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NKS-B NORCO
Nordic freshwater ecosystem microcosms study

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Abstract

Ecosystem response due to radiation exposure depends on the different species sensitivities and the multitude of direct and indirect pathways by which individual organisms can be affected, including the potential for complex interactions across multiple trophic levels. Multi-species model ecosystems (microcosms) were used to investigate effects of ionizing radiation on a model aquatic ecosystem including indirect effects caused by ecological interactions. Microcosms were exposed for 22 days to a gradient of gamma radiation with 4 decreasing dose rates (20, 8, 2, 0.8 mGy/h). A range of endpoints were measured at different time points in order to capture effects on individual components of the ecosystems as well as whole-ecosystem processes. Individual and population growth was measured for all species; species interactions were measured in the form of grazing rates, whole ecosystem respiration and production were quantified; and measurements ecosystem elements, nutrients status and cycling were collected. Plant growth rates were generally lower in the irradiated treatments. Several photosynthetic parameters were negatively affected by radiation in a dose-dependent manner and ROS production increased with radiation dose in *L. minor*. Primary production decreased in all treatments during the first week and remained low for the duration of the experiment. Primary consumers were not effected by dose rates, however their impact on primary producers was significant. Abiotic measurements revealed simialr conditions between all microcosms. By taking an ecosystem approach, this study shows that the net effect of radiation in a simple aquatic ecosystem is a combination of the direct effects of radiation to the individual species with different relative radiosensitivity, and the indirect effects mediated by ecological and abiotic processes.

Key words; radioecology, radiation, microcosm, freshwater, ecosystem

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NKS-B NORCO Final report

The importance of ecological processes and indirect effects in determining an aquatic ecosystem's response to ionising radiation.

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1. Introduction

In the last decade, a number of national and international organisations have recognised the need for specific protection of the environment and non-human organisms from radiation (ICRP 2007) and advocated an ecosystem-based approach (Bréchignac *et al* 2011). However, in practice many of today's radiation protection frameworks rely heavily upon reference organisms like the ICRP Reference Animals and Plants to predict an environmental response, or are based on data from single species experiments, partly due to the lack of data on higher-level effects. By extrapolating single species data to an ecosystem, researchers and risk assessors may overlook the important multiple interactions that exist between individuals and populations of different species (Bradshaw et al 2014). The observed responses of an ecosystem due to radiation exposure depend on the different species sensitivities and the multitude of direct and indirect pathways by which individual organisms can be affected, including the potential for complex interactions across multiple trophic levels. Radioecological research is therefore shifting focus towards studying impacts on the structure and functions at population, community and ecosystem levels. However, investigations have generally been restricted to laboratory experiments using single species, and our knowledge of radiosensitivities of aquatic plants and animals is very limited (UNSCEAR 2008). An ongoing literature review by our research group within the Norwegian Centre for Excellence for Environmental Radioactivity (CERAD) revealed less than 20 radioecology based microcosm studies.

The nuclear testing of the 1950's and 60's resulted in a huge interest in the effects of radiation on the environment. There was large funding bodies during this time, especially in the USA, that investigated ecosystems through microcosm studies (Odum *et al* 1970, Patten and Witkamp, 1966). Some even using large outdoor facilities (McCormick & Platt, 1962), which, with today's restrictions, would be impossible to recreate. The end of the nuclear testing period in the late 60's resulted in an abrupt halt in radioecology studies, as is evident in the gap of ecosystem radioecological research from this time to the late 90's. Recent microcosm studies have mostly focused on radionuclide transfer in terrestrial ecosystems (Fritsch *et al* 2008 ,Tuovinen *et al* 2016), or radiation studies using extremely high doses. Numerous microcosm studies by Fuma *et al* (1998, 2009, 2010) have investigated the indirect effects of ionizing radiation in aquatic communities with more than one trophic level. However, their studies are mostly using microbial communities, which are known to be highly radiation resistant (Whicker and Shulz, 1982) and they have used acute dose rates of up to 5000Gy.

The NKS-B NORCO project was initiated in 2016 with the aim of applying an ecosystems approach to radioecology. We created aquatic microcosms with multiple species from several trophic levels that represented some of the numerous pathways and interactions that exist between species in an ecosystem (Fig. 1).

The study investigated the effects of external gamma irradiation and ecological processes on an experimental aquatic ecosystem. A large range of endpoints, from molecular- to ecosystem-level, were measured in order to try and capture potential direct and indirect effects of radiation. This experiment is the first microcosm study, to our knowledge, to have used experimental dose rates similar to those experienced by organisms in the field during the acute and intermediate phases of a nuclear accident. The clearest radiation effects were seen in the primary producers and at molecular rather than individual or population level. Effects on species interactions and indirect effects were harder to determine. This could be due to the natural variability between microcosms, the insufficient duration of the experiment and possibly buffering effects within the ecosystem. Below we discuss our results and also suggest a number of recommendations for future microcosm studies with radiation. This study has been accepted for publication;

Hevroy TH, Golz AL, Xie L, Hansen EL, Bradshaw C (2019) Radiation effects and ecological processes in a freshwater microcosm. Journal of Environmental Radioactivity.

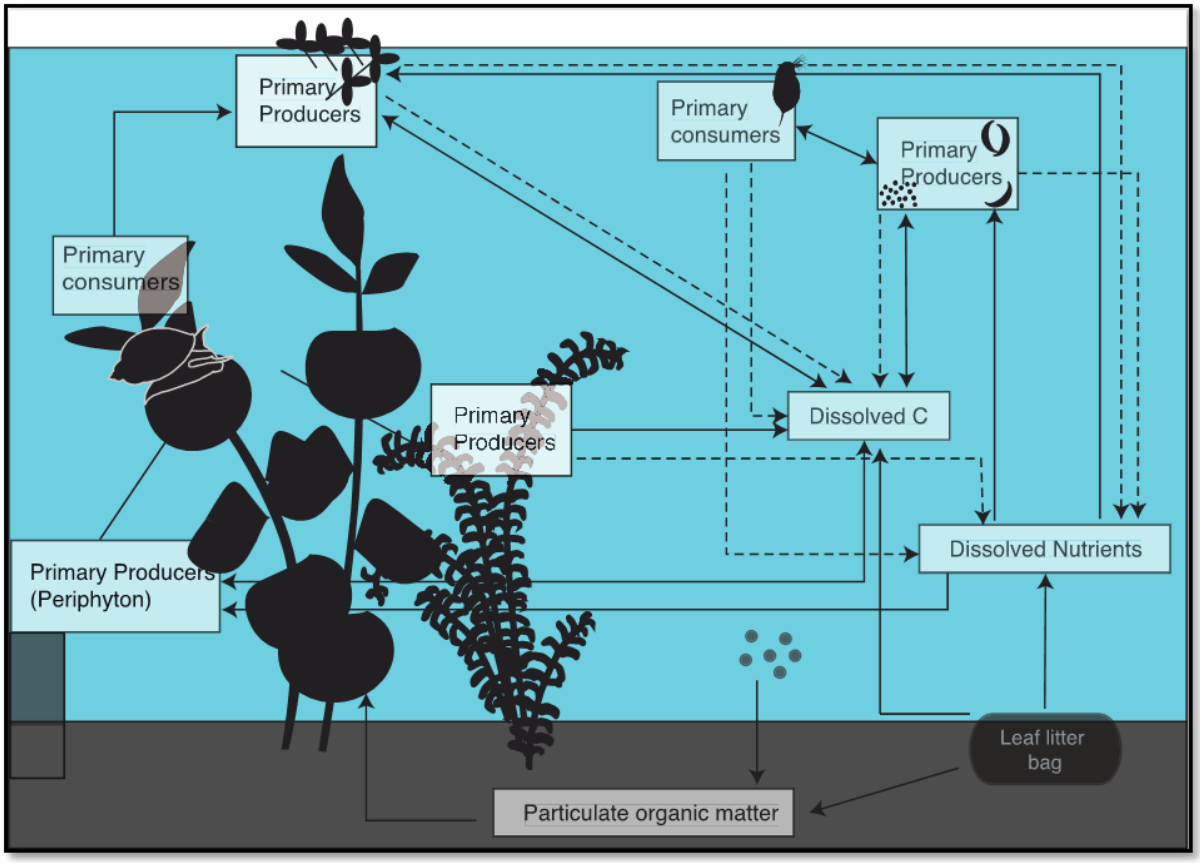

Figure 1. Conceptual model of the NORCO microcosm, illustrating each species and component of the aquatic ecosystem and the interactions between them. Solid lines = trophic transfer and dotted line = excretion/decay.

