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Gamma Radiation Induced Changes in the Biochemical Composition of Aquatic Primary Producers and Their Effect on Grazers

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Changes in the biochemical composition of primary producers can alter their food quality, influencing their consumers and further propagating through the food web. Gamma (ɣ) radiation is an environmentally important type of ionizing radiation as it can damage macromolecules such as DNA, proteins, and lipids due to its high frequency, short wavelength, and high energy photons. Here, we investigate whether short-term ɣ-radiation changes the biochemical composition of primary producers and if radiation-induced changes affect higher trophic levels. Two phytoplankton species were exposed to two doses of ɣ-radiation and compared to a control. The metabolic profile and total protein content of the algae were measured at five time points within 24 h. Additionally, we measured carbon incorporation rates of *Daphnia magna* fed with the exposed algae. Gamma radiation had a significant effect on phytoplankton biochemical composition, although these effects were species-specific. The changes in phytoplankton biochemical composition indicate that ɣ-radiation induced the production of reactive oxygen species (ROS). *D. magna* incorporated more carbon when fed with algae previously exposed to ɣ-radiation; this could be due to radiation-induced changes in nutritional quality, algal anti-grazing defenses, or chemical feeding stimuli.

Keywords: untargeted metabolite profiling, effects of ionizing radiation, phytoplankton, food quality, biochemical changes

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INTRODUCTION

The biochemical composition, or food quality, of primary producers can affect the productivity of aquatic food webs (Sterner and Hessen, 1994; Vrede et al., 2004; Malzahn et al., 2010). One of the challenges in aquatic ecology is to predict how changes in primary production propagate up the food chain. Food quality can be defined as all attributes of the food that influence edibility as well as nutritional quality (Dickman et al., 2008; Sommer et al., 2012), including the elemental composition, biochemical make-up, particle size, and morphological characteristics of the food item (Sommer et al., 2012). As primary producers synthesize many biochemical compounds, such as essential fatty and amino acids that cannot be synthesized by primary consumers like crustaceous zooplankton, the food quality of primary producers has been shown to be important for reproduction and growth in copepods and cladocerans (Elser and Hassett, 1994).

Exposure to contaminants has been shown to change the biochemical composition of microalgae. In the presence of pesticides, the fatty acid (FA) profile of various microalgae changed

to one with fewer polyunsaturated FA (PUFAs), especially eicosapentaenoic acid (EPA) and linoleic acid, but more monounsaturated FA (MUFAs), particularly palmitoleic acid and oleic acid (Filimonova et al., 2016). Exposure to heavy metals resulted in significant biochemical changes in phytoplankton, with a decrease in PUFAs and an increase in saturated fatty acids (SFAs) and MUFAs (Rocchetta et al., 2006; Filimonova et al., 2016). Higher concentrations of PUFAs in phytoplankton have been linked to higher somatic growth and higher fecundity in crustaceous zooplankton (Ahlgren et al., 1990; Lang et al., 2011). Other macromolecules also change when phytoplankton species are exposed to certain contaminants. For instance, exposure to copper and salicylic acid led to the amino acid composition of *Scenedesmus quadricauda* being altered to include a higher concentration of arginine, histidine, methionine, and proline (Kovacik et al., 2010).

Ecosystems are continuously exposed to ionizing radiation from cosmic rays and naturally occurring radioactive isotopes, which are found in many natural resources, such as igneous rocks and ores (IAEA, 2003). While naturally occurring radionuclides can pose a potential radiological threat locally, the risk of environmental contamination from anthropogenic activities has spread globally due to natural resource exploitation, nuclear weapon production and testing, as well as nuclear power plant operations and accidents (Hu et al., 2010). Gamma (ɣ) radiation is an environmentally important type of ionizing radiation. Due to its high frequency, short wavelength, and high-energy photons it can interact with biological matter and cause ionization and excitation of macromolecules (Vanhoudt et al., 2014). Gamma radiation has been shown to cause DNA damage and induce detrimental effects on growth, reproduction, and morphology (Dallas et al., 2012; Jan et al., 2012; Parisot et al., 2015). These effects can be caused by either direct effects of ɣ-radiation on biomolecules, such as single or double strand DNA breaks, or indirect effects, for example through the production of radiolytic water products, including reactive oxygen species (ROS) (Spitz et al., 2004).

The production of ROS is the most likely effect of ɣ-radiation in organisms, since cells and organisms as a whole consist primarily of water (c. 90%) (Spitz et al., 2004). When the concentration of ROS exceeds the capacity of cellular antioxidants to maintain normal steady state redox potential, the cell is under oxidative stress which, if prolonged, can lead to damaged biomolecules, mutagenesis, and ultimately cell death (Mallick and Mohn, 2000; Choo et al., 2004; Spitz et al., 2004). When a cell is under oxidative stress, the antioxidant defense from ROS scavenging is not effective enough and ROS can readily attack PUFAs in the cell membrane (Mylonas and Kouretas, 1999). This in turn initiates a self-propagating chain reaction that can potentially damage cells or tissues, even if only a few lipids in the cell membrane were oxidized in the first place (Mylonas and Kouretas, 1999). Lipid peroxides can directly induce physiological changes in an organism by collapsing the membrane structure, which reduces membrane fluidity and ion transport (Won et al., 2014a). At the (primary) producer-consumer interface, oxidation of PUFAs can affect the food quality of the primary producer and may therefore indirectly affect the consumer.

