Permafrost thaw stimulates primary producers but has a moderate effect on primary consumers in subarctic ponds

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Abstract. Frozen tundra soils hold one of the Earth’s largest pools of organic carbon. Climate warming and the associated permafrost thaw release a large fraction of this carbon into circumpolar lakes, inducing extreme browning that fuels the heterotrophic microbial food web. How this permafrost carbon affects organisms higher in the food chain remains unknown. Using dissolved organic matter properties, total phosphorus, chlorophyll $a$, fatty acids, and stable isotopes, we investigated the influence of thawing permafrost on primary producers and primary consumers of the planktonic food web. We sampled four subarctic thaw ponds that were affected by permafrost carbon and another four ponds that were not. Our results highlight the stimulating influence of eroding and degrading ice-rich permafrost on nutrients and planktonic algae. Relative to the non-thaw ponds, the permafrost thaw-influenced freshwaters had higher total phosphorus concentrations (14.8 vs. 70.4 µg/L, respectively). This in turn led to a higher chlorophyll $a$ (2.7 vs. 45.2 µg/L) and seston omega-3 fatty acid concentrations (7.3 vs. 53.5 µg/L) despite significantly reduced light for primary production. Differences between the thaw and non-thaw ponds were less marked at the primary consumer level. Daphnia pulex, which dominated the crustacean zooplankton community, did not respond to the higher omega-3 availability in the thaw ponds but rather assimilated the high-quality fatty acids equally in all ponds, possibly because their metabolic needs were already saturated. However, some lower quality terrestrial carbon compounds from permafrost ended up in the D. pulex body mass, resulting in a median allochthony of 18% based on fatty acid mixing model; non-thaw ponds had median allochthony mixing model estimates of 8%. The high availability of algal resources seemed to prevent extensive zooplankton allochthony in subarctic thaw ponds.

Key words: allochthony; browning; Daphnia; fatty acid; food web; mixing model; permafrost thaw; stable isotope; terrestrial carbon; thaw ponds; thermokarst; zooplankton.

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INTRODUCTION

Permafrost is defined as a perennially frozen ground and represents a major constituent of the northern landscape. These frozen regions also hold one of the Earth’s largest pools of organic carbon (Schuur et al. 2015). Due to climate warming, these frozen soils are thawing at an accelerated rate. The erosion and collapse of permafrost-rich terrain is forming small and shallow freshwaters (Vonk et al. 2015), hereafter referred to as thaw ponds. Over the past decades, these ponds have become increasingly numerous at northern latitudes and now represent one of the most abundant types of freshwater systems in arctic and subarctic regions (Grosse et al. 2013). They act as recipients of the large amounts of carbon released and
transported to aquatic ecosystems from the thawing permafrost (Schuur et al. 2008). A recent study of the influence of degrading permafrost on dissolved organic matter (DOM) composition in northern freshwaters highlighted the stronger terrestrial imprint in thaw ponds, emphasizing a shift toward an increasing dominance of land-derived organic carbon (Wauthy et al. 2018). The increasing terrestrial influence in arctic and subarctic ponds is an example of browning, a global phenomenon that refers to an increase in DOM concentrations and a change in water optical properties, which affect particularly northern freshwaters (Creed et al. 2018, Wauthy et al. 2018). Transparent arctic ponds and lakes—characterized by high oxygen concentrations and benthic primary production (Rautio et al. 2011a) and a general net autotrophy (Bogard et al. 2019)—are being outnumbered by anoxic turbid ponds that receive increasing amounts of terrestrial organic material from the changing watershed. This shift has consequences for ecosystem metabolism (Deshpande et al. 2015, Vonk et al. 2015). The greater influx of terrestrial carbon and nutrients stimulates bacterial production and respiration (Roiha et al. 2016) and decreases light availability for pelagic (Karlsson et al. 2009) and benthic primary production (Forssström et al. 2013). As a result, an increased number of circumpolar freshwaters have become net heterotrophic systems (Roiha et al. 2015, Creed et al. 2018) as well as significant sources of carbon dioxide and methane emissions to the atmosphere (Walter et al. 2006).

These emissions of greenhouse gases from northern freshwaters have brought attention to the carbon cycle in tundra ecosystems and more precisely to heterotrophic bacteria that are responsible for the main turnover of terrigenous carbon in natural waters (Roiha et al. 2016, Wurzbacher et al. 2017). However, changes in allochthonous DOM are also expected to have a strong effect on internal lake processes and the pelagic food web, from phytoplankton at the base of the trophic chain to higher levels such as zooplankton. Given the absence of fish, zooplankton have a very high biomass in most arctic and subarctic freshwaters (Rautio and Vincent 2006). This high abundance of zooplankton is even increasing in the context of permafrost thaw (Bégin and Vincent 2017), and the influence of degrading watershed on primary consumers and higher trophic levels would depend on the quantity and quality of available food (Creed et al. 2018). Phytoplankton are expected to respond to a DOM-controlled decrease in light penetration (Williamson et al. 1996), a greater thermal stability (Houser 2006), and an increase in the total nutrient pool (Solomon et al. 2015). The current assumption is that phytoplankton would be stimulated in initially oligotrophic lakes due to the nutrient enrichment, but they would be affected adversely in dystrophic systems that receive further loading of terrestrial DOM (Kelly et al. 2018, Bergström and Karlsson 2019), shifting the phytoplankton community from autotrophic to mixotrophic/heterotrophic algae (Roiha et al. 2015) and concurrently reducing the total availability of algal-originating essential fatty acids (FA) in seston (Senar et al. 2019).