2. Experimental setup

The experiment was conducted in the FIGARO facility at NMBU (Ås, Norway). The facility contains a climate controlled chamber with a gamma radiation source (12 Ci Co-60), which provides a continuous dose rate field from 3 Gy/hr directly at the source, down to 400 µGy/hr at the furthest end of the 20 m hall. The width and height of the beam constrained the overall microcosm dimensions to 20*20*10 cm (width*height*depth). Furthermore, we needed to achieve a high enough dose as to expect biological impacts (FIGARO individual species tests have not shown any effects below 10mGy/h), take into account shielding effects and rate of loss of gamma dose through water (47% reduction through 10cm water). Our experiment had four nominal dose rates; 20mGy/h, 8mGy/h, 2mGy/h and 0.8mGy/h, five control microcosms and six positive control microcosms were placed behind a wall of lead shielding (Fig. 2). The positive controls included three high Cd doses, with nominal concentrations of 44.3 µgCd/L and three low dose Cd treatments with nominal concentrations of 8.8 µgCd/L.

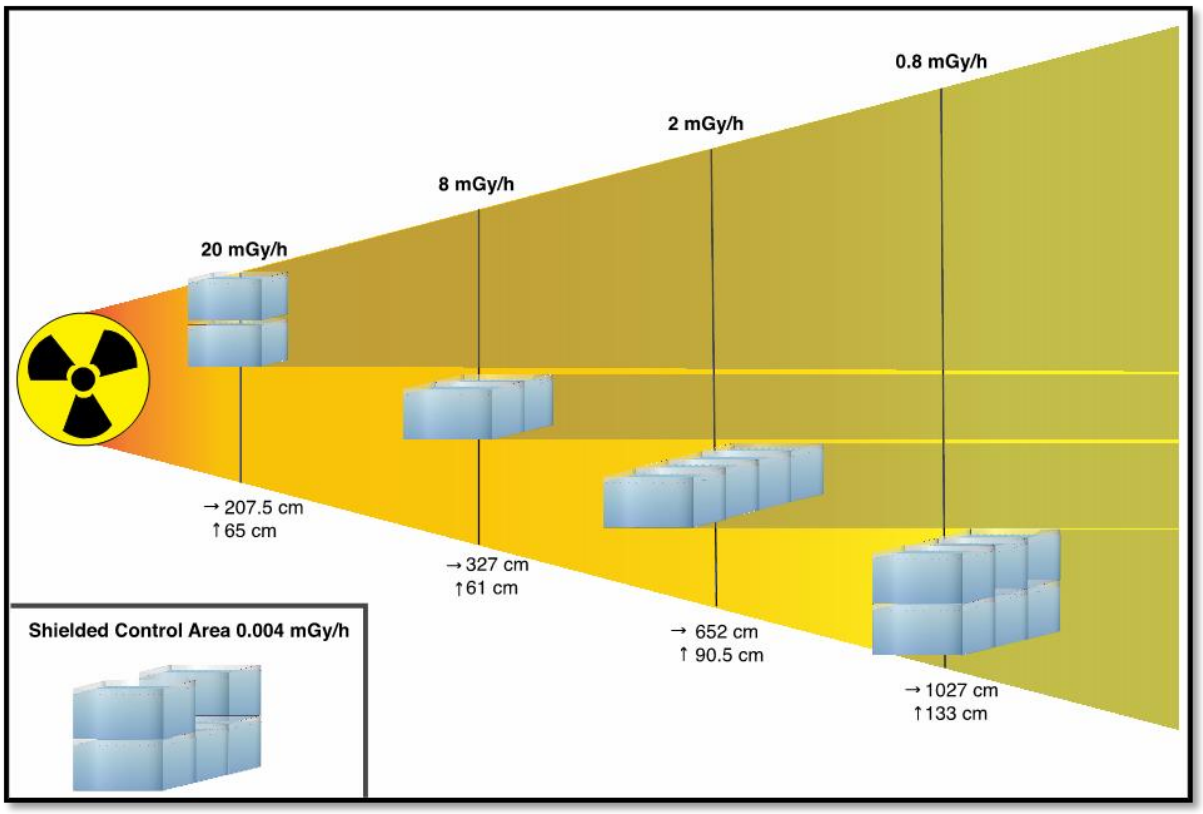

Figure 2. Microcosms set up in FIGARO climate chamber, arranged to avoid shielding. At 2 meters from the beam the field of width is only 40cm expanding to almost 3m at 15 meters from source, therefore the number of microcosms placed at each dose depends on space available at the distance.

2.1 Dosimetry

The dosimetry was conducted according to the guidelines of a newly developed exposure characterization and dosimetry framework for FIGARO (Hansen et al. 2019). The exposure setup was planned based on reference measurements of air kerma rates by the Secondary Standard Dosimetry Laboratory (SSDL) at the Norwegian Radiation Protection Authority (Bjerke and Hetland, 2014) and verified by measurements of air kerma rates with nanoDot dosimeters (Landauer, Inc., Greenwood, IL) placed over the front and back faces of empty microcosms (see Supplementary Information).

The dosimetry framework for FIGARO includes a Geant4 Monte Carlo radiation transport model of the exposure hall and source that is used for simulating air kerma rates and absorbed dose rates to experiments (Fig. 3). A Geant4 model of the exposure setup for microcosms have been described in Hansen et al. (2019) for simulating average whole-setup absorbed dose rates to water for each microcosm. The median value for the relative

deviation between air kerma rates simulated by the model and measured with nanoDots across the front and back faces of empty microcosms was 4.0% (Hansen et al. 2019).

In the current work, we also simulated absorbed dose rates to plants that occupy the midplane of microcosms (*E. densa* and *L. nummularia*) and to plants that occupy the water surface (*L. minor*). We estimated that absorbed dose rates to *D. magna* are well approximated by the average absorbed dose rates to water for each microcosm because *D. magna* utilise the full microcosm volume. All organisms were modelled with the same density and composition as water.

After 22 days exposure, the measured dose, using nanodots and exact exposure times at each nominal treatment level was;

- 20 mGy/h = 8 Gy
- 8.0 mGy/h = 3 Gy
- 0.8 mGy/h = 0.8 Gy
- 0.2 mGy/h = 0.3 Gy

In the rest of the report, results are given based on nominal dose rates.

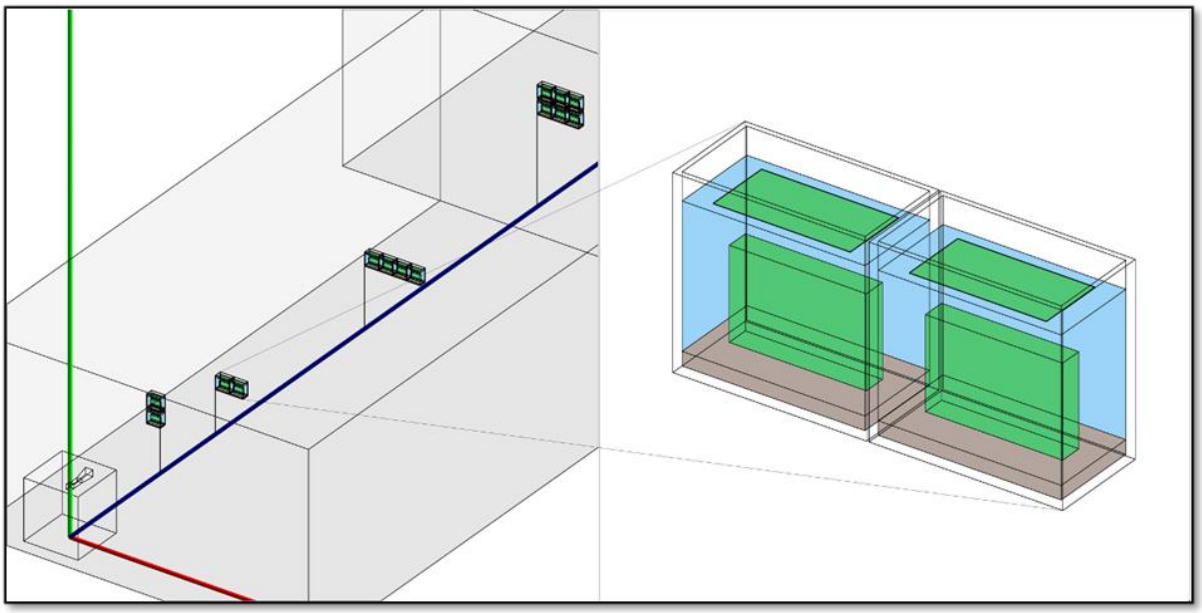

Figure 3. The Geant4 model of the setup of microcosms inside the FIGARO exposure hall. The source chamber is located in the lower left corner and microcosms are arranged over four dose levels at increasing distances to the source focus. The red, green and blue axes indicate the positive x-, y- and z-directions of the exposure hall. The z-axis coincides with the projection of the central field axis onto the xz-plane. Microcosms at the highest dose level were placed to the left of this axis (as seen from the source) whereas microcosms at all other dose levels were placed to its right. The number of replicate microcosms per dose level were limited by the size of the field and by the requirement that no microcosm should experience shielding of the gamma field from any of the microcosms at the higher dose levels. b) A close-up of the Geant4 model of two microcosms. Grey areas are sediments, blue areas are water and the two green areas are modelled plants.

