability of the TDN:TDP ratio was greater, ranging from 18.4 to 121.1. Similar to the trend in the distribution of high and low values of the TN:TP ratio, high values of TDN:TDP occurred in spring and summer, while low values mainly occurred in winter (**Figure 3**), The monthly mean values of the particulate N:P ratios ranged from 11.9 to 103.1 in the study area. Unlike the above trends in the distribution of high and low values of TN:TP and TDN:TDP ratios, the high values of PN:PP appeared mainly in winter (**Figure 4**). This may suggest that the mechanisms driving the variation in particulate N:PP ratios in pond waters differ from those of TN:TPN and TDN:TDP ratios in the study area.

The TN:TP, TDN:TDP and PN:PP ratios of the watershed cascade ponds were further classified into five classes and the spatial distribution of the three ratios was output using ArcGIS software. The results showed that the ratios of the three nitrogen and phosphorus components had similar spatial distribution patterns. In general, the TN:TP and TDN:TDP ratios in the upstream ponds were relatively higher than those in the downstream ponds. Moreover, the ratios of

Group ID	Pond ID	Location	TN:TP	TDN:TDP	PN:PP	Land use	Management
1	P6	Upstream	14.6 ± 5.8	24.6 ± 6.7	13.6 ± 7.0	Forest	Fish farming
	P10	Downstream	28.5 ± 3.6	35.5 ± 11.5	64.8 ± 19.8	Paddy	Fish farming
2	P20	Upstream	24.4 ± 7.4	57.2 ± 6.1	17.3 ± 8.0	Residential	Fish farming
	P17	Downstream	18.6 ± 1.4	49.8 ± 5.8	17.36 ± 1.5	Residential	Fish farming
3	P29	Upstream	26.8 ± 12.1	99.3 ± 2.06	17.01 ± 9.87	Forest	Fish farming
	P24	Downstream	50.6 ± 42.5	73.6 ± 18.3	55.47 ± 109.12	Paddy	Fish farming
4	P30	Upstream	13.7 ± 5.1	47.9 ± 4.3	9.92 ± 4.36	Forest	Non-fish farming
	P27	Downstream	33.0 ± 33.5	90.1 ± 5.2	20.3 ± 12.5	Forest	Fish farming
5	P45	Upstream	26.2 ± 16.1	52.6 ± 5.2	18.2 ± 21.7	Forest	Fish farming
	P44	Downstream	20.9 ± 11.3	34.5 ± 5.6	25.2 ± 16.9	Forest	Fish farming
6	P49	Upstream	15.6 ± 6.9	29.6 ± 2.9	12.9 ± 6.6	Forest	Non-fish farming
	P48	Downstream	22.3 ± 16.7	32.9 ± 8.4	87.7 ± 23.5	Residential	Fish farming
7	P54	Upstream	25.3 ± 15.1	61.9 ± 5.6	25.1 ± 2.8	Forest	Non-fish farming
	P56	Downstream	23.2 ± 6.4	45.5 ± 7.9	22.3 ± 18.8	Paddy	Non-fish farming
8	P60	Upstream	19.0 ± 7.2	30.1 ± 2.9	21.4 ± 15.2	Forest	Fish farming
	P58	Downstream	21.2 ± 8.7	31.9 ± 11.8	28.7 ± 33.8	Paddy	Fish farming
9	P61	Upstream	25.2 ± 7.6	61.2 ± 13.4	19.2 ± 11.2	Forest	Non-fish farming
	P62	Downstream	29.4 ± 12.4	46.3 ± 15.3	24.0 ± 15.0	Residential	Fish farming
10	P68	Upstream	18.9 ± 1.0	26.1 ± 21.2	18.3 ± 3.3	Forest	Non-fish farming
	P70	Downstream	20.3 ± 3.7	46.3 ± 16.2	17.0 ± 6.2	Paddy	Fish farming
11	P75	Upstream	17.9 ± 14.9	50.1 ± 6.5	12.3 ± 10.0	Paddy	Non-fish farming
	P74	Downstream	26.1 ± 25.5	44.3 ± 14.2	22.2 ± 18.8	Forest	Non-fish farming
12	P80	Upstream	26.7 ± 7.8	47.6 ± 6.2	22.5 ± 19.7	Forest	Fish farming
	P73	Downstream	28.7 ± 11.9	57.1 ± 10.3	31.9 ± 3.2	Paddy	Fish farming
13	P84	Upstream	13.3 ± 2.1	50.1 ± 13.0	11.1 ± 12.5	Forest	Fish farming
	P82	Downstream	15.6 ± 9.5	32.3 ± 11.8	14.8 ± 16.5	Forest	Fish farming
14	P97	Upstream	47.1 ± 4.2	54.8 ± 5.9	40.3 ± 5.3	Forest	Fish farming
	P95	Downstream	21.5 ± 10.1	42.2 ± 13.5	16.5 ± 8.7	Forest	Fish farming
15	P100	Upstream	16.1 ± 7.3	45.9 ± 23.0	10.7 ± 5.6	Residential	Fish farming
	P99	Downstream	38.0 ± 31.2	53.8 ± 12.1	37.2 ± 72.4	Residential	Fish farming
16	P86	Upstream	13.6 ± 5.5	14.6 ± 5.5	17.1 ± 11.8	Paddy	Non-fish farming
	P117	Downstream	23.9 ± 4.4	53.4 ± 8.2	26.6 ± 21.9	Residential	Non-fish farming
17	P88	Upstream	23.3 ± 5.4	127.8 ± 4.6	23.5 ± 27.0	Forest	Non-fish farming
	P116	Downstream	55.6 ± 6.2	38.5 ± 139	26.0 ± 13.1	Forest	Fish farming
18	P114	Upstream	30.6 ± 18.4	76.2 ± 12.2	10.3 ± 8.2	Forest	Non-fish farming
	P112	Downstream	26.3 ± 11.4	56.2 ± 19.5	18.4 ± 8.0	Paddy	Non-fish farming