In this study, we explored the influence of thawing permafrost on the planktonic food web of subarctic ponds. We characterized the DOM, light absorbance, and nutrients available for primary producers in the context of permafrost thaw. We also estimated the effect of degrading permafrost on FA composition in the seston and zooplankton body mass. We then used stable isotopes (SI) as well as mixing models based on FA to estimate how permafrost thaw has affected zooplankton feeding. Given the higher terrestrial imprint of DOM in subarctic freshwaters exposed to degrading permafrost (Wauthy et al. 2018), we hypothesized that permafrost thaw induces an increase in DOM and nutrient concentrations as well as greater light absorption, thereby adversely affecting the phytoplankton community in these already dystrophic ponds. We further hypothesized that zooplankton in these thaw ponds assimilate a high proportion of terrestrial compounds into their biomass (i.e., more allochthonous in ponds subjected to permafrost thaw) and that their omega-3 FA concentrations are lower in ponds affected by permafrost degradation, leading to poorer nutritional condition. To remove the confounding effects of interspecies differences, we focused on Daphnia pulex (hereafter referred to as Daphnia), an abundant and cosmopolitan filter-feeding cladoceran in subarctic lakes (Rautio et al. 2011a, Bégin and Vincent 2017).
METHODS

Study sites

Fieldwork was carried out during August 2014 and 2015 in the vicinity of Kuujjuarapik-Whapmagoostui, Nunavik, Québec (55°17' N, 77°47' W). In this area, permafrost is sporadic and thawing at an accelerated rate. We selected eight small freshwaters distributed across two subarctic sites based on their exposure to thawing permafrost: (1) four waterbodies which are not influenced by degrading permafrost soils, hereafter referred as non-thaw ponds; and (2) four thermokarstic waterbodies directly in contact with thawing permafrost, hereafter referred as thaw ponds. Photographs of sampled ponds and sites are presented in Appendix S1: Fig. S1.

The non-thaw ponds are surrounded by grass-, shrub-, and bush-tundra vegetation that partly covers the rocky substrate watershed. These small (mean surface area 375 m$^2$) and shallow (mean depth 0.9 m) waterbodies are oligotrophic, unstratified, and transparent (Rautio et al. 2011a). Benthic algae represent more than 99% of the biomass and primary production in these freshwater ponds (Rautio et al. 2011a). In contrast, the thaw ponds—characterized by a small size (mean surface area 70 m$^2$), cylinder shaped with steep banks and flat bottoms with depth ranging from 2.4 to 2.9 m—displayed very different properties due to their exposure to degrading permafrost soils. Located in a peatland mainly colonized by Sphagnum and the semiaquatic macrophyte Carex (Arlen-Pouliot and Bhiry 2005), thaw ponds are strongly stratified, eutrophic waterbodies having very turbid water and no light reaching the anoxic bottom (Deshpande et al. 2015). No active benthic algae grow on the bottom of the dark and anoxic thaw ponds (<1% of incoming surface photosynthetically active radiation light left at 1.1 m depth, as measured with an underwater radiometer; LI-COR Biosciences, Lincoln, Nebraska). The strong stratification in thaw ponds was also believed to limit the exchange between the bottom and surface waters (Matveev et al. 2016). Thus, the benthic algae in thaw ponds were considered insignificant.

Sample collection

Water was collected at different depths using a 2-L transparent Plexiglas water sampler (Limnos, Turku, Finland) in order to get a representative sample of the entire water column. In addition, seston and Daphnia were collected using the same water sampler as for the integrated water column and a 50-µm plankton net (opening diameter of 25 cm, length of 50 cm), respectively. The samples were transferred to plastic bottles pending subsequent treatments in the laboratory. Laboratory analyses were performed within 3 h of sample collection. A subsample of zooplankton (three replicates per pond) was preserved in formaldehyde (4% final concentration) for later identification. In order to investigate and quantify the source contribution to Daphnia, we collected various sources, including pelagic and benthic algae, surrounding soils, macrophytes, and Sphagnum. Phytoplankton was collected as a seston sample and brought to the laboratory in a dark 4-L plastic bottle for algal FA filtration. We collected the upper horizon (0–5 cm) of soils along the pond edge. In the non-permafrost sites, these samples were a thin layer of organic matter on the rock substrate close to the ponds, whereas in the thermokarst system, samples were the surface soils from small mounds of organic-rich frozen soil that were thawing and collapsing into the ponds. Benthic algae were sampled by scraping the epibenthic material from the surface of submerged rocks in the non-thaw waterbodies. Decaying, submerged Sphagnum and macrophytes were collected from the immediate area surrounding the thaw ponds.

In the laboratory, integrated water was filtered through pre-rinsed cellulose acetate filters (0.2 µm) to analyze dissolved organic carbon concentrations (DOC) and perform optical analyses on chromophoric DOM (CDOM). For total phosphorus (TP) analyses, we also added 67 µL of H$_2$SO$_4$ 30% to 20 mL of unfiltered integrated water. The DOC, CDOM, and TP samples were stored in acid-washed glass vials at 4°C in the dark. We also filtered integrated water on GF/F filters to determine chlorophyll a (Chl a) concentrations. Live Daphnia were sorted and picked under binocular microscope at 12× magnification. Two replicates, each with approximately 100 individuals, were prepared for each pond. Seston samples were passed through a 50-µm sieve to remove zooplankton and then filtered onto pre-combusted and pre-weighted GF/F filters to collect the seston. Unless stated otherwise,
samples were stored at −80°C until freeze-drying and further analyses.