2.2 Microcosms

Twenty-five Plexiglas 4l microcosms were established with 3.8 litres of artificial freshwater, based on the MWC algal culturing media, and 630 grams wet weight pre-washed sediment (red sand). Different components of the microcosms were added the days leading up to the start of the experiment (T0) for stabilization (Fig. 4). Next two plant species (*Egeria densa* and *Lysimachia nummuleria*) were cut into 12 and 5cm shoots (with roots intact), two shoots of each species were weighed and photographed before planting them in the sediment.

After rooting the plants, 44-48 fronds of *Lemna minor* were added to each microcosm. We allowed the microcosm to settle overnight, then added the primary consumers *Daphnia magna* and *Lymnaea peregra*. Six big and six small *D. magna* were added to each cosm. Ten *D. magna* of each size class were photographed and preserved in Lugol to determine exact size range of each size class. Four snails (*L. peregra*) were weighed and measured following OECD guidelines (OECD, 1984), and added at the same time as the *D. magna* to each microcosm. To ensure *D. magna* had sufficient amount of food (phytoplankton), we calculated amount of carbon consumed per daphnia per day (OECD guidelines), resulting in 0.25mg C/L ml *Raphidocelis subcapitata* and 0.25mg C/L *Eustigmatos* sp, added per microcosm. Litter bags were composed of dried leaves of 4 Nordic tree species (birch, maple, willow and oak) and pre-weighed. Lastly, we added a microscope slide to each microcosm, fastened in the sediment to establish biofilm communities, which we could later analyse. We let the microcosm stabilize for 48 hours before starting measurements for T0.

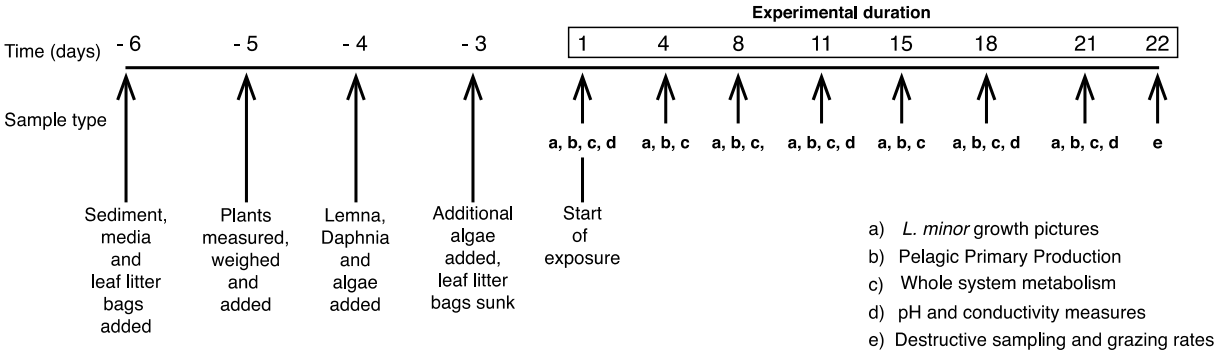

Figure 4. Timeline of construction and stabilization of microcosms, followed by experimental duration and endpoints sampled throughout.

The climate chamber was set to 18 °C, with each microcosm receiving light from a LED light source (c. 1000 lux) on a 16-h light:8-h dark cycle. Both light and temperature were monitored throughout the experiment using temperature and light automatic loggers (HOBO Pendant® Data Logger). No significant deviations were observed (Table 1).

Table 1. Abiotic parameters (mean ± st.dev), based on measurements taken with a WTW Multimeter 350i.

Average dose rate	n	t0			t10			t21		
Conductivity (µS/cm)										
0.004 mGy/h	5	150,72	±	22,26	163,06	±	18,1	191,94	±	15,93
0.72 mGy/h	6	170,73	±	31,96	179,82	±	25,38	208,63	±	23,71
1.78 mGy/h	4	129,88	±	22,07	149,83	±	18,03	184,4	±	14,12
7.85 mGy/h	2	170,55	±	33,3	181,75	±	27,22	211,15	±	23,83
18.5 mGy/h	2	143,65	±	44,05	157,8	±	35,36	189,2	±	32,24
Temperature (°C)										
0.004 mGy/h	5	19,14	±	0,13	18,3	±	0,1	18,76	±	0,11
0.72 mGy/h	6	19,18	±	0,04	18,22	±	0,04	18,52	±	0,1
1.78 mGy/h	4	19,18	±	0,05	18	±	0,08	18,53	±	0,1
7.85 mGy/h	2	19,25	±	0,07	18,15	±	0,07	18,7	±	0
18.5 mGy/h	2	19,25	±	0,07	18,2	±	0,14	18,75	±	0,07
pH										
0.004 mGy/h	5	7	±	0,09	7,2	±	0,16	7,32	±	0,21
0.72 mGy/h	6	7,03	±	0,06	7,21	±	0,07	7,24	±	0,09
1.78 mGy/h	4	6,98	±	0,07	7,17	±	0,08	7,21	±	0,13
7.85 mGy/h	2	7,05	±	0,02	7,16	±	0,09	7,22	±	0,07
18.5 mGy/h	2	7,04	±	0,11	7,21	±	0,01	7,25	±	0,01
Light (lux)										
0.004 mGy/h	5	1144	±	150						
0.72 mGy/h	6	1204	±	118						
1.78 mGy/h	4	1229	±	124						
7.85 mGy/h	2	1184	±	46						
18.5 mGy/h	2	1216	±	168						

2.3 Start of the experiment (T0)

At T0, the day before we switched on the ionizing radiation source, we measured a range of endpoints for each microcosm. Particulate carbon, nitrogen and phosphorus (CNP), dissolved nutrients (DN), dissolved organic carbon (DOC), pelagic primary production (Chlorophyll a and 14C incorporation), whole ecosystem respiration and production (oxygen measurements made both in the light and the dark), pH and conductivity were measured. Photos of *L. minor* to estimate growth rates were taken and additional water samples for dissolved elemental analysis using ICP-MS (a type of mass spectrometry capable of detecting metals and several non-metals at low concentrations).

We also added 155 ml of the Cd stocks to the positive control microcosms at T0 to achieve nominal concentration in the microcosms of c. 9ug/L and 44ug/L.

2.4 Time series sampling

Throughout the experiment we sampled for whole ecosystem metabolism, pelagic primary production (using both 14C incorporation and chl a measurements), *L. minor* growth rates and water chemistry (CNP). From the oxygen measurements we calculated net ecosystem production (NEP) and whole system respiration (R). Gross ecosystem production was then calculated as the sum of these two measurements (NEP + R).

After each sampling, the microcosms were refilled with fresh media to compensate for the samples taken as well as accounting for evaporation. Once a week, phytoplankton was added to ensure sufficient food concentration for *D. magna*. To account for *D. magna* population growth, the phytoplankton concentrations added were doubled at day 10 from 0.5 mg C/L to 1.0 mg C/L.

