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Sinking of floating plastic debris caused by biofilm development in a freshwater lake

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HIGHLIGHTS

- Biofilm development on floating plastics was studied in a freshwater lake.
- Biofilm showed different growth rates and algae composition in different seasons.
- Floating plastics lost buoyancy due to density change caused by biofilm development.
- Sinking of plastics was affected by the plastic size, temperature and water chemistry.

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ABSTRACT

Plastic pollution has been increasingly reported in both marine environment and inland waters, but their fate is not well understood. Several studies have showed that the surface of plastic debris can be colonized by microbes, leading to the sinking of floating plastic debris in marine environment. In this work, development of biofilm on polypropylene sheet (squares with a side length of 5 and 10 mm) and their buoyancy changes were studied in a freshwater lake in four seasons. Results showed that biofilm development have different growth rate and distinct algae composition in different seasons, which are mainly related to the difference in temperature, nutrient levels, and suspend solids in lake water. Biofilm development was much quicker on small plastics in all seasons. At the end of the experiment, all plastics lost buoyancy in summer while only a small portion lost buoyance in other seasons. Sinking of the floating plastics can be attributed to the development of biofilm and the trapped minerals. Our results demonstrated that biofilm development can cause the sinking of floating plastics in fresh lakes but the time required to lose buoyance can differ seasonally. Floating plastics will remain in water for a longer time in cold season but sink in a short time in warm season. Future research is required to determine the influence of plastic types and shapes, and quantitative relation between environmental variables and the sinking behavior of the fouled plastics should be established for a better prediction of their fate in the freshwater environment.

1. Introduction

Pollution of plastic debris in the aquatic environment has become an emerging issue worldwide due to the extensive use of plastic products and the mismanagement of plastic waste (Browne et al., 2007). It has been estimated that about 12,000 million metric tons of plastic waste will end up in landfills or in the natural environment by 2050 (Geyer et al., 2017). Once released to the environment, plastics will experience physical, chemical, and biological degradation processes, which break large plastics into small debris gradually. Plastic debris <5 mm in size is usually considered as microplastics, which has been found to be ubiquitously presented in marine environment and freshwater habitats such as lakes and rivers (Barboza and Gimenez, 2015; Wagner et al., 2014).

In the aquatic environment, microplastics can be mistakenly ingested by many aquatic organisms including zooplankton (Cole et al., 2013), fish (Jabeen et al., 2017), invertebrates (Rochman

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et al., 2015), and marine mammals (Lusher et al., 2018). Transfer of microplastics along the aquatic food chain has also been documented (Setälä et al., 2014). Exposure to microplastics may adversely affect the health of those aquatic organisms. Effects such as abrasion and ulcers, blockage of the digestive tract, increase in mortality, decrease in energy reserve, reproductive disruption, and changes in behavior have been experimentally demonstrated (da Costa et al., 2016; Li et al., 2016). Therefore, microplastic pollution has raised increasing concerns in recent years.

Most of plastics used in the daily life are less dense than water such as polyethylene (PE), polypropylene (PP), and expanded polystyrene (PS) (PlasticsEurope, 2018). However, these plastics with lower than water density have also been commonly detected in sediment from lakes and rivers (Corcoran et al., 2015; Wang et al., 2018), which pose potential risks to benthic organisms. Presence of microplastics in benthic organisms has been reported from the coastal area and even polar regions (Bour et al., 2018; Fang et al., 2018). Ingested and egested by aquatic organisms, wrapped by organic detritus, and biofouling have been indicated to be main reasons for the sedimentation of microplastics with lower than water density (Cole et al., 2016; Kaiser et al., 2017). However, mechanisms and influence factors for the sedimentation of microplastics in aquatic environment are still unclear.

In the aquatic environment, microplastics provide surfaces for the attachment of microorganisms. Microbial biofilm developed rapidly on PE plastic submerged in seawater, which became less hydrophobic and more neutrally buoyant in 3 weeks (Lobelle and Cunliffe, 2011). Microplastics from marine environment was found to host distinct bacterial communities compared with the source community, and the attached microbial communities depended on season, geographical location and plastic polymer types (Oberbeckmann et al., 2014; Ogonowski et al., 2018). Meanwhile, microorganism attachment and the formation of biofilm change the sinking behavior of plastic debris (Rummel et al., 2017). Previously, it has been demonstrated that biofouling enhanced the deposition of microplastics in the marine environment and the rate of biofouling is related to the size of microplastics (Fazey and Ryan, 2016; Kaiser et al., 2017). Theoretical model was also developed to simulate the effect of biofouling on the fate of microplastics, which predicted size-dependent vertical movement of microplastics and a maximum concentration at intermediate depths in the ocean (Kooi et al., 2017), but field observation supporting this prediction is still lacking.

However, only few works have been carried out to investigate the fate of microplastics in freshwater habitats. Floating microplastics entering rivers can be transported all way to the ocean, which is considered as a major source of marine plastic pollution (Siegfried et al., 2017). Ingestion of microplastics by fishes from lakes and rivers has also been documented (Biginagwa et al., 2016; Sanchez et al., 2014). Freshwater bivalves was found able to accumulate microplastics from surrounding environment (Su et al., 2018). Laboratory experiment showed that PP microplastics can be colonized and aggregated rapidly by freshwater microalgae (Lagarde et al., 2016).

