Lake Phytoplankton Assemblage Altered by Irregularly Shaped PLA Body Wash Microplastics but not by PS Calibration Beads

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Abstract: Microplastics are an emerging environmental pollutant, whose global ubiquity is becoming increasingly evident. Conventional wastewater treatment does not completely remove them, and there are growing concerns about microplastics in source water and post-treatment drinking water. Microplastics have been reported to alter the development, physiology, and behavior of various aquatic organisms; however, limited knowledge exists on their effect on natural phytoplankton communities. Many studies also use uniformly spherical plastic beads, while most scrub particles in consumer products and secondary microplastics in the environment have various shapes and sizes. We tested the effects of two types of microplastics, 50 μm polystyrene (PS) calibration beads and polylactic acid (PLA) plastic body wash scrub particles, and one type of plant-derived body wash scrub particle on a natural phytoplankton assemblage through a 7-day incubation experiment in a temperate, mesotrophic lake. The calibration beads and the plant-derived particles generally did not alter the taxonomic composition of the phytoplankton in the mesocosms, while the PLA body wash microplastics eliminated cryptophytes ($p < 0.001$) and increased chrysophytes ($p = 0.041$). Our findings demonstrate differential effects of irregularly shaped PLA body wash microplastics vs. PS calibration beads on lake phytoplankters and empirically support potential bottom-up alteration of the aquatic food web by secondary microplastics.

Keywords: plastic pollution; body wash; wastewater; water

1. Introduction

Microplastics (MPs), or plastic pieces that are typically <5 mm, have come to be widely recognized as an emerging pollutant in recent years. While the earliest major scientific report of aquatic microplastic pollution dates back to 1971 [1], MPs did not draw much attention as a research topic until the 21st century. Since then, we have gained substantial knowledge on the extent of the global MP pollution and its impact on the development, physiology, and behavior of various marine and freshwater organisms [2–7]. We have also learned that conventional wastewater treatment plants do not completely remove MPs and can be point sources of aquatic MPs [8–11]. Simultaneously, MPs in source water and processed or bottled drinking water have been a growing concern for water utilities and the general public [12]. Controlled lab studies are elucidating the molecular pathways of organismal responses to aquatic MPs, including nanoplastics (NPs) [13–15]. Data are severely limited, however, on how MPs affect natural phytoplankton communities instead of lab monocultures

where a single, well-studied species from a culture collection is grown in defined growth media at constant temperature and regular light/dark cycles. Such studies have revealed physiological effects of MPs on phytoplankton cells and their gene expression using certain enzymes and reactive oxygen species as responses [16]. The influences of MPs at the population and community levels, however, are yet to be studied to the same extent, although remarkable advances are being made for biofilm communities [17,18]. Many of these MP studies used uniformly spherical and fluorescent plastic beads, while many scrub particles in consumer products [16,19–21] and MPs found in environmental samples [22,23] have various shapes and sizes. Non-spherical MPs, including those resulting from fragmentation of macroscopic plastic debris, are expected to be the majority of MPs encountered by aquatic organisms. Yet, non-spherical MPs are rarely used in MP bioassays because analytic-grade MP beads are readily available and facilitate straight-forward study design and data interpretation.

We aimed to investigate whether analytic-grade MP beads are a reasonable surrogate to other types of anthropogenic microparticles that may be released in household wastewater and interact with lake phytoplankton. A novel, 7-day summertime in situ incubation experiment was conducted in a temperate, mesotrophic lake where different types of spherical and non-spherical microparticles were added to mesocosms containing natural lake phytoplankton. We first hypothesized that MPs have quantitative and/or qualitative effect(s) on the lake phytoplankton, while plant-derived particles have no such effect. Plant-derived scrub particles from a body wash were included as one of the experimental treatments. They are often chosen by personal care product manufacturers as alternatives to plastic microbeads, which have been banned or are being voluntarily phased out of production by manufacturers [24,25]. We also hypothesized that planktonic cyanobacterial colonies become smaller and more numerous with MPs. In previous years, cyanobacteria such as *Aphanizomenon*, *Microcystis*, *Dolichospermum*, and *Planktothrix* were regularly observed in summer at the study site, where they only formed very localized and/or diffuse "blooms". The same cyanobacterial taxa, however, often caused harmful blooms in other local lakes, and MPs may have an effect on colony formation and maintenance in these taxa, which can cause harmful blooms in eutrophic lakes.

