Interactive comment on **"Experimental tests of phytoplankton response to ornithological eutrophication in Arctic freshwaters"** by **Heather L. Mariash et al.**

Heather Mariash et al.

Reviewer 1

1. This study provides interesting new knowledge on the important role of goose droppings in affecting water quality in Arctic freshwaters. It shows that these droppings have a greater short-term effect on water quality than a sedge plant. These results are not unsurprising. The paper is well written and clear. The experimental design is simple and straightforward. The parameters measured are basic water quality parameters, although chlorophyll-a concentrations were not measured.

   Thank you for the positive feedback on several aspects of the manuscript. We underestimated the amount of organic matter in the goose treatments when designing the experiment, as such several aspects such as chlorophyll-a and primary productivity failed to be realized.

2. The findings from using small containers should not be over-interpreted. These small containers have a high surface area to volume ratio which can become important in terms of biofilm growth on walls. So the effect in the first few days is the most ecological relevant. This limitation should be discussed.
   We have included an additional sentences to P.9 to acknowledge the potential container effects: "Our experimental design was not without issues: more replicates would have made the results clearer, and the use of small containers has the potential to contribute technique-related artefacts (e.g., biofilm growth, altered physiochemical conditions, and species interactions due to container area-to-volume relationship; Liber et al., 2007), effects that we attempted to mitigate through the use of s short experimental duration. Despite these caveats, the responses in the phytoplankton communities were pronounced."

3. Nitrogen versus phosphorus limitation is only relevant if concentrations are low. Therefore N:P ratios should be with caution. Additionally, N fixing cyanobacteria are only promoted if N concentrations are low, not just because N:P ratios are low.

   On P. 10 of the Discussion we have a sentence that highlights when low nitrogen concentrations promote cyanobacteria, not just low N:P ratios.
   "This is an environmental concern from a water quality perspective, because when N is limiting, N2-fixing cyanobacteria are competitively favoured (Guildford and Hecky, 2000; Schindler et al., 2008)"
   To decrease the emphasizes on the N:P ratios in this same paragraph, we have removed this sentence ("In the wintering grounds with highest goose densities, TN:TP ratios of the waterbodies had a mean of 15 (Kitchell et al., 1999), indicating N- limitation. While the"
   Lastly we adjusted the last sentence to include that we have both low N and decreasing N:P ratios. "The wetlands across Southampton Island have relatively low nitrogen concentration and TN:TP ratios of approximately 30 (Mariash et al. 2018), on par with other shallow Arctic freshwaters (Rautio et al., 2011), there is an indication that these wetlands are becoming more N-limited with decreasing TN:TP ratios (Mariash et al., 2018)."

4. The study did not measure primary production, that is a rate process. So the paper should be explicit that what was measured was accumulation of biovolume.
   We are now more explicit throughout the manuscript using biovolume and not the rate of primary productivity. Also Reviewer 2 made several suggestions in this regard, please refer to those comments for specific amendments.

Reviewer 2

1. In this manuscript Mariash and co-workers investigate the impact of goose faeces on water chemistry and phytoplankton communities in arctic freshwaters. The authors present results from a mesocosm experiment in which goose faeces and Carex leaves were added in different treatments. In the experiment the development of water chemistry (C, P and N) and phytoplankton community were followed. Estimated goose faeces loading rates, water chemistry and chlorophyll data (unpublished data) from a previous study by the authors in the Southhampton area were compared to with results from other studies in USA and Canada. Given the increasing goose populations in the arctic and the impact they have on the arctic environment the subject of the manuscript is highly relevant. This is especially true considering that the main focus within this subject so far has been on goose impacts in the terrestrial environment while little attention has been devoted to impacts on the freshwater environment. The manuscript is well written and the language fluent.

   We appreciate the reviewer's recognition of the value and relevance of the data that we report.

2. A major comment concerns the focus of the manuscript as related to the results presented. The title gives the impression that this is a story about goose/bird mediated impact on phytoplankton communities in arctic freshwaters. Furthermore, a large part of the text is devoted to phytoplankton. However, in my mind the strong part of the data presented are the results on changes in water chemistry in response to addition of goose faeces. In fact, it is quite interesting to see how rapid the nutrients from the faeces are released into the water. Due to the limitations outlined below, I do not think that the phytoplankton data presented are substantial enough to back up a main focus on effects on the phytoplankton community.

   We will modify the manuscript using the reviewer's suggested improvements to shift the focus more to the water chemistry and less emphasis on the phytoplankton results.

3. 3 out of 10 (or 8?) phytoplankton samples from the experiment could not be counted due to insufficient fixation. This is a significant part of the samples, especially since the missing samples were from day 1 in the goose faeces treatment and from day 3 and 5 in the Carex treatment, respectively. This made it impossible to compare the phytoplankton response between treatments on day 1, 3 and 5, which is the most important period of the experiment in my mind.

   We agree with the reviewer that the loss of three critical samples does impact the extent to which we can draw conclusions regarding changes to the phytoplankton community. However, the relative comparison between the treatments is still valuable as they indicate the magnitude and compositional difference between goose droppings and carex treatments.

4. The experimental design is clear. However, a consequence of the phytoplankton sampling, where three replicates (per sampling day/treatment) were pooled, there is just one observation/replicate on phytoplankton biovolume/composition per sampling day/treatment. Considering the focus of the manuscript on phytoplankton responses, it would have been good with more observations/replicates to get an idea about the variation in the phytoplankton response.

We agree with the reviewer and appreciate the recommendation. Including more replicates would have allowed for more variability to be seen within the phytoplankton response between and within treatments. In the future, our experimental set up will include higher sample volumes to accommodate for replication and higher concentrations of fixative to compensate for the organic material in the samples.

5. It is stated that one of the aims was to measure changes in phytoplankton productivity. In the methods it is explained that phytoplankton production rates were estimated from changes in biovolumes between different sampling days. Thus, it is not the phytoplankton productivity that is measured but phytoplankton biovolumes. In the results section no production estimates are presented. Here, only the changes in the biovolumes are mentioned. I guess, this is due to the lacking phytoplankton samples mentioned above making comparison between treatments impossible throughout most of the period that phytoplankton was sampled in the experiments.

   Yes, as mentioned above, the loss of samples made direct comparisons between treatments challenging. We will edit the manuscript to discuss "phytoplankton response" rather than "phytoplankton productivity" as we did not present true primary productivity results. We have changed the aim from "phytoplankton production" to changes in "phytoplankton biovolume".