**Limnological analyses and zooplankton identification**

The quantification of DOC was carried out using an Aurora 1030W TOC Analyzer (OI Corporation, College Station, Texas, USA) in the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Ottawa, Ontario, Canada). CDOM concentrations were estimated as the absorption coefficient of DOM at 320 (a$_{320}$) and 440 nm (a$_{440}$) following Blough and Del Vecchio (2002). The concentration of TP was determined using persulfate digestion and ascorbic acid following the standard methods section 4500-P.E. (APHA 1998). Chl $a$ concentrations were calculated according to Nusch (1980). Daphnia community was identified and enumerated using the Utermöhl sedimentation method (Utermöhl 1958). Counts were made under an Axio Observer.A1 inverted microscope (Carl Zeiss, Oberkothen, Germany) at 100× magnification.

**Fatty acid analyses**

FA from *Daphnia* and their potential diet sources (seston, benthic algae, soils, macrophytes, and *Sphagnum*) were extracted as per Mariash et al. (2011) using a modified extraction method from Bligh and Dyer (1959). FA were transmethylated according to a protocol adapted from Lepage and Roy (1984) at our Fatty Acid Analytical Laboratory. First, a mix of methanol/toluene and acetyl chloride (4:1:0.125) was added to the samples with internal standard (nonadecanoic acid C19:0, available from Sigma-Aldrich, N5252, Saint-Louis, Missouri, USA). After centrifugation, the samples were incubated at 90°C for 20 min. Trans-esterified FA were extracted with hexane and were analyzed by gas chromatography–mass spectrometry (GC-MS) using a 7890A GC (Agilent, Santa Clara, California, USA) coupled to a 5975C MS with triple-axis detector (Agilent) and a J&W DB-23 column (Agilent). Identification and quantification were carried out using the calibration curves based on different concentrations of a standard mixture (37 FA components, Supelco 47885-0, and 2 additional bacterial FA, Sigma-Aldrich).

Both the production and the transfer of high-quality FA to zooplankton through the food web are good indicators of food quality. Lipids and FA are critical for the survival and fitness of all vertebrates and, quite probably, of almost all invertebrates as well (Napolitano 1999). Therefore, FA can be used as chemical proxies to investigate the health of an ecosystem or its organisms, such as zooplankton (Arts and Wainman 1999). Moreover, FA can also be used as trophic markers to study food webs (Napolitano 1999). The polyunsaturated fatty acids (PUFA) are particularly valuable for metazoans, playing an important role in cellular membrane structure and function. They are also precursors of bioactive compounds and are required for somatic growth, survival, and reproduction (Parrish 2009). A subcategory of PUFA, the omega-3, is essential for metazoans and must be included in their diet (Ahlgren et al. 2009). These omega-3 fatty acids are also typical for aquatic systems, most of them produced only by phytoplankton (Ahlgren et al. 2009). On the other hand, the saturated fatty acids (SAFA) are considered as low-quality FA that are of less interest for zooplankton, but they can have potentially severe impacts on zooplankton health when abundant in the diet.

Several FA proxies were calculated for *Daphnia* and seston: the amount of PUFA, omega-3, SAFA, and specific algal, terrestrial, and bacterial FA, respectively. In addition, the PUFA:SAFA ratio was used as an index to investigate FA quality (Napolitano 1999). The lists of identified FA and specific FA biomarkers are presented in Appendix S2: Table S1.

**Stable isotope analyses**

To investigate the relative carbon contribution of both pond types, we carried out SI analyses on *Daphnia* and its potential carbon sources. *Daphnia*, soils, *Sphagnum*, and macrophyte samples were analyzed for δ13C using a FLASH 2000 OEA interfaced with a Delta V Plus MS (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at the RIVE Research Center (Université du Québec à Trois-Rivières, Trois-Rivières, Quebec, Canada). Before the δ13C analyses, soil samples were acid fumigated for 96 h to remove carbonates, as described in Rammarine et al. (2011). To estimate the phytoplanktonic and benthic algal isotopic signature, we ran δ13C analyses on the specific algal FA 16:1n7, 18:2n6, 18:3n3, and 20:5n3 extracted from bulk seston and benthos.
(Taipale et al. 2015, Grosbois et al. 2017a, Grosbois et al. 2017b). The δ^{13}C analyses on FA were performed in the Stable Isotope Laboratory of Memorial University (Memorial University of Newfoundland, St. John’s, Newfoundland and Labrador, Canada), using a 6890N GC (Agilent) linked to a Delta V Plus MS (Thermo Fisher Scientific). In addition, δ^2H analyses were done on Daphnia in the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, Arizona, USA) following Doucett et al. (2007), using a CONFLO II coupled to a Delta Plus XL MS (Thermo Fisher Scientific).

**Mixing models**

We performed the Bayesian mixing model “FA Sources Tracking Algorithm in R” (FASTAR) based on the percentage of 14 FA (see list in Appendix S2: Table S1) in each sample, considering several potential sources (end-members) contributing to Daphnia following the method of Galloway et al. (2014). First, we calculated FA trophic modification as calibration coefficients. Similarly to SI where trophic fractionation is different for δ^{13}C and δ^{15}N isotopes and trophic levels, there occurs trophic modification for FA, which varies depending on the FA and source. These values were estimated using FA profiles available in Galloway et al. (2014), Lang et al. (2011), and this study. In brief, Galloway et al. (2014) reported FA profiles for different Daphnia populations that had each been fed with pure diets ranging from algae to terrestrial carbon, Lang et al. (2011) reported FA profiles for different algal diet cultures, and in this study, we measured terrestrial organic matter FA profiles. Using these data, the trophic modification was calculated as a ratio between a FA (%) in Daphnia and the source it had consumed in the feeding trial. We assumed the terrestrial carbon source in Galloway et al. (2014) and in our study is similarly trophically modified in Daphnia. We also assumed the FA trophic modification of macrophytes and Sphagnum to be similar to terrestrial carbon source due to the similarities these sources had with soils (lack or very low percentage of omega-3 FA, δ^{13}C values close to −27‰). Benthic source and its algae being made of single cells or colonies of cells were further assumed to be trophically modified as phytoplankton. The FASTAR assumes FA modification by consumer is accounted for; therefore, the source FA values were multiplied with the respective FA ratios, that is, calibration coefficients prior running the model (data available in Appendix S2: Table S2). In comparison with SI mixing models, the end-members in FASTAR are not source-specific values of certain individual FA but differences in the overall composition of 14 FA.

