



Freezing prevents $^{60}\text{Co}-\gamma$ irradiation artefacts in DOM concentration, composition and $\delta^{13}\text{C}$

A study of the effects of $^{60}\text{Co}-\gamma$ irradiation on stable isotopes

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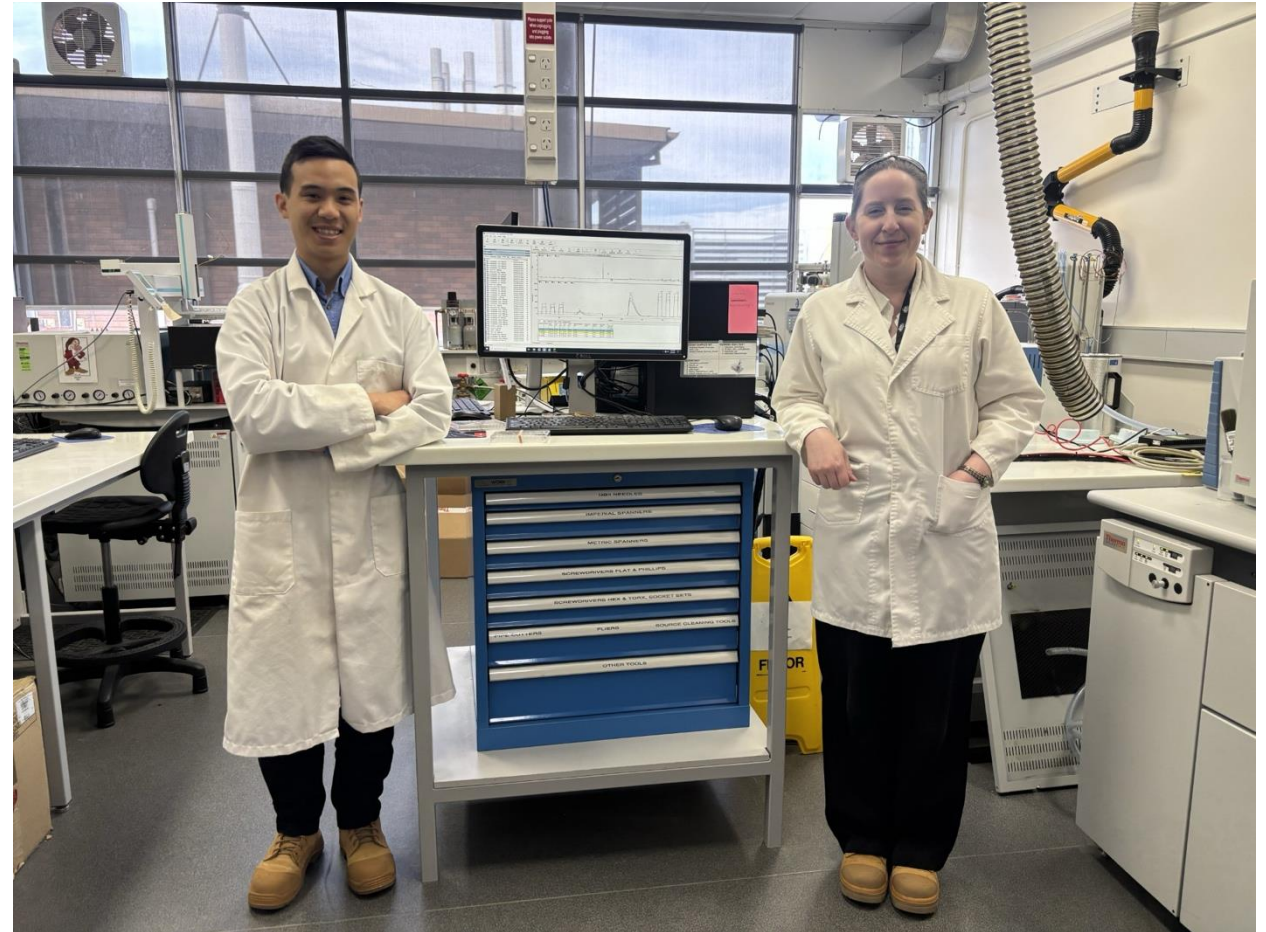
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Science. Ingenuity. Sustainability.

Background

- Stable Isotope Lab at the Australian Nuclear Science and Technology Organisation (ANSTO)
- Part of the Environment Research and Technology Group (ERTG)





- Environmental samples are brought back by researchers from field work

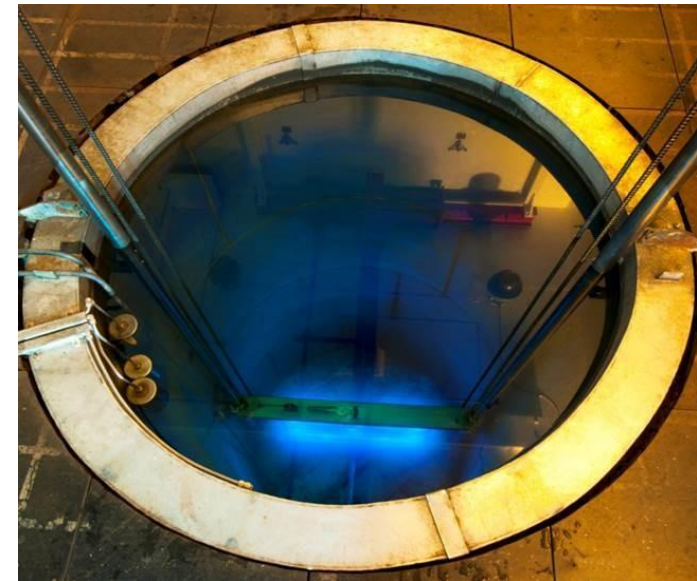


- Some samples are collected from outside Australia



Gamma Technology Research Irradiator (GATRI)

- Australian biosecurity requires sterilisation of samples
- GATRI is a cobalt-60 irradiator
- Consisting of 100 sealed sources in a 1 sqm rack, stored in a pool 5 metres deep.
- For irradiation, the source rack is raised out of the pool into a bunker room with concrete walls 1.5 metres thick.