6. Phytoplankton taxa were identified to genera. Species within the same genera may show different responses to a given environmental change, e.g. eutrophication. Hence, a better taxonomical resolution in the identification would have made it possible also to get an idea about the species-specific responses to the experimental treatments. For reasons outlined above, I do not think the data presented on phytoplankton justifies the focus in the manuscript on phytoplankton response to goose impacts in arctic freshwaters.

   Although species-specific response would have been best, identification to genera still provides a good comparison between treatments. We have taken the reviewer's suggestions on how to better present the phytoplankton results throughout the manuscript.

7. However, the data presented on the changes in water chemistry in the goose treatment are very interesting. Especially the rapid release of nutrients from the goose faeces brings new insights. I suggest the authors rework the manuscript to "Experimental tests of water chemistry to goose mediated (or ornithological?) eutrophication in Arctic freshwaters", leaving out the phytoplankton data from the manuscript.

   We appreciate the reviewer's suggestion and will change the title to include "water chemistry and planktonic response" to better reflect the water chemistry focus of the manuscript. We will rework the manuscript to focus on the water chemistry results more; however, the phytoplankton results are a valuable part of the data demonstrating how the biotic component reacts to the changes in chemistry. We will keep the phytoplankton data in the manuscript using amendments suggested from both reviewers to shift the focus towards the water chemistry results.

8. Minor comments: In the introduction there is a focus on the nutrient enrichment as the mechanistic explanation for the goose impact on arctic freshwaters. I agree that this is likely an (the most) important mechanism in explaining goose impacts. Still, dispersal effects in the experiments cannot be completely excluded due to the way goose faeces additions were done (no treatment of the faeces was done, e.g. heating, before addition to the experimental beakers). Hence, differences in phytoplankton composition between treatments could also, at least partly, be caused by input of phytoplankton spores/cysts with the goose faeces. This is not a criticism of the experimental setup, but I think this issue should be mentioned in the manuscript (introduction and discussion) as long as the phytoplankton aspect is included in the manuscript.

We agree that the main mechanism of goose impacts is through nutrient inputs, but species dispersal is also possible. We have added a couple of sentences to introduction and discussion to include this aspect.

In Introduction P. 2. Inserted "Birds can also act as vectors for the dispersal of plants, phytoplankton, and zooplankton, when propagules are spread through their faeces eggs (Figuerola and Green, 2002; Hessen et al., 2019).

In discussion, P. 10. Amended the 3rd paragraph on P10, now includes: "Geese are therefore acting as biovectors on the landscape, consuming large amounts of terrestrial nutrients bound in vegetation and excreting these nutrients in form that is bioavailable for freshwater ecosystems. Goose faeces could also contribute to the dispersal of aquatic species, altering aquatic communities in this direct manner (Figuerola and Green, 2002). Tested phytoplankton species were not viable under cultured conditions once passed through waterbirds (Atkinson 1980), however tests of this mechanism have not yet been carried out for geese in the Arctic."

9. P. 5 l. 15-16: "(for sampling details see Mariash et. al. 2018, Table 3)" instead of "(for sampling details see Mariash et. al. 2018)(Table 3)".
   Added a semi colon instead of the double bracket.
10. P. 5 l. 27-28: Production rates are not included in the results section.
    Correct, this sentence has now been removed.
11. P. 5 l. 28: include identification literature.
    Now in the methods:
    "Phytoplankton taxonomy relyed on the following literature Cox, 1996; Millebrand et al, 1999; Whitton and Brook, 2002; Komárek and Anagnostidis, 2000; Guiry and Guiry, 2017; Taylor and Archibald, 2007; and Wehr and Kociolek, 2015."

12. P. 6 l. 4-5: "To model the effect of our treatments on primary productivity, we used a linear mixed effects model implemented in lme4 (Bates et al., 2014)." As far as I can see these results are not included in the results section, likely due to the lacking phytoplankton data.
    True, these sentences correspond to data that we no longer present in this paper, and thus these sentences should be deleted.

13. P.7 l. 8-16: This text passage seems a bit strange due to the lacking phytoplankton data. This also applies to l. 24-26 on p. 7.
    We have amended to read:
    "While the biovolumes were similar, the phytoplankton communities were different between treatments."
14. P. 7 l. 23: delete "(". P. 9. L. 19: "We had expected phytoplankton production to be higher in the goose dropping treatment vs. the Carex treatment". As noted above phytoplankton itself is not measured. Thus, it seems safer to use "phytoplankton biovolume".
    We have changes "phytoplankton production" to phytoplankton biovolumes"
15. P. 9 l. 28-32: Also, this text passage seems a bit strange due to the lacking phytoplankton data.
    We have amended the sentence to be more cautious and include suggestions from both reviewer's. The paragraph now reads:
    "Community composition of the phytoplankton responded to increased nutrient availability in both treatments. Our experimental design was not without issues: more replicates would have made the results clearer, and the use of small containers has the potential to contribute technique-related artefacts (e.g., biofilm growth, altered physiochemical conditions, and species interactions due to container area-to-volume relationship; Liber et al., 2007), effects that we attempted to mitigate through the use of s short experimental duration. Despite these caveats, the responses in the phytoplankton communities were pronounced."

16. Figure 4: it is difficult the distinguish the colors used for some of the phytoplankton classes, e.g. dinophyceae, euglenophyceae and fragilariophyceae. Maybe use different patterns in grey tones instead.
    With 13 classes it is difficult to get a lot of distinction when the layers are so small. Since it is for online publication, we prefer to use color rather than grey-scale. We custom built the color pallet to improve the distinction between colors.

Associate Editor Decision: Reconsider after major revisions (07 Aug 2019) by Perran Cook
Comments to the Author:
Dear Dr. Mariash
Thank you for submitting your work to Biogeosciences. Both reviewers see the significance of your work and are broadly supportive of publication. They do however, note a number of issues, for which you have outlined a satisfactory response. In particular, I agree with your point about retaining the phytoplankton data in response to reviewer 2.

Please revise the manuscript as outlined, clearly noting the changes you have made in response to each point. I will ask one of the reviewers to assess whether you have addressed their comments to their satisfaction.

In addition, I have two minor comments.

pg 9, line 6. Please provide a little more detail on your calculation and loading rate for the comparison with the work of Schindler.

We added a sentence to help clarify how the nutrients were calculated: "In comparison, Schindler et al. (2008) added on average 298 kg of N and 24 kg of P per year to a small boreal lake annually for the first 6 years of their classic whole-lake eutrophication experiment. Since the amounts of N and P additions varied from year to year, an average of nutrient additions for the first 6 years was used, and then divided by 365 to get a daily loading rate. The daily load per lake area of N and P additions from their whole-lake experiment was smaller than nutrient loads produced by goose colonies (Table 3)."