Due to the grouping of the diets in the feeding trials in Galloway et al. (2014), we also split the phytoplankton putative source into two groups that were made of Chlorophyceae and Cyanophyceae, and of Cryptophyceae. Further, due to the under-representation or absence of Chrysophyceae, dinoflagellates, and diatoms in the Lang et al. (2011) algal FA database, they were not included in the model. The putative sources in our model were therefore soils, a phytoplankton source that included Chlorophyceae and Cyanophyceae, another phytoplankton source that included Cryptophyceae, and benthic algae for non-thaw ponds. For the thaw systems, we targeted macrophytes and Sphagnum instead of benthic algae due to the different occurrences of these sources in thaw and non-thaw ponds (see Study Sites section). For each source group (end-member) included in the model, we calculated their FA means ± SD (in %) that were measured from our samples for benthos, soils, macrophytes, and Sphagnum, but taken from the literature (Lang et al. 2011) for phytoplankton. Based on the FA mixing model results, allochthony was estimated as the mean soil contribution (in %) to Daphnia. Mixing model was also considered for SI. However, the Daphnia isotopic signature based on δ^{13}C and δ^2H fell outside the polygon of potential sources in thaw systems (Appendix S3: Fig. S1), compromising the full results from the isotope mixing model. Therefore, the SI mixing model was not kept in this paper (more information available in Appendix S3). All mixing models were run in R v 3.5.1. (R Development Core Team 2018).

**Statistical analyses**

Limnological variables were analyzed by one-way analyses of variance (ANOVA) or Kruskal–Wallis rank tests when the assumptions of normality and homogeneity of variance were not fulfilled. Given the multiple sampling of ponds for zooplankton, FA and SI, Daphnia abundance, FA proxies, and δ^{13}C isotopic signatures were analyzed by nested ANOVA, preceded by rank
transformation and followed by pair-wise comparison using a post hoc test (Bonferroni) if needed. All FA profiles of *Daphnia* were combined and ordered according to pond type by non-metric multidimensional scaling (NMDS) with Wisconsin transformation and a Euclidean index. To test the influence of pond type on *Daphnia* SI and FA composition, we performed nested permutational multivariate analyses of variance (PERMANOVA) on isotopic tracers (δ^{13}C and δ^{2}H) and every identified FA (list available in Appendix S2: Table S1), respectively. The data were normalized (SI) or Wisconsin transformed (FA), using Euclidean distance as a dissimilarity index. The number of permutations was fixed at 999, and the multivariate homogeneity of group dispersions was verified by performing permutational analyses of multivariate dispersions (PERMDISP). To test the algal and terrestrial source contribution to *Daphnia* in non-thaw vs. thaw ponds, we carried out nested ANOVA on rank-transformed proportional FA mixing model results. Data were expressed as mean ± standard error (SE), and all statistical analyses were run in R v 3.5.1. (R Development Core Team 2018).

**RESULTS**

**Limnological properties and Daphnia abundance**

The limnological parameters measured for the integrated water column illustrated a strong effect of permafrost thaw (Table 1) with significant differences between pond types for all variables (*P* < 0.05, one-way ANOVA or Kruskal–Wallis by rank test). The amount of DOC varied between 4.6 and 48.3 mg/L with the highest values in thaw ponds. The CDOM concentrations followed the same trend, as indicated by the a_{250} and a_{440} values. TP and Chl a concentrations were also higher in thaw ponds, reflecting a more enriched trophic state. The abundance of *Daphnia* was significantly different between pond types (nested ANOVA after rank transformation, *F* = 21.3, *P* = 0.004), displaying higher values in thaw ponds compared to non-thaw ponds (mean 4.1 vs. 0.4 ind./L, respectively).

**Characteristics of seston and primary consumers**

While the FA proxies between non-thaw and thaw ponds differed markedly for seston, we observed less difference for *Daphnia* between pond types (Table 2). All FA proxies were significantly different in seston, except for terrestrial-specific FA and PUFA:SAFA ratio (*P* < 0.05, nested ANOVA after rank transformation). Thus, the amount of PUFA, omega-3, and algal- as well as bacterial-specific FA in seston were considerably higher in thaw ponds. Contrariwise, in *Daphnia*, only terrestrial-specific FA were significantly different and higher in non-thaw ponds (*P* < 0.001, nested ANOVA).