Compared with marine environment, freshwater habitats have different hydrological, hydrodynamic, physico-chemical, and species composition characteristics. Therefore, biofilm attachment on the surface of microplastics in the freshwater may show different features. In addition, how different environmental factors affect the biofouling-driven deposition process of microplastics is still unclear. Therefore, the sedimentation of plastics driven by biofilm attachment in a freshwater lake in different seasons was investigated in this work. Characteristics of the attached biofilm and changes in density of the fouled plastics were determined overtime. The relations between environmental conditions, biofilm development, and the buoyancy of plastic debris were discussed. This work contributes to a better understanding of the fate of plastic debris in freshwater lakes.

2. Materials and methods

2.1. Experimental setup

Experiment was carried out in East Lake, which is a mesotrophic to eutrophic urban lake located in Wuhan, China. The surface area of the lake is 33 km^2, and the average water depth is 2.5 m. PP was chosen for investigation due to its versatility and a high detection frequency in the aquatic environment. PP sheet with a thickness of 0.3 mm and a density of 0.91 g/cm^3 was cut into 5 × 5 mm (5S) and 10 × 10 mm (10S) squares. The experimental devices were assembled using expandable PE floating plate and steel wire. The steel wire was made into a square shape with a side length of 40 cm and the expandable PE floating plate was fixed about 20 cm above the square. PP squares were tied to the steel wire using a 10 cm long PE fishing line. A total of 30 plastic squares were fastened to each device (15 for each shape), and 10 devices were prepared for the experiment. Then, the experimental devices were deployed in the lake with one end fixed to a steel frame by ropes. Plastic squares were suspended in the water about 10 cm below the surface. Picture of the experimental setup and the devices were provided in the supplementary Fig. S1.

Experiment was carried out in spring (11 May to 12 June 2018), summer (30 July to 29 August 2018), autumn (17 October to 14 November 2017), and winter (20 December 2017 to 19 January 2018). One experimental device was retrieved about every three days. PE fishing lines were cut, and the buoyancy of the fouled plastic squares were determined in a 1 L beaker filled with 500 mL lake water. The number of sunk samples was recorded. Biofilm from 1 to 3 pieces of plastic squares was scraped into 10 mL falcon centrifuge tubes using a scalpel, dispersed with 5 mL deionized water, and preserved with Lugol's Iodine Reagent for algae identification and quantification. The rest of the plastic squares were stored at −25 °C for other analysis. Water temperature (T), pH, and dissolved oxygen (DO) were measured in situ using a Hach HQ40 d m (Loveland, CO, USA), Secchi depth (SD) was also measured in situ using a Secchi disk. Water samples were collected for the analysis of total phosphorus (TP), total nitrogen (TN), ammonia (NH_3-N), total organic carbon (TOC), chlorophyll a (Chl.a), and total suspended solids (TSS). Weather conditions during the experiment were also recorded.

2.2. Sample analysis

Concentrations of TP, TN, TSS, and NH_3-N in water were measured following standard methods (MEPC, 2002). TOC was analyzed using an elementar Vario cube TOC analyzer (Langenselbold, Germany). For the analysis of Chl.a content, an aliquot of 100 mL water sample was filtered with the 0.45 μm glass fiber filter. The filter was subsequently extracted with 5 mL 90% acetone for 24 h at 4 °C in dark. After extraction Chl.a was determined using an Agilent Cary 60 UV–Vis spectrophotometer (Santa Clara, CA, USA).

Three plastic squares were randomly selected to determine the biomass of the biofilm by gravimetric method. Dry weight (DW) was measured after being dried at 105 °C in an oven to a constant weight. Ash weight (AW) was measured after being burned at 400 °C for 3 h in a Muffle furnace. Ash free dry weight (AFDW) was calculated by subtracting AW from DW. Chl.a content of the biofilm was determined using an Agilent Cary 60 UV–Vis spectrophotometer after extraction with 90% acetone. Algae composition was examined under a light microscope and identified by referring to

Hu and Wei (2006).

2.3. Data analysis

Density of the biofilm was calculated using equations (1) and (2) by referring to Tchobanoglous and Burton (1991), where D_d is the dry biofilm density (mg/cm^3); D_w is the wet biofilm density (mg/cm^3); M_s is the dry mass of a biofilm (mg); D_f is the density of fixed mineral solids in a biofilm (assuming a density of 2500 mg/cm^3); M_f is the dry mass of any fixed mineral solids in a biofilm (mg); D_v is the density of any volatile solids in a biofilm (assuming a density of 1000 mg/cm^3); and M_v is the dry mass of volatile solids in a biofilm (mg). W_{ds} is the biofilm dry solids content (%); W_{tw} is the biofilm water content (%); and ρ_w is the density of water (assuming a density 1000 mg/cm^3). In this experiment, $M_s = DW$, $M_f = AW$, and $M_v = AFDW$.

$$\frac{M_s}{D_d} = \frac{M_f}{D_f} + \frac{M_v}{D_v} \quad (1)$$

$$\frac{1}{D_w} = \frac{W_{ds}}{D_d} + \frac{W_{tw}}{\rho_w} \quad (2)$$

Density of the fouled plastic squares was calculated using equation (3), where D_c is the density of the fouled plastic squares (mg/cm^3); MP is the mass of the plastic square (mg); V_B is the volume of the biofilm (cm^3); V_P is the volume of the plastic square (cm^3).

$$D_C = \frac{DW + MP}{V_B + V_P} \quad (3)$$

Principal component analysis (PCA) was used to explore differences between the environmental parameters in different seasons, which was performed by using CANOCO software Version 4.5 (Ithaca, NY, USA). Other graphs were plotted using Origin 2017 (Northampton, MA, USA). Differences in the biomass and Chl.a content of the biofilm were compared using paired-samples *t*-test by SPSS software Version 18.0 (SPSS Inc, Chicago, IL, USA). A confidence level of 95% was used.