 Table 1. Ratios of nitrogen and phosphorus components in water bodies and their surrounding land use at different pond observation sites.



Figure 2. Monthly variation of TN:TP in water bodies of cascade ponds.



Figure 3. Monthly variation of TDN:TDP in water bodies of cascade ponds.



Figure 4. Monthly variation of PN:PP in water bodies of cascade ponds.

the three nitrogen and phosphorus components were generally higher in the ponds surrounded by forests than in the ponds surrounded by farmland and residential areas (Figure 5). This may suggest that Non-point source nitrogen and phosphorus pollution from agricultural lands and residential sewage affected the ratios of nitrogen and phosphorus components in the study area.



Figure 5. Spatial distribution patterns of nitrogen to phosphorus ratios in water bodies of cascade ponds. ((a): TN:TP, (b): TDN:TDP and (c): PN:PP).

3.3. Effect of Agricultural Land Use and Fish Farming on the Ratios of Nitrogen and Phosphorus Components

Fish farming is the main use of ponds in the study area and is an important factor influencing the nitrogen and phosphorus component content of pond waters. Compared to ponds without fish farming, the contents of the three nitrogen and phosphorus components (total, dissolved and particulate), increased significantly in fish-farmed ponds (**Figure 6**). The concentrations of TN, TP, TDN, TDP, PN and PP increased by 37.5%, 47.1%, 66.3%, 66.7%, 66.3% and 80.0%, respectively.

In order to investigate the influence of the surrounding landscape types on the ratios of the nitrogen and phosphorus fractions in the ponds, the ponds were further subdivided into three types of ponds: forest, agricultural and residential. In the same pond utilization, the types of land use around the ponds affected the nitrogen to phosphorus ratios. When ponds were used for fish farming, the ranking of the magnitude of the three N:P ratios under different land uses followed the same pattern, *i.e.* forest (29.8 to 49.0) > agricultural land (23.4 to 47.8) > residential area (20.5 to 40.7) (**Figure 7**). When the ponds were not fish-farmed, the ranking of the magnitude of the N and P ratios under different land uses were similar, *i.e.* forest > agricultural land and residential land. This suggests that agricultural land use and domestic pollution discharges exacerbate the imbalance in nitrogen and phosphorus accumulation in the pond water columns.

Hierarchical variance decomposition was further used to estimate the contributions of land use and pond management to the variance in the ratios of nitrogen



Figure 6. Comparison of the contents of nitrogen and phosphorus components under different pond managements with and without fish farming.