Background

- Samples imported from outside Australia need irradiation to comply with biosecurity requirements (γ -irradiation, 50 kGy)
- γ -irradiation is widely used for sterilisation of water, biological materials, and environmental samples across multiple disciplines
- Scientists conducting nutrient analysis may sterilise samples to prevent bacterial activity in storage.
- Irradiation has the potential to affect stable isotopic signatures
- The structure of the organic matter molecules can affect the degree of fractionation

A study was conjured up

- Quantify the magnitude of $\delta^{13}\text{C}$ fractionation in DOC and DIC between unirradiated water samples and those γ -irradiated at a standard biosecurity dose (50kGy)
- Compare the effects of γ -irradiation on isotopes in water when frozen or liquid
- Why/how fractionation occurs
- Determine the best practice to sample, preserve, and store samples







Water Research
Volume 292, 15 March 2026, 125308



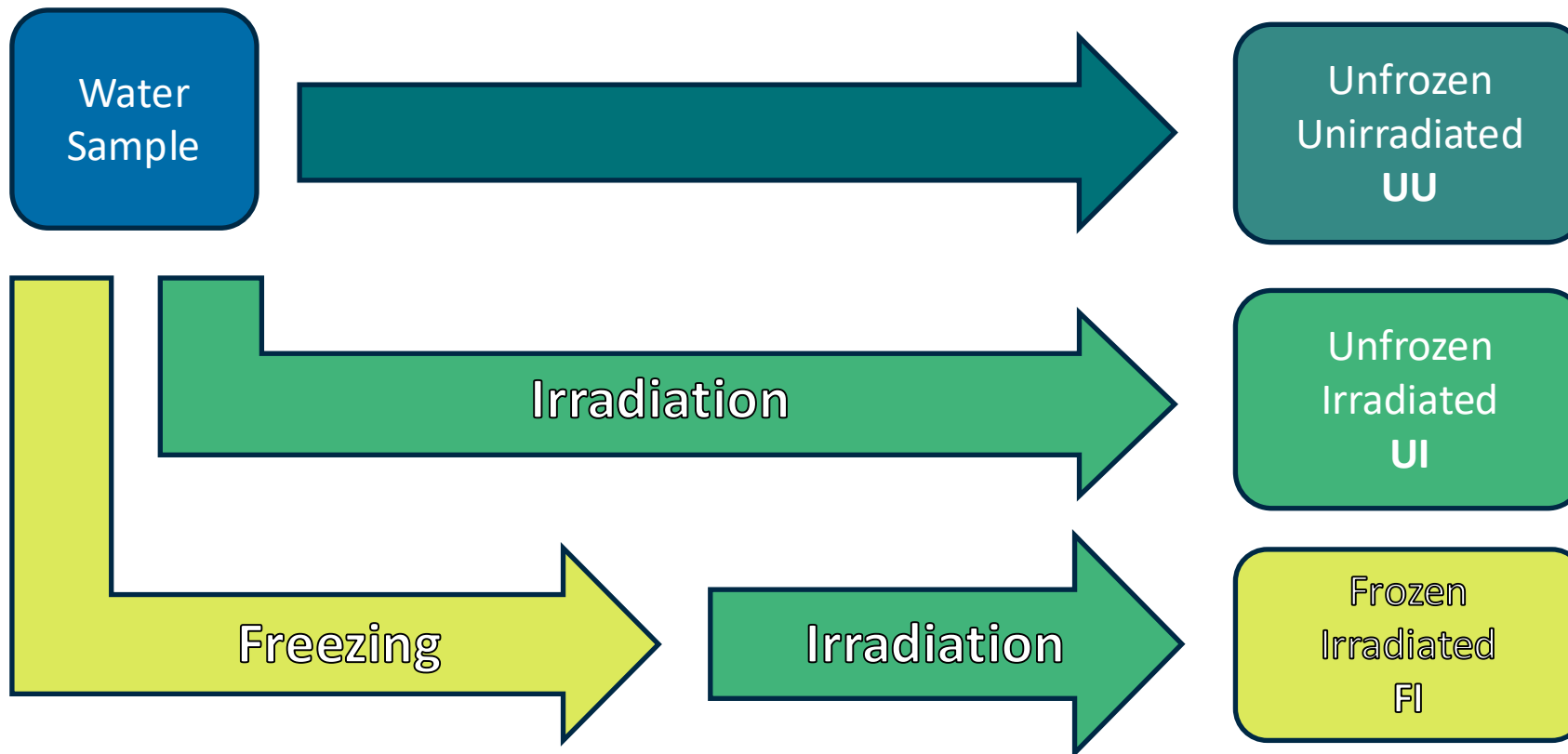
Freezing prevents ^{60}Co - γ irradiation artefacts in DOM concentration, composition and $\delta^{13}\text{C}$

<https://doi.org/10.1016/j.watres.2025.125308>

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Experiment

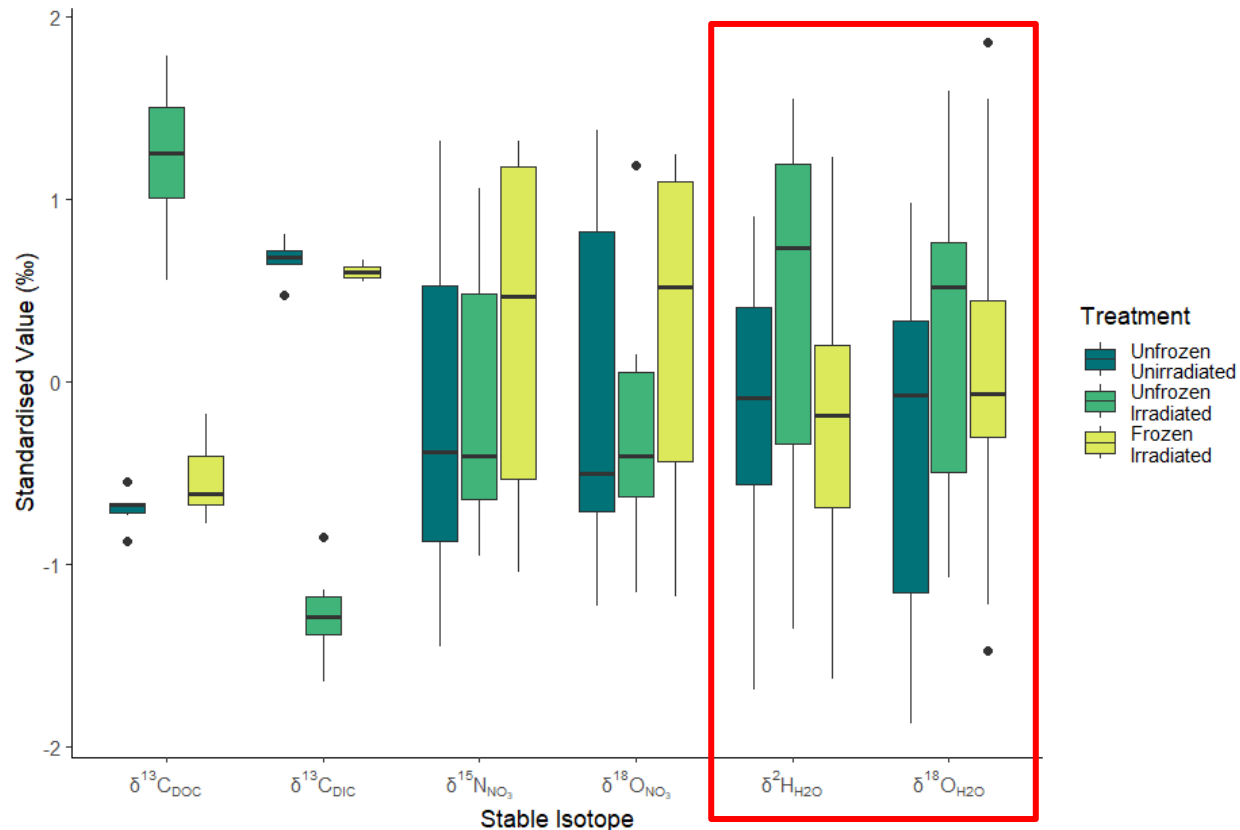
- Samples made up from river water and IHSS organic matter standards mixed into Milli-Q.
- Samples γ -irradiated at 50 kGy ^{60}Co source at GATRI ANSTO