3. Results and discussion

3.1. Development of biofilm on plastic squares

Biofilm was visibly appeared on the surface of the plastic squares on day 3 in spring and summer, on day 6 in autumn, and on day 9 in winter (Supplementary Fig. S2). Biofilms developed on the surface of the plastic squares in different seasons showed distinctive features at the end of the experiment as presented in Fig. 1. Plastic squares were more densely covered by the biofilm in summer with a dark green color. In winter, the biofilm was less dense with a brown color. In autumn and winter, plastic squares in 10S were not fully covered at the end of the experiment. Partially cover of 10S in autumn and winter can be attribute to a slow development of the biofilm on large plastic squares. The appearance of the biofilm can be related to the microbial as well as the inorganic mineral composition in the biofilm. Different temperature, illumination, and hydrodynamic conditions in different seasons might have caused the difference in composition and structure of the biofilm (Arnon et al., 2007; Zhao et al., 2018).

Changes in the biomass of the biofilm on plastic squares in four seasons during the experiment are presented in Fig. 2. Biofilm biomass (DW) is divided into AFDW and AW, which represents organic and inorganic components, respectively. In all samples, the biofilm biomass showed an increasing trend overtime except in autumn, when the highest biomass was observed on Day 22 for 5S and then decreased. This might be due to the partial detachment of

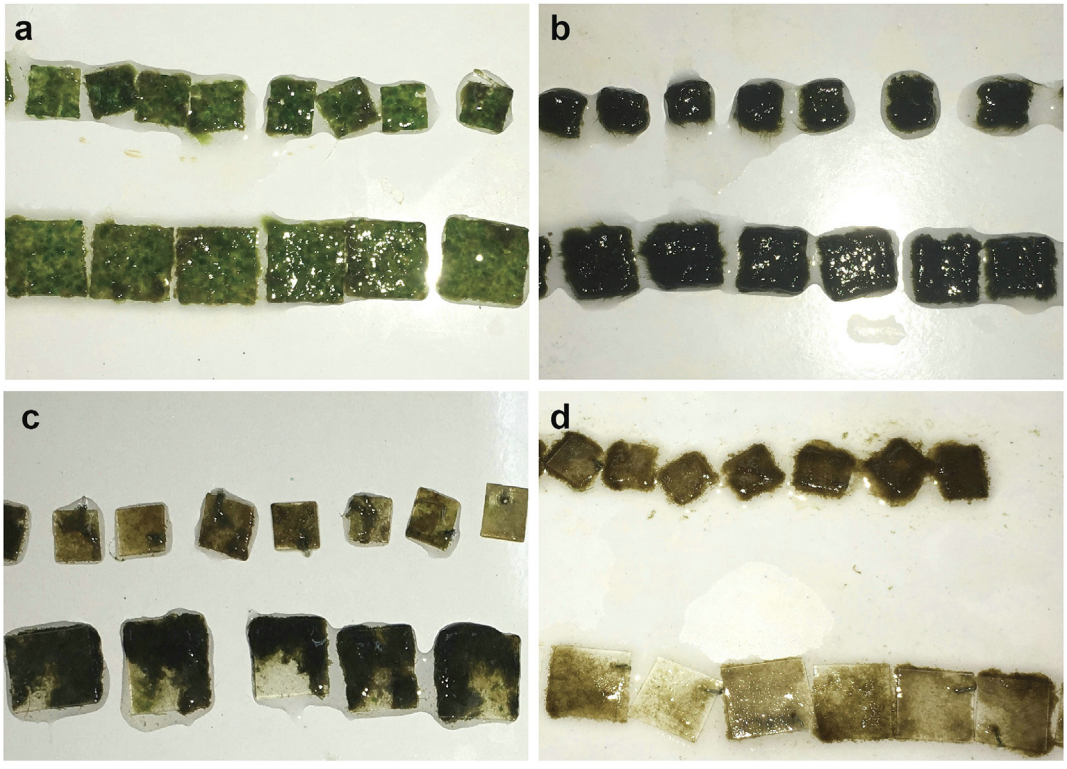

Fig. 1. The appearance of the biofilm developed on plastic squares at the end of the experiment in spring (a), summer (b), autumn (c), and winter (d).

the biofilm caused by the windy conditions. Additionally, loss of periphyton biomass can be caused by grazing of grazers such as crustaceans and trichopteran larvae (Hillebrand, 2009). However grazer control of periphyton should not only be limited to autumn, grazer control might not be the major reason causing the decrease of periphyton biomass after D22 in autumn.

Biofilm biomass per unit area was higher for 5S than 10S in all seasons ($p < 0.006$), which agrees with the result of a previous research in marine environment (Fazey and Ryan, 2016). Substrates with a lager surface area to volume ratio is beneficial to the attachment of microbes. In different seasons, biofilm developed at a different rate. Biofilm biomass per unit area in summer was higher than in spring and winter for 5S ($p < 0.05$), and was higher than in all other seasons for 10S ($p < 0.05$). Biofilm biomass per unit area in winter was lower than in summer and autumn for 5S ($p < 0.05$), and was lower than in all other seasons for 10S. The average water temperature during the experiment was 25.5, 29.1, 18.8, and 9.8 °C for spring, summer, autumn, and winter, respectively. Temperature is correlated with the reaction rates of enzymes, which is associated development of the cells (Garrett et al., 2008). Within the optimum temperature range, higher temperature increases cell metabolism resulting in a more rapid development of the attached microbes consequently (Cao et al., 2017; Mahdy et al., 2015).