Regarding the suggestion for the title given by reviewer 2. I think this is an excellent point, but I would suggest using a more specific term like 'nutrient concentrations' or 'nutrient release' rather than the more generic term 'water chemistry'.
In the title, "nutrient release" replaced "water chemistry".

Regards
Perran Cook

Experimental tests of nutrient release and planktonic responses to ornithological eutrophication in Arctic freshwaters

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Abstract. Many populations of Arctic-breeding geese have increased in abundance in recent decades, and in the Canadian Arctic, Snow (*Chen caerulescens*) and Ross's Geese (*Chen rossii*) are formally considered overabundant by wildlife managers. The impacts of these overabundant geese on terrestrial habitats are well documented, and more recently, studies have suggested impacts to freshwater ecosystems as well. The direct contribution of nutrients from goose faeces to water chemistry could have cascading effects on biological functioning, through changes in phytoplankton biovolumes and community composition. We demonstrated previously that goose faeces can enrich ponds with nutrients at a landscape scale. Here, we show experimentally that goose droppings rapidly released nitrogen and phosphorus when submerged in freshwater, increasing the dissolved nitrogen and phosphorus in the water. This resulted in both a decrease in the nitrogen:phosphorus ratio and an increase in cyanobacteria in the goose dropping treatment. In contrast, this pattern was not found when we submerged cut sedge (*Carex* sp.) leaves. These results demonstrate that geese act as biovectors, causing terrestrial nutrients to be bioavailable in freshwater systems. Collectively, the results demonstrate the direct ecological consequences of ornithological nutrient loading from hyperabundant geese in Arctic freshwater ecosystems.

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1 Introduction

Arctic regions are important breeding grounds for a wide range of migratory species. With 22.1 million geese, belonging to five species, breeding in the Canadian Arctic (Fox and Leafloor, 2018), along with substantial numbers of non-breeders, geese are ubiquitous on the Arctic landscape during the summer. Goose populations have been increasing since the 1950s, primarily due to changes in agricultural practices that have increased food availability in the southern wintering grounds, but also because of increased survival from increased use of wildlife reserves and protected areas, as well as milder winters (Abraham et al., 2005).

Increases in abundance have been especially pronounced for several populations of Snow (*Chen caerulescens*) and Ross's Geese (*Chen rossii)* (Fox and Leafloor, 2018). These large and increasing populations have caused considerable change to the Arctic habitats that they use for staging, breeding and brood-rearing (Abraham et al., 2012). Geese provide both deleterious and beneficial ecosystem services to tundra habitats (Buij et al. 2017). In a negative role, repeated overgrazing of graminoid forage plants weakens them, and grubbing of the below-ground plant parts compromises vegetation regrowth and the stability of pond edges (Jefferies et al., 2006). However, geese also rapidly liberate nutrients in an otherwise nutrient-poor landscape. Because geese digest only a fraction of the plant material they ingest, they compensate with a high turn-over from feeding to faeces (Cadieux et al., 2005). This nutrient enrichment of the terrestrial environment can lead to enhanced primary productivity (reviewed in Buij et al. 2017, but see Gauthier et al. 1995). Geese predominantly graze around ponds, especially with broods and when they are moulting their flight feathers; the ponds are essential to escape predation. As a result, pond perimeters in areas used heavily by geese are notably mossy, brown and muddy due to the heavy localized grazing. At these pond margins and indeed throughout the catchment, geese have the potential to influence freshwater ecosystems indirectly through this mobilization of nutrients.

While considerable research has outlined the effects of grazing and grubbing on terrestrial habitats, very few studies have focused on the associated freshwater habitats. Shallow freshwaters are highly connected to their catchments by a high surface area to volume ratio (Rautio et al., 2011). Thus, ponds are very susceptible to habitat changes within the catchment, from increased terrestrial organic matter flowing into the pond due to heavily grazed pond edges, through the decomposition of goose droppings, and through sediment bioturbation that brings nutrients back into suspension. Increased terrestrial organic matter leads to a more bacteria-based production rather than photosynthetic (Ask et al., 2009). On the other hand, increased nutrients can cause shifts in trophic status and increased phytoplankton productivity, as demonstrated in nutrient addition experiments (Schindler et al., 2008), or in systems where seabirds act as biovectors transferring marine nutrients to ponds (Michelutti et al., 2009). Arctic-breeding geese could be acting as biovectors, causing an accumulation of terrestrial

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nutrients in ponds, but so far there is little published information on rates of nutrient loading into freshwater systems in the Arctic (Dessborn et al., 2016).

Additions of nitrogen and phosphorus in temperate waters can clearly contribute to eutrophication, resulting in pronounced shifts in community composition and ecosystem function (Pace et al., 2010; Schindler et al., 2008). While it has been demonstrated that goose droppings are a significant source of total nitrogen (N) and phosphorus (P) to nearby ponds (Côté et al., 2010; Mariash et al., 2018; Olson et al., 2005), only a few studies have attempted to quantify the magnitude of ornithological nutrient loading (Liu et al., 2014; Post et al., 1998), and relate increased nutrients to broader limnological affects (Van Geest et al., 2007; MacDonald et al., 2015; Unckless and Makarewicz, 2007). Birds can also act as vectors for the dispersal of plants, phytoplankton, and zooplankton, when propagules are spread through their faeces (Figuerola and Green, 2002; Hessen et al., 2019). Currently, almost no measures of these broader ecosystem-level effects exist for Arctic ponds.

Arctic ponds, typically characterized as oligotrophic transparent waters, are increasingly represented by turbid, mesotrophic waterbodies (Wauthy et al., 2018; Wrona et al., 2016). Increasing Arctic temperatures, and increased permafrost thaw play an important role in these changes (Vonk et al., 2015; Wauthy et al., 2018). However, geese are another potential vector of change in these ecosystems through direct (faeces) and indirect (bioturbation) nutrient release. Arctic freshwater wetlands used by geese are vital feeding and breeding grounds for many migratory bird species, including many sympatric and declining species of shorebirds (Flemming et al., 2019). Given the crucial ecological role played by freshwater wetlands in the Arctic, an understanding of goose-related habitat change in Arctic freshwaters has been identified as a research priority by goose population managers.