While pesticides and heavy metals have been shown to change the biochemical composition of phytoplankton, to our knowledge such effects have not yet been demonstrated as a response to ɣ-radiation exposure. Nascimento and Bradshaw (2016) showed that *Daphnia magna* fed with the green algae *Raphidocelis subcapitata* that had been exposed to varying doses of ɣ-radiation incorporated more carbon than *D. magna* fed with algae that had not been exposed to ɣ-radiation. While this could indicate a change in the food's biochemical composition, no differences in FA composition at the highest exposure (100 Gy) were observed (Nascimento and Bradshaw, 2016). Here, we investigate whether external, short-term ɣ-radiation changes the biochemical composition of two primary producers over time and if radiation-induced changes in the primary producers can propagate to or affect the next trophic level. We exposed two species of phytoplankton—one chlorophyte (*R. subcapitata*) and one eustigmatophyte (*Eustigmatos magnus*)—to two doses of external, short-term ɣ-radiation (5 Gy, 25 Gy). We then screened for changes in the metabolic profile and biochemical parameters (total protein content) and compared these to an unexposed control. Based on the phytoplankton exposure and changes in biochemical composition, we chose one time point and ɣ-radiation exposure and fed the exposed and control algae to a primary consumer (*D. magna*) and measured carbon incorporation in both the primary producers, as a proxy for primary production, and in the consumers, as a measure for grazing rates.

METHODS

Culturing Conditions

The chlorophyte *R. subcapitata* and the eustigmatophyte *E. magnus* (SAG 36.89) were cultured in artificial freshwater enriched with modified Woods Hole (MWC) medium (Guillard and Lorenzen, 1972) in a climate-controlled chamber set at 20°C and 16:8 h light: dark cycle. Two weeks prior to the experiment, batch cultures were set up from the same monoclonal stock culture in triplicates per species and treatment. To keep the cultures in exponential growth, fresh media was supplied every other day. These species were chosen due to their different food qualities for zooplankton species. Eustigmatophyceae, such as *E. magnus*, are known to be rich in PUFAs and are therefore regarded as high-quality food for zooplankton, while Chlorophyceae, such as *R. subcapitata*, generally lack PUFAs and are therefore regarded as poorer quality food (Lang et al., 2011). Additionally, *R. subcapitata* serves as a model species in many ecotoxicology studies and has been used in effect studies of ɣ-radiation and effects of radionuclide exposure (Neves et al., 2015; Nascimento and Bradshaw, 2016; Nascimento et al., 2016); (Gomes et al., 2018).

Gamma Radiation Exposure

Raphidocelis subcapitata and *E. magnus* cultures were exposed to short-term ɣ-radiation using a 6.22 Gy/min ^{137}Cesium source (Gammacell 1,000). Due to the size of the exposure chamber,

the cultures were pooled, well-mixed, and then divided into 6 * 500 mL bottles, duplicates of which were exposed to 5 Gy (for 48 s) and 25 Gy (for 4 min), resulting in total doses of 5.0 and 24.9 Gy. The two controls were handled in the same way as the exposed samples and were placed next to the ɣ-radiation wrapped in aluminum foil to simulate the darkness during exposure. These doses were chosen in the lower range at which effects on *R. subcapitata* have been seen in previous studies by this group (e.g., Nascimento and Bradshaw, 2016). Since no previous data exists for the radio-sensitivity of *E. magnus*, we used these as fixed dose rates for both algal species. After the exposure, the cultures from each treatment were again pooled and then split into three replicates per dose and time point. Each replicate contained 200 mL of the respective algae culture and 50 mL of fresh modified MWC medium. Destructive samples were taken at five time points (3, 6, 9, 12, and 24 h) after exposure, leading to a total of 45 experimental units per species. At each time point, the 3 replicates per treatment were divided into 5 * 50 mL samples, which were then stored in −80°C until further analyses.

Metabolic Profiling

Sample preparation, derivatization, and gas chromatography–mass spectrometry (GC-MS) analyses were performed in accordance to Gullberg et al. (2004). In short, 100 µl of extraction buffer (20/20/60 v/v chloroform/water/methanol) including internal standards were added to 1–7 mg of freeze-dried algae. The samples were then shaken in a mixer mill at 30 Hz for 3 min with tungsten beads, and afterwards the samples were centrifuged at +4°C, 14 000 rpm, for 10 min. Subsamples of 200 µL of the supernatant were transferred to a micro vial and solvents were evaporated. For the derivatization of the samples 30 µL of methoxyamine were added to the dried samples, which were then shaken for 10 min and left to react at room temperature. After 16 h, 30 µL of MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) were added and left to react for another hour. Before the analysis, 30 µL of methyl stearate were added and 1 µL of the derivatized samples was injected into splitless mode by a CTC Combi Pal autosampler (CTC Analytics AG, Switzerland) into an Agilent 6,890 gas chromatograph. For more details of the GC-MS analysis see **Supplementary Material** and Gullberg et al. (2004).

For the GC-MS data, all non-processed MS-files from the metabolic analysis were exported from the ChromaTOF software in NetCDF format to MATLAB® R2016a (Mathworks, Natick, MA, USA), where all data pre-treatment procedures, such as base-line correction, chromatogram alignment, data compression, and multivariate curve resolution were performed using custom scripts. The extracted mass spectra were identified by comparisons of their retention index and mass spectra with libraries of retention time indices and mass spectra (Strelkov et al., 2005). Mass spectra and retention index comparisons were performed using NIST MS 2.0 software. Annotation of mass spectra were based on reverse and forward searches in the library. Masses and ratio between masses indicative for a derivatized metabolite were especially notified. If the mass spectrum was with highest probability indicative of a metabolite and the retention index between the sample and library for the suggested metabolite was ± 5 (usually < 3), the deconvoluted "peak" was annotated as an identification of a metabolite in accordance with the Swedish Metabolomics Center standard practice.

The data were then standardized to the original dry weight and the volume of the subsample analyzed to obtain a relative concentration of each metabolite.

Protein Content

Phytoplankton samples were analyzed for total protein content according to the procedure of Spáčil et al. (2010) and Annadotter et al. (2015) with minor modifications. The samples were dissolved in 80% methanol (80/20 methanol/water, v/v). Cell lysis was achieved by sonication for three 45-s cycles at 70% intensity (Sonopuls, Model HD 20170; Bandelin Electronic, Berlin, Germany). To avoid protein degradation, the samples were kept on ice and allowed to cool between each cycle. Protein concentrations were determined using the Bio-Rad RC/DC kit (Bio-Rad, Sundbyberg, Sweden). The spectrophotometric measurements were performed at 750 nm in mQuant Monochromatic Microplate Spectrophotometer at room temperature (BioTek, Winooski, VT, USA). The total protein content was then calculated using a standard dilution series and standardized to sample dry weight.