2. Materials and Methods

The overall design of our in situ incubation experiment was based on [26], utilizing 15.2 cm × 15.2 cm Bitran® polyethylene bags (Thermo Fisher Scientific, Waltham, MA, USA) as mesocosms, deployed in floating racks at a near-shore lake surface (Figure 1). We collected ~20 L and 2 L of surface lake water containing phytoplankton from mesotrophic Otsego Lake, New York, USA, on 6 and 13 July 2016, respectively. The pre-incubation sample (20 L) was filtered through a 118 μm Nitex® (Nitex, Singapore) mesh to exclude most large grazers before treatment. Three types of microparticles constituted treatments in this experiment: 50 μm diameter polystyrene calibration beads (DC) (Dri-Cal® Particle Size Standards Beads, DC-50, Thermo Fisher Scientific, Waltham, MA, USA), plant-derived scrub particles from Softsoap® (Softsoap, Chaska, MN, USA) Coconut Scrub body wash (CS), and plastic scrub particles from Dial® (Dial, Scottsdale, AZ, USA) Power Scrub body wash (DP). Figure 2 shows images of representative microparticles used. CS and DP (Supplementary Materials) were harvested in a lab from June to August 2013 through repeated rounds of dilution with deionized water and centrifugation. They were subsequently dried and stored at room temperature. The detailed harvesting method and size distributions for CS and DP are described in [20]. CS burned and DP melted, respectively, when exposed to heat similar to the hot needle test used in [27], meaning that DP was a plastic and CS was not. CS was most likely an apricot kernel powder, as it was the only scrubbing agent listed on the bottle. Both DC and DP sank when mixed into deionized water. Fourier transform infrared (FTIR) analysis by a Frontier FT-IR/NIR spectrometer with a built-in standard reference library (PerkinElmer, USA) was performed on CS and DP for further characterization. Full FTIR spectra were compared to reference spectra by Open Specy [28] with default preprocessing parameters (smoothing polynomial = 3, baseline correction polynomial = 8) and a spectral range of 650 to 4000 cm^{-1}. Each treatment was applied at 67 mg L^{-1} (~30 mg particles per

450 mL pre-incubation ambient lake water per mesocosm) with 4 replicates per treatment. Control mesocosms did not receive any microparticle addition but otherwise were prepared in the same manner as the treatment mesocosms. With the control group, a total of 16 mesocosms were incubated for 7 days. A 7-day incubation was selected as it was long enough for the phytoplankton population to build up in the mesocosms, yet no signs of CO_2 limitation nor a shift from planktonic to attached growth were observed.

(**a**)

(**b**)

Figure 1. (**a**) Experimental mesocosms. (**b**) Deployment site.

(**a**) CS

(**b**) DC

(**c**) DP
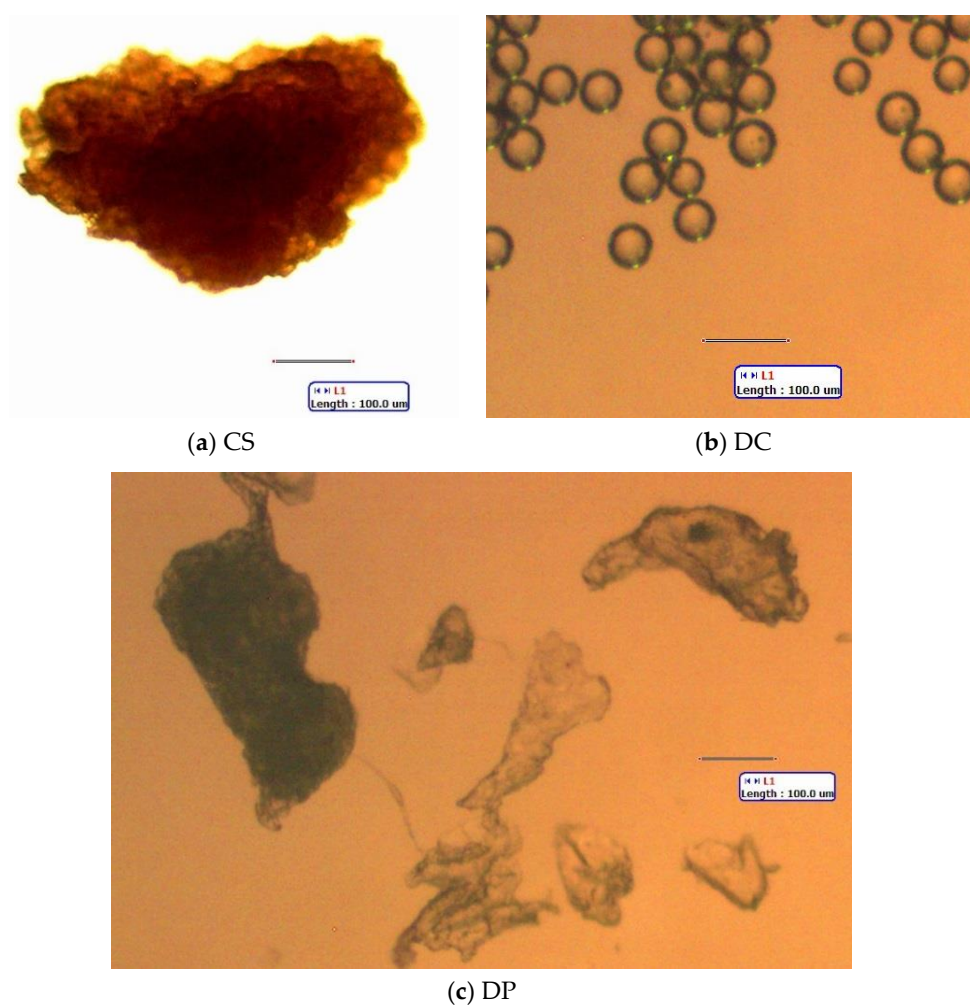
Figure 2. Representative microparticles added to lake phytoplankton. (**a**) CS (apricot kernel powder from a "coconut scrub" body wash). (**b**) DC (Dri-Cal polystyrene (PS) beads, 50 µm). (**c**) DP (polylactic acid fragments from a body wash). All scale bars: 100 µm. Standard light microscopy.