Analyses:

- DOC concentration and characterisation.
- Major ions
- **Stable Isotopes**

$\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^2\text{H}_{\text{H}_2\text{O}}$



- Analysed at ANSTO on a Picarro L2130-I Isotopic H₂O Cavity Ring-Down Spectrometer

- No significant difference between irradiated and unirradiated samples

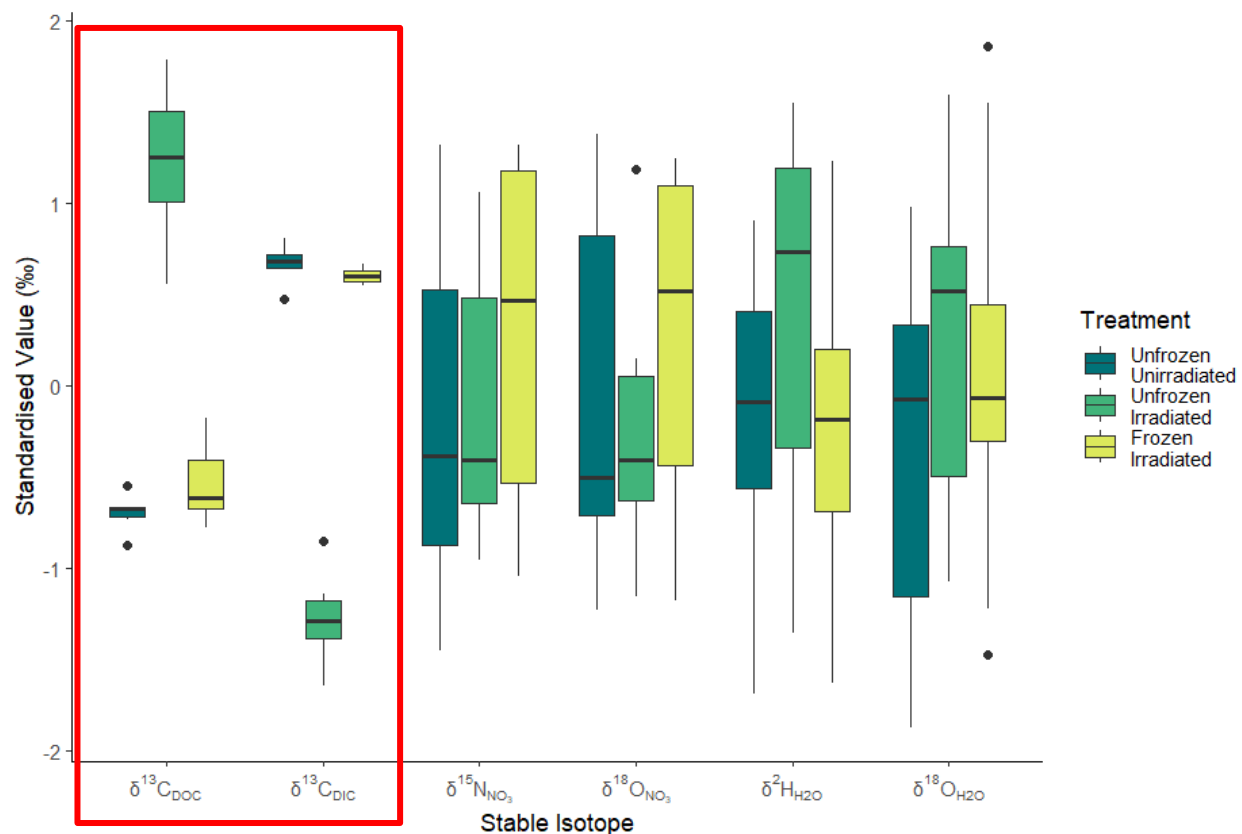
- Follow normal good practice

Unfrozen
Unirradiated
UU

Unfrozen
Irradiated
UI

Frozen
Irradiated
FI

$\delta^{13}\text{C}_{\text{DOC}}$ and $\delta^{13}\text{C}_{\text{DIC}}$



- $\delta^{13}\text{C}_{\text{DIC}}$ Analysed at ANSTO on GasBench II coupled Delta V Advantage IRMS

$\delta^{13}\text{C}_{\text{DOC}}$

- UI samples were on average 5.0 ‰ and 4.7‰ higher than UU and FI

$\delta^{13}\text{C}_{\text{DIC}}$

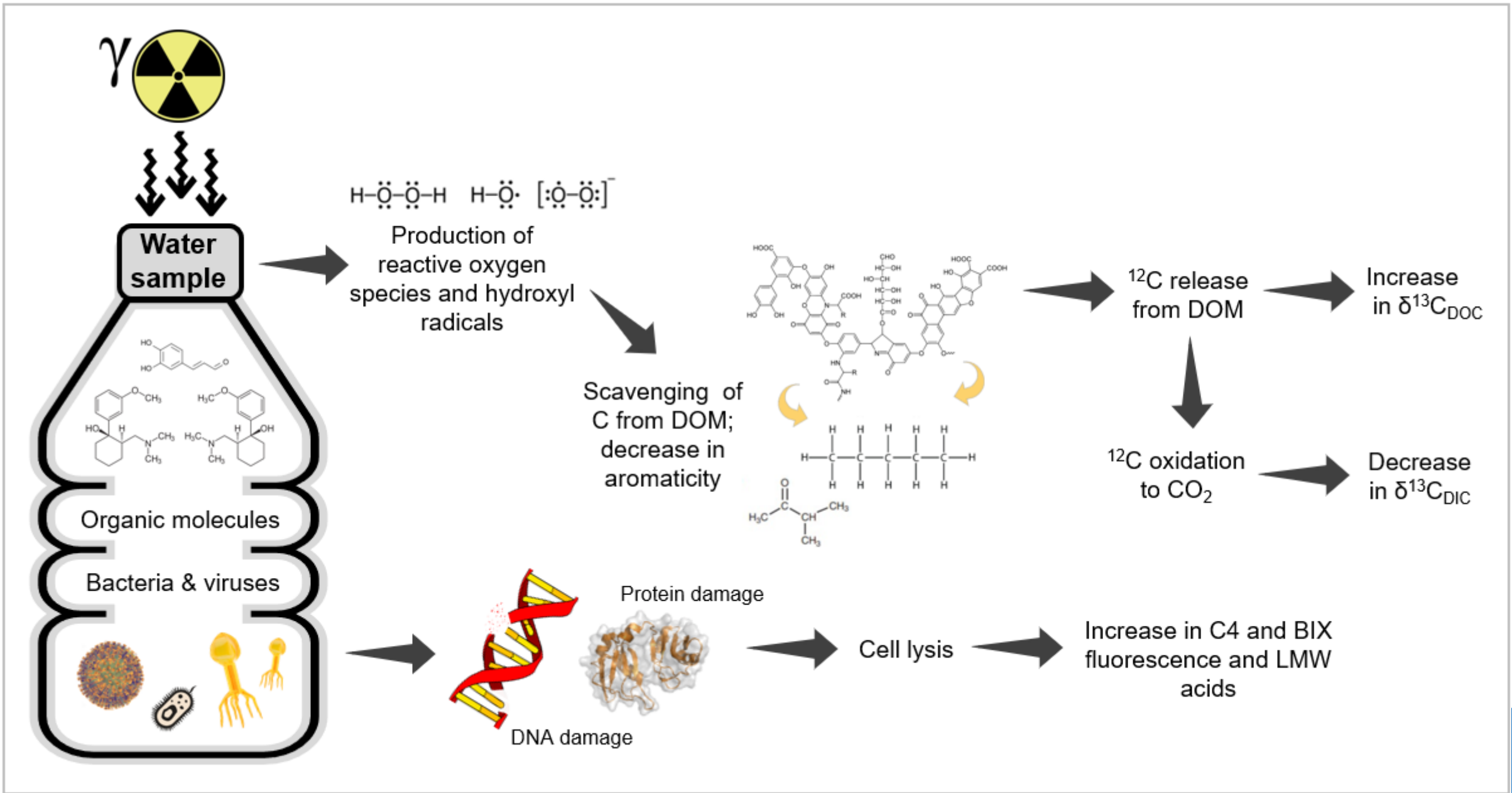
- UI samples were on average 9.0‰ and 8.6‰ lower than UU and FI