The isotopic signatures of *Daphnia* highlighted a strong difference (nested PERMANOVA, *F* = 11.24, *P* = 0.006) between the thaw and non-thaw ponds (Fig. 1a, Table 3). The δ^{13}C signatures of *Daphnia* were significantly more depleted (nested ANOVA, *F* = 3.20, *P* = 0.001) in thaw ponds (−34.50 ± 0.46_{o/oo}) than non-thaw ponds (−30.65 ± 0.45_{o/oo}). SI signatures of *Daphnia*, however, had similar δ^{2}H values for both pond types, ranging between −148.5_{o/oo} and −195.7_{o/oo}. Regarding phytoplankton, its isotopic signatures displayed a similar pattern, with significantly more depleted δ^{13}C of specific algal FA in thaw ponds (nested ANOVA after rank transformation, *F* = 95.81, *P* = 0.01) (Table 3). The NMDS ordination based on *Daphnia* FA composition also separated the two pond types (nested PERMANOVA, *F* = 7.13, *P* = 0.001) and suggests an association with the NMDS2 axis (Fig. 1b, Table 2).

**Source contributions to primary consumers**

Compared to the putative food sources, *Daphnia* δ^{13}C isotopic signatures were similar in both pond types, and not different from planktonic and benthic algae but more depleted compared to allochthonous sources, regardless of the influence of thawing permafrost (Fig. 2). Thus, in non-thaw ponds, the δ^{13}C values of *Daphnia* and algae oscillated between −29.34_{o/oo} and −32.29_{o/oo} and were significantly different from the terrestrial organic matter source (−24.90 ± 0.82_{o/oo}) (nested ANOVA after rank transformation, *F* = 6.54, *P* = 0.015). Also in thaw ponds, *Daphnia* and phytoplankton had similar δ^{13}C values (−35.29 ± 0.46_{o/oo} vs. −35.74 ± 1.26_{o/oo}, respectively), which were significantly different compared to the allochthonous sources (from −25.52 to −27.70_{o/oo}) (nested ANOVA after rank transformation, *F* = 48.34, *P* < 0.001).
Table 1. Integrated water column values (mean ± SE) and associated statistical test results of limnological properties in subarctic non-thaw (N = 4) and thaw (N = 4) ponds.

| Variables | Non-thaw ponds | Thaw ponds | Statistical test | F or χ² value | df | P       |
|-----------|----------------|------------|------------------|---------------|----|---------|
| DOC (mg/L) | 9.23 ± 1.79 | 31.42 ± 7.86 | Kruskal–Wallis | 5.33 | 1 | 0.021* |
| a_{320} (m⁻¹) | 29.07 ± 5.01 | 102.03 ± 18.29 | ANOVA | 14.81 | 1, 6 | 0.008** |
| a_{440} (m⁻¹) | 4.40 ± 0.73 | 17.04 ± 2.51 | ANOVA | 23.32 | 1, 6 | 0.003** |
| TP (µg/L) | 14.75 ± 6.45 | 70.35 ± 15.41 | ANOVA | 11.07 | 1, 6 | 0.016* |
| Chl a (µg/L) | 2.66 ± 1.05 | 45.21 ± 13.84 | Kruskal–Wallis | 5.33 | 1 | 0.021* |

Notes: a_{320}, absorption coefficient of dissolved organic matter at 320 nm; a_{440}, absorption coefficient of dissolved organic matter at 440 nm; Chl a, chlorophyll a concentration; DOC, dissolved organic carbon concentration; TP, total phosphorus concentration.

Significant differences are indicated by asterisks. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

Table 2. Values (mean ± SE; values in parentheses are sample sizes) and associated statistical test results of fatty acid composition in seston and *Daphnia pulex* from non-thaw and thaw ponds.

| Variables | Non-thaw ponds | Thaw ponds | Statistical test | F | df | P       |
|-----------|----------------|------------|------------------|---|----|---------|
| Seston PUFA (µg/L) | 16.69 ± 1.09 (24) | 77.55 ± 13.96 (36) | RT-nested ANOVA | 18.94 | 1, 6 | 0.005** |
| Seston omega-3 (µg/L) | 7.30 ± 0.67 (24) | 53.49 ± 12.27 (36) | RT-nested ANOVA | 12.04 | 1, 6 | 0.013* |
| Seston algal FA (µg/L) | 70.15 ± 2.24 (24) | 168.16 ± 18.65 (36) | RT-nested ANOVA | 23.15 | 1, 6 | <0.001*** |
| Seston terr FA (µg/L) | 7.00 ± 0.54 (24) | 5.78 ± 0.99 (36) | RT-nested ANOVA | 2.14 | 1, 6 | 0.194 |
| Seston bact FA (µg/L) | 8.12 ± 0.02 (24) | 28.85 ± 3.50 (36) | RT-nested ANOVA | 151.40 | 1, 6 | <0.001*** |
| Seston PUFA:SAFA | 0.16 ± 0.02 (24) | 0.21 ± 0.04 (36) | RT-nested ANOVA | 0.36 | 1, 6 | 0.571 |
| Daphnia PUFA (µg/mg DW) | 69.75 ± 7.54 (12) | 58.15 ± 5.47 (9) | Nested ANOVA | 0.70 | 1, 6 | 0.435 |
| Daphnia omega-3 (µg/mg DW) | 45.96 ± 6.75 (12) | 43.20 ± 4.14 (9) | Nested ANOVA | 0.05 | 1, 6 | 0.836 |
| Daphnia algal FA (µg/mg DW) | 78.08 ± 8.17 (12) | 64.41 ± 5.45 (9) | Nested ANOVA | 0.78 | 1, 6 | 0.413 |
| Daphnia terr FA (µg/mg DW) | 0.24 ± 0.02 (12) | 0.06 ± 0.01 (9) | Nested ANOVA | 15.89 | 1, 6 | <0.001*** |
| Daphnia bact FA (µg/mg DW) | 5.39 ± 0.78 (12) | 6.02 ± 1.05 (9) | RT-nested ANOVA | 0.16 | 1, 6 | 0.703 |
| Daphnia PUFA:SAFA | 1.01 ± 0.07 (12) | 1.07 ± 0.14 (9) | Nested ANOVA | 0.16 | 1, 6 | 0.699 |
| Daphnia FA profiles | NA (12) | NA (9) | Nested PERMANOVA | 7.13 | 1, 13 | 0.001*** |

Notes: algal FA, specific algal fatty acids; bact FA, specific bacterial fatty acids; DW, dry weight; FA profiles, complete fatty acid profiles (see list of fatty acids in Appendix S3: Table S1); PUFA, polyunsaturated fatty acids; PUFA:SAFA, ratio polyunsaturated:saturated fatty acids; RT, rank transformation; terr FA, specific terrestrial fatty acids.