Chlorophyll *a* (chl. *a*) was analyzed by filtration onto Whatman GF/F filters (nominal pore size: 0.7 μm) (GE Healthcare, Chicago, IL, USA), followed by extraction by acetone and fluorometry on a TD-700 laboratory fluorometer (Turner Designs, Sunnyvale, CA, USA) [29,30]. Sample volumes that were filtered onto the GF/F filters were 150 mL for each experimental replicate and 500 mL for the pre- and post-incubation ambient lake water samples (all were pre-filtered through a 118 μm mesh). All chl. *a* filters were prepared as soon as possible, within an hour of ambient lake water collection and within two hours of mesocosm takedown, and then kept frozen until the acetone extraction step. Phytoplankton samples from the ambient lake water and experimental mesocosms were preserved with Lugol's iodine and stored at 4 °C. They were settled in 10 mL Utermöhl chambers overnight before identification and quantification under an inverted light microscope (Axiovert, Zeiss, Dublin, CA, USA). At 400X, the first 100 identifiable cells (i.e., >5 μm in the largest dimension) were counted along a transect across the bottom of the Utermöhl chamber. Examination of 5 to 17 fields of view were typically required to reach 100 cells. Phytoplankters were first identified to the genus level whenever possible and then grouped into major taxonomic groups. We examined the following variables as responses to the treatments: species richness (total number of taxa per replicate), Shannon–Wiener (SW) diversity index [31], and taxon-specific cell counts. Statistical analyses were performed on the open-source statistical software R [32] and RStudio [33] with packages multcomp [34], multcompview [35], tidyverse [36], and ggplot2 [37]. All response variables except chl. *a* were square-root (sqrt) transformed. Their normality was visually inspected in Q-Q plots, and normality of residuals in ANOVA models was confirmed by Shapiro–Wilk test. Assumption of equal variance among treatments was justified by the results of Bartlett's K-squared test.

3. Results

3.1. FTIR Analysis of Body Wash Particles

FTIR analysis confirmed that CS was a plant material; all top 10 matches (r ≥ 0.93) on Open Specy [28] were plant materials, with the top three (fiber poplar down, fiber kapok, and fiber jute) sharing the best match at r = 0.96 (spectra source: [38]) (Figure 3a). The top matches for DP were two spectra for polylactic acid (PLA) (r = 0.99 and 0.98) and fiber polylactide (0.98) (spectra source: [38]) (Figure 3b).