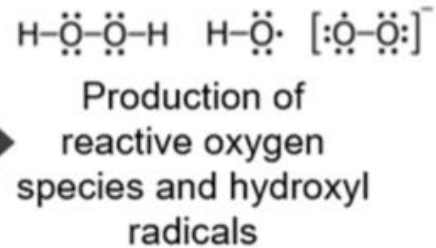
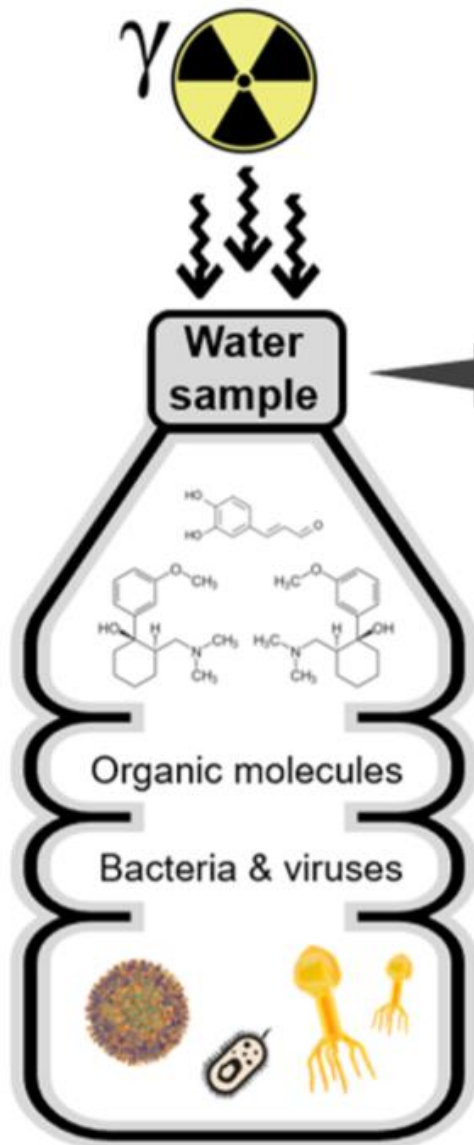
- No significant difference between Carbon isotopes of UU and FI