Isotopic Labeling and Carbon Incorporation

^{14}C-incorporation (from ^{14}C-labeled phytoplankton to *D. magna*) was used as a short-term measure of food assimilation. Twenty-four hours was chosen as the grazing period since it is long enough to obtain measureable C-assimilation values without risking substantial changes to the food quality of the algae cells during the grazing period or loss of ^{14}C through respiration.

The cladoceran *D. magna* was cultured in COMBO media and fed with radiolabeled *R. subcapitata* or *E. magnus*. Based on the results from the microalgae exposure experiment, we exposed *R. subcapitata* and *E. magnus* to 25 Gy external ɣ-radiation and allowed these and a non-exposed control culture to grow for 24 h in triplicates.

The two algae species were incubated with $NaH^{14}CO_3$-spiked (1.48 MBq) MWC medium for 7 days. After the incubation period, half of the *R. subcapitata* and *E. magnus* were exposed to the ɣ-radiation treatment while the other half was kept as an non-irradiated control. After 24 h cells from each treatment and species were harvested by centrifugation at 10,000 g for 5 min. After centrifuging the samples, the supernatant was discarded and the microalgae were rinsed until the supernatant was 0.01% of the initial activity added (Nascimento and Karlson, 2008). The algae pellets were then frozen at −20°C.

Before the experiment, samples of each of the concentrated *R. subcapitata* and *E. magnus* suspensions were thawed and observed under a microscope to confirm cell integrity. To quantify the ^{14}C concentrations in the phytoplankton, 500 µl samples were taken, preserved in 1 mL of 70% ethanol, and solubilized in 1 mL Soluene-350 for 2 weeks in the dark at room temperature. Three-day old *D. magna* neonates, which were reared on the corresponding algae species, were transferred into experimental units, which consisted of 50 mL centrifuge tubes. Each treatment was set up as triplicates per exposure and algae species, with 40 mL fresh COMBO media and 15 *D.*

magna individuals per unit. To ensure that food was not limited, *D. magna* were fed *R. subcapitata* or *E. magnus* at a ratio of ∼0.1 mg C/daphniid and were allowed to graze for 24 h. After 24 h, *D. magna* were transferred to 5 mL of clean COMBO media to clear their guts and ensure that the ^{14}C detected was the ^{14}C incorporated into the tissues and not ^{14}C from undigested microalgae in the gut. After 30 min, they were picked out and preserved in 70% ethanol.

The preserved daphniids from each replicate were then pooled and solubilised in 1 mL Soluene-350 for 2 weeks in the dark at room temperature. After the addition of 10 mL Ultimate Gold XR to each scintillation vail, the radioactivity in the samples *D. magna*, *R. subcapitata,* and *E. magnus* was measured in a liquid scintillation counter (Tri Carb, 2,910 TR, Perkin Elmer). The scintillation counts (Bq) were converted to μg ^{14}C by using the specific activity of ^{14}C (1 Bq equals 6.1^{-06} μg ^{14}C) and standardized to the number of phytoplankton cells or daphniids in the sample.

Statistical Analyses

The metabolic profile dataset was analyzed using constrained analysis of principal coordinates (CAP) based on Bray-Curtis dissimilarity. First, the overall multivariate metabolite data for both species was constrained by species, ɤ-radiation dose and time after exposure. An interaction between the terms was also included to test potential differences in the effects between species, time after exposure and doses. The minimal adequate model (containing only significant parameters) was identified, but due to the nestedness of the factors, non-significant factors (either time or dose) were included in the model as a condition to partial out their effects. Then separate CAP analyses were performed per species to discern interactive effects. Exposure to ɤ-radiation and time after exposure were used to constrain the multivariate metabolite data for each species. The analyses were performed using the "capscale" function from the {vegan} R package (Oksanen et al., 2016). Significance of the models, terms, and axes were assessed using a permutation procedure with 999 permutations. A similarity percentage test (SIMPER) was used to identify which metabolites drove the metabolic profile effects.

The effects of time and exposure on protein content of the algae, as well as on carbon incorporation and grazing rates of *D. magna*, were analyzed using two-way analysis of variance (ANOVA) followed by a Tukey's *post-hoc* test. Interactive effects between time and exposure were also tested and, if significant, are noted in the results section. Prior to analyses, the homogeneity of variance and normality of each variable were tested, and data transformations were applied when necessary.

All statistical analyses were performed in R version 3.3.3 (R Core Team, 2018). The significance levels were set to $\alpha = 0.05$.

RESULTS

Metabolic Composition

The metabolic profiles of the two phytoplankton species were significantly different from each other [$F_{(1,80)} = 1206.8$, $p = 0.001$] and were also significantly influenced by dose [$F_{(2,80)} = 3.42$, $p = 0.03$], and the interaction between species and dose was also significant [$F_{(2,80)} = 3.32$, $p = 0.03$; **Figure S1**]. To discern the interactive effect of species and dose the effect of time and dose was investigated