3.2. Chl. a and Diversity Indices

Chl. *a* concentrations in untreated pre- and post-incubation ambient lake water were 8.6 μg L^{-1} and 8.1 μg L^{-1}, respectively. After the 7-day incubation, mean chl. *a* concentrations in the mesocosms were 100.9, 137.1, 106.6, and 95.4 μg L^{-1} for control, CS, DC, and DP, respectively, with no significant difference across treatments (Figure 4a). Similarly, the mean SW index was 1.88, 2.24, 1.96, and 1.46; the mean species richness was 12.2, 14.8, 10.8, and 10.2, respectively. No significant difference was detected among treatments in SW index or species richness (Figure 4b,c).
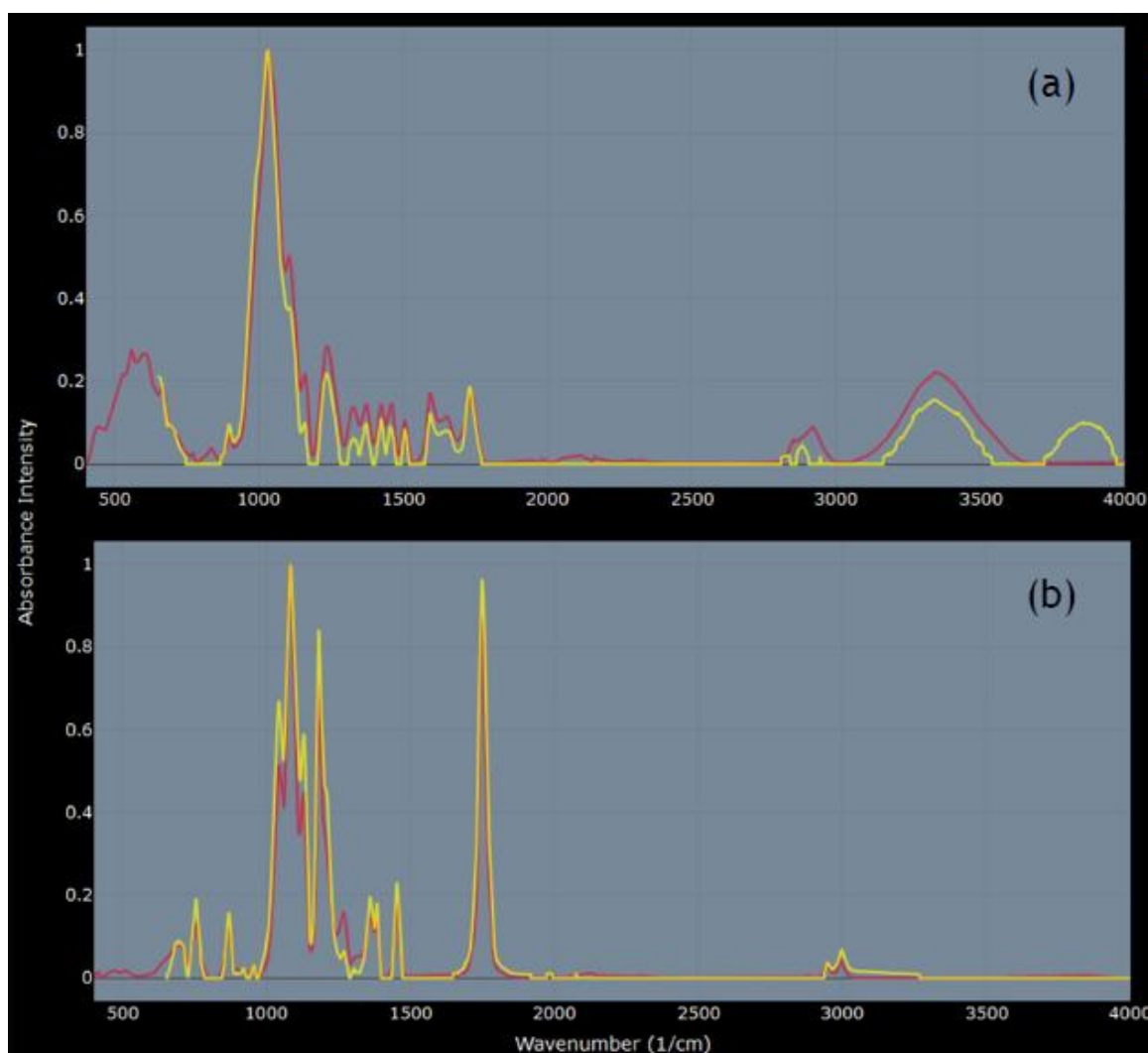
Figure 3. Comparisons of FTIR spectra on Open Specy [28]. (**a**) Yellow line: CS (plant-derived microparticles); red line: fiber poplar down [38], r = 0.96. (**b**) Yellow line: DP (body wash microplastic (MP)); red line: polylactic acid (PLA) [38], r = 0.99.