Unfrozen
Unirradiated
UU

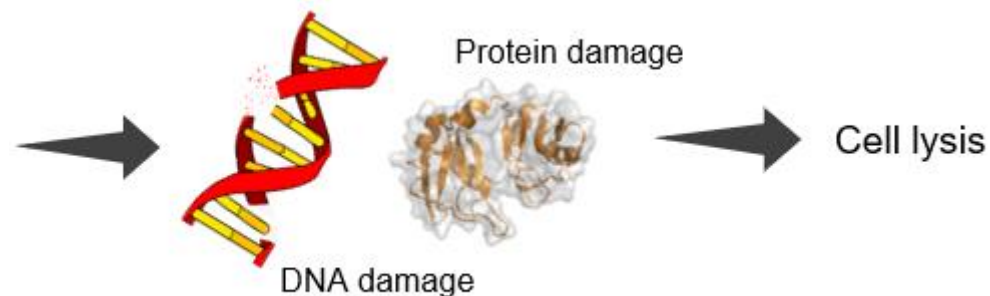
Unfrozen
Irradiated
UI

Frozen
Irradiated
FI

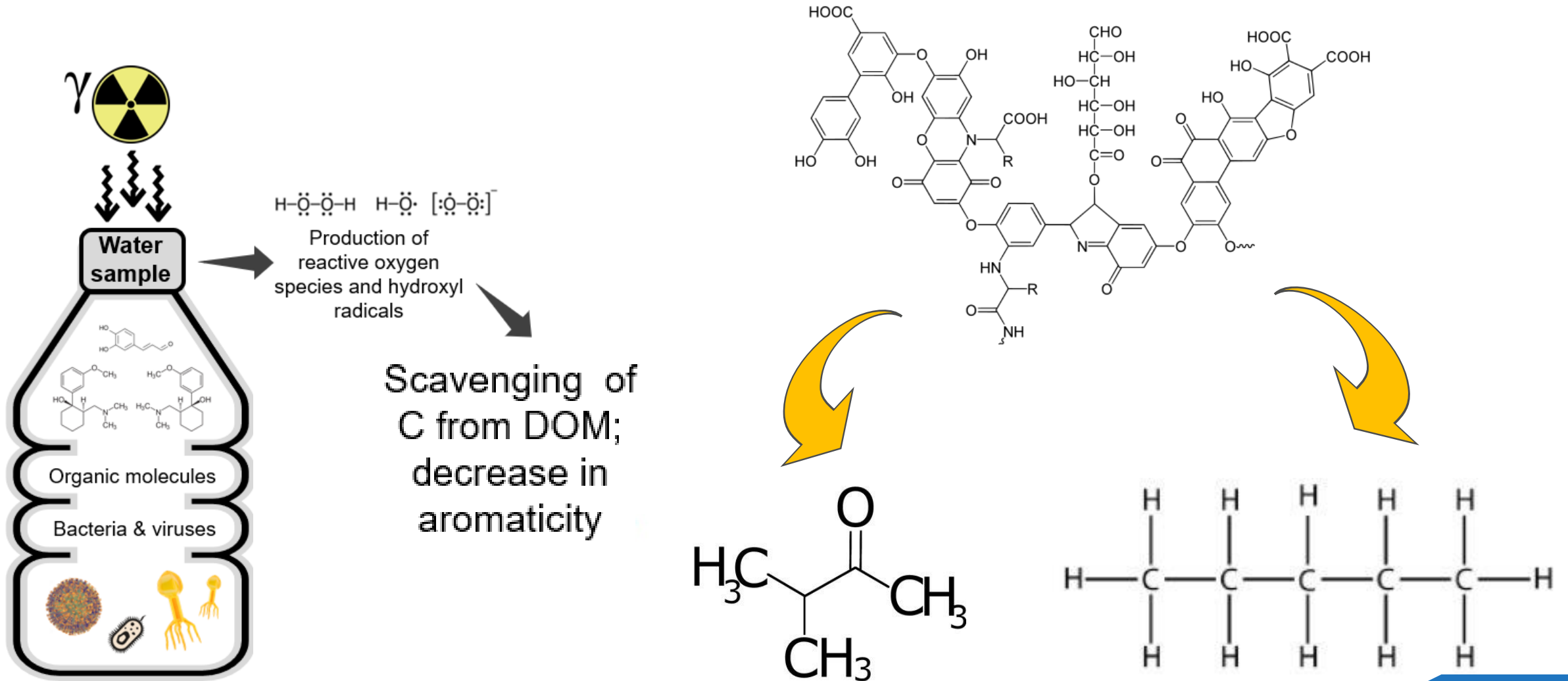




- Environmental water samples carry organic molecules as well as bacteria & viruses
- Irradiation of sterilises the samples, but also produces reactive oxygen species and hydroxyl radicals

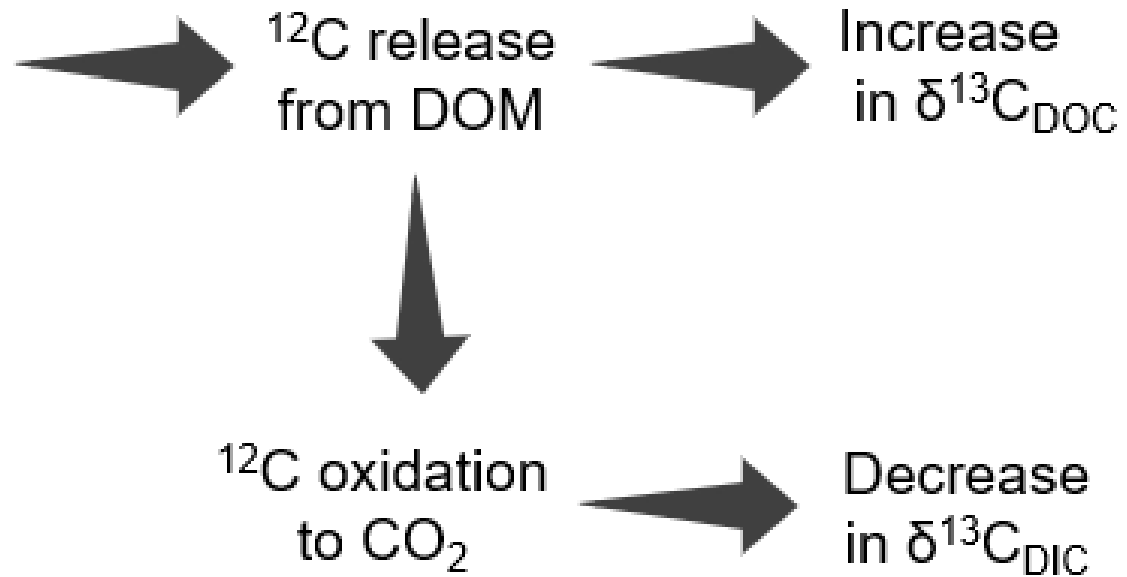
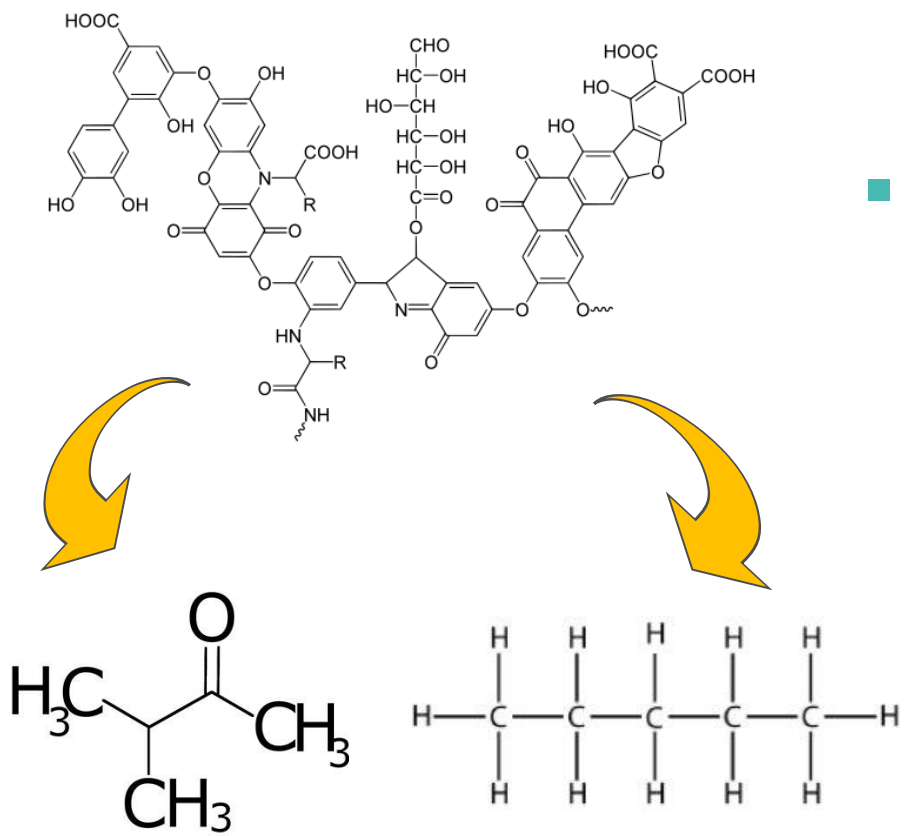