To better understand how ornithological nutrient loading affects both water chemistry and biological functioning, we designed a study to: i) measure the nutrients released from submerged goose droppings, ii) measure the concentration of dissolved nutrients in the water over time in the presence of these droppings, and iii) measure the resultant changes in phytoplankton biovolume and community composition. We hypothesized that goose droppings would increase nutrient loading in the water, specifically total nitrogen and phosphorus, and that this increase in nutrients would increase algal biovolume and change phytoplankton community composition, with a shift towards increased presence of cyanobacteria.

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2 Methods

2.1 Mesocosm experiment

We measured the primary production and phytoplankton community composition response to nutrients released into the water column by submerged goose droppings and *Carex*, using an *in situ* mesocosm experimental approach. In order to differentiate between nutrients released from the plant detritus and nutrients cycled through geese, we compared the nutrients released from undigested graminoid clippings, goose droppings, and pond water only treatments. The *in situ* mesocosm experiment was established in the wetlands of Southampton Island, Nunavut, Canada, at the East Bay long-term field station (63˚59'N, 81˚40'W). Fresh goose droppings containing both faeces and uric acid from lesser snow geese and fresh clippings of the dominant graminoid (*Carex sp.*), herein simply referred to as *Carex*, were all collected on 8 July 2015. The goose droppings were all less than 24 h old, moist and green in color; the droppings were pooled and homogenized, while the *Carex*, including the bulb and blade, was clipped into 2 cm long pieces. We placed 10 ± 0.05 g of goose droppings or 4 g ± 0.05 g of fresh *Carex* (approximately equivalent to 1.1 g dry mass), into plastic cups, which were filled with 200 mL of pond water, that had been passed through a < 50 µm sieve. The cups were placed in a floating wooden frame to keep each container upright and floating in the pond, in order to retain some natural turbulence (Fig 1). The cups were covered with plastic wrap to allow light through but prevent evaporation or overfilling. The experiment was conducted for 17 days in the pond at ambient temperatures (average temperature in the cups 8.2 °C) and natural light conditions (approximately 21 h daylight, 3 h of dusk).

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Treatments were goose droppings and *Carex* with five replicates of each treatment for each sampling day, along with control cups containing only pond water. Water parameters were sampled on day 1, 3, 5, 10, and 17. On sampling days, we sampled both the overlying water and the organic matter from the five cups per treatment to measure the rate of nutrients released into the water and decomposition of the *Carex* and goose droppings. For nutrient samples, the water from two of the five cups from each treatment was passed through 50 µm sieve and poured into prepared vials for total phosphorous (40 mL volume with 116 µl of 30% H_2SO_4) and total nitrogen (24 mL volume with 230 µl HCL). The water from the remaining three replicates was pooled for phytoplankton community composition (150 mL amber glass bottle preserved with Lugol's iodine solution). Phytoplankton were only sampled during the first 10 days of the experiment, to limit the cup-effect on phytoplankton community dynamics.

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2.2 Identifying an appropriate loading rate

To compare our results to those published from elsewhere, it was necessary to establish a "faecal loading rate" with consistent units. We define nutrient loading rate within the catchment as the concentration of nitrogen and phosphorus measured from goose droppings, scaled up using rates of defecation and the density of geese per km² reported for each site used in our comparisons, for a final unit of kg of nutrients per km² per day. The loading rate for the region in which our study took place, Southampton Island, was calculated based on nutrient concentrations from the goose droppings used in the experiment, combined with a conservative estimate of faecal production based on weight and defecation rate per day (Unckless & Makarewicz, 2007) and the average density of geese breeding in Southampton Island goose colonies (Kerbes et

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al., 2014). We view this estimate as conservative because non-breeding geese are numerous across Southampton Island (perhaps outnumbering breeding geese), and goslings make a substantial contribution to dropping densities. Both Kitchell et. al. (1999) and Olsen et. al. (2005) used the daily excretion rate for N and P as reported in Post et. al. (1998). For these studies, values for Table 3 used the minimum levels reported in Post et. al. (1998), which were 0.001 kg N day^{-1} and 0.0002 kg P day^{-1} per goose.

2.3 Laboratory analysis

The particulate organic matter, goose droppings or *Carex*, from each cup was frozen in the field and later freeze dried and weighed, ground, then subsampled for carbon, nitrogen, and phosphorus content. Carbon and nitrogen content were analysed at the University of Ottawa's G.G. Hatch Isotope Laboratory, using an elemental analyser (Elementar Isotope Cube, Germany), from samples (*Carex* 2.5 ± 0.4 mg; goose droppings 4.5 ± 0.5mg) and standards that were weighed into tin capsules and loaded into the elemental analyser. The phosphorus from the solid samples was first processed by dissolving 0.1 g of each sample in 5 mL concentrated HNO_3 for 1 h at 95 ˚C, with an additional 1 mL of H_2O_2 30% added to each sample then incubated for another 2 hr at 95 ˚C. Ultra-pure water was added to complete the sample volume to 50 mL. These samples were then analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES: Varian Vista AX, Palo Alto, California, USA). The amounts of C, N, and P are expressed relative to the initial amounts of nutrients (%; Table 1). For the water samples, total dissolved nitrogen (TN) and total phosphorous (TP) were analysed using catalytic combustion with a Shimadzu VCPH (Kyoto, Japan), including 3 blanks of ultra-pure water, at the Institut National de la Recherche Scientifique Centre-Eau Terre Environment (INRS, Québec, Canada).

For the comparison of faecal loading rate and the subsequent effects on water chemistry at a landscape scale, we report previously unpublished chlorophyll-a values from a large-scale survey of lakes across Southampton Island (for sampling details see Mariash et. al. 2018; Table 3). Water samples from these lakes were filtered onto GF/F filters (in duplicates), frozen at -80˚C, and later analysed using a Cary Eclipse fluorescence spectrophotometer (Aglilent, Santa Clara, USA) using standardized extraction methods and calculations (Holm-Hansen and Riemann, 1978; Jeffrey et al., 1997).

2.4 Phytoplankton biovolumes and community composition

Phytoplankton biovolumes, volume of cells per volume of water, and community composition were measured from Lugol-preserved samples using Utermöhl settling chambers (Utermöhl, 1958), and an inverted phase contrast microscope (Zeiss Axio Observer.A, Germany). A minimum of 400 cells per sample were counted, using 400x magnification until 200 cells were counted and 100x magnification for the remaining 200 cells. This ensured that both larger and smaller cells were accounted for. A minimum of 10 fields were counted with each magnification. Biovolume estimates were based on geometrical models and cell measurements using photography and the AxioVision software (Zeiss, Germany) and converted using carbon to volume relationships (Menden-Deuer and Lessard, 2000). Phytoplankton taxonomy relied on the following

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literature: Cox, 1996; Hillebrand et al., 1999; John et al., 2002; Komárek and Anagnostidis, 2000; Guiry and Guiry, 2017; Taylor et al., 2007; and Wehr et al., 2015. Taxa were identified to genera when possible but later grouped by class for comparisons. One phytoplankton sample (150 ml. was taken for each sampling, Day 1, 3, 5, 10. Three samples had insufficient preservation and could not be quantified.