Significant differences are indicated by asterisks. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

Fig. 1. *Daphnia pulex* grouping visualization according to pond types and based on their (a) δ¹³C and δ²H signatures and (b) non-metric multidimensional scaling (NMDS) carried out on all identified fatty acids.
The FA mixing models showed similar patterns for non-thaw and thaw ponds, the FA composition of *Daphnia* being mainly from phytoplankton, and more precisely from the algal planktonic group encompassing the Chlorophyceae and Cyanophyceae (Fig. 3). However, the phytoplankton source contribution to *Daphnia* was significantly higher in non-thaw vs. thaw ponds (means of 77.8 ± 0.7% vs. 64.9 ± 5.1%, nested ANOVA after rank transformation, $F_{1.6} = 6.96$, $P = 0.039$). In non-thaw ponds, the other sources had a minor contribution (means of 9.5 ± 0.6% and 7.7 ± 0.2% for benthic and terrestrial sources, respectively). In the thaw ponds, however, the terrestrial contribution to *Daphnia* was significantly higher than in non-thaw ponds (means of 21.5 ± 5.7% vs. 7.7 ± 0.2%, nested ANOVA after rank transformation, $F_{1.6} = 131.7$, $P < 0.001$), whereas macrophytes and *Sphagnum* were secondary contributors (means of 5.5 ± 0.1% and 2.1 ± 0.1%, respectively).

**Effect of permafrost thaw on food web**

Overall, permafrost thaw altered the physicochemical properties of water and the different levels of the food chain from those measured in non-thaw ponds (Fig. 4). Except for the lower
amount of omega-3 in Daphnia (although not statistically different; Table 2), all other variables were 13% to 1602% higher in the thaw ponds. While light absorption increased up to 287%, the availability of nutrients was also higher in thaw ponds (TP increased by 377%). This TP increases most likely more than compensated for the adverse effect of less light and explained the higher omega-3 content (633% increase) and Chl a concentration (1602% increase) in seston. Overall, while the percentage of increase in the context of degrading permafrost was high for the limnological properties and the primary producers, the changes in Daphnia were less marked (Fig. 4), within a range from −6% (Daphnia omega-3 concentration) to 177% (Daphnia allochtony).

**DISCUSSION**

Our results show clearly that permafrost thaw alters both the physicochemical environment and the biomass composition of the planktonic food web in subarctic ponds; however, specific environmental variables and food web compartments were regulated very differently by the increased quantities of terrestrial carbon. While the non-living environment and the pelagic organisms were both stimulated by permafrost thaw, within the food web, permafrost thaw had a greater effect on primary producers than primary consumers. Our results are in line with other studies, indicating that browning in subarctic ponds influences biogeochemistry (Wauthy et al. 2018), and further show that increasing nutrients and phytoplankton create eutrophic conditions that can be considered part of Arctic greening (Michelutti et al. 2005). Contrary to the boreal lakes, where brownification has been shown to substantially increase the allochtony of zooplankton (Wilkinson et al. 2013), our study indicated that permafrost thaw-caused browning only leads to a moderate allochtony. This was likely due to the large quantity of nutrients that were associated with permafrost thaw and hence the greater phytoplankton resources for zooplankton. We further showed that permafrost thaw...
does not only increase the phytoplankton biomass (as Chl \textit{a}) but also additionally stimulates rather than suppresses the production of high-quality omega-3 in the food web—within a mean DOC increase from 9 to 31 mg/L—a pattern opposite to our hypothesis.
Permafrost thaw impact on light, nutrients, and plankton biomass

Permafrost thaw induced important changes in the chemical properties of waters (Table 1, Fig. 4). The higher DOC and CDOM concentrations, supporting recent publications on DOM in the context of permafrost thaw (Vonk et al. 2015, Wauthy et al. 2018), strongly decreased light availability for primary producers. In the thaw ponds, only the uppermost meter remained sufficiently illuminated, and the majority of the water column received <1% of the incident PAR. Such high absorption is common in thaw ponds (Watanabe et al. 2011) and can shift primary production from benthic-dominated to pelagic-dominated production (Vadeboncoeur et al. 2008, Forström et al. 2013). In oligotrophic arctic ponds surrounded by rocky catchments, this shift often leads to an overall decrease in total primary production— and hence lower algal biomass— because nutrient-limited phytoplankton cannot attain the high production levels of benthic algae (Rautio and Vincent 2006). In our study sites, the non-thaw ponds were oligotrophic and mesotrophic freshwaters and comparable to other clear-water arctic ponds (Rautio et al. 2011a). In our studied thaw ponds, the situation was different. Phosphorus concentrations were more typical of mesotrophic and eutrophic systems, with phosphorus likely originating from the degrading watershed, as observed in recent studies (Larsen et al. 2017). Consequently, the Chl a concentrations were higher, suggesting a stimulated pelagic primary production from this greater nutrient influx (Vonk et al. 2015), whereas the lower TP concentrations in the non-thaw systems led to Chl a values similar to other circum-polar rock basins (Rautio et al. 2011a).