2.5 End of the experiment (T22)

In addition to the endpoints that were measured and sampled throughout the experiment, on the last day, the microcosms were broken down and each individual compartment was sampled (see Table 2 for complete overview of endpoints measured). A range of parameters that evaluate plant stress through photosynthetic capacity was measured for all three plant species using a PAM-2000 (portable chlorophyll fluorometer). Production of reactive oxygen species (ROS) in *L. minor* fronds was also measured as an estimate of oxidative stress. We calculated change in biomass for *L. nummularia* and *E. densa* shoots by weighing and measuring individuals for each treatment.

Ten adult *D. magna* from each treatment were used to measure grazing rates using 14C-labelled *R. subcapitata* suspension. Similarly, four adult *L. peregra* from each treatment were used to measure grazing rates (following Crichton et al. 2004). Both *D. magna* and *L. peregra* were left to graze for 24 hours. Reproduction rates for *L. peregra* were estimated from the number of egg capsules per microcosm and the number of eggs per capsule.

To test bacterial activity within the microcosms, leaf litter bags comprising teabags filled with a known dry weight of leaves of 4 Nordic tree species (birch, maple, willow and oak, dried at 50°C) were placed on the sediment surface.

Lemna minor fronds and whole plants of *L. nummularia* and *E. densa*, *D. magna*, *L. peregra* and sediment samples were frozen for CNP analysis. We collected all individuals of *D. magna* and *L. peregra* for population size and structure analyses. Leaf litter bags were collected and weighed to estimate the microbial mineralization rates of the leaves. Lastly, water samples for dissolved elemental analysis using ICP-MS were taken again.

Table 2. List of all endpoints measured in NORCO microcosms.

Endpoints Measured
Ecosystem Endpoints
NPP
R
GPP
Total Primary Production
Bacterial Production
Elements (ICPMS)
Carbon, Nitrogen and Phosphorus
Ecological Endpoints
***L. peregra* grazing rates**
***D. magna* carbon incorporation**
Chlorophyll *a*
Individual species endpoints
***L. minor* growth**
***L. nummularia* growth**
***E. densa* growth**
L. minor: ETR, ΦPSII, qP & qN
***L. nummularia:* ETR, ΦPSII, qP & qN**
E. densa: ETR, ΦPSII, qP & qN
***L. minor* MDA**
***L. nummularia* MDA**
***E. densa* MDA**
***L. peregra* growth**
***L. peregra* egg capsual production**
***L. peregra* number per egg capsual**
***L. peregra* number juveniles**
***L. peregra* MDA**
***D. magna* length**
***D. magna* abundance**
***D. magna* MDA**
Leaf litter loss

3. Primary Producers

Significant plant stress was evident in all three plants in a dose dependent relationship (Fig. 5). Several parameters of photosynthesis were measured to determine the health of the photosynthetic systems in plants. Firstly, we measured chlorophyll fluorescence in Photosystem II and the rate of photosynthesis via the electron transport rate (Fig. 5 Ia). We also measured both photochemical quenching, that measure the distribution of light energy in the plant, and nonphotochemical quenching (Fig 5. IVa), which is a protective mechanism to dissipate excess energy.

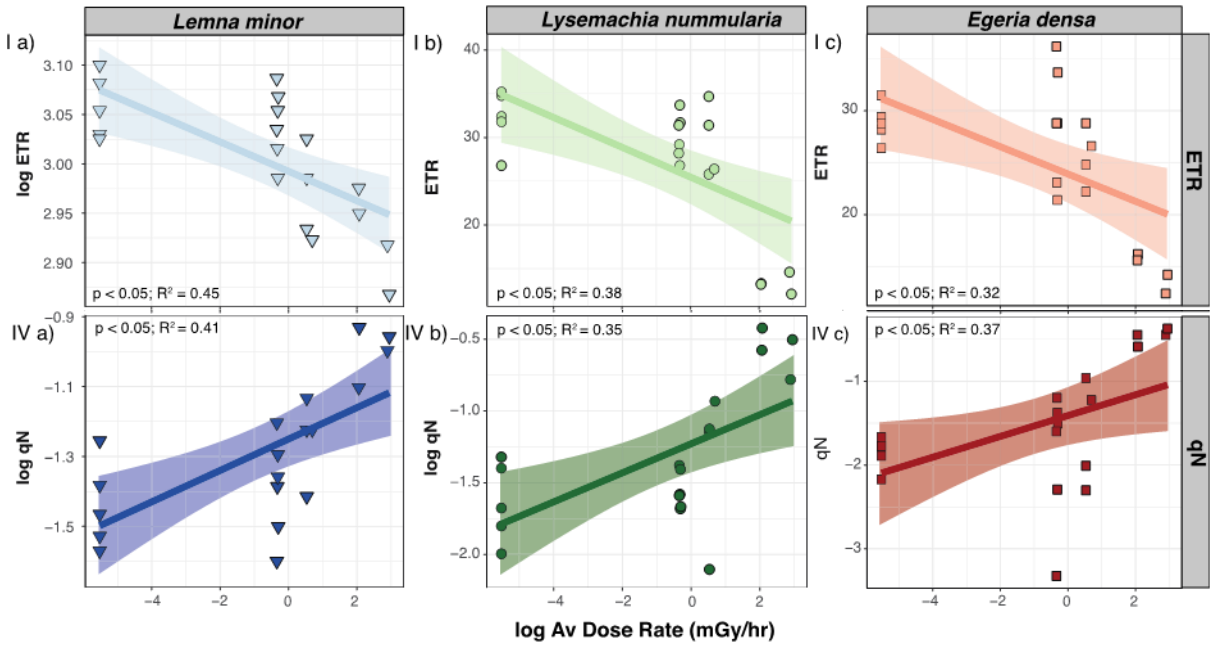

Figure 5. Plant stress measured in selected parameters for *L. minor*, *E. densa* and *L. nummuleria* (±S.E), after gamma irradiation in a dose rate range 0.8-20 mGy/h I a) electron transport rate and, IV a) non photochemical quenching.

Reactive oxygen species (ROS) are a natural by-product of metabolism. However, during times of environmental stress, ROS levels can increase dramatically which can result in significant cell damage. ROS are generated by external forces such as ionizing radiation, and are therefore often studied in radiobiology and radioecology. We measured ROS in *L. minor* and observed a significant dose dependent relationship between increased ROS formation with increasing dose (Fig. 6). In addition, the growth rates of *L. minor* were significantly less in the exposed treatments than the control (Fig. 7b) which correspond to previous radiation studies on *L. minor*, using similar doses (van Hoeck *et al* 2015). However, the growth rates did not show a dose-dependent response.

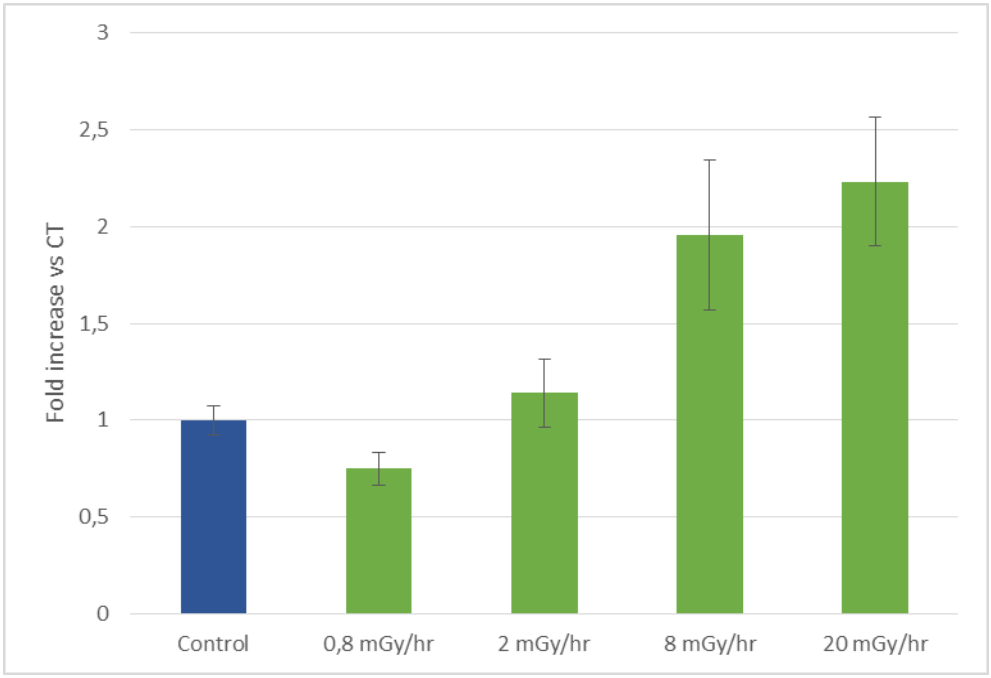

Figure 6. Average ROS production (as a fold change compared to the control) in *L. minor* (±S.E), after gamma irradiation in a dose rate range 0.8-20 mGy/h.