2.5 Data analysis

A General Linear Model was used to test for differences in the rate of nutrient loss from our organic matter treatments (goose droppings or *Carex* clippings. Time (days), time^2, treatment and their interactions were included as predictors, and nutrients (C, N, P, N:P ratio) measured in the organic matter were the response variables. A similar analysis was conducted for the corresponding changes to nutrient concentrations in the experimental water. To visualize results, we fit a quadratic function with a 95% confidence interval (CI) to the relationship between TP and TN concentrations in the water and time, throughout the experiment, using *ggplot2* (Wickham, 2009). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality (Zuur et al., 2010). P-values were obtained by likelihood ratio tests. Phytoplankton biodiversity was calculated using the Shannon-Wiener (alpha diversity) index. All analyses were conducted using R software (R version 3.1.1; (R Core Team, 2016) , and all means are reported ± SD unless otherwise noted.

3 Results

3.1 Nutrients released from submerged organic matter

The initial composition of the solid material showed that goose droppings had higher water content compared to *Carex*, a significantly higher percentage of nitrogen and phosphorous, and a significantly lower content of carbon and N:P ratio (Table 1, 2a,b,c). Once submerged, the carbon remained largely intact, showing a small but statistically significant loss of around 2% for goose droppings and 5% for *Carex* (Fig. 2a, Table 2a). Losses of nitrogen and phosphorus were much more rapid for the goose dropping treatment. After only 1 day, the goose droppings had released 48% of the original nitrogen and 43% of the original phosphorous, with no additional significant release of nutrients throughout the rest of the 17d experiment (Fig. 2b,c). In contrast, there was no net loss of nitrogen or phosphorus from *Carex* over the experimental period (Fig. 2b,c). The N:P ratio stayed between 8 – 10 for the goose dropping treatment, while the N:P ratio for *Carex* fluctuated between 11 – 14 (Fig 2d). Despite the rapid loss of nutrients from the goose droppings, phosphorous remained higher in the goose droppings compared to *Carex* at the end of the experiment (Fig. 2).

3.2 Nutrients released into the water

The nutrients released from the organic matter caused reciprocal changes in water chemistry during the experiment. The dissolved TN and TP in the water of the goose dropping treatment were orders of magnitude higher than the concentrations found in the water of the *Carex* treatment (Fig. 3). By the end of the experiment (Day 17), the water in the goose treatment

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contained 108 mg L^{-1} TN, while the water in the *Carex* treatment had 1.6 mg L^{-1} TN, compared with 0.1 mg L^{-1} in the pond water initially, or the 0.6 mg L^{-1} average TN from Southampton lakes (Mariash et al., 2018). Similarly, for TP, higher concentrations were found in the water of the goose treatment compared to the *Carex* treatment (16 mg L^{-1} TP compared to 0.2 mg L^{-1} TP, respectively). The initial pond water concentration was 0.002 mg L^{-1} TP, compared to an average TP concentration for local ponds of 0.01 mg L^{-1}. For total dissolved nitrogen, there was a significant main effect of treatment (*Carex* vs. droppings; Table 2d). There was also a significant interaction between treatment and time (and time^2; Table 2d, Fig. 3a), indicating a larger increase over time for the goose droppings treatment. Total dissolved phosphorus showed similar patterns, with a significant treatment effect, and significant time^2 and time^2*treatment effects (Table 2e, Fig. 3b).

3.3 Phytoplankton biovolumes and composition

Each treatment contained diverse phytoplankton communities and showed increased biovolumes over the course of the experiment. The initial phytoplankton biovolume was low at only 0.1 mm^3 L^{-1}. For both treatments, phytoplankton biovolumes increased during the experiment reaching 4.6 ± 0.2 mm^3 L^{-1} by Day 10 (Fig. 4). While the biovolumes were similar, the phytoplankton communities were different between treatments. In the *Carex* treatment, phytoplankton growth on Day 1 had risen to 1.2 mm^3 L^{-1}, which was mainly from the production of chlorophytes (0.64 mm^3 L^{-1}; 50 % of total biovolume), picoplankton (0.21 mm^3 L^{-1}; 16%), and bacillariophytes (0.16 mm^3 L^{-1}; 13 %, diatoms) (Fig. 4b). In the goose dropping treatment, phytoplankton biovolumes had doubled in the *Carex* treatment, to a total of 2.5 mm^3 L^{-1} on Day 3 (Fig. 4a). There was no rise in phytoplankton biovolume between Day 3 and Day 5, but biovolume doubled again between Day 5 to Day 10 to 4.6 mm^3 L^{-1}. Dominant phytoplankton in the goose dropping treatment were mainly picoplankton accounting for 2.3 mm^3 L^{-1} (92%), chlorophytes 0.1 mm^3 L^{-1}, and 0.06 mm^3 L^{-1} Chrysophyceae on Day 3, but rapidly changed to cyanobacteria (75%), on Day 5 (Fig. 4b). Cyanobacteria was no longer the main class but remained high on Day 10.

The goose treatment had less taxa than the *Carex* treatment, with only 10 taxa present, represented by 8 classes. The most abundant taxa were *Chlorella, Ochromonas, Aphanocapsa, and Gonyostomum*, the latter being attributed to nuisance algal blooms. For the *Carex* treatment, the phytoplankton community was diverse at Day 10, with 19 taxa, represented by 12 classes, but was clearly dominated by *Chlamydomonas,* a green algae (Chlorophyceae; 50%) on Day 10 (Fig. 4b). Cyanobacteria were not observed in the phytoplankton community of the *Carex* treatment.

These differences in phytoplankton communities among treatments were also confirmed with diversity indices. Initially, the pond water had a Shannon-Wiener index of 1.9 with 16 taxa present. This increased to 2.2 and 2.3 on Day 1 and 10, respectively for the *Carex* treatment. For the goose droppings treatment, the Shannon-Wiener index declined to 1.8 on Day 3 and 5, then returned to 1.9 on Day 10.