3.3. Phytoplankton Composition

The pre-incubation phytoplankton community was represented by five major taxonomic groups: Cryptophyta (including Synurophyta) (42.0% by cell count), Bacillariophyta (diatoms) (37%), Chlorophyta (11.0%), and Cyanophyta (8.0%) (Figure 5). Pyrrophyta (dinoflagellates) were not counted in the pre-ambient sample but were detected in post-incubation samples at low frequencies (means: 5.5, 1.3, 0.5, and 0.5% for control, CS, DC, and DP, respectively). Cyanophytes were predominantly small colonies of picocyanobacteria such as *Aphanocapsa* and *Gloeocapsa*.
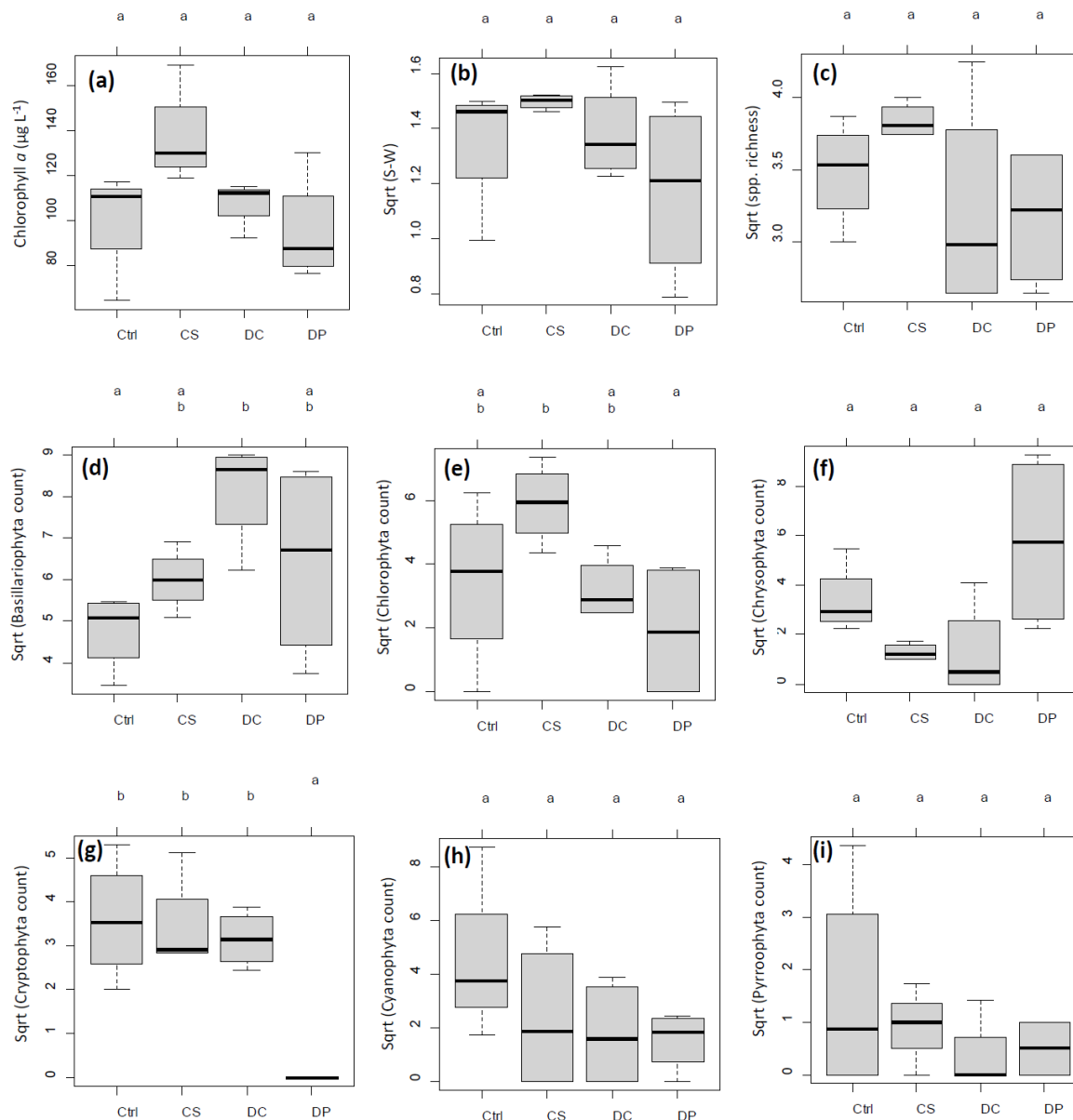
Figure 4. Boxplots of major experimental responses after a 7-day incubation by treatment groups. n = 4 each for control (Ctrl), plant particles (CS), calibration beads (DC), and body wash MPs (DP), except chlorophyll *a* (chl. *a*) for DC (n = 3 due to sample loss). All responses except chl. *a* were square-root (sqrt) transformed to better meet model assumptions. Letters a and b on top of each panel indicate groupings by the results of multiple pairwise comparisons among the treatment groups by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. (**a**) chl. *a* ($\mu g\ L^{-1}$), (**b**) Shannon–Wiener (SW) index, (**c**) species richness, (**d**) Bacillariophyta count, (**e**) Chlorophyta count, (**f**) Chrysophyta count, (**g**) Cryptophyta count, (**h**) Cyanophyta count, and (**i**) Pyrrophyta count.

Consistent with the chl. *a* results above, there was no noticeable difference in general abundance of identifiable phytoplankton cells among treatments after the 7-day incubation. Cell counts by major taxonomic groups, however, showed a treatment effect, most evidently for cryptophytes (one-way ANOVA, $p < 0.001$), followed by chrysophytes ($p = 0.041$) and marginally by bacillariophytes ($p = 0.051$) and chlorophytes ($p = 0.066$) (Figure 6). DP had such a strong inhibitory effect on cryptophytes that it was totally separated from the other three treatment groups (Tukey's honestly significant difference (HSD), $p < 0.0025$, Figures 4g and 6). DP was also marginally distinct from DC and CS for chrysophytes