There were no significant changes in biomass of the plants *E. densa* or *L. nummuleria* (Fig. 7a). Similar results on plant biomass has been witnessed in terrestrial studies, Jones *et al* (2015) did not see an effect on plant biomass till above 20 Gy, a dose which our study did not meet (highest dose = 8 Gy). In light of these results, when compared to the photosynthetic parameters (Fig. 5) suggests that the study was too short to see the effects of damage to photosynthetic parameters on plant growth and biomass or the plants were able to growth regardless of the photosynthetic damage. Reports of different radiosensitivites within plant cells has been reported in other plant studies (Mitsuhashi *et al* 1998), where damage is repaired over time by action of intracellular mechanisms. There is however, a trend for competition in the results of biomass, with *E. densa* growing more at dose ranges where *L. nummuleria* grew less. Perhaps our nutrient analyses, in combination with other growth rates data, will reveal this pattern more clearly.

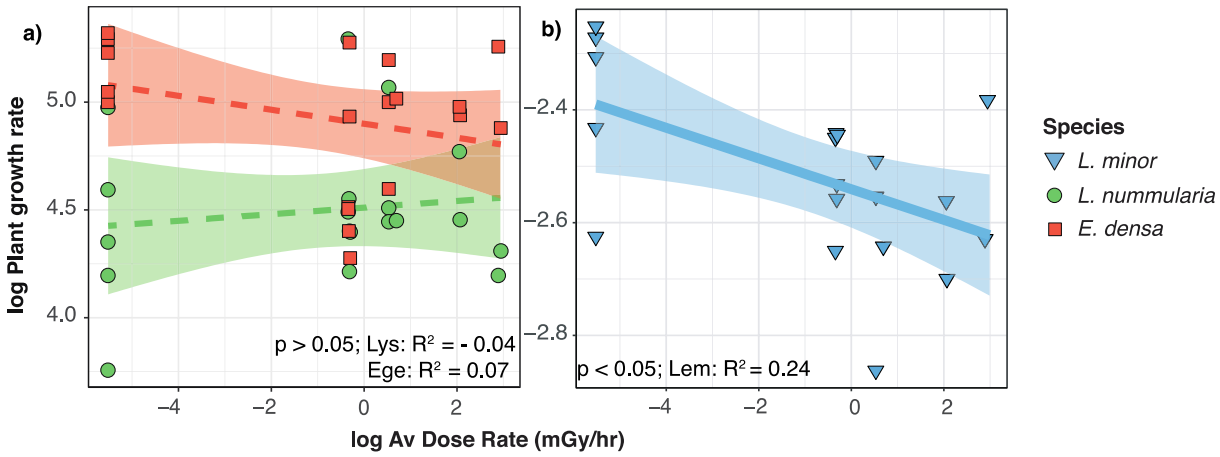

Figure 7. Average growth rate, expressed as milligram wet weight per day (±S.E), of *L. minor*, *E. densa* and *L. nummuleria* after gamma irradiation in a dose rate range 0.8-20 mGy/h.

The chlorophyll a measurements (Fig. 8) reflect the pelagic primary production measurement, where primary production decreased in all treatments during the first week and remained low for the duration of the experiment (Fig. 8). Differences between treatments varied during the course of the experiment, sometimes being highest in the controls or lower irradiation treatments, sometimes in the higher dose treatments. These temporal variations and treatment differences were also seen in measurements of whole ecosystem production and respiration. However, there were no significant differences in Chl a measurements between treatments over the course of the experiment.

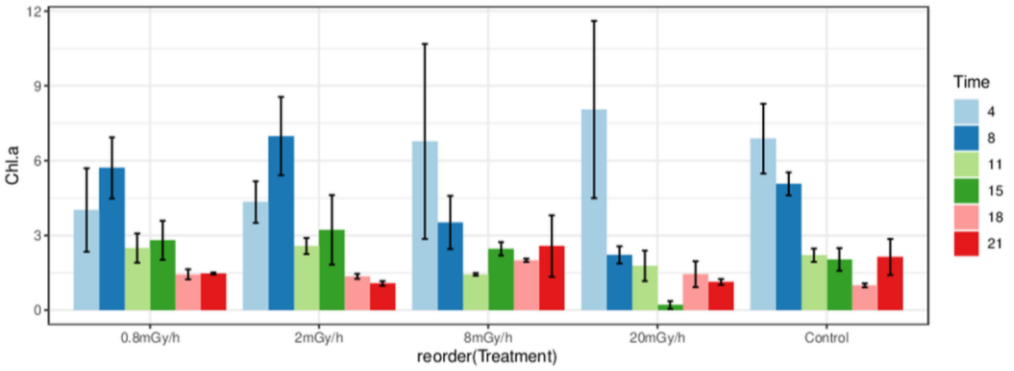

Figure 8. Average chlorophyll a concentrations (±S.E), per dose rate per sampling day throughout the exposure.

4. Primary Consumers

Lymnaea peregra populations grew in all the microcosms and there was a trend for decreasing population with increasing radiation dose (Fig. 9a). Interestingly however, there was no apparent decrease the in number of

eggs per capsule, or number of capsules per cosm (Fig. 9a). Furthermore, we measured the grazing rates of our primary consumers, *D. magna* and *L. peregra* (Fig. 10), which showed no significant differences between treatments for either species, but a negative trend for *L. peregra* was visible (Fig. 10). A reduction in grazing rates would indirectly affect adult snail health, and potentially reproduction, as well as the nutrients available in the microcosms.

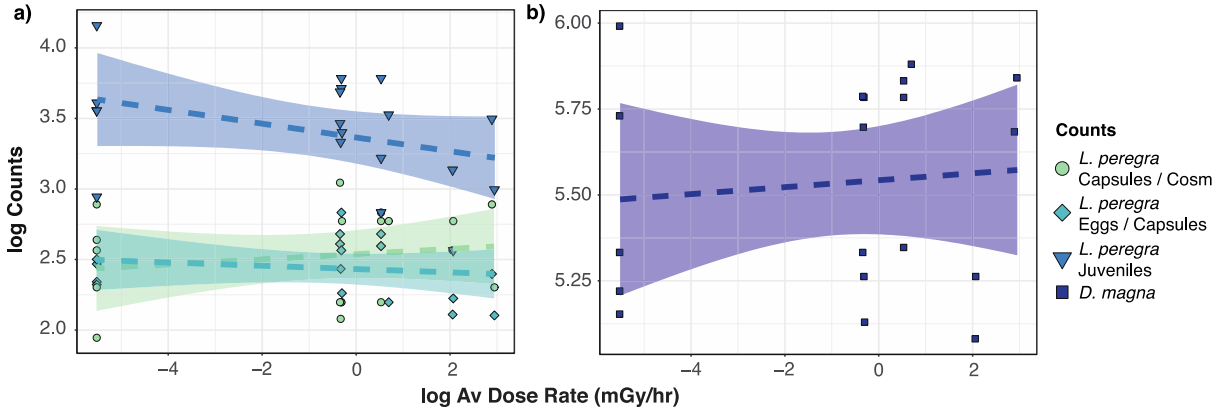

Figure 9. Average number of capsules/cosm and eggs/cosm, juvenile snails (±S.E), at control (0) and Number of D. magna (±S.E), at each treatment level after gamma irradiation in a dose rate range 0.8-20 mGy/h.