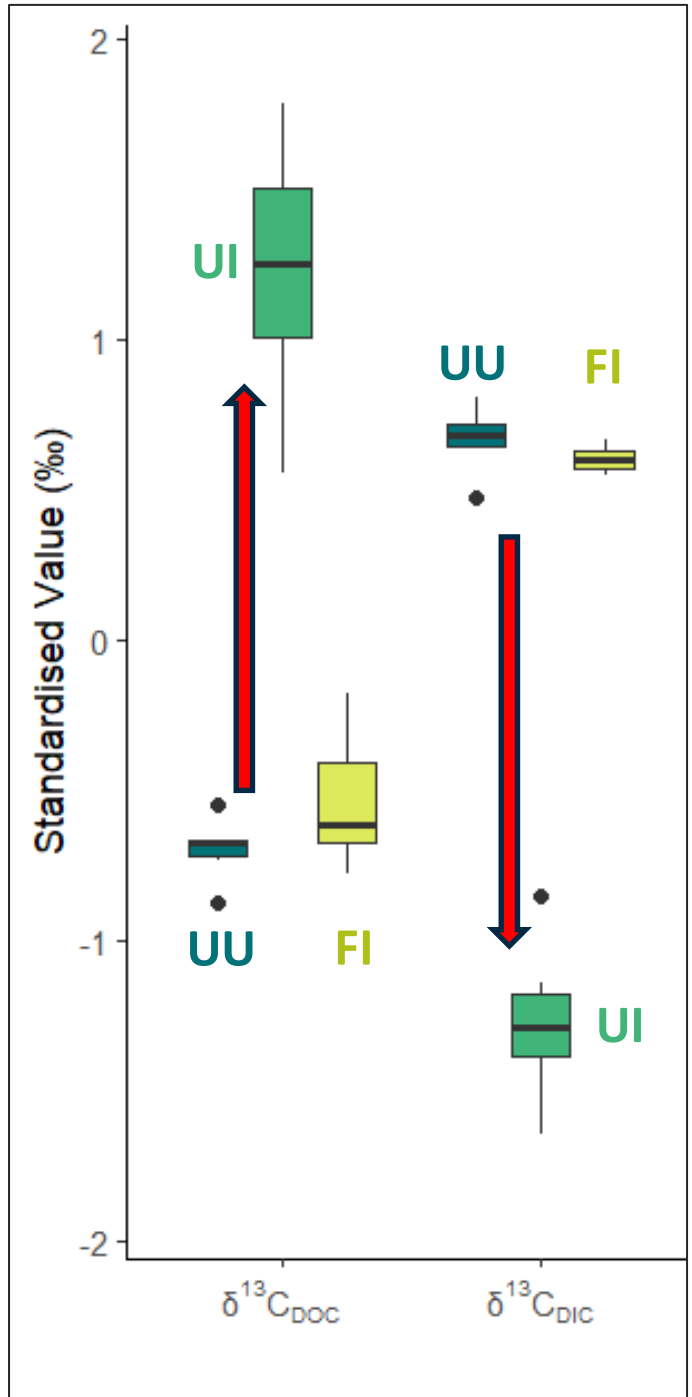
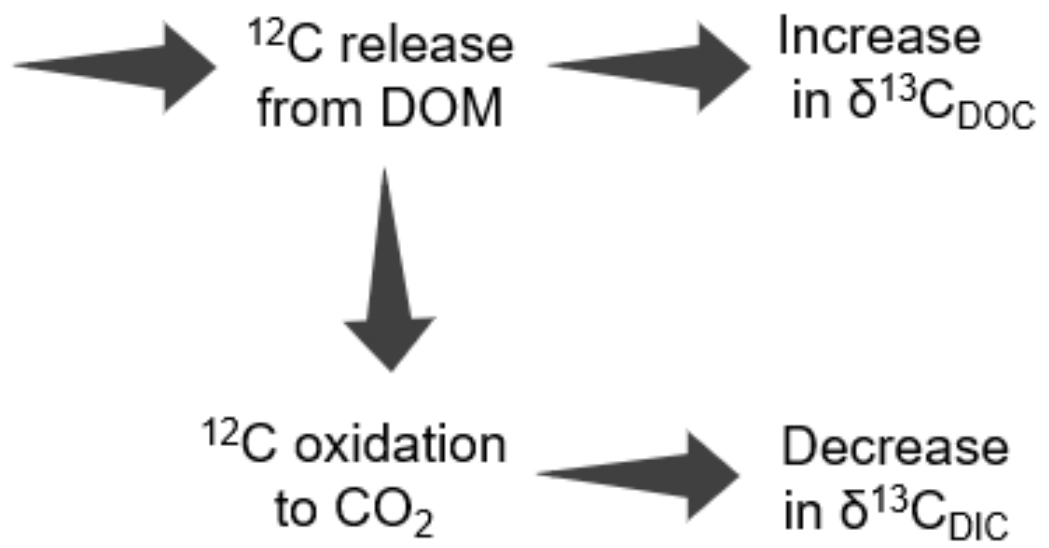
- Complex organic molecules are broken apart by reactive oxygen species and hydroxyl radicals



- Lighter ^{12}C is preferentially released from DOM and oxidised to make CO_2 , Resulting in decreased DIC isotope ratios

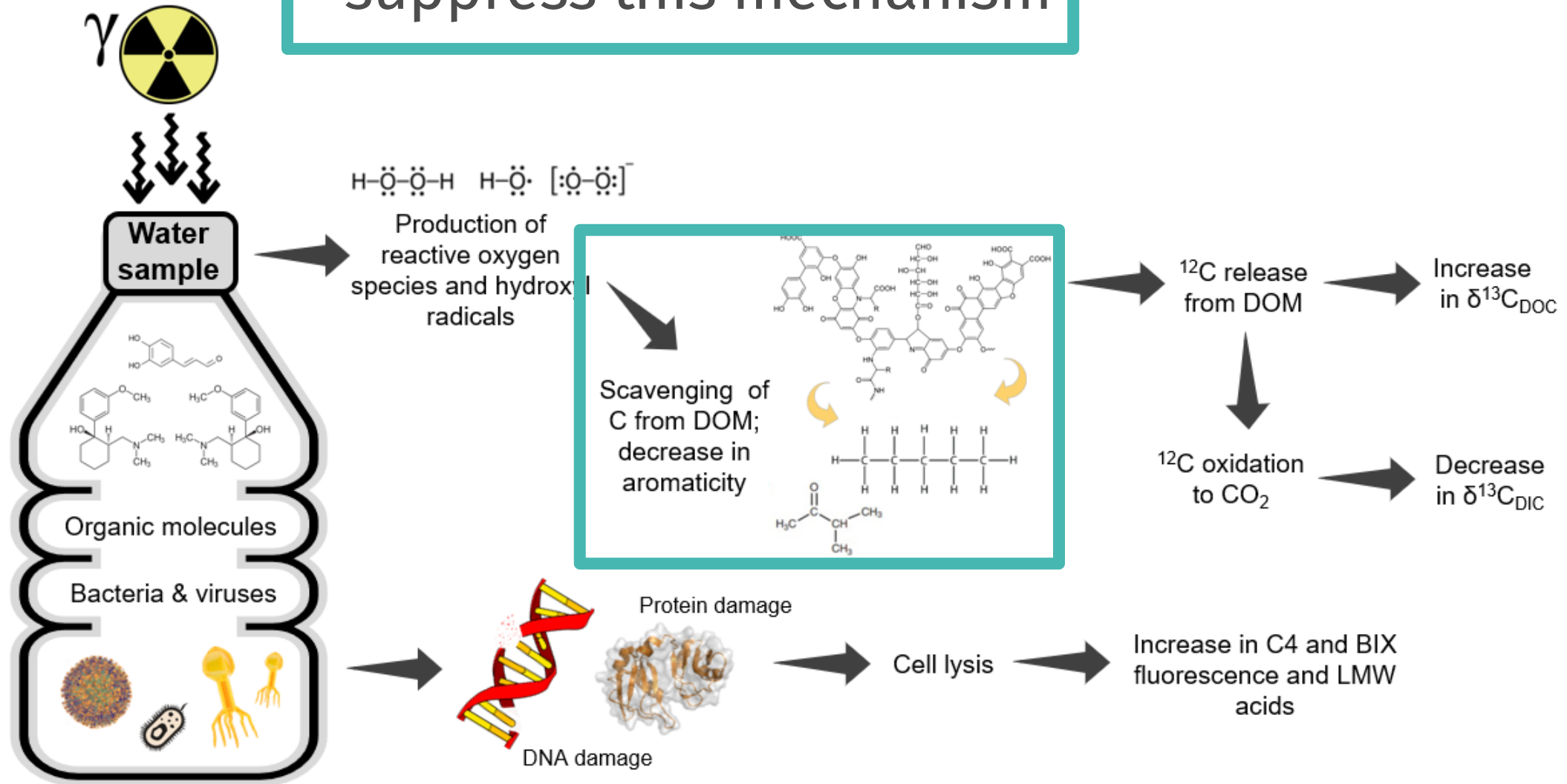
- The remaining DOC is left with heavier ^{13}C , resulting in an increase in DOC isotope ratios