Changes in the Chl.a content of the biofilm on plastic squares are presented in Fig. 3. Chl.a content of the biofilm in all seasons showed an increasing trend first but turned to decrease in the last few samplings. Decrease in the Chl.a content of the biofilm toward the end of the experiment maybe due to the shading of the inner layer of the biofilm, which caused the apoptosis of algae. Similar to biofilm biomass, Chl.a content per unit area of the biofilms on plastic squares was higher for 5S than 10S ($p < 0.05$). In different seasons, Chl.a content per unit area of the biofilms in spring and summer was not significantly different ($p > 0.75$) but both higher than in autumn and winter ($p < 0.007$), and Chl.a content per unit area of the biofilms in autumn and winter was not significantly different ($p = 0.052$). Chl.a is usually used as an estimate of algal biomass. Most algae have an optimum growing temperature range 20 °C–30 °C (Singh and Singh, 2015). Therefore, algal growth in the biofilm was favorable in spring and summer while algae growth could be inhibited in autumn and winter with an average water temperature lower than 20 °C.

Although Chl.a content of the biofilm in spring and summer was similar but the biofilm biomass in spring was lower than in summer ($p < 0.05$), while Chl.a content of the biofilm in autumn is lower than in spring but the biomass was even higher ($p = 0.014$) in autumn. This discrepancy could be attributed to the proportion of inorganic materials in the biofilm. AW was averaging 47.6% and 43.4% in autumn for 5S and 10S, respectively. Whereas AW was averaging 20.0% and 21.6% in spring for 5S and 10S, respectively. Proportion of inorganic materials in the biofilm was significantly higher in autumn than in winter ($p < 0.05$). This result indicates that the amount of inorganic materials trapped in the biofilm

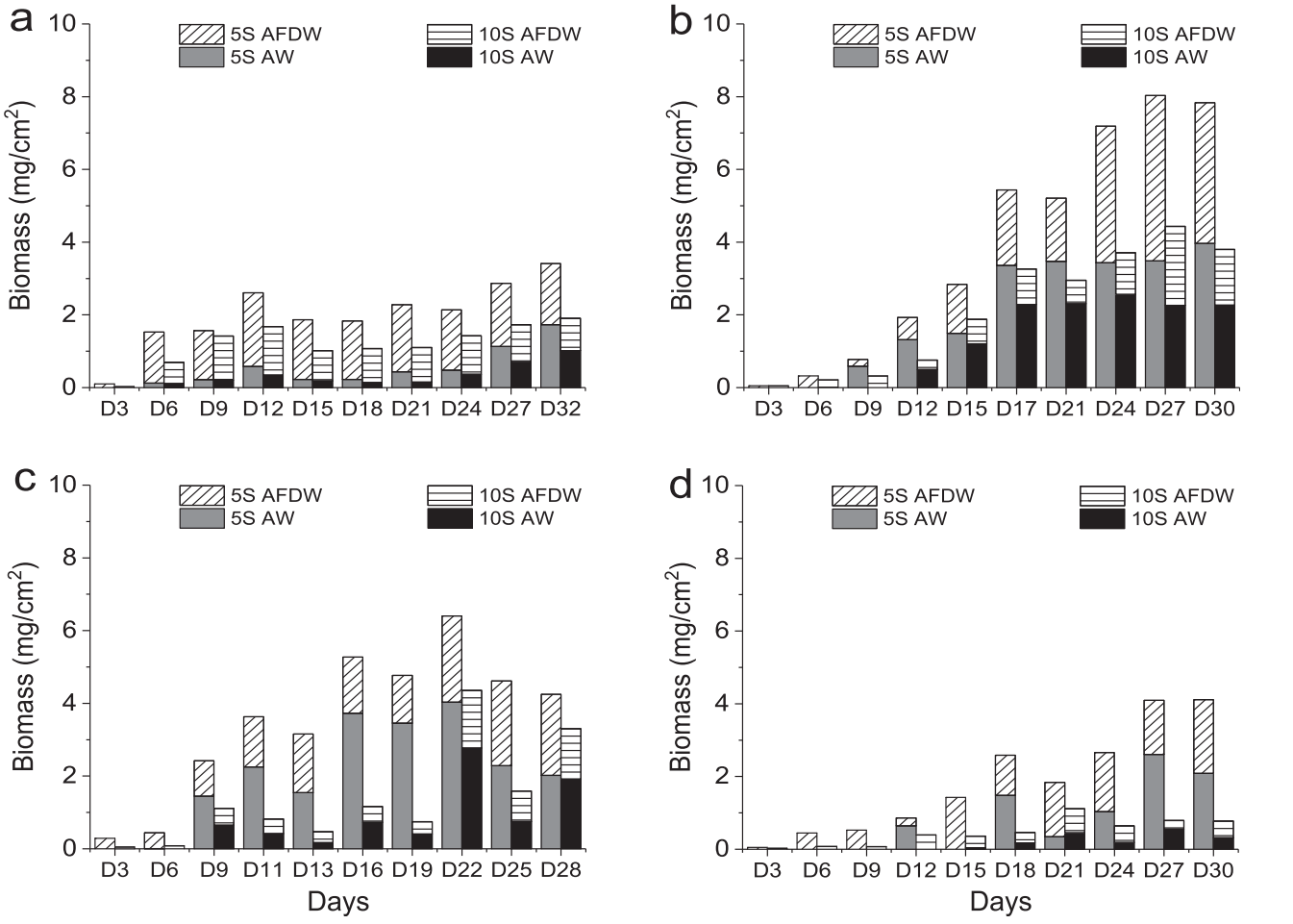

Fig. 2. Changes in ash free dry weight (AFDW) and ash weight (AW) of the biofilm developed on the plastic squares in spring (a), summer (b), autumn (c), and winter (d) during the experiment.

during its formation is also important in determining the total biofilm biomass.