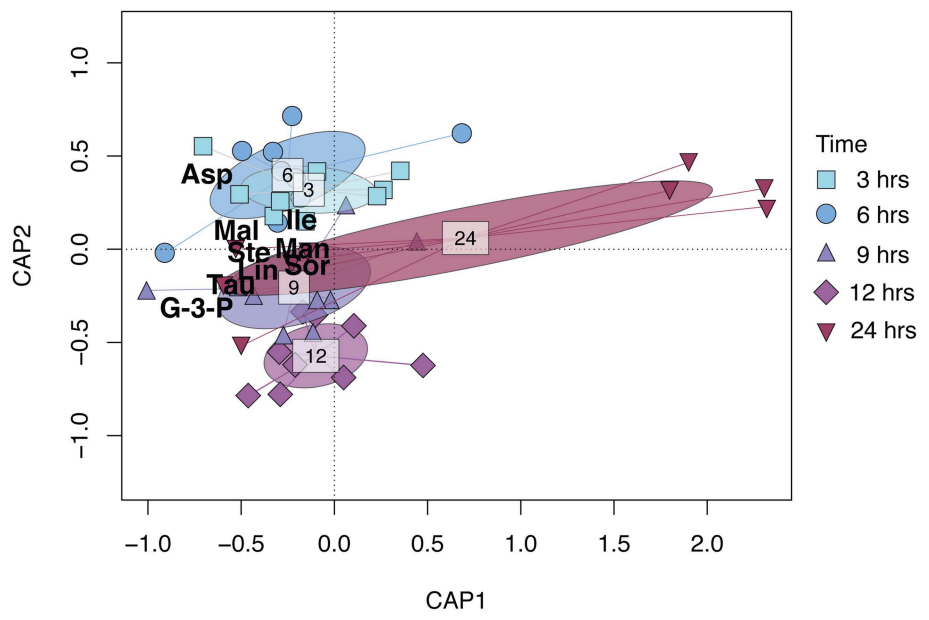

FIGURE 1 | CAP (Constrained Analysis of Principal coordinates) biplot based on the relative metabolite concentration matrix of *R. subcapitata* constrained by time after ɤ-radiation exposure. Points represent individual samples and are shaped and colored by time after exposure (see figure legend). The ellipsoids represent the standard deviation for each time after exposure. The metabolites shown in the figure—Asp (aspartic acid), Tau (taurine), G-3-P (glycerol-3-phosphate), Mal (malic acid), Lin (linoleic acid), Ste (stearic acid), Man (mannitol), Sor (sorbitol), and Ile (isoleucine)—contributed 80% to the dissimilarity as identified in the SIMPER analysis (**Table S1**).

on each species separately. Six metabolites contributed to 80% of the dissimilarity between the species (**Table S1**). Sorbitol, mannitol, and inositol (*scyllo*) characterized the metabolic profile of *E. magnus*, while Glycerol-3-phosphate, taurine and aspartic acid characterized the metabolic profile of *R. subcapitata*.

The metabolic composition of *R. subcapitata* was influenced by time after exposure [$F_{(4,38)} = 2.37$, $p < 0.05$; **Figure 1**], but not by dose. Glycerol-3-phosphate and taurine characterized the metabolic profile in *R. subcapitata* at 9, 12, and 24 h after exposure, while aspartic acid and isoleucine were more characteristic of the metabolic profile at 3 and 6 h after exposure (**Figure 1**). A total of 9 metabolites contributed to 80% of the dissimilarity between time points: three amino acids, aspartic acid, taurine and isoleucine; two fatty acids, linoleic acid and stearic acid; one lipid, glycerol-3-phosphate; two alcohol sugars, mannitol and sorbitol; and an organic acid, malic acid (**Figure 2** and **Table S2**). Even though there was no significant effect of