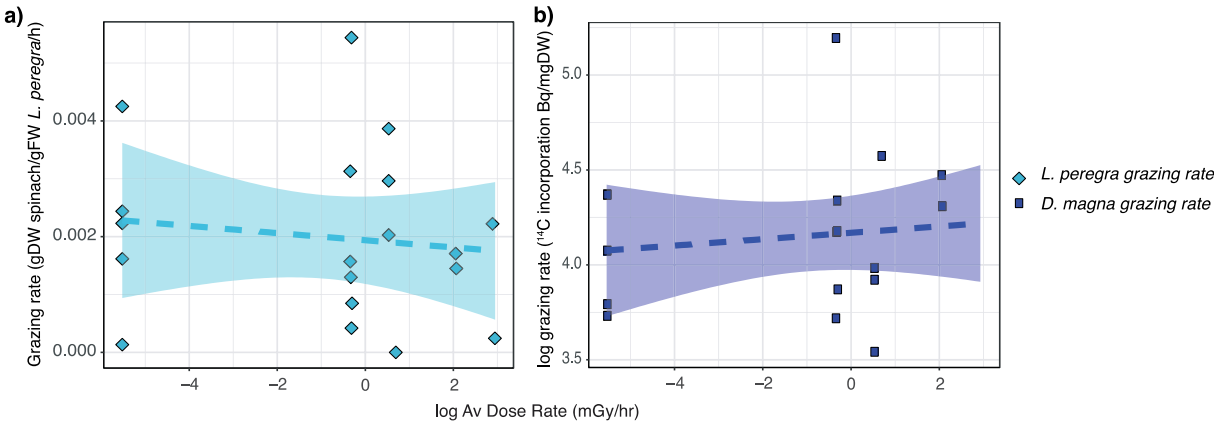

Figure 10. Grazing rates of a) *L. peregra* and b) *D. minor* predicted by average dose rate (mGy/h). Dashed lines indicate non-significant relationships.

Daphnia abundance showed a significant increase over the course of the experiment, starting at 12 individuals per microcosm and reaching population sizes clustered into two distinct groups, one with a mean number of individuals of 186±8 (8 microcosms, +/- SD) and the other with a mean number of individuals of 330±31(11 microcosms). However, there were no significant differences between the treatments (Fig. 9 b) and the two size groups were not related to dose rate. Size distributions of *D. magna* populations revealed different features (monomodal or bimodal distributions), however these differences were within the range of the controls size distributions (Fig. 11). We believe the abundance of *Daphnia magna* and rapid disappearance of phytoplankton witnessed in the microcosms at all treatments are best explained by the overgrazing of phytoplankton due to *D. magna* not having a natural predator, and not due to radiation. This relationship is clearly illustrated in by the inverse relationship between *D. magna* numbers and phytoplankton biomass, measured as chlorophyll a concentration (Fig. 9).

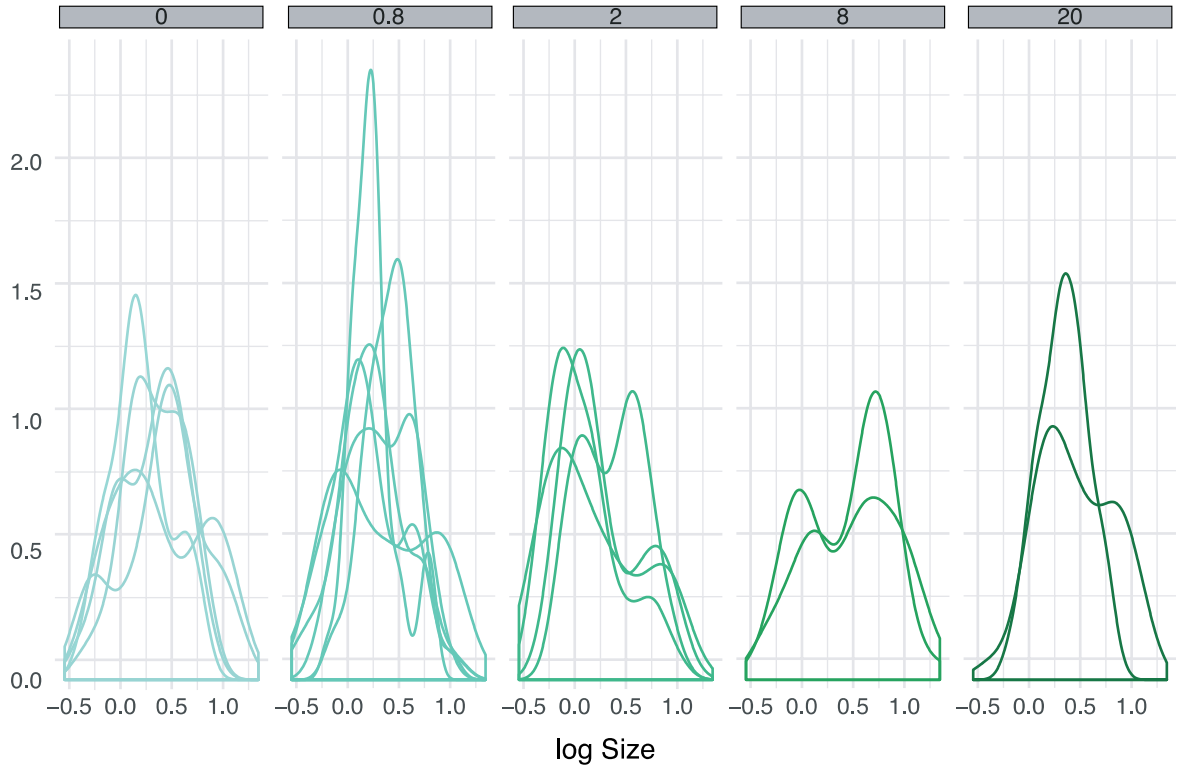

Figure 11. Size distributions of *Daphnia magna* per microcosm at treatments (nominal dose rate mGy/hr).

5. Whole ecosystem production

There were no significant differences between the dose rates treatments and the controls on the production and respiration of the microcosms (Fig. 12). Net ecosystem production (NEP) is the amount of primary production after the costs of respiration by plants, heterotrophs and decomposers are included, and it is clear that all the microcosms shifted significantly from a production ecosystem to a respiration ecosystem. At each treatment and the control, microcosms showed a significant increase in respiration, which together with an unbalanced relationship between production and respiration can signify a stressed system (Taub, 1997). However, our calculated values of net primary production were often negative, which is the result of large *Daphnia* populations and bacteria respiring. In addition, a biofilm had developed on the microscope slides, which we believe to be evidence of a bacterial community forming. In aquatic systems, phytoplankton are a major primary producer, using photosynthesis to produce essential nutrients and oxygen for the higher trophic levels. Quantification of oxygen production and CO_2 assimilation are measures for estimating the primary productivity of phytoplankton. This is commonly done using ^{14}C radiolabelling method, which estimates assimilated carbon by using a tracer to quantify amount of uptake and assimilation of dissolved inorganic carbon (DIC). This method is based on the assumption that ^{14}C labelled DIC is proportional to the more commonly non-radioactive ^{12}C DIC.

For our 14C production measurements, both phytoplankton and some bacteria will be able to incorporate C from the water. To separate the two, incubations are done in both light (phytoplankton and bacterial production) and dark (bacteria only). There was a significant shift of primary production to bacterial production over the course of the experiment (Fig. 13), which is most likely due to the explosion of *D. magna* populations (Fig. 9b), resulting in overgrazing of the phytoplankton. There was no significant difference between the different treatments.

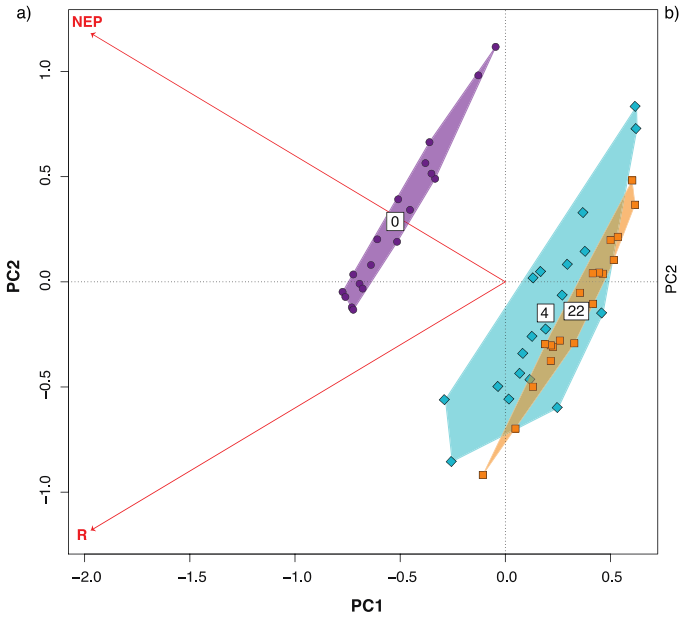

Figure 12. Net Ecosystem Production (NEP) and Respiration (R) at the start of the experiment in purple (T0), 4 days after exposure at in blue and the end of exposure in orange (T22) (±S.E), measured in milligrams of oxygen per litre per hour at each treatment level in a dose rate range 0.8-20 mGy/h.