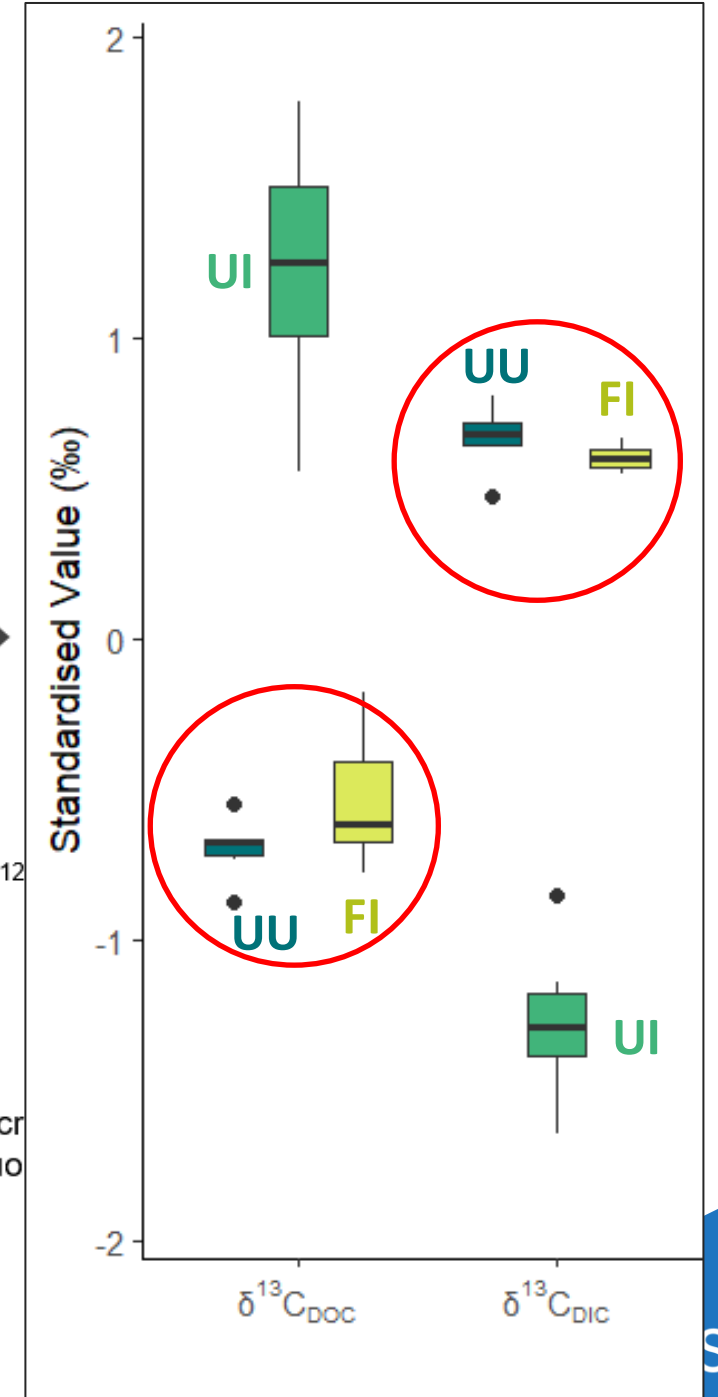
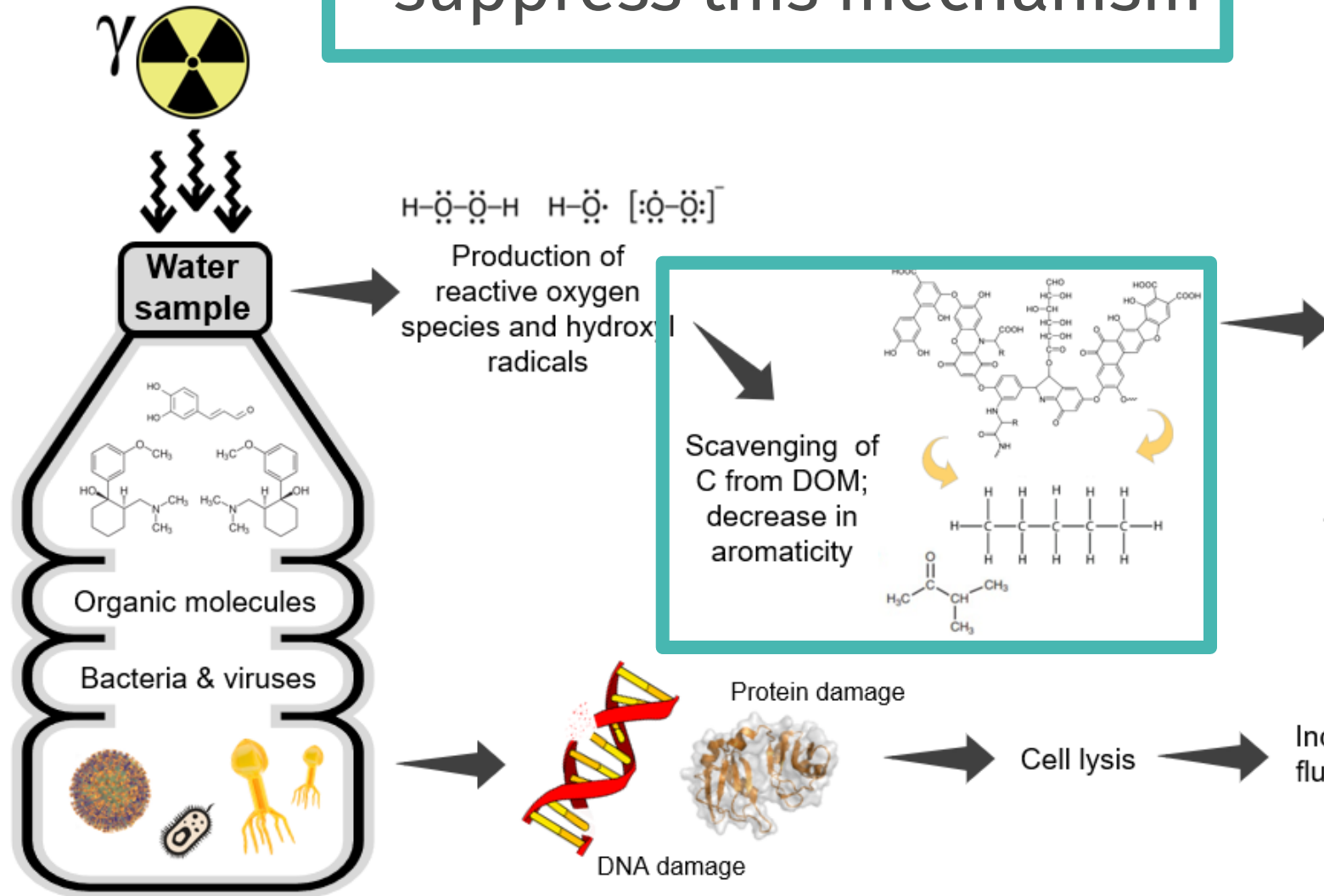