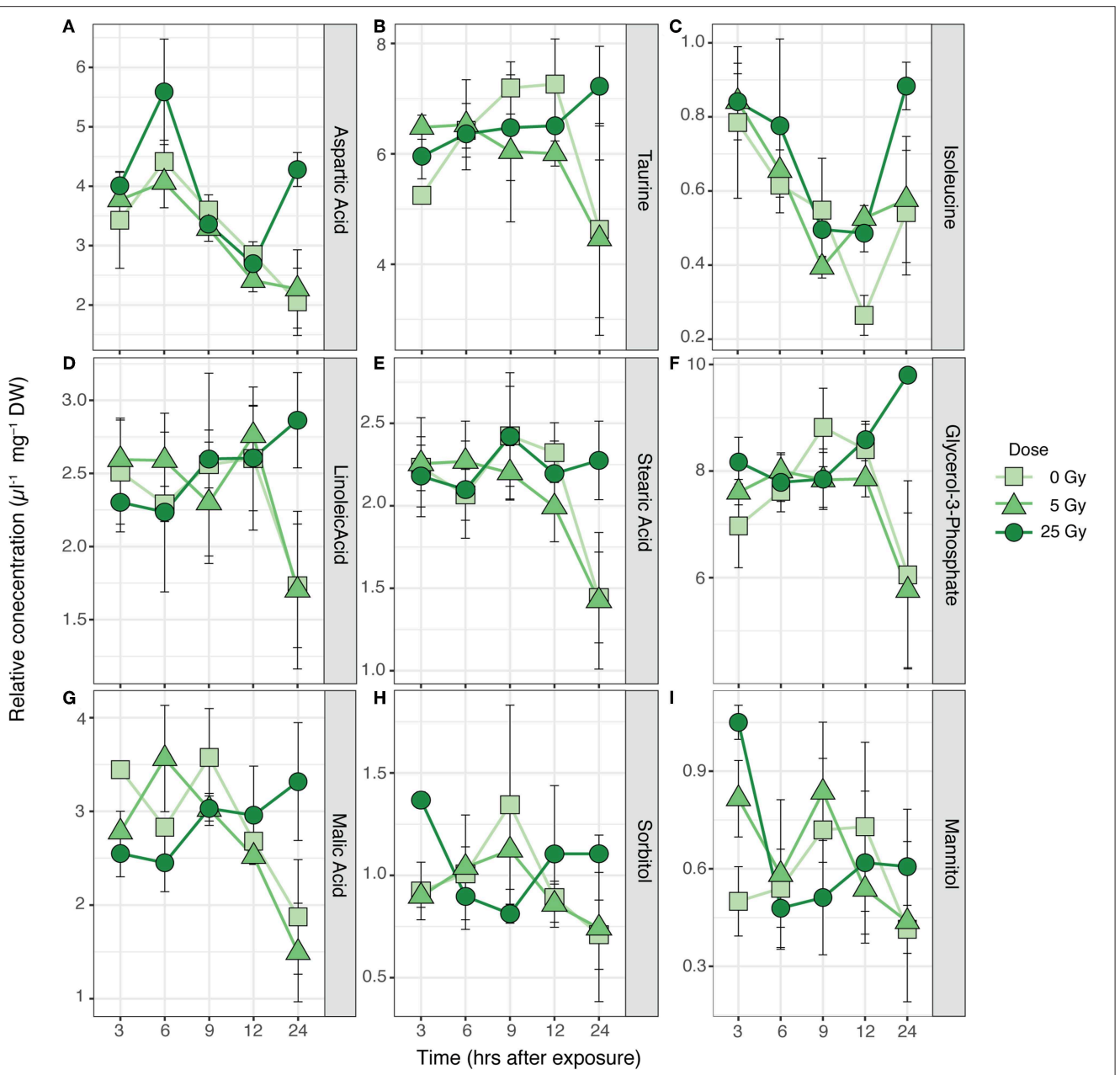

FIGURE 2 | Time series of average relative concentrations (± SE) of the metabolites identified by the SIMPER analysis as contributing 80% to the cumulative dissimilarity between times after exposure (**Table S1**) in *R. subcapitata* ($n = 3$). **(A)** Aspartic acid, **(B)** taurine, **(C)** isoleucine, **(D)** linoleic acid, **(E)** stearic acid, **(F)** glycerol-3-phosphate, **(G)** malic acid, **(H)** sorbitol, and **(I)** mannitol.

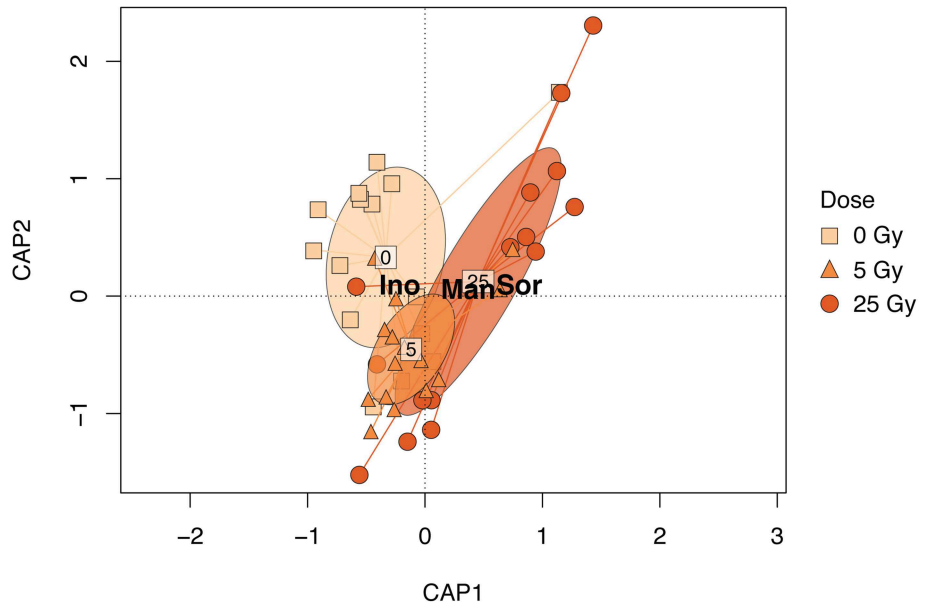

FIGURE 3 | CAP biplot based on the relative metabolite concentration matrix of *E. magnus* constrained by dose. Points represent individual samples and are shaped and colored by exposure (see figure legend). The ellipsoids represent the standard deviation for each exposure. The metabolites shown in the figure—Sor (sorbitol), Man (mannitol), and Ino (scyllo- inositol)—contributed 80% to the dissimilarity as identified in the SIMPER analysis (**Table S2**).

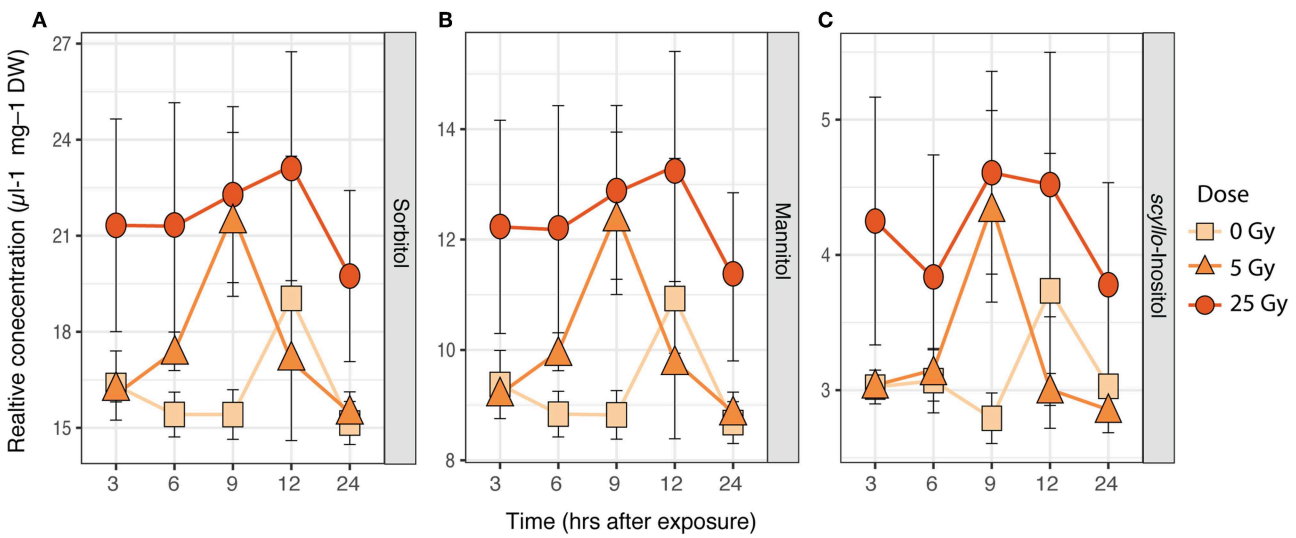

FIGURE 4 | Time series of average relative concentrations (± SE, $n = 3$) of metabolites identified by the SIMPER analysis as contributing 80% to the cumulative dissimilarity between doses (**Table S2**) in *E. magnus*. **(A)** Sorbitol, **(B)** mannitol, and **(C)** inositol (scyllo).

treatment, there was a trend for higher concentrations at 24 h at the 25 Gy dose compared to 5 Gy and the control (**Figure 2** and **Figure S2**).