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4 DISCUSSION

Previous studies have suggested that geese can act as an important vector of nutrients (especially N and P) from terrestrial to aquatic systems, and this nutrient transfer might be especially important in otherwise oligotrophic Arctic ponds (Dessborn et al. 2016). However, studies from the Arctic are rare (Mariash et al. 2018, Mallory et al. 2006), and none to date have assessed the ecosystem response of goose-related eutrophication by linking the nutrient levels to phytoplankton growth and community responses. Our experimental field trials in wetlands on Southampton Island, Nunavut, provided evidence that submerged goose droppings leach a significant amount of nitrogen and phosphorous, immediately elevating the nutrient concentrations in the water. These leached nutrients were bioavailable, rapidly increasing phytoplankton biovolume and altering community composition. Both treatments showed diverse phytoplankton communities, however only in the goose dropping treatment did cyanobacteria become dominant. Collectively, these results demonstrate the direct ecological consequences of ornithogenic nutrient loading in Arctic freshwater ecosystems.

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4.1 Nutrients released from submerged organic matter into the water

Once submerged, goose droppings released approximately 45% of the nitrogen and phosphorus that they contained on the first day. In a previous study, Lui et al. (2014) demonstrated that most nutrients were released from goose droppings in the first 10 days, but they did not measure the nutrient concentrations in the water. Also, our mesocosm approach allowed for natural light, temperature, and some mixing, along with more comprehensive tracking of the nutrients released from the goose droppings into the water, and the resultant effects on primary producers. We showed that this rapid release of N and P from goose droppings resulted in a rapid increase in TN and TP concentrations in the water column. The nutrient concentrations in the water continued to increase until a peak at Day 10, then concentrations of TN and TP showed signs of decreasing on Day 17, when nutrients were presumably assimilated by phytoplankton.

Our mesocosm approach demonstrated that under natural light and temperature conditions, the release of nutrients from *Carex* and goose droppings, and the final dissolved nutrient concentrations in the water column, were quite different. While the *Carex* clippings themselves were relatively N-rich, measuring 18 mg N g^{-1} (nearly half of the concentration of N in goose droppings), there was no net change in nitrogen in the water column from *Carex* during our experiment. This was similar to the nutrient release dynamics found in Lui et al (2014), where the *Carex* at 10 °C had an immobilization phase within the first 5 days. In contrast, submerged goose droppings had a peak of nutrient release in the first day, while much of the remaining nutrients in the goose droppings were retained over the next 17 days. This high retention of the remaining P, approximately 50% over 17d, has also been observed in other experiments (Liu et al., 2014). These retained nutrients in residual organic matter can settle into the sediment (Unckless and Makarewicz, 2007), where they can build up and later be re-suspended by a strong wind event. Additionally, the differences in the fractions of labile and recalcitrant nutrients may play a crucial role in the degree to which the nutrients contained in droppings versus *Carex* are available to influence

productivity. Both a quick release of half the nutrients and sedimentation of the other half of the nutrients, in combination with high evaporation and low flushing rates, in Arctic wetlands may further concentrate the nutrients leading to late summer eutrophic conditions (Lewis et al., 2015; Mariash et al., 2018).

While the experiment was helpful to demonstrate the rate of nutrients leaching into the water, the concentrations of goose droppings used were higher than natural loading rates calculated for Southampton Island goose colonies. To demonstrate loading rates on a landscape scale, we compared natural loading rates and water chemistry changes from studies in goose colonies (Table 3). These studies reported a wide range of nutrient concentrations arising from natural droppings into the environment and experimental additions. The highest reported natural loading rates were found in the southern wintering grounds, with 9-15 kg N km^{-2} d^{-1} and 0.9-1.5 kg P km^{-2} d^{-1}. In comparison, Schindler et al. (2008) added on average 298 kg of N and 24 kg of P per year to a small boreal lake annually for the first 6 years of their classic whole-lake eutrophication experiment. Since the amounts of N and P additions varied from year to year, an average of nutrient additions for the first 6 years was used, and then divided by 365 to get a daily loading rate. The daily load per lake area of N and P additions from their whole-lake experiment was smaller than nutrient loads produced by goose colonies (Table 3). Nutrient loads from geese translated into increases in both TN and TP in the water along with high Chl-a (Table 3) for all studies compared. Clearly, after entering the waterbody, many factors affect nutrient dynamics in the water, including residence time, depth, stratification, and algae biovolume (Anderson et al., 2017). Nonetheless, despite the differences in climate and hydrology in these studies, dissolved TN and TP were highest where the nutrient loads were highest. Moreover, at the landscape level, these nutrient load changes related to geese were occurring at a much faster pace than water chemistry changes caused by climate variables (Mariash et al., 2018).

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4.2 Phytoplankton response

Chlorophyll-a (Chl-a) concentrations in shallow freshwaters averaged 1.9 µg L^{-1} across the circumpolar arctic (Rautio et al., 2011), concentrations in pristine ponds in southwestern Greenland were lower, at 0.5 µg L^{-1} (Mariash et al., 2014), while ponds on Southampton Island were slightly above that circumpolar average with 2.2 µg L^{-1} Chl-a. In more southerly temperate wetlands, with higher nutrient loads from geese, Chl-a is orders of magnitude higher, with concentrations between 27 - 800 µg L^{-1} (Kitchell et al., 1999). We had expected phytoplankton biovolumes to be higher in the goose dropping treatment vs. the *Carex* treatment; however, the phytoplankton biovolume was similar between the two treatments by the end of Day 10, showing that the input of nutrients derived from either treatment was enough for a phytoplankton response in these oligotrophic waters. Temperature may be another important factor limiting productivity; given enough nutrients, both light and temperature can restrict phytoplankton growth (Fanesi et al., 2016). Also, high concentrations of submerged *Carex* would occur primarily only along pond margins during periods of inundation, such as during the spring freshet, or when shoots are pulled by grazing geese to consume the starchy base of the leaves. Thus, nutrients released from *Carex* may be insignificant compared to goose droppings at a landscape scale.

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Community composition of the phytoplankton responded to increased nutrient availability in both treatments. Our experimental design was not without issues: more replicates would have made the results clearer, and the use of small containers has the potential to contribute technique-related artefacts (e.g., biofilm growth, altered physiochemical conditions, and species interactions due to container area-to-volume relationship; Liber et al., 2007), effects that we attempted to mitigate through the use of s short experimental duration. Despite these caveats, the responses in the phytoplankton communities were pronounced. Initially dominated by diatoms, the phytoplankton community of the *Carex* treatment changed to having a more diverse community with the dominant class being green algae. The phytoplankton community in the goose dropping treatment initially had only 10 taxa present represented by 8 classes, but by Day 5 the community was dominated (98%) by cyanobacteria (*Aphanocapsa* and *Pseudanabaena*) and chrysophytes (*Ochomonas*). A similar pattern in dominance of cyanobacteria and cryophytes is consistent with results in other nutrient-enrichment studies (Paerl et al., 2016; Przytulska et al., 2017). Cyanobacteria can outcompete other taxa when both nitrogen and phosphorous concentrations are high (Paerl et al., 2016; Schindler et al., 2008). High abundance of cyanobacteria will negatively affect species richness and diversity, as seen in the diminishing presence of other phytoplankton classes in our experiment.