Changes of the algae community structure of the biofilm on 5S in four seasons are presented in Fig. 4. The algae community structure of the biofilm on 10S was similar to that on 5S. Algae identified from the biofilm belong to 6 phyla and 38 genera in spring, 4 phyla and 31 genera in summer, 6 phyla and 48 genera in autumn, and 5 phyla and 25 genera in winter. Algae community was more diverse at the initial stage of the experiment but became dominated by Cyanophyta toward the end of the experiment especially in summer and autumn while a relatively high proportion of Chlorophyta and Bacillariophyta was identified at the end of the experiment in spring and winter, respectively. Previously, several works have studied the characteristics of bacterial community of the biofilm developed on plastic debris and bacterial communities are found to be related to the polymer type, sampling season and location (Frère et al., 2018; Miao et al., 2019; Oberbeckmann et al., 2014). Algae are also important components of the biofilm, especially when light is available. On the surface of microplastics from marine environment, algae such as diatom and cyanobacteria have also been commonly observed in the attached biofilms (Kaiser et al., 2017; Zettler et al., 2013). Complex interactions exist between algae and bacteria in the biofilm, where heterotrophic bacteria can grow on organics secreted by algae and recycle nutrients from organics for algae growth (Kouzuma and Watanabe, 2015). The difference in algae community structure of the biofilm can be related to the variation of water temperature and nutrient levels in different seasons (dos Santos and Ferragut, 2013; Wu et al., 2017). Different algae community structure may also affect the biomass growth of the biofilm. Algae interact with bacteria to produce extracellular polymeric substances (EPS), which bind algae and bacteria to form the internal structure of the biofilm and trap inorganic materials from the surrounding environment (Ramanan et al., 2016).

3.2. Effect of the biofilm on the buoyancy of plastics

Proportion of plastic squares lost buoyancy during the experiment in different seasons is summarized in Table 1. For 5S, loss of buoyancy was initially observed on the fourth sampling in autumn, the fifth sampling in summer, the ninth sampling in winter, and the last sampling in spring. Whereas, loss of buoyancy was initially observed on the sixth sampling in both summer and autumn but no sample lost buoyancy in spring and winter for 10S. Proportion of plastic squares lost buoyancy generally increased over time but fluctuation was also observed especially in autumn, which was likely caused by the windy conditions causing biofilm detachment. At the end of the experiment, all plastic squares sank in summer while 14.3% and 20% of the plastic squares sank in autumn for 5S and 10S, respectively. No plastic square lost buoyancy in spring and winter for 10S while 13.3% and 20% of the plastic squares sank in spring and winter for 5S. Plastic squares lost buoyancy at different