The metabolic composition of *E. magnus* was influenced by the ɣ-radiation exposure [$F_{(2,38)} = 7.01$, $p < 0.01$; **Figure 3** and **Figure S3**]. The sugar alcohols sorbitol, mannitol, and inositol (*scyllo*) contributed to 80% of the dissimilarity between doses (**Figures 4A–C** and **Table S3**). Higher concentrations of all three sugar alcohols characterized the 25 Gy dose, while the concentrations were generally lower in the 5 Gy dose and the control (**Figure 4**).

Protein Content

The total protein content of *R. subcapitata* did not change significantly over time or with dose (**Figure 5A**), while in *E. magnus* both time after exposure [$F_{(4,37)} = 12.72$, $p < 0.001$] and dose [$F_{(2,37)} = 12.90$, $p < 0.001$] affected the protein content (**Table 1**). In general, the protein content in *E. magnus*

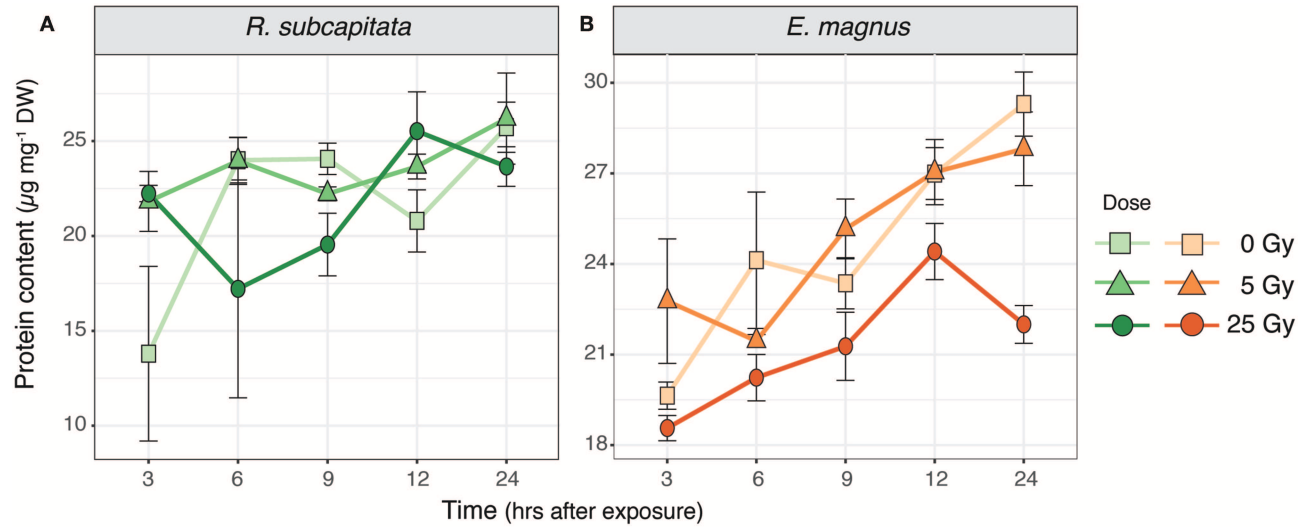

FIGURE 5 | Protein content in **(A)** *R. subcapitata* and **(B)** *E. magnus* over time per dose (mean ± SE; $n = 3$).

increased over time and the algae exposed to 25 Gy had lower protein content compared to both the control and the 5 Gy dose (**Figure 5B**).

Carbon Incorporation

There was no difference in carbon incorporation into microalgae between the control and the 25 Gy dose, but *E. magnus* incorporated significantly more ^{14}C than *R. subcapitata* [$F_{(1,22)} = 24.81$, $p < 0.001$; **Figure 6A**]. When these algae were fed to *D. magna*, the cladocerans incorporated more ^{14}C when both algae species were exposed to ɣ-radiation compared to the control [$F_{(1,9)} = 24.09$, $p < 0.001$; **Figure 6B**]. Furthermore, *D. magna* incorporated more ^{14}C when fed with *R. subcapitata* compared to *E. magnus* [$F_{(1,9)} = 24.36$, $p < 0.001$; **Figure 6B**].

DISCUSSION

We investigated the effects of external, short-term ɣ-radiation on the biochemical composition of two phytoplankton species and whether radiation-induced effects on primary producers also affected their suitability as food for primary consumers. Our results indicate that ɣ-radiation had a significant effect on the biochemical composition and primary production of phytoplankton, but these effects were species specific. The overall metabolic profile of both phytoplankton species was affected by ɣ-radiation and also by an interaction of ɣ-radiation and time after exposure (**Figure S1**). However, species specific analysis indicated that the metabolic profile (**Figures 1**, **2**), protein content (**Figure 5A**) and ^{14}C incorporation (**Figure 6A**) of *R. subcapitata* were not affected by dose. In contrast, the metabolic profile of *E. magnus* was affected by dose (**Figures 3**, **4**), as were the total protein content (**Figure 5B**) and ^{14}C incorporation (**Figure 6A**).

The metabolic profile of *R. subcapitata* was significantly affected by time after exposure, but no significant differences could be detected between doses (**Figure 2** and **Table S2**). Two sugar alcohols (sorbitol and mannitol), taurine, malic acid, aspartic acid, and glycerol-3-phospate showed a time dependent change with a trend to higher relative concentrations at 25 Gy and 24 h after exposure (**Figure 1**). Similar to sugar alcohols, taurine has been shown to act as a ROS scavenger in various organisms (Shimada et al., 2015; Tevatia et al., 2015). Malic acid is part of the tricarboxylic acid (TCA) cycle, which is part of the energy metabolism being responsible in driving the oxidation of respiratory substrate for ATP synthesis (Sweetlove et al., 2010). This response was similar in other metabolites of the TCA cycle (**Figure S2**). Previous studies have shown that the synthesis of

TABLE 1 | Differences from Tukey's all-pair comparison of means shown for protein content of *E. magnus* for time after exposure and dose.