- Unfrozen Unirradiated UU
- Unfrozen Irradiated UI
- Frozen Irradiated FI

- Freezing the sample will suppress this mechanism



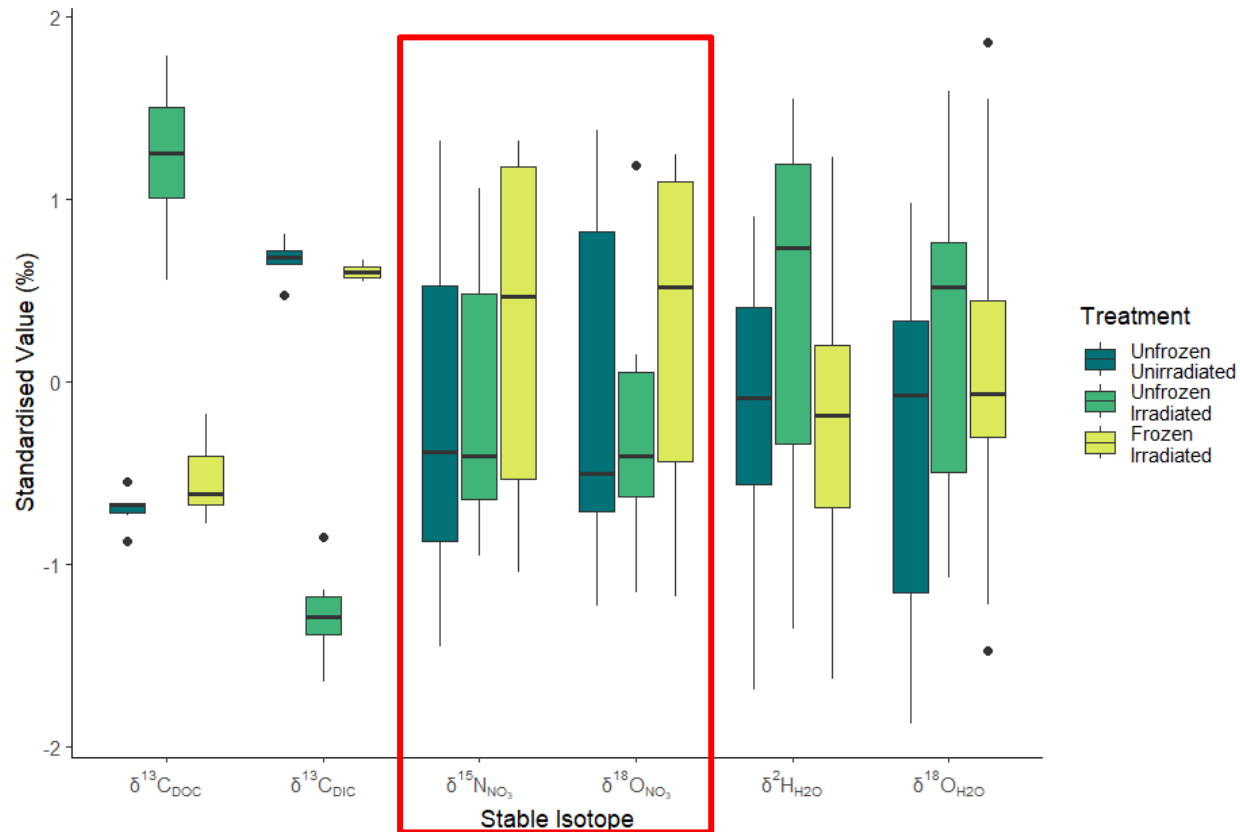
Freezing the sample will suppress this mechanism



Summary of Results

- Stable water isotopes were not significantly impacted by γ -irradiation
- γ -irradiation heavily impacts the isotopic composition of carbon analytes ($\delta^{13}\text{C}_{\text{DOC}}$, $\delta^{13}\text{C}_{\text{DIC}}$). Affected results could be misinterpreted for natural, environmental processes
- **Freezing the sample during irradiation preserves the composition**
- Other forms of irradiation may have similar albeit smaller impacts (such as sunlight)
- This study demonstrates an exaggerated example of improper sample handling and storage

$\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$



- Analysed at a university lab using a bacterial denitrification method
- NO_3 Isotopes inconsistent changes in isotopic values across treatments and replicates.
- Statistical insignificance presented in the graph
- Further in-depth look required

Unfrozen
Unirradiated
UU





Unfrozen
Irradiated
UI

Frozen
Irradiated
FI

Acknowledgements

- Study was supported by ARC SRIEAS grant and Securing Antarctica's Environmental Future (SAEF)
- Chris Dimovski (ANSTO)
- Narelle Hegarty (ANSTO)
- Chris Vardanega (ANSTO)
- Patricia Gadd (ANSTO)
- Andy Baker (UNSW)
- Martin Andersen (UNSW)
- Helen Rutledge (UNSW)
- Elizabeth Bell (ANSTO)
- Liza McDonough (ANSTO)

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Australian Government