N- limitation of algae growth can occur at TN:TP ratios < 20 (Findlay et al., 1994; Guildford and Hecky, 2000). In Schindler et al. (2008), the presence of cyanobacteria peaked within weeks after reducing the TN:TP from 12 to 4. Goose droppings in this study had an N:P ratio of 10, and when placed in water in our experimental treatment, they lowered the TN:TP ratio to 5.4, demonstrating goose droppings have the potential to significantly alter the ambient nutrient balance. The relatively phosphorous-rich goose droppings can cause nitrogen limitation in freshwaters (Mariash et al., 2018; Post et al., 1998; Schindler et al., 2008). This is an environmental concern from a water quality perspective, because when N is limiting, N_2-fixing cyanobacteria are competitively favoured (Guildford and Hecky, 2000; Schindler et al., 2008). The wetlands across Southampton Island have relatively low nitrogen concentration and TN:TP ratios of approximately 30 (Mariash et al. 2018), on par with other shallow Arctic freshwaters (Rautio et al., 2011), there is an indication that these wetlands are becoming more N-limited with decreasing TN:TP ratios (Mariash et al., 2018).

Geese are very inefficient herbivores, excreting approximately 60% of their ingested nutrients (Kitchell et al. 1999), and these nutrients are quickly released into the aquatic environment as demonstrated by the rapid release of nutrients from the organic matter in our goose dropping treatment. Once released these nutrients are bioavailable, altering water chemistry and the phytoplankton communities of the watershed. On Southampton Island, graminoids such as *Carex* spp. are the primary diet source for the geese, and our experiment demonstrates that only when passed through geese was the nitrogen and phosphorus bound in the *Carex* released into the water. Geese are therefore acting as biovectors on the landscape, consuming large amounts of terrestrial nutrients bound in vegetation and excreting these nutrients in form that is bioavailable for freshwater ecosystems. Goose faeces could also contribute to the dispersal of aquatic species, altering aquatic communities

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in this direct manner (Figuerola and Green, 2002). Tested phytoplankton species were not viable under cultured conditions once passed through waterbirds (Atkinson 1980), however tests of this mechanism have not yet been carried out for geese in the Arctic.

As geese are long distance migrants, and as many circumpolar Arctic goose populations have increased substantially (Fox and Leafloor, 2018), their movement and effects on the aquatic habitats have implications across Arctic North America and Europe, at locations where geese congregate in large numbers. Management strategies for hyper-abundant geese currently emphasize the need for maintaining the ecological integrity of terrestrial habitats. Our results demonstrate that the impacts of geese extend to freshwater Arctic ecosystems, and future management strategies should better acknowledge these aquatic impacts.

Data archive statement: Once accepted, the data will be made freely available on the Government of Canada's OpenData system.

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Tables and Figures

Table 1. Initial composition of *Carex* sp. leaves and goose droppings, and the pond water used in the experiment. *Percent water is calculated from wet weight versus dry weight, while the other parameters are calculated as a percentage of dry weight (DW). Solid material had 5 replicates, while water chemistry assays were run in duplicates.

	Carex clippings		Goose Droppings		Pond water	
	Mean		Mean		Mean	
Material	%	SD	%	SD	mg L^{-1}	SD
Percent						
Water*	71.7	0.0	87.9	0.0		
Carbon	46.6	0.4	43.6	0.3		
Nitrogen	1.8	0.4	4.0	0.3	0.13	0.0
Phosphorus	0.1	0.4	0.4	0.1	0.002	0.0

Table 2. Summary of results from General Linear Models comparing a) carbon b) nitrogen and c) phosphorus between the solid organic matter of *Carex* and goose dropping treatments and d) total dissolved nitrogen e) total dissolved phosphorous in the overlaying water. Significant values are in bold.

	Source	Degrees of Freedom	MS	F	p-value
a) Carbon	treatment	1	4543.1	26.4	**< 0.001**
	time	1	837.4	4.9	**0.03**
	time^2	1	0	0.01	0.99
	treatment:time	1	6	0.03	0.85
	treatment:time^2	1	240.3	1.3	0.244
	Residuals	54	173.8		
b) Nitrogen	intercept	1			
	treatment	1	548	20.58	**< 0.001**
	time	1	66.6	2.5	0.11
	time^2	1	234.14	8.7	**0.004**
	treatment:time	1	194	7.2	**0.008**
	treatment:time^2	1	270	10.1	**0.002**
	Residuals	54	26.62		
c) Phosphorus	treatment	1	30.1	85.9	**< 0.001**
	time	1	0.17	0.38	0.49
	time^2	1	1.6	4.7	**0.03**
	treatment:time	1	0.67	1.9	0.17
	treatment:time^2	1	3.9	11.2	**0.001**
	Residuals	54	0.35		
d) TN dissolved	intercept				0.953
	treatment	2	35553	107.3	**< 0.001**
	time	1	3572	21.6	**< 0.001**
	time^2	1	2162	13.1	**0.001**

	treatment:time	2	5749	17.4	**< 0.001**
	treatment:time^2	1	2185	13.2	**0.002**
	Residuals	20	3312		
e) TP dissolved	treatment	2	616.6	59.6	**< 0.001**
	time	1	49.7	4.8	**0.04**
	time^2	1	92.5	8.9	**0.007**
	treatment:time	2	39.5	3.8	**0.04**
	treatment:time^2	1	94.6	9.1	**0.007**
	Residuals	20	10.4		

Table 3. Comparison of the loading rates of nitrogen (N) and phosphorus (P) from geese and these nutrients in dissolved form (TN, TP) in the water along with the Chlorophyll-a (Chl.a) concentrations found in the waterbodies. Loading rates are in kg nutrients per the density of geese per km^2 at a given site per day.