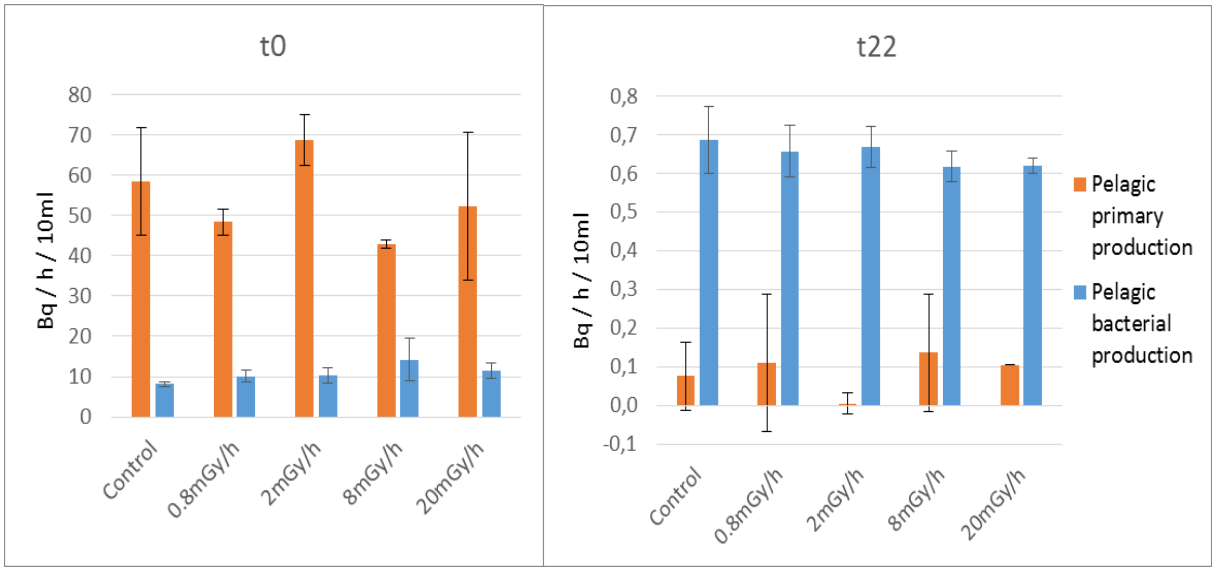

Figure 13. Pelagic primary production and bacterial production at the start (t0) and after gamma irradiation (t22) in a dose rate range 0.8-20 mGy/h (±S.E). Note the different scales on the y-axis. Production is quantified as Bq 14C assimilated per hour by a 10 ml water sample from each cosm.

Benthic results analysed so far show leaf litter degradation was slower in all radiation treatments compared to the controls (Fig. 14), which will lead to differences in the release of particulate and dissolved nutrients into the sediment and the water column, with potential knock-on effects for the biota. Again, we hope the nutrients data in combination with CNP analyses will resolve these patterns.

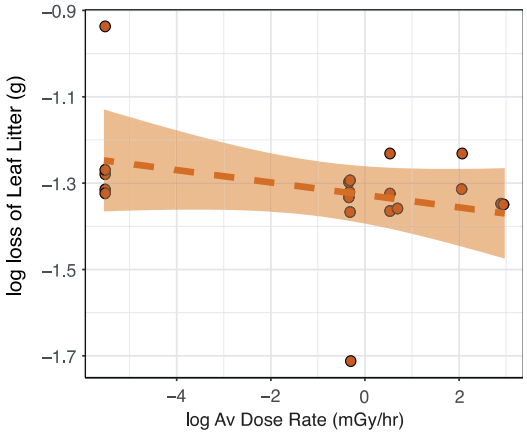

Figure 14. Leaf Litter loss (g) predicted by average dose rate (mGy/h). Dashed lines indicate non-significant relationships.

6. Elements

The ICP-MS analyses revealed no significant differences between concentrations of elements in the microcosms at different treatment levels (Fig. 15). There were however differences in concentrations between the start and the end of the experiment. The dominating elements were Na, Mg, Si, P, S, K and Ca, which are all essential elements in water, structure and biochemistry of all living organisms. Apart from Na and Ca, none of these concentrations changed over time (Fig. 15a). Trace elements Mn, Co and Cd appeared to increase over time (Fig. 15c), whereas Zn and Fe decreased over time (Fig. 15b). All of these elements are essential to aquatic primary producers (Bradshaw et al 2012). The fluctuations are therefore closely tied to the biotic compartments of the cosms. Thorium decreased over time, whereas uranium increased (Fig. 15d). In addition, there was a higher concentration of Th in the highest dose treatments at the end of the experiment.

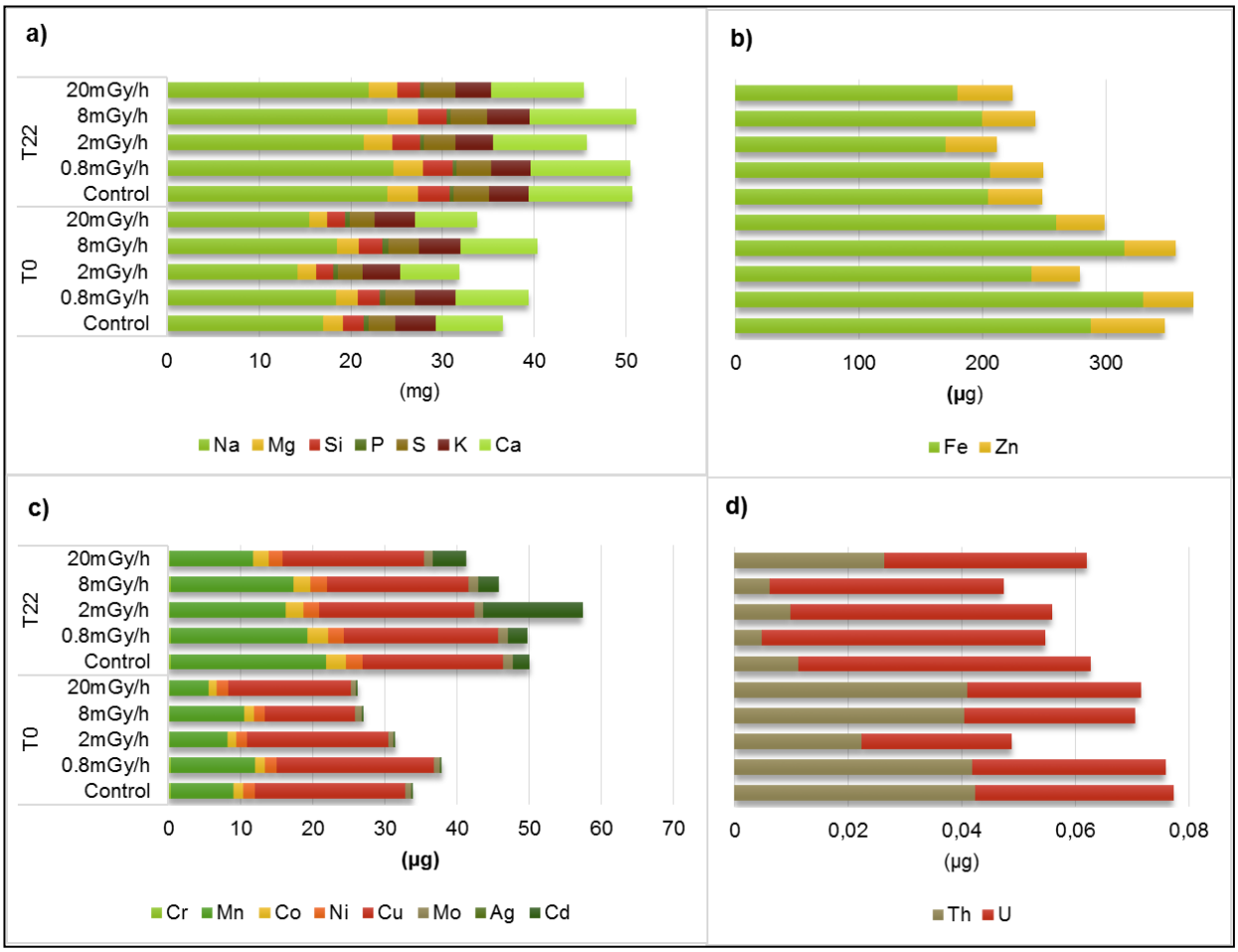

Figure 15. Relative amounts of elements at the different dose levels, 0.8-20 mGy/h, at start of experiment (T0) and end of experiment (T22). Note the different units of measurement, a) milligrams b),c) and d) in micrograms.