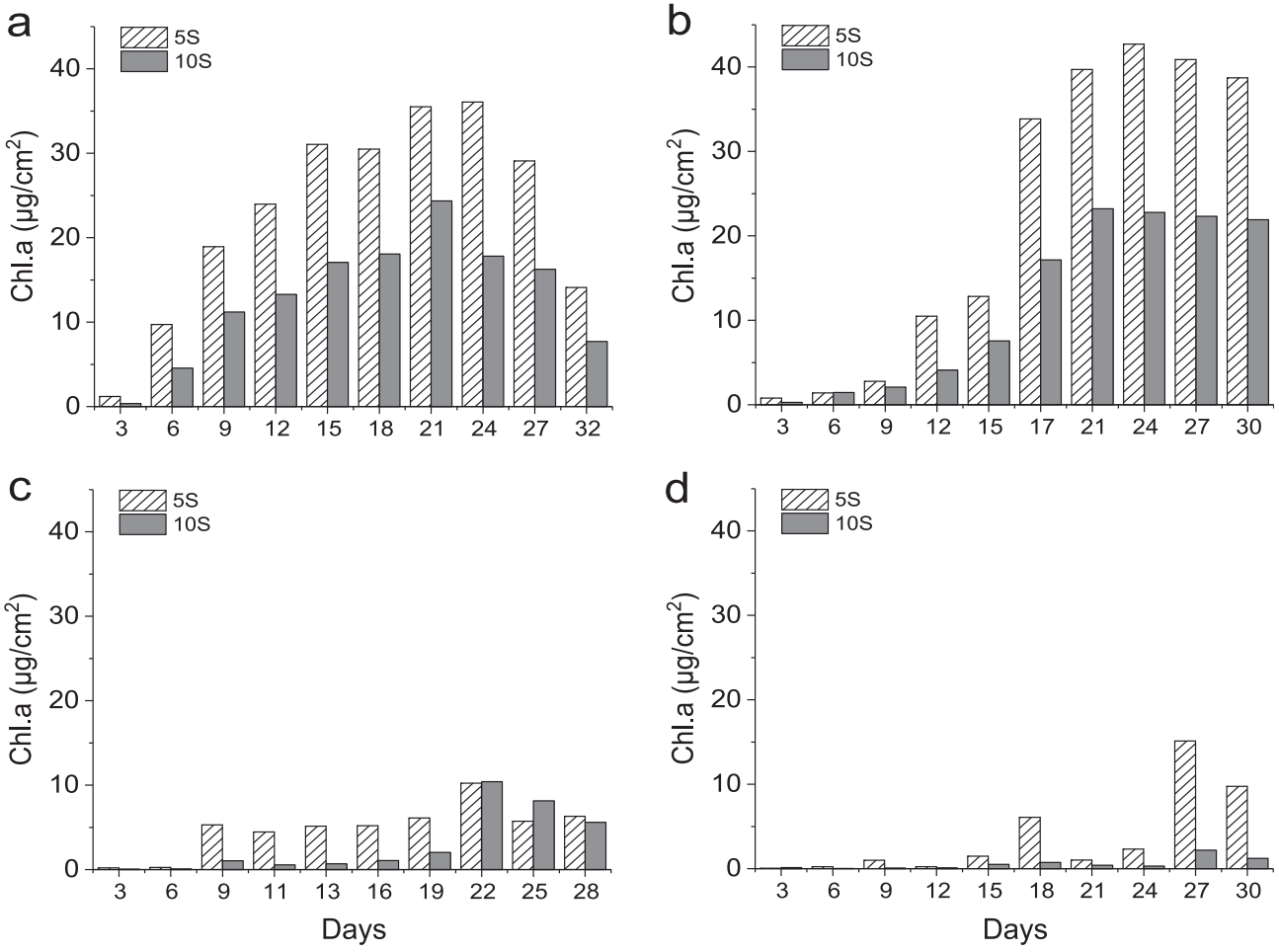

Fig. 3. Changes in the Chl.a content of the biofilm developed on the plastic squares in spring (a), summer (b), autumn (c), and winter (d) during the experiment.

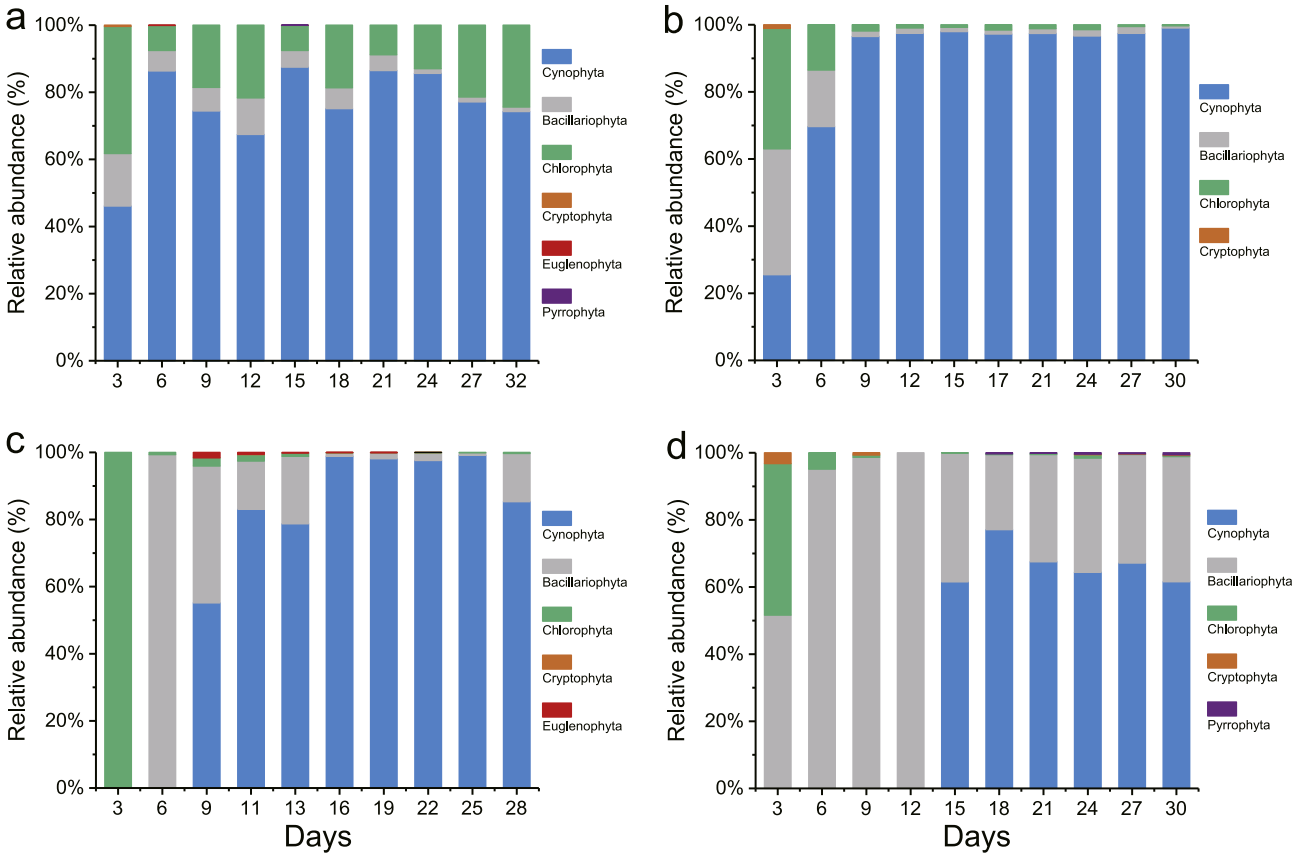

Fig. 4. Changes of algae composition of the biofilm on 5S during the experiment in spring (a), summer (b), autumn (c), and winter (d).

rate in different seasons, which can be related to the difference in biofilm development rate. Biofilm developed much quicker in summer resulted in an earlier sinking phenomenon of the fouled plastic squares. Biofilm development was slower in spring and winter and the density change caused by the attached biofilm was not sufficient to alter the buoyancy of the plastic squares. Biofilm development was also slower in 10S than in 5S. Therefore, 5S lost buoyancy earlier than 10S in all seasons.