		Nutrient Load			Water			
		N	P	N:P	TN	TP	TN:TP	Chl.a
		kg km^{-2}	kg km^{-2}			mg		
Location	Study	day^{-1}	day^{-1}		mg L^{-1}	L^{-1}		µg l-1
Rio Grande River, USA	Kitchell et. al. 1999	15.68	1.52	10.3	35.00	2.50	15.0	800.0
Middle Creek Reservoir, USA	Olson et. al. 2005	8.90	0.86	10.3	4.00	0.08	53.3	94.3
Southampton Island, Canada	Mariash et. al. 2018	4.46	0.50	8.9	0.45	0.02	30.1	2.2
Ontario, Canada	Schindler et. al. 2008	2.72	0.22	12.4	0.83	0.04	20.8	27.0

High Bird density loads are reported from Kitchell et. al. 1999. For Olson 2005, the average nutrient loads for inflow and outflow are reported. For Southampton Island (SHI), values are the average of the 26 shallow waterbodies surveyed in 2015 (see Mariash et. al. 2018 for survey details). Schindler et. al. 2008 values are the average nutrient addition from the first 6 years when TN and TP were added with a similar ratio to the ratio found in goose droppings (after year 6, only P was added).

Figure 1. Mesocosm set up in the pond, sample cups had either fresh *Carex* clippings or fresh goose droppings submerged in 200 mL of pond water. The organic matter and the water from experimental cups were sampled throughout the 17-day experiment.

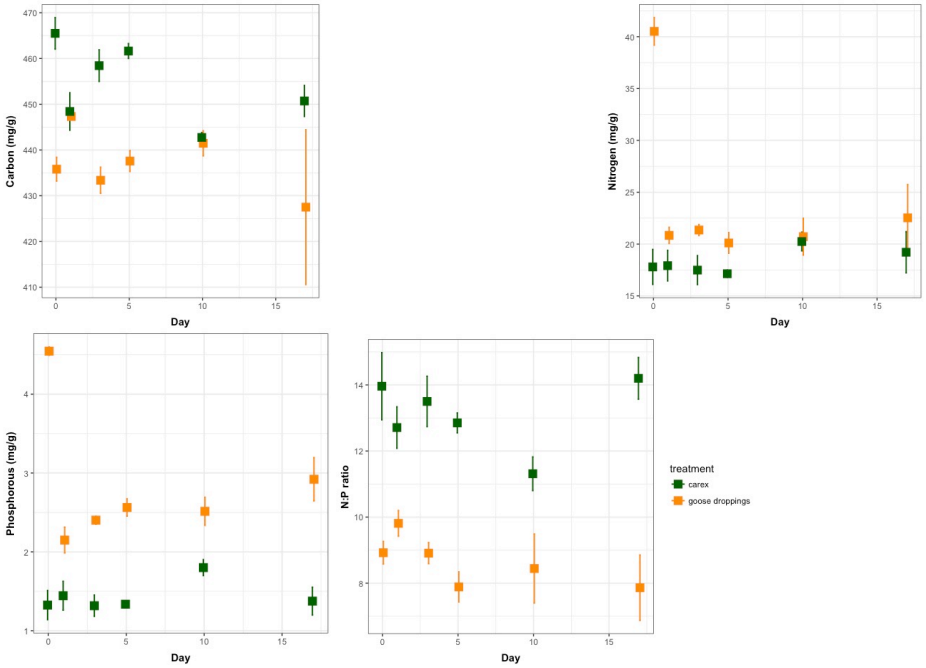

Fig 2. The decomposition of a) carbon, b) nitrogen, c) phosphorus and d) nitrogen:phosphorous ratio, from submerged organic material of *Carex* clippings and goose droppings, reported as mean ± SE across replicated (n=5) throughout the 17-day experiment.

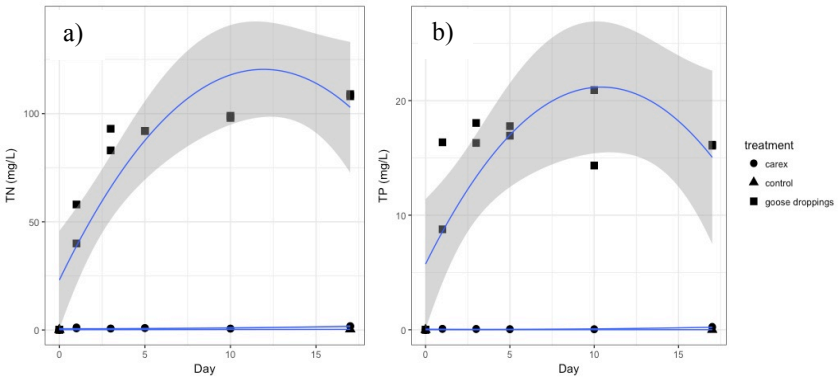

Figure 3. The cumulative nutrient concentrations released from goose droppings and *Carex* sp. into the water column a) total dissolved nitrogen (TN) and b) total dissolved phosphorus (TP) in mg L^{-1} over the 17-day mesocosm experiment. Dots represent individual samples, on each day duplicate samples were taken. Quadratic function used to fit data with shaded area representing the 95% confidence interval.

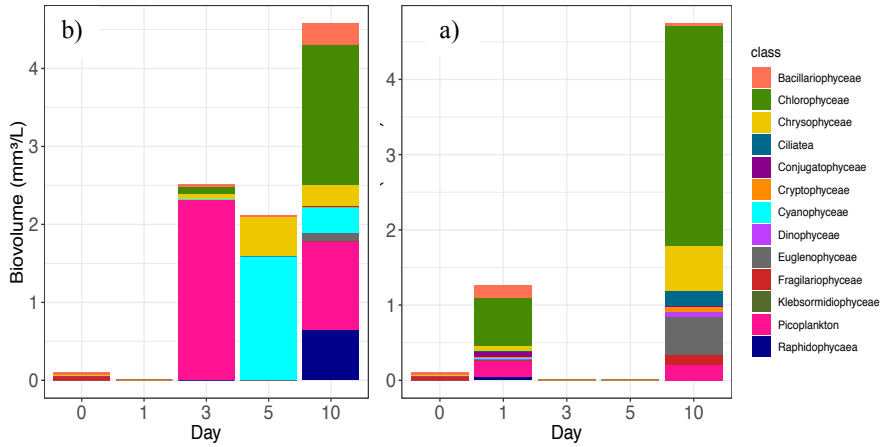

Figure 4. Change in phytoplankton community composition by biovolume (mm^3 L^{-1}) grouped by class, measured over the first 10 days of the mesocosm experiment that used either submerged a) goose droppings or b) *Carex* sp. to stimulate phytoplankton production in lake water. Missing values were due to incomplete preservation of those samples.