The abundance of *D. pulex* was different between pond types, displaying higher values in thaw ponds compared to non-thaw ponds (mean 4.1 vs. 0.4 ind./L, respectively). This higher abundance of cladoceran probably comes from the higher production in the water column of ponds affected by degrading permafrost, compared to the subarctic oligotrophic freshwaters characterized by low terrestrial inputs. Bégin and Vincent (2017) also emphasized the higher biomass of zooplankton in thaw than non-thaw freshwaters.

Increased supply of omega-3 fatty acids in thaw ponds

When terrestrial subsides of DOM increase, the food webs may shift from a reliance on autochthonous and autotrophic production to one based on heterotrophic production supported by terrestrial carbon (Rautio et al. 2011b, Mariash et al. 2018). In such conditions, a decline in the production (Senar et al. 2019) and transfer (Creed et al. 2018) of high-quality omega-3 is expected due to a reduction in phytoplankton biomass or dilution of the omega-3 by an increase in bacterial biomass. However, in the environmental setting of this study, Chl a, PUFA, omega-3, and specific algal FA had higher concentrations in the seston of the thaw ponds (Table 2, Fig. 4). Indeed, whereas previous studies illustrated a high abundance of bacterial biomass in thaw ponds (Roiha et al. 2015), our results here show that the phytoplankton are able to concurrently sustain a high biomass. This may result from the dominance of flagellated (i.e., motile) and mixotrophic species that are known to dominate in thaw ponds (L. Forssström, personal communication; Roiha et al. 2015) and are rich in high-quality FA. Some other studies have highlighted a low production of omega-3 in the context of browning, however, in these studies the browning was accompanied with a shift toward dominance of cyanobacteria low of essential PUFA (Senar et al. 2019). Mixotrophic organisms compensate for low light by phagotrophy on bacteria, and they can migrate daily to the deeper layers (Jones 1991) where higher nutrient concentrations occur below the thermocline (Roiha et al. 2015). The specific bacterial FA were also higher in thaw ponds (Table 2), corroborating previous studies highlighting the huge abundance of bacteria in thaw freshwaters (e.g., Roiha et al. 2015). However, it is important to notice that the bacterial FA concentration could have been underestimated; our FA extraction method did not allow for the identification of FA biomarkers specific to methanotrophic bacteria (e.g., C16:1n5t, C16:1n6c, C18:1n5t, C18:1n6c) that are known to compose an significant part of the bacterial community in thaw ponds (Crevecoeur et al. 2015).

The different phytoplankton SI values between the pond types revealed that the algal communities, or their metabolism, differed; this may also contribute to the differences in seston PUFA concentrations.
concentrations. The seston $\delta^{13}C$ signatures were more depleted in thaw ponds (Table 3, Fig. 2). One explanation could be the higher fractionation during carbon fixation by phytoplankton in an environment particularly rich in CO$_2$ (Hinga et al. 1994, Laurion et al. 2010). This may also result from the high concentration of methane in thaw ponds (Matveev et al. 2016) and its bioaccumulation in mixotrophic algae. Methane has a more negative $\delta^{13}C$ value due to fractionation during methanogenesis; it can be metabolized by methanotrophic bacteria and then enter the food web (Bastviken et al. 2003). The phytoplankton community in the thaw ponds of the region is mainly mixotrophic (Bégin and Vincent 2017) and can rely on these methanotrophic bacteria as an energy source, explaining the more depleted $\delta^{13}C$ signature of phytoplankton and hence Daphnia. Algal metabolism based on methanotrophic bacteria cannot, however, explain the higher PUFA, as bacteria lack these essential FA. We propose that the higher concentration of omega-3 in the thaw-pond seston is a combination of a high algal biomass, sustained by the high concentration of nutrients originating from the thawing permafrost, and a community composition dominated by motile mixotrophic algae that are able to access both nutrients and sufficient light.

**Fatty acid composition of Daphnia**

The FA composition of Daphnia appeared to be different between non-thaw and thaw ponds (Fig. 1b), but this difference is not shown in the FA proxies except for the specific terrestrial FA (Table 2). Considering the strong terrestrial imprint in thaw ponds (Wauthy et al. 2018), the higher amount of specific terrestrial FA in the non-thaw ponds for Daphnia was unexpected and is inconsistent with the results of the FA mixing models (Fig. 3). However, most limnological studies involving FA are carried out in boreal, temperate, or tropical regions having forested catchments. Consequently, FA considered as exclusively terrestrial (including C20:0, C22:0, C23:0 and C24:0) are mostly FA specific to trees (Taipale et al. 2015). Yet, the subarctic landscape is very different, and while the studied thermokarstic site is deprived of most vascular plants and colonized mainly by small herbaceous plants and mosses, the sampled non-thaw ponds are surrounded by some bush and shrub vegetation. Therefore, finding higher amount of FA specific to vascular plants in non-thaw ponds is not surprising, and using these FA as a proxy for terrestrial matter might not fit in the context of permafrost and arctic regions.