Factor	Coefficient	SE	*p*-value
TIME (HOURS)			
3–6	−0.077	0.041	0.356
3–9	−0.138	0.041	0.016
3–12	−0.257	0.041	<0.0001
3–24	−0.249	0.043	<0.0001
6–9	−0.061	0.041	0.593
6–12	−0.179	0.041	0.001
6–24	−0.172	0.043	0.002
9–12	−0.119	0.041	0.051
9–24	−0.112	0.043	0.090
12–24	0.007	0.043	1.000
DOSE (Gy)			
0–5	−0.016	0.033	0.875
0–25	0.136	0.033	0.001
5–25	0.152	0.032	<0.0001

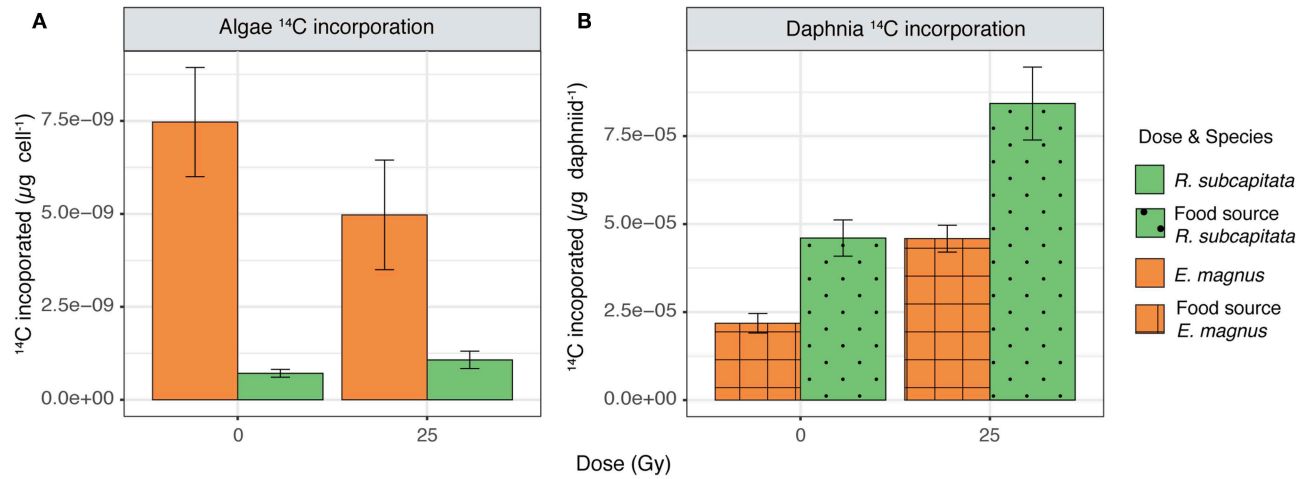

FIGURE 6 | ^{14}C incorporation in **(A)** *R. subcapitata* and *E. magnus* (mean ± SE; $n = 6$) and **(B)** *D. magna* when reared on *R. subcapitata* and *E. magnus* under control conditions and exposed to 25 Gy of ɣ-radiation (mean ± SE; $n = 3$).

ATP through the TCA cycle varies with physiological demand of the plants. However, when ATP, and therefore energy demand, increases, most of the ATP is synthesized through the TCA cycle (Sweetlove et al., 2010). Aspartic acid and/or members of the Aspartate family, such as isoleucine, have been shown to feed into the TCA cycle. Under non-stressed conditions, photosynthesis is the main energy source for plants during the day, while during the night, the TCA cycle is used to generate energy, among other pathways (Galili, 2014). The relatively high concentrations of metabolites associated to the TCA cycle at the 25 Gy treatment could indicate that the photosynthetic capacity of *R. subcapitata* was impaired by ionizing radiation.

Previous studies on another green algae, *Chlamydomonas reinhardtii*, detected adverse effects of ɣ-radiation. The photosynthetic machinery of *C. reinhardtii* was impaired, especially at higher dose rates (Gomes et al., 2017). The authors attributed these effects to the production of ROS, which was induced in a dose-dependent manner (Gomes et al., 2017). Similar trends were seen in ɣ-exposed *R. subcapitata* by Bradshaw et al. (2019), who showed a stimulation of antioxidant production (catalase, thiamine diphosphate) and photoprotective mechanisms (xanthophyll cycle). The metabolic profile of *E. magnus* was directly affected by ɣ-radiation exposure (**Figures 3**, **4**). The changes in the metabolic profile were mostly characterized by a ∼1.5-fold increase in carbohydrates in the highest exposure (25 Gy), namely sorbitol, mannitol, and *scyllo*-inositol (**Figure 4**). One of the many cellular roles carbohydrates play is the provision of non-enzymatic antioxidant defense (Demidchik, 2015). Previous studies have shown an increase in carbohydrates and total soluble sugars in plants as a response to ɣ-radiation, using similar dose rates to our study (El-Beltagi et al., 2011; Moussa, 2011). Since no enzymatic scavenging mechanism for hydroxyl radicals (•OH) exists in plants, they rely on non-enzymatic antioxidants to detoxify these highly damaging ROS (Gechev et al., 2006; Keunen et al., 2013). In plants, mono- and disaccharides have been shown to act as •OH scavengers as follows: maltose -> sucrose -> fructose -> glucose -> deoxyribose -> sorbitol (Morelli et al., 2003; Couée et al., 2006; Demidchik, 2015). Additionally, mannitol has been linked to an increased resistance to oxidative stress in transgenic tobacco plants (Shen et al., 1997). The increase in sorbitol, mannitol, and *scyllo*- inositol may therefore indicate that the exposure to ɣ-radiation induced a state of oxidative stress in *E. magnus*.