($p < 0.059$, Figure 6) but had an overlap with the control. This weak trend was recognized but not indicated as statistically significant in Figure 4f at $\alpha = 0.05$.
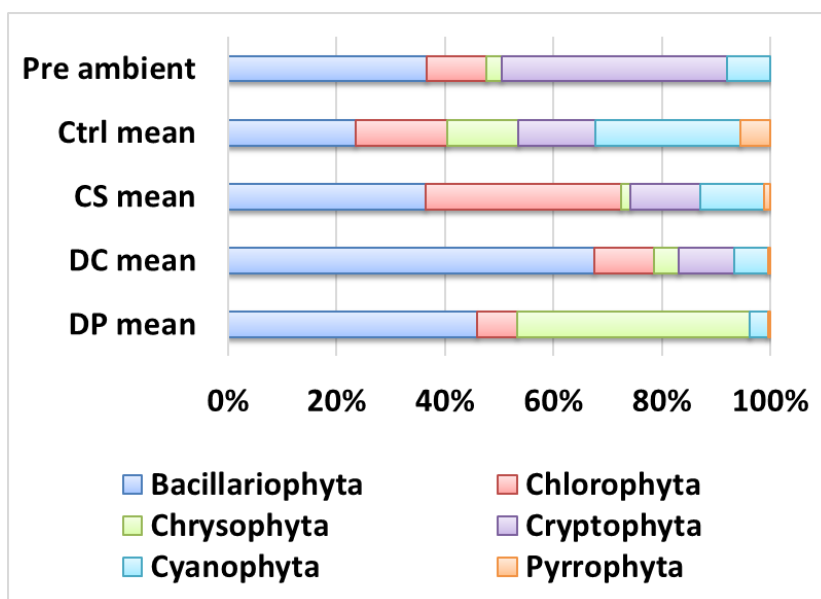

Figure 5. Mean taxonomic composition of the phytoplankton in the untreated pre-incubation lake water ("Pre ambient") and in the experimental mesocosms after 7 days of in situ incubation. Ctrl: control; CS: plant particles; DC: calibration beads; DP: body wash MPs.
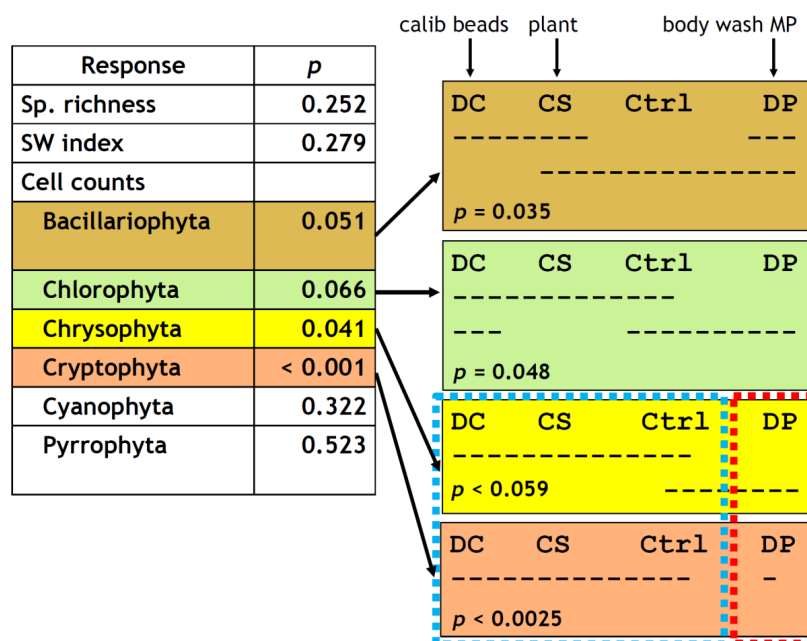

Figure 6. Summary of one-way ANOVA (**left**) and grouping by Tukey's HSD test (**right**) on the taxonomic responses of the phytoplankton to the treatments. n = 4 for all four treatment groups except for chl. *a* for DC (n = 3 due to sample loss). Dashed horizontal lines in the right panel indicate Tukey's HSD groupings; for example, for Bacillariophyta (top right box), DC, CS, and DP formed one group and CS, Ctrl (control), and DP formed another group. While an overlap existed between the two groups, DC and Ctrl never belonged to the same group and therefore had statistically different responses in this case ($p = 0.035$ for the DC–Ctrl contrast). Similarly, CS–DP contrast for Chlorophyta was significant ($p = 0.048$), DP–DC and DP–CS contrasts had $p < 0.059$, and DP–DC, DP–CS, and DP–Ctrl contrasts had $p < 0.0025$.