The amount of omega-3 in zooplankton was the same in the thaw and non-thaw ponds, despite the higher concentration of omega-3 in the seston of thaw ponds (Table 2, Fig. 4). This reduction in the transfer of omega-3 through the food chain could be explained by the dilution of these high-quality FA by the higher abundance of Daphnia in thaw vs. non-thaw ponds (4.1 vs. 0.4 ind./L, respectively), an increase in bacterial biomass, or a shift in algal community composition for the benefit of phytoplanktonic species of lower FA quality. While the latter has been suggested by Creed et al. (2018), the higher PUFA and omega-3 content of the seston in thaw ponds (Table 2) does not indicate a presence of a poorer quality algae. It may be that the phytoplankton production of PUFA in both pond types was sufficient to saturate the metabolic needs of Daphnia; therefore, the higher PUFA concentration in seston in thaw ponds was not displayed by Daphnia. This is supported by the higher amount of PUFA, omega-3, algal FA biomarkers, and PUFA:SAFA ratio in Daphnia compared to seston for both thaw and non-thaw ponds (Appendix S2: Fig. S1). This pattern emphasizes the ability of Daphnia to selectively accumulate and retain higher quality FA. The specific terrestrial FA also highlighted the selective assimilation, showing a reverse pattern with smaller amounts of these low-quality FA in Daphnia tissue than in seston.

**Allocation of permafrost carbon to Daphnia biomass**

Although the $\delta^{13}C$ signature (Fig. 2) and the PUFA content (Table 2) of Daphnia appeared not to be altered by exposure to permafrost carbon, the overall FA composition showed that some organic material from the permafrost was assimilated by Daphnia. We used FA-based mixing models to quantify the contribution of different organic material sources to Daphnia. An important assumption of these FA mixing models is that the differences in the FA content of Daphnia are driven by diet and the retention of lipids from this diet (Galloway et al. 2014). Other
factors, such as temperature (Masclaux et al. 2009), zooplankton growth, and egg production (Vargas et al. 2006, Schneider et al. 2017), can also directly influence consumer lipids. Our sampling, however, was carried out over two short time windows, and consequently, the effects of different life-history stages on Daphnia FA content are assumed to be negligible.

The FA mixing model outcomes indicated that allochthony, although significantly higher in thaw ponds, was lower than the 30–70% range known for temperate and boreal freshwaters affected by brownification (Cole et al. 2006, Solomon et al. 2011, Grosbois et al. 2017a). Allochthony has also been reported to be high in subarctic ponds of Kangerlussuaq, Greenland (Mariash et al. 2018); it was >75% for D. pulex in high DOC (39.4 ± 6.1 mg/L) but ≤25% in low DOC (10.9 ± 3.4 mg/L) ponds. The allochthony in these previous studies was based on SI, and method-specific differences need to be taken into account; for example, it is possible that the fingerprint of terrestrial FA is modified when processed through the DOM-microbial loop, which would underestimate the terrestrial contribution to Daphnia especially in thaw ponds dominated by terrestrial DOM (Wauthy et al. 2018). While we are currently unable to take into account any trophic upgrading impacts, we argue that the differences in allochthony were more importantly affected by nutrient conditions. The systems studied by Cole et al. (2006), Solomon et al. (2011), Grosbois et al. (2017a), and Mariash et al. (2018) were oligotrophic; for example, even in the high DOC Greenland ponds, the mean TP concentration was 14.1 µg/L. In our phosphorus-rich thaw ponds, nutrient availability and abundant algal resources seemed to prevent D. pulex from becoming highly allochthonous, as was not only suggested by the FA mixing model but also suggested by the actual FA concentrations in Daphnia (Table 2).

Our results were consistent with other FA studies (e.g., Galloway et al. 2014) and testified the importance of algae in the diet of Daphnia in both thaw and non-thaw ponds (Fig. 3). Moreover, the significant contribution of phytoplankton of high biochemical quality (Cryptophyceae), despite their relatively low abundance in these freshwaters (L. Forsström, personal communication), highlighted the ability of filter-feeding zooplankton to select higher quality food present in the water column. The FA-based models displayed similar patterns between non-thaw and thaw ponds; however, the FA emphasized the higher terrestrial imprint in these ponds surrounded by degrading permafrost. This is consistent with Wauthy et al. (2018) who highlighted the shift toward an increasing dominance of terrigenous organic carbon in freshwaters subjected to ongoing permafrost thaw. Nonetheless, compared to the DOM allochthony in Wauthy et al. (2018), Daphnia in this study showed less terrestrial influence, again suggesting their ability to select higher quality food items in the water column. The similar ratio PUFA:SAFA also implied no influence of degrading permafrost on the nutritional condition of filter-feeding Cladocera, Daphnia being able to grow and reproduce while relying on poor-quality diets as long as high-quality food was available (Taipale et al. 2012).

**Conclusions**

Thawing permafrost induces rapid changes in northern freshwaters, releasing stocks of organic carbon that end up in aquatic systems and lead to a decrease in water column transparency. This in turn affects biogeochemical processes and the balance of autotrophic and heterotrophic production (Wauthy et al. 2018) and will favor algal species in the plankton at the expense of benthic algae (Karlsson et al. 2009, Forsström et al. 2013). In addition to the massive influence of eroding and degrading permafrost on water chemistry, light attenuation, and algae at the base of the trophic chain (Fig. 4), our results highlight a moderate but significant influence of this extreme version of browning on higher levels of the food web. Although the contribution of terrestrial organic matter to the biomass of the filter-feeding Daphnia increased, our results show that phytoplankton remain the key resource that fuels the food web in arctic ponds brown by permafrost thaw, and emphasize the ability of zooplankton to select a higher quality diet even in sites where the high-quality phytoplankton are diluted by abundant bacterial and terrestrial organic matter. Future work is needed to determine whether Daphnia selectively forage on phytoplankton, or whether the high-quality
FA are retained preferentially in zooplankton tissue.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3099/full