7. Carbon, Nitrogen and Phosphorus

Carbon data will be used to quantify changes in biomass and feed into the network analyses. In combination with the grazing rate and production/repsiration measurements they will enable us to quantify C and energy cycling in the ecosystem. Nitrogen and phosphorus will provide information about the nutrient status and cycling in the system.

We are currently analysing a structural network to illustrate the roles that the most affected species have within the community, as well as the cascading effects of radiation resulting from changes in different species (Fig. 16). The network uses the carbon and oxygen data we collected for each compartment of the ecosystem, and by manipulating various parameters, we can investigate which compartments of the ecosystem are the most sensitive or robust, and which interactions have the largest effects. This study will hopefully provide much-needed detailed empirical evidence on how ecological network dynamics change in response to radiation.

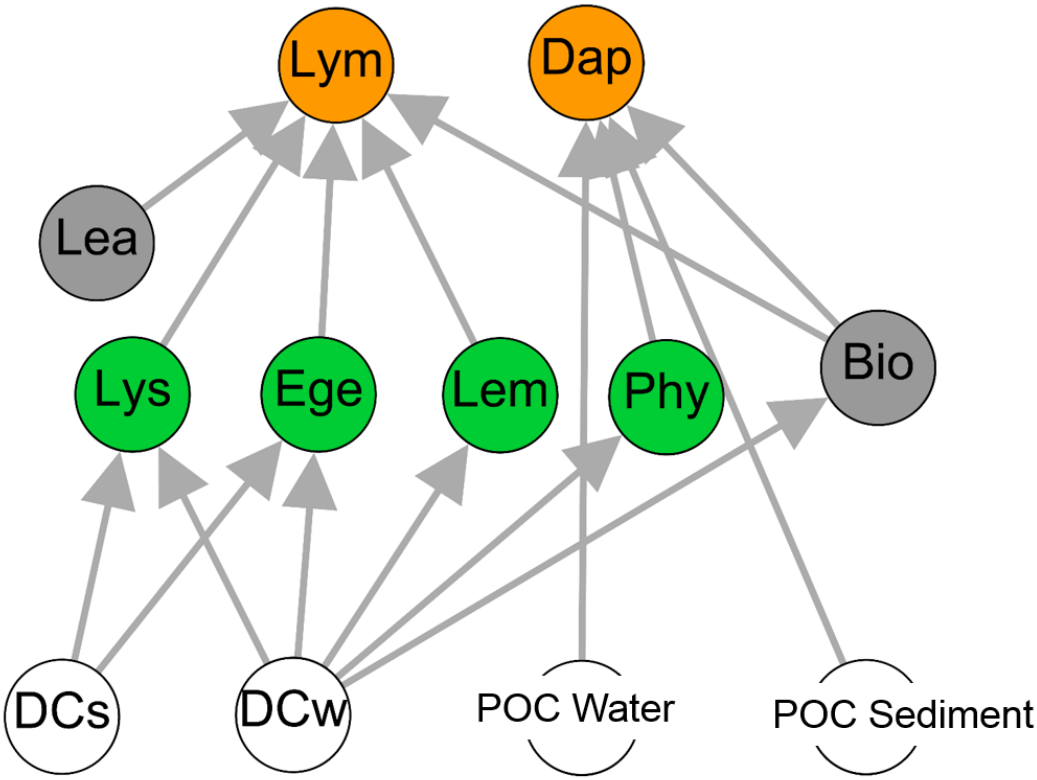

Figure 16. Illustration of the NORCO aquatic ecosystem as a network of carbon pools and fluxes, which will be used in future network analysis.

8. Conclusion

The main advantage of using microcosms is it combines a degree of natural interactions together with experimental control, allowing us to minimize the amount of unexplained variability (Giesy and Odum 1980). For radioecological studies, microcosms are especially beneficial because the inclusion of interactions between organisms/species and abiotic-biotic interactions have been lacking in radioecology research in recent decades. Including these elements in our study means we could investigate the mechanisms that ultimately determine effects of a stress in an ecosystem.

Ecological processes may both constrain, or override the effects of a stress (Taub, 1997). An ecosystem's ability to cope with stress has been linked to processes that interact. For example, biodiversity is correlated with ecosystem processes and functions, so that loss in biodiversity can result in altered structures (Ferens and Beyers 1972), followed by functional or regime shifts (Folke et al. 2004, Baert et al. 2016). The degree to which this occurs depends on the ecosystem's ''buffering capacity" (Elmqvist et al. 2003, Fester et al. 2015), which may be provided by abiotic parameters, like sediment, that plays a key role in nutrient cycling (Søndergaard et al. 2003), or biotic parameters such as biodiversity and species interactions. We observed a significant stress response of plants at the molecular level that was not so apparent at individual or population level, and different effects in *D. magna* and *L. minor* to those seen in previous single species studies using the same or similar dose rates. We argue that when species are exposed to radiation in single species tests, they are without a "buffering capacity" which can make them more or less sensitive.

Both ecosystem level and individual measurements from microcosm tests are important for assessing the potential effects of a stress to the environment. The lack of correspondence between individual and population level responses in our study support the hypothesis that effects at one level cannot always be used to infer effects at another level (Stay et al. 1988). Indeed, microcosms, with their multi-species approach, have different interactions, processes and mechanisms that single species tests fail to encompass. Microcosms allowed us to isolate specific relationships between interacting species in an ecosystem and test the effects of radiation on them, both direct and indirect. In addition, the dose range we used was within the range of those seen at contaminated sites (Geras'kin, 2016). This kind of experimentation is vital for future radioecological studies that have been very much limited to high dose rates and single species studies.

The few irradiation exposure facilities that exist for radioecological studies are generally based on a similar design – a radiation point source producing a radiation field that is strongest closest to the source and weakest furthest away. Thus, the geometry of the radiation field always means that there will be less space for the higher dose treatments, and thus less possibility for extensive replication. However, higher dose treatments are important since, even if they are not so environmentally relevant, they enable a larger radiation gradient (i.e., more of the dose response curve) to be investigated, which is essential for drawing wider conclusions about radiation effects.

More radioecology microcosm studies are needed in order to build up a larger knowledge base on the ranges of dose rates where effects of radiation on species interactions and community- and ecosystem-level endpoints can be measured.

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Title	Final report for Nordic freshwater ecosystem microcosms pilot study.
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Abstract max. 2000 characters	Ecosystem response due to radiation exposure depends on the different species sensitivities and the multitude of direct and indirect pathways by which individual organisms can be affected, including the potential for complex interactions across multiple trophic levels. Multi-species model ecosystems (microcosms) were used to investigate effects of ionizing radiation on a model aquatic ecosystem including indirect effects caused by ecological interactions. Microcosms were exposed for 22 days to a gradient of gamma radiation with 4 decreasing dose rates (20, 8, 2, 0.8 mGy/h). A range of endpoints were measured at different time points in order to capture effects on individual components of the ecosystems as well as whole-ecosystem processes. Individual and population growth was measured for all species; species interactions were measured in the form of grazing rates, whole ecosystem respiration and production were quantified; and measurements ecosystem elements, nutrients status and cycling were collected. Plant growth rates were generally lower in the irradiated treatments. Several photosynthetic parameters were negatively affected by radiation in a dose-dependent manner and ROS production increased with radiation dose in *L. minor*. Primary production decreased in all treatments during the first week and remained low for the duration of the experiment. Primary consumers were not effected by dose rates, however their impact on primary producers was significant. Abiotic measurements revealed simialr conditions between all microcosms. By taking an ecosystem approach, this study shows that the net effect of radiation in a simple aquatic ecosystem is a combination of the direct effects of radiation to the individual species with different relative radiosensitivity, and the indirect effects mediated by ecological and abiotic processes.
Key words	radioecology, radiation, microcosm, freshwater, ecosystem