4. Discussion

Our first hypothesis that MPs, but not plant-derived particles, affect the lake phytoplankton was partially supported. Microparticle addition to the lake phytoplankton did not lead to any statistically significant increase or decrease in chl. *a* in the control nor any of the three microparticle treatments (one-way ANOVA and Tukey's HSD at $\alpha = 0.05$), which was interpreted as no quantitative effect on overall phytoplankton biomass. Responses of each taxonomic group to different types of microparticles, however, showed previously unreported qualitative effects: Cryptophytes were eliminated (Figures 4g and 5) and chrysophyte counts increased (Figures 4f and 5) in response to the addition of irregularly shaped and sized MP fragments recovered from a body wash (DP). Plant-derived scrub particles (CS) had no effect ($\alpha = 0.05$) on cell counts of any of the taxonomic groups included in this study (Figures 4–6), supporting the idea that plant-derived microparticles are a more environmentally sustainable alternative to MPs as scrubbing agents in personal care products. Yet, the effects of the perfectly spherical calibration beads (DC) on the phytoplankton were either nondetectable or indistinguishable from those of CS in this study. Other studies have reported negative effects of analytic calibration beads on aquatic primary producers [39–41]. If these experiments were repeated with MPs that are more representative of secondary MPs found in the environment, more pronounced and/or different results may be observed. Such studies are going to be critical as we seek to predict long-term effects of secondary MPs in our aquatic ecosystems and to mitigate them at strategic points in the modern hydrologic cycles such as wastewater treatment plants. Our second hypothesis that planktonic cyanobacterial colonies become smaller and more numerous with MPs was not testable as the cyanobacteria observed in this study were predominantly small colonies of picocyanobacteria. With certain NPs being reported to increase microcystin production in cyanobacteria [42], repeating this experiment with a more eutrophic source water and in late summer to early fall when the water temperature is higher would be interesting and may potentially have an applied implication to management of harmful cyanobacterial blooms, an increasing concern for water utilities with respect to cyanotoxins as well as taste and odor compounds [43].

The differential effects of the calibration beads (DC) vs. irregularly shaped body wash MP particles (DP) on cryptophytes and chrysophytes were totally unexpected, as they were both "sinking" MPs (i.e., not made of "floating" high- or low-density polyethylene or polypropylene), and their size difference was within one order of magnitude. DP had an extremely close ($r = 0.99$) match with PLA, a family of plant-based biodegradable plastics widely used in medicine as suture threads, bone fixation hardware, and drug delivery devices and also noted for its brittleness and slow degradation [44]. PLAs derived from corn-based starch are also widely used in biodegradable/compostable single-use clamshell food containers [45] and tableware [46]. *Chroomonas* and *Rhodomonas* are flagellated, "naked" unicellular algae, and small cryptophytes resembling them (definitive species identification is not possible with light microscopy) comprised the majority of our cryptophytes (Figure 7), the group eliminated by DP (Figures 4g and 5). Cryptophytes have an external covering called periplast, which is composed of a plasma membrane and plate(s) embedded underneath [47–49]. This would be expected to offer some extra support, but this reinforcement appears limited [50] and easily compromised by trypsin digestion [47]. Cryptophytes therefore do not have physical protection for their plasma membranes against degradation products from MPs, including proteolytic metabolites from surface biofilm. Trypsin has been detected in coastal surface seawater [51], and proteolytic enzymes in general have been associated with both live and dead bacteria in lakes [52].
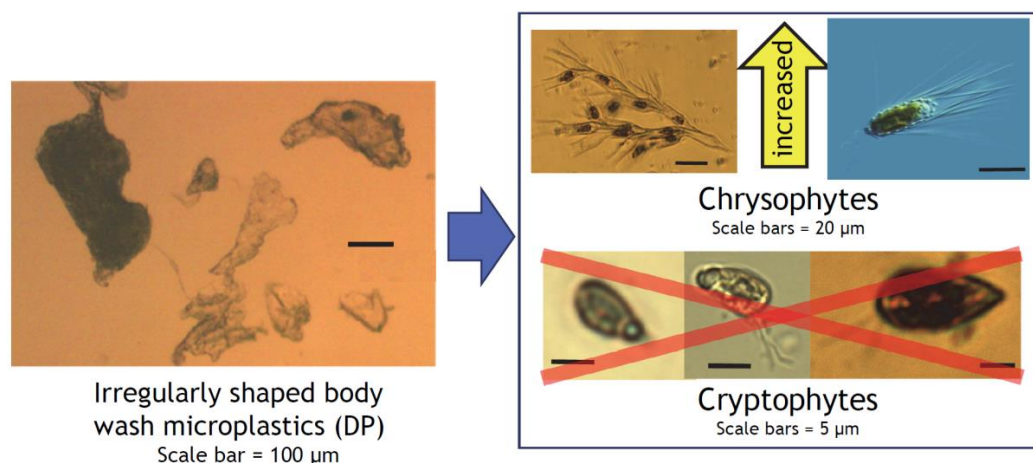
Figure 7. Conceptual diagram of the differential effects of irregularly shaped body wash MPs on Chrysophytes and Cryptophytes, with images of representative taxa identified in this study. Images are not to scale. Inside the right box: *Mallomonas* sp. image (**upper right**) is licensed under CC BY-NC-SA 2.0 [53]; images of *Dinobryon divergens* (**upper left**), *Chroomonas-* (**lower left** and **middle**) and *Rhodomonas*-like (**lower right**) cryptophytes are from this and past studies from Otsego Lake phytoplankton.