Density change of the fouled plastic squares are presented in Fig. 5. The trend of density change was similar to that of the biofilm biomass in different seasons. Density of the fouled plastic squares was less than water in spring and winter for both 5S and 10S, became greater than water in summer after 17 days for both 5S and 10S, and fluctuated greatly during the experiment in autumn. The density of the fouled plastic squares needs to become greater than 1 mg/cm^3 for them to sink in lake water. The result of the density change are generally in accordance with the result of the buoyancy test. Loss of buoyance was observed in spring and winter although the calculated density was less than 1 mg/cm^3. This might be due to the within group variation and error of the calculation as the density was estimated using equation (1) with the assumption that the density of any volatile solids in a biofilm is 1 mg/cm^3 and the density of fixed mineral solids in a biofilm is 2.5 mg/cm^3. Fixed mineral solids in a biofilm are critical to sink the plastic squares.

Biofilm formation on substrates can be divided into three stages including surface conditioning, propagule dispersal and settlement, and population growth (Larned, 2010). A conditioning layer is formed by the deposition of organic and inorganic substances on the surface of the substrate from the surrounding environment, which facilitates the attachment and provides nutrients for the growth of bacteria. Mucilage produced by bacteria provides binding site for other organic and inorganic compounds and algae. The attached bacteria and algae spread outward with growth and form aggregate through the binding of the excreted EPS. During the development of biofilm, suspended minerals can be trapped and imbedded in the biofilm and some algae are able to precipitate carbonate minerals such as calcite on the micro-surface of the biofilm (Lu et al., 2016). With a much higher density than water, increase in minerals content with the development of biofilm eventually leads to the sink of the fouled plastic squares. Previously, calcareous fouling organisms such as barnacles and mussels were observed on the surface of plastics submerged in marine environment, which have a great influence on the mass of the fouled plastics (Fazey and Ryan, 2016). However, no calcareous fouling organisms were observed and inorganic minerals trapped during biofilm development should be mainly responsible for the loss of buoyancy in this experiment.

Table 1
Percentage of sunk plastic squares during the experiment in different seasons.

Season	Sampling times	1	2	3	4	5	6	7	8	9	10
Spring	5S	0	0	0	0	0	0	0	0	0	13.3
	10S	0	0	0	0	0	0	0	0	0	0
Summer	5S	0	0	0	0	6.7	93.3	80.0	100	100	100
	10S	0	0	0	0	0	100	80.0	100	100	100
Autumn	5S	0	0	0	26.7	0	33.3	35.7	40.0	20.0	14.3
	10S	0	0	0	0	0	6.7	0	80.0	0	20.0
Winter	5S	0	0	0	0	0	0	0	0	60.0	20.0
	10S	0	0	0	0	0	0	0	0	0	0