Figure 7. The ratios of nitrogen and phosphorus components under the different treatments of agricultural land use and fish farming.

and phosphorus components in pond waters. The results showed that there was a difference in the contributions of land use (forest, farmland and residential) and pond management (fish farming) to the variance of the nitrogen and phosphorus component ratios. The contribution of land use was significantly smaller than that of pond management. The co-contribution of both to the TN:TP, TDN:TDP and PN:PP, were 71.2%, 55.8% and 40.2%, respectively (**Figure 8**). This further demonstrates that agricultural land use and pond fish farming are key factors driving changes in the ratios of nitrogen to phosphorus components in pond waters in the study area.

4. Discussion

In this paper, the spatial and temporal dynamics of nitrogen to phosphorus ratios in cascade ponds were analyzed in the subtropical hilly watershed of Jinjing. The results shown that the annual mean values of N:P ratios in the cascade ponds were greater in the downstream than in the upstream, which was related to the differences of surface runoff and pollutant acceptance between the upstream and downstream ponds. The cascade ponds in the study area are all artificial ponds along the sloping topography, with woodland occurring around the upper ponds (78%) and agricultural and residential areas occurring around the lower ponds (67%). The upstream and downstream ponds are connected by storm water runoff, dry ditches and grassed canals. These ponds are temporary "sources" of non-point source pollutions as they receive surface runoff from woodlands, farmland or villages [16] [18] [20]. When it rains, water flows out of the upstream ponds and into the upstream ponds, which become an output "source" of nitrogen and phosphorus pollutants to the downstream ponds [25]. In addition to receiving upstream pollution from stormwater runoff, fish farming is also present in the downstream ponds [26], making them more polluted than upstream.



Figure 8. Contributions of land use and pond management to variance in the ratios of nitrogen and phosphorus components in pond waters.

Nitrogen to phosphorus ratios (TN:TP and TDN:TDP) in the study area generally showed a general trend towards higher values in summer and spring than in winter. This may be related to rainfall and biological activity. On the one hand, algae grow vigorously in ponds during spring and summer, and their growth consumes nitrogen and phosphorus nutrients in the ponds. On the other hand, nitrogen and phosphorus nutrients in pond waters can be replenished by nitrogen and phosphorus inputs from surrounding land surface runoff [25]. However, phosphorus is mainly lost in particulate form and is easily trapped, which leads to a relatively higher accumulation of nitrogen than phosphorus in pond waters [27] [28] [29]. This partly explains the high TN:TP and TDN:TDP ratios during the spring and summer seasons. In addition, large proportion of agricultural land in the study area and the use of large amounts of nitrogen fertilizer in spring cultivation also lead to higher TN:TP and TDN:TDP in the ponds. Finally, Higher precipitation in summer and higher water levels in the ponds reduce nitrogen and phosphorus concentrations by dilution, resulting in greater ratios of TN:TP and TDN:TDP.

In southern China agricultural ponds are not only important hydraulic nodes for agricultural irrigation [1] [2], but also for freshwater fishery farming and the consumption of nitrogen and phosphorus pollutants from surrounding agriculture [30]. As a result, agricultural pond waters are high in nitrogen and phosphorus and severely eutrophic. The eutrophication of water bodies in the cascade ponds in the study area is high, and the nutrient status of the water bodies is generally governed by phosphorus. From the perspective of nitrogen and phosphorus pollution management, the cascade ponds are an important ecological transitional zone, which not only slow down the flow of water and prolong the residence time, but also play an ecological role in the storage, filtration and removal of nitrogen and phosphorus pollutants [31]. In addition, building and maintaining ponds are inexpensive and have some economic benefits. Ecological construction of cascade ponds is a long-term development strategy for solving non-point source environmental problems. Ecological regulation of cascade ponds can not only effectively control nitrogen and phosphorus pollution in water bodies, but can also play a role in inhibiting nitrogen and phosphorus pollution downstream.

5. Conclusion

The spatio-temporal patterns of the ratios of TN:TP, TDN:TDP and PN:PP were studied in water bodies of cascade ponds in an agricultural watershed. Agricultural land use and pond fish farming are important factors triggering water quality pollution and influencing the changing of N:P ratios in the cascade ponds. Strengthening the ecological management of agricultural non-point source pollution and residential sewage is an important measure to reduce nitrogen and phosphorus pollution in the ponds in the study area, especially to control phosphorus pollution.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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