In contrast, chrysophytes, mostly *Dinobryon* and *Mallomonas* in our samples, increased their occurrence with DP addition. These taxa have protective siliceous lorica or plates [48,54] that may afford them protection from MPs and potentially harmful chemicals leaching from or adsorbed to MPs. At the same time, DP did not affect cell counts of Bacillariophytes (diatoms), which are protected by siliceous frustules (valves) and mucilage [48,55] and considered resistant to abrasion and disturbance in stream periphyton [56]. The vast majority of diatoms observed in this study were non-colony forming, and MP effects on diatom colony sizes could not be evaluated. We thus postulate that a physically protective cellular outer covering is an important factor for phytoplankters to avoid negative effects from irregularly shaped and brittle aquatic MPs with large and uneven surface areas. The negative effects could include physical abrasion, electrostatic interaction, and chemical leaching from jagged and constantly eroding surfaces. Perfectly spherical MPs (DC), which are smaller in particle size and collectively have a large but smooth surface area, are more likely to roll along the surface of a cell than to damage it, which may explain them having no effect on cryptophyte counts in this study (Figure 4g). Plant-derived CS particles also tend to have rounded edges due to their cellular composition, as visible along the outline of the representative particle in Figure 2a. DP particles, on the other hand, have many projections and sharp edges that seemed to have resulted from exuding of melted plastic through a fine mesh-like structure or mechanical shredding or shaving of larger pieces of plastic. Sinking rates of diatoms have been reported to decrease with formation of diatom-microbead aggregates, while those of cryptophyte-microbead aggregates increased their sinking rate [39]. This can lead to more diatoms remaining in the photic zone while the flagellate and motile cryptophytes may experience higher mortality due to sinking into the aphotic zone. If the motile cryptophytes sense increased sinking, this may also manifest as a subtle decrease in fitness due to increased energy expenditure to swim against gravity in order to remain in the photic zone.

The findings from this study also point to the potential danger of extrapolating results from precision-manufactured calibration beads and nanoparticles to secondary MPs found in the environment. We fully acknowledge the fact that harvesting microparticles from consumer products can be extremely labor intensive and that obtaining the raw particles from a vendor is often difficult, as the exact entity of the scrub particles is not necessarily disclosed in the ingredients list. Vendors are also unlikely to respond to researcher inquiries in general. Whenever possible, however, comparison between calibration beads and scrub particles in consumer products that are expected in wastewater effluents or lab-generated secondary particles that mimic those in the environment (e.g., [57]) is advised in

studies of biotic responses to MPs. Use of MPs in personal care products is either banned or being voluntarily phased out in the European Union, United Kingdom, and North America and assumed to be responsible for a relatively small portion of the overall aquatic MP pollution [24,25]; however, in this study the MPs harvested from a body wash served as a superior surrogate for secondary MPs found in source and wastewater than uniformly sized and shaped calibration beads (primary MPs). The body wash MPs better mimicked the products of fragmentation of larger pieces of plastic waste and were most likely still associated with chemical residue of the body wash. Such residue would contain chemicals commonly expected in household effluent and potentially adsorbed by aquatic MPs, e.g., surfactants, fragrances, colorants, moisturizers, stabilizers, and preservatives. Effects of MPs on aquatic organisms are expected to be driven by the physical (size and shape) and chemical characteristics of MPs themselves as well as externally associated chemicals from the surrounding water.

This study focused mainly on the effects of MPs that would be classified as fragments in environmental samples. Fibers are the most irregularly shaped MPs with a large surface-to-volume ratio and a miniscule mass, resulting in a very slow sinking rate. Fibers are therefore more likely to interact with living cells that they encounter in the environment than beads and fragments. With our increasing awareness of their true ubiquity [9,23,58], further investigation of the effects of MPs, including fibers on phytoplankton communities, and potential bottom-up effects on aquatic food webs are warranted.

5. Conclusions

This study focused on the interaction between a freshwater lake phytoplankton community and 50 μm polystyrene calibration beads, PLA-based MP from a body wash, and plant-derived scrub particles from another body wash through a 7-day, summertime, in situ incubation experiment in a mesotrophic, northern temperate lake. Taxonomic composition of the phytoplankton in the 450 mL mesocosms were not affected by the calibration beads and the plant-derived particles, while the body wash MPs eliminated cryptophytes ($p < 0.001$) and increased chrysophytes ($p = 0.041$). These differential effects of irregularly shaped body wash MPs on certain taxa of lake phytoplankters suggest potential bottom-up alteration of aquatic food webs by secondary MPs, including those considered biodegradable. Increased monitoring data and sample sharing between utilities operators and researchers will accelerate our understanding of how environmental MPs interact with freshwater biotas before they enter the ocean.

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