Gamma radiation had no significant effect on the total protein content of *R. subcapitata*, whereas *E. magnus* contained significantly less proteins at 25 Gy compared to the control and 5 Gy (**Figure 5**). While only a rough measure, a decrease in protein content could indicate a decrease in antioxidants, which in turn could be indicative of RNA damage that would subsequently lead to failure in protein synthesis (Bajaj, 1970; El-Beltagi et al., 2011). The two FAs linoleic acid and stearic acid contributed in part to the dissimilarity in the metabolic profile of *R. subcapitata* with time after exposure. These PUFAs are the major FAs in the lipids of plant membranes, built into galactolipids in the thylakoid membranes and phospholipids in all other membranes (Møller et al., 2007). One of the most common and most severe effects of oxidative stress is lipid peroxidation, which can lead to membrane damage (Gill and Tuteja, 2010; Won et al., 2014b). An increase in lipid peroxidation has been shown in *R. subcapitata* exposed to ɣ-radiation (Bradshaw et al., 2019). PUFAs are especially susceptible to ROS, since the double bond weakens the C-H bond and facilitates H^+ substitution. We would have therefore expected to find higher concentrations of the saturated stearic acid and lower concentrations of the PUFA linoleic acid in ɣ-irradiated microalgae. Similar trends in *R. subcapitata* were observed in a previous study (Nascimento and Bradshaw, 2016), where no changes in FA concentrations were found after exposure to short-term ɣ-irradiation. However, glycerol-3-phosphate concentrations were highest in *R. subcapitata* at 25 Gy after 24 h, which could indicate a repair mechanism of cell membranes, as

glycerol-3-phosphate is the starting building block for glycerol lipids (Cao et al., 2006).

Nascimento and Bradshaw (2016) investigated the direct and indirect effects of ϒ-radiation on a grazer-phytoplankton interaction using *R. subcapitata* and *D. magna* as their model species and exposing them to short-term ϒ-radiation, both together and separately. When unexposed *D. magna* were fed with *R. subcapitata* that had been exposed, the daphniids showed the highest ^{14}C incorporation. This increase in carbon incorporation was attributed to an increased grazing rate of *D. magna*, which in turn could have been caused by a lower food quality of the exposed *R. subcapitata*. However, the FA profile did not show any changes after exposure (Nascimento and Bradshaw, 2016). Concurring with these results, *D. magna* in our study also incorporated more ^{14}C when the phytoplankton had been exposed to ϒ-radiation compared to the control (**Figure 6B**). This trend was the same irrespective of phytoplankton species, i.e., regardless of the original (control) suitability of these species as food; *D. magna* incorporated more ^{14}C when fed with *R. subcapitata* than with *E. magnus*, both when irradiated and non-irradiated. These two phytoplankton species were chosen due to their different FA profiles, which was used as a proxy for overall food quality. Chlorophyta, like *R. subcapitata*, are generally considered to be of lower food quality for zooplankton, since they contain very little or lack FAs such as DHA and EPA (Lang et al., 2011; Taipale et al., 2013). These FAs have been linked to increased somatic growth in crustaceous zooplankton (Brett and Müller Navarra, 1997).

The higher ^{14}C incorporation of *D. magna* fed with *E. magnus* exposed to 25 Gy might also be explained by an increase in feeding stimuli. Chemosensory studies in crustaceans have shown that carnivore species primarily detect amino acids, nucleotides and amines, while herbivores and omnivores are additionally sensitive to carbohydrates (Corotto and O'Brien, 2002; Eriksson Wiklund et al., 2014). These biochemicals can act as cues for location and identification of preferred food items (Corotto and O'Brien, 2002). In *Daphnia*, sugar receptors have been identified, which suggests that sugars could be used as a feeding stimulus (Peñalva-Arana et al., 2009; Eriksson Wiklund et al., 2014). In our study, *E. magnus* had higher sugar alcohol content at 25 Gy and, in *R. subcapitata*, the amino acid content was also higher at 25 Gy compared to the control. These changes in the biochemical composition of *E. magnus*, and to a certain extent also in *R. subcapitata*, could have resulted in higher feeding rates due to a higher release of chemical stimuli. However, an increased feeding of *D. magna* on *E. magnus* exposed to 25 Gy might have had some adverse effects on the daphniids. Certain sugars have been shown to lead to heart arrhythmia in *Daphnia* (Campbell, 2004). These changes in heart rate may have in turn also contributed to an increased feeding rate, as heart rate and feeding rate are usually tightly coupled (Eriksson Wiklund et al., 2014).

Another factor that could explain the increased ^{14}C incorporation by the daphniids feeding on *R. subcapitata* could be that the algae anti-grazing defense mechanisms were decreased due to exposure to ϒ-radiation (Nascimento and Bradshaw, 2016). The metabolic profile of *R. subcapitata* shows an increase of metabolites that are part of glycolysis (glucose, glucose-6-phosphate, and fructose-6-phosphate; **Figure S2**) and the citric acid cycle (e.g., malic acid; **Figure 2G** and **Figure S1**). This could indicate a shift from photosynthesis to photorespiration, which is less efficient and could therefore lead to a shift in energy allocation toward processes that are needed for cellular functions and away from energy-demanding processes such as grazing defenses.

In conclusion, our results indicate that external, short-term ϒ-radiation can change the biochemical composition of aquatic primary producers. Changes in the metabolic profile indicated that this could be due to responses to oxidative stress, or changes in photosynthetic capacity. However, the response to ϒ-radiation is species specific. Our results also suggest that these biochemical changes can affect primary consumers, such as *D. magna*, through changes in food quality, increases in chemical feeding stimuli, or decreased grazing defenses by the algae.

ETHICS STATEMENT

This study was carried out using *D. magna* as study animal, for which no ethics approval was required.

AUTHOR CONTRIBUTIONS

A-LG preformed the experiment, analyzed the data, and wrote the first draft of the manuscript. CB and A-LG designed the study and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00100/full#supplementary-material

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