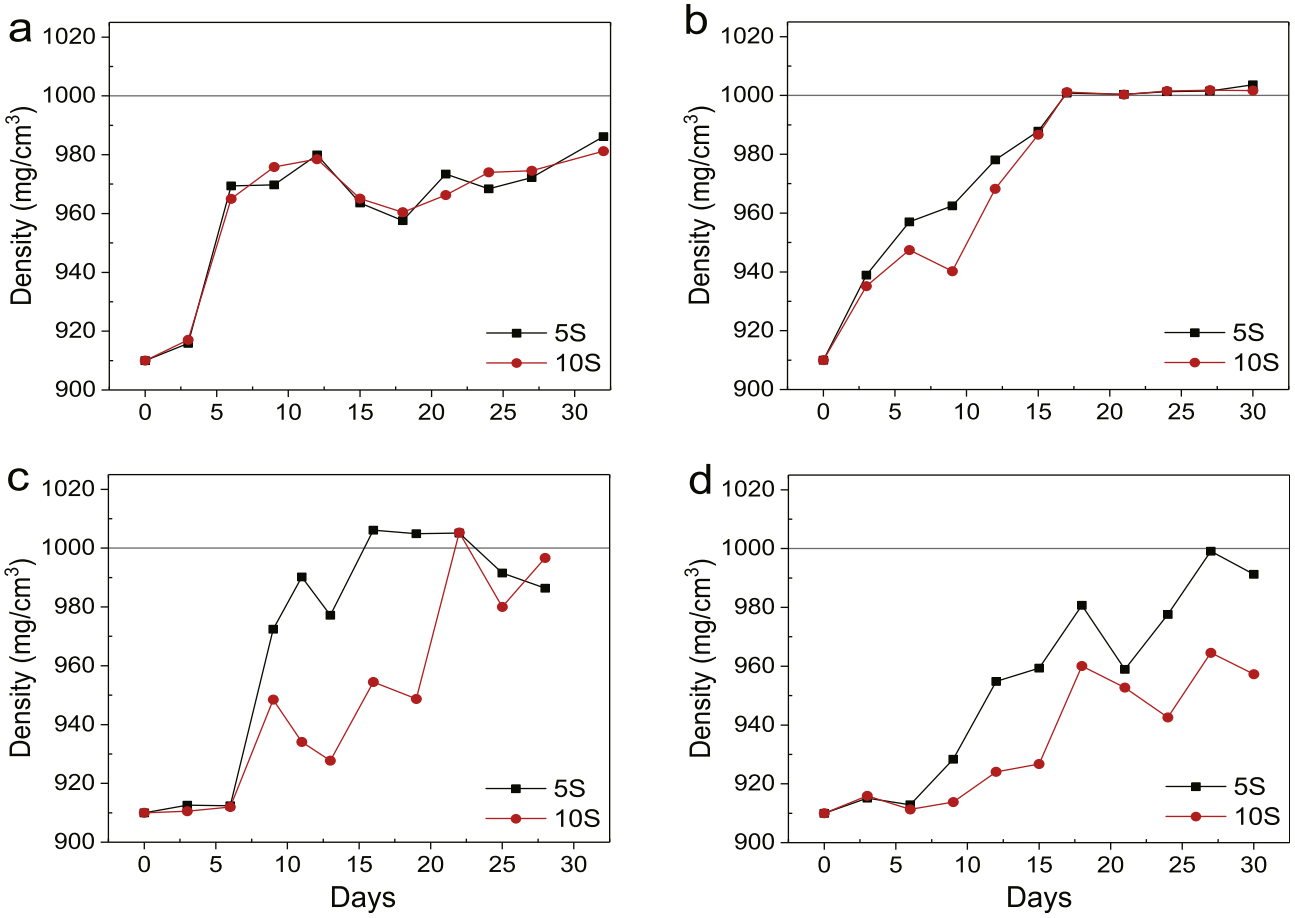

Fig. 5. Density change of the fouled plastic squares during the experiment in spring (a), summer (b), autumn (c), and winter (d).

3.3. Relationship between environmental condition and biofilm development

Environmental parameters recorded during the experiment was provided in supplementary Table S1. Environmental parameters showed distinct characteristics in different seasons as indicated by PCA (Fig. 6). PC1 and PC2 explain 49.9% and 17.2% of the total variance, respectively. Spring samples were characterized by high nutrient contents but low TSS, summer samples were characterized by high temperature and high nutrient and Chl.a contents, autumn samples were characterized by high TSS, while winter samples were characterized by high SD, DO, and pH but low temperature and nutrient contents.

The relation between biofilm and environmental conditions has been studied in many previous research. Warmer condition resulted in a higher biomass of biofilm due to direct effects of temperature on the growth of microorganisms and indirect effects on sediment phosphorus release (Kazanjian et al., 2018). Nutrient is essential for the growth of microorganisms, higher nutrient level favors the development of biofilm (DeNicola et al., 2006). High TSS allows more mineral to be trapped in biofilm but high TSS also indicates more turbulent hydrological condition, which can have both positive and negative effects on biofilm biomass accretion. Light is important for the development photosynthetic microorganisms in biofilm. Light availability strongly limits the propagation of biofilm and can have interactive effects with other environmental conditions such as nutrients and temperature (Sanches et al., 2011; Zhao et al., 2018).

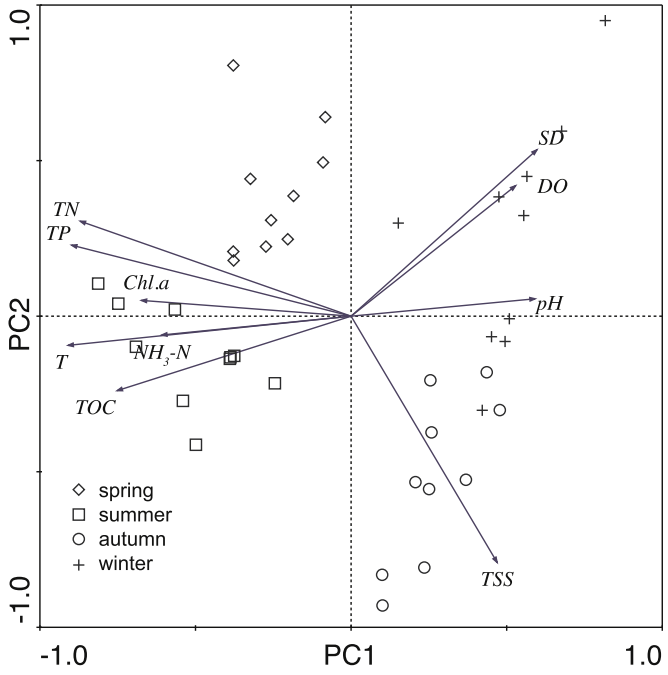

Fig. 6. An ordination biplot of environment variables and different sampling seasons obtained by PCA (Arrows depict environmental variables and symbols depict samplings).

In summer, high temperature, elevated nutrient level, and long illumination duration all favor the development of the attached biofilm on plastic squares, resulting in the loss of buoyancy in a short time. In winter, low temperature, low nutrient content, and short illumination duration were all against biofilm development. In spring, temperature was moderate and nutrient level was high but the accretion of biofilm was relatively slow, which could be related to a low TSS. In contrary to spring, TSS was high in autumn and the accretion of biofilm was quicker although the growth of algae was relatively slow.

4. Conclusions

In this work, we demonstrated that plastics in a freshwater lake can lose their buoyancy due to the development of biofilm on their surface. Biofilm showed different growth rate with distinctive characteristics in different seasons, which can be related to the difference in temperature, nutrient levels, and TSS. Biofilm growth was more rapid on small plastics similar to the observation from marine environment. The difference in biofilm development and corresponding density changes resulted in different amounts of time required for the sinking of the floating plastics. In general, floating plastic debris will remain longer on the surface of water in cold seasons than in warmer seasons and smaller plastics can be removed more quickly than larger plastics from the surface in lakes. Our results also indicate that sediment is an important sink of plastics even for polymers with a lower than water density in lakes, which agree with the results of field investigation (Sruthy and Ramasamy, 2017; Su et al., 2016). Therefore, more research is required to focus on the fate and effects of plastic debris in lake sediment. Features and impact of biofilm on plastics of other types, shapes, and sizes should also be further investigated in future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.02.015.

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