

The impacts of light and season on isopyrazam degradation in river microcosms

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Background

OECD testing

- ▶ Developed by the **O**rganisation for **E**conomic **C**ooperation and **D**evelopment and carried out by industry
- ▶ Provide a number of tests to determine the **risks** of chemicals to the **environment** and **human health**
- ▶ Test **308** specifies water and sediment are incubated in the **dark**



Source: ibacon.com

OECD testing

- ▶ How relevant and **reproducible** are the OECD tests?
- ▶ No adequate consideration of:
 - **Light**
 - **Microbial diversity**
 - **Temporal variation**
- ▶ Do these processes need to be taken into **consideration** when carrying out the OECD tests?





Experimental aims and objectives

Experimental aims and objectives

1. How is **isopyrazam degradation** affected by;
 - **Non-UV light**?
 - **Temporal variation**?
2. What is the role of the **microbial community** in these interactions?





Materials and methods

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- ▶ Sample water and sediment from the River Dene, Wellesbourne, UK
- ▶ Every 3 months from June 2014 to April 2016



September 2014



January 2016



Materials and methods

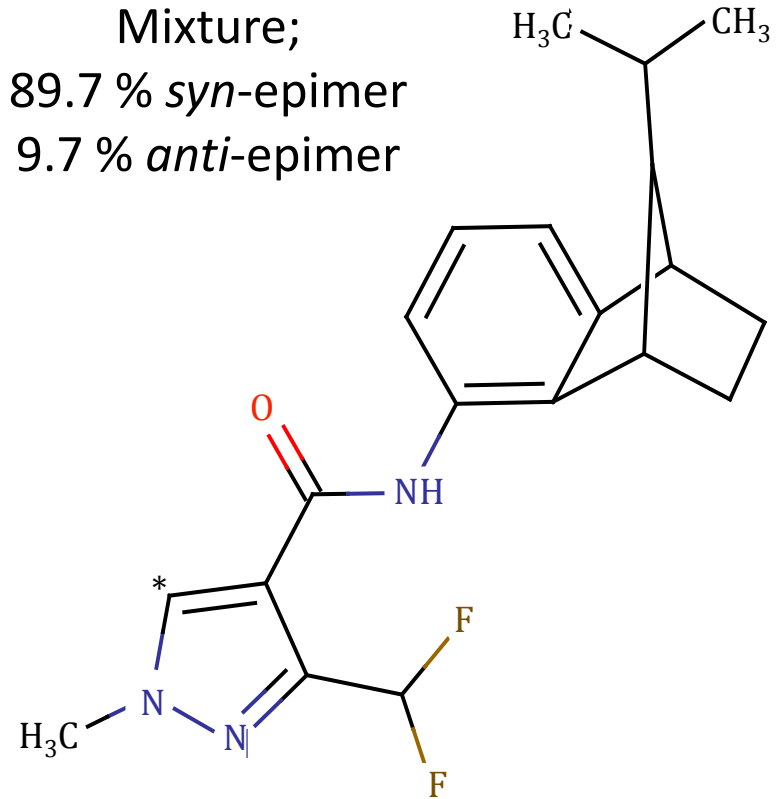
- ▶ All 8 sampling points were assessed using **identical methods...**
- ▶ Pre-incubate the water and sediment for 9 days
 - 16 hour non-UV light cycle, 50 rpm shaker, 20 °C incubation temperature
- ▶ Two separate treatments;
 - **Dark water-sediment**
 - **Illuminated water-sediment**



Materials and methods

- ▶ After 9 days, replace with fresh water and amend with **0.1 mg/L ^{14}C -labelled isopyrazam**
- ▶ Labelled in the **pyrazole ring**
- ▶ Destructive harvests in triplicate at days **9, 18, 27,** and **36**

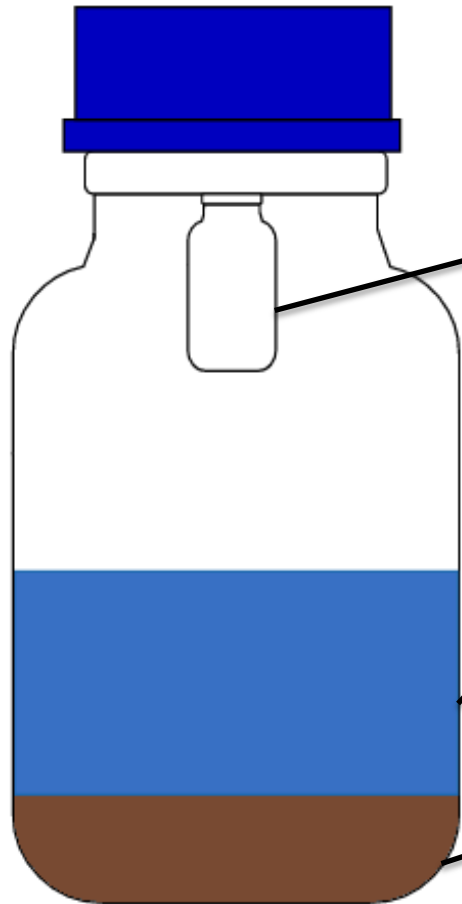
Mixture;
89.7 % *syn*-epimer
9.7 % *anti*-epimer



Created using ChemDraw

Isopyrazam analysis

Carried out at each destructive harvest;



Made using youidraw.com

Gaseous fraction

- ▶ LSC of NaOH traps, which capture $^{14}\text{CO}_2$

Water fraction

- ▶ LSC and HPLC analysis of the pesticide

Sediment fraction

- ▶ Extraction, LSC, and HPLC analysis of the pesticide
- ▶ Combustion to analyse bound residues

Microbial analysis

- ▶ Microbial **DNA extraction** on;
 - a) water and sediment from the **sample site**
 - b) water and sediment from the **microcosms** at the **end** of the experiment
- ▶ Amplification of DNA using 16S and 23S rRNA genes to amplify both **bacteria** and **phototrophs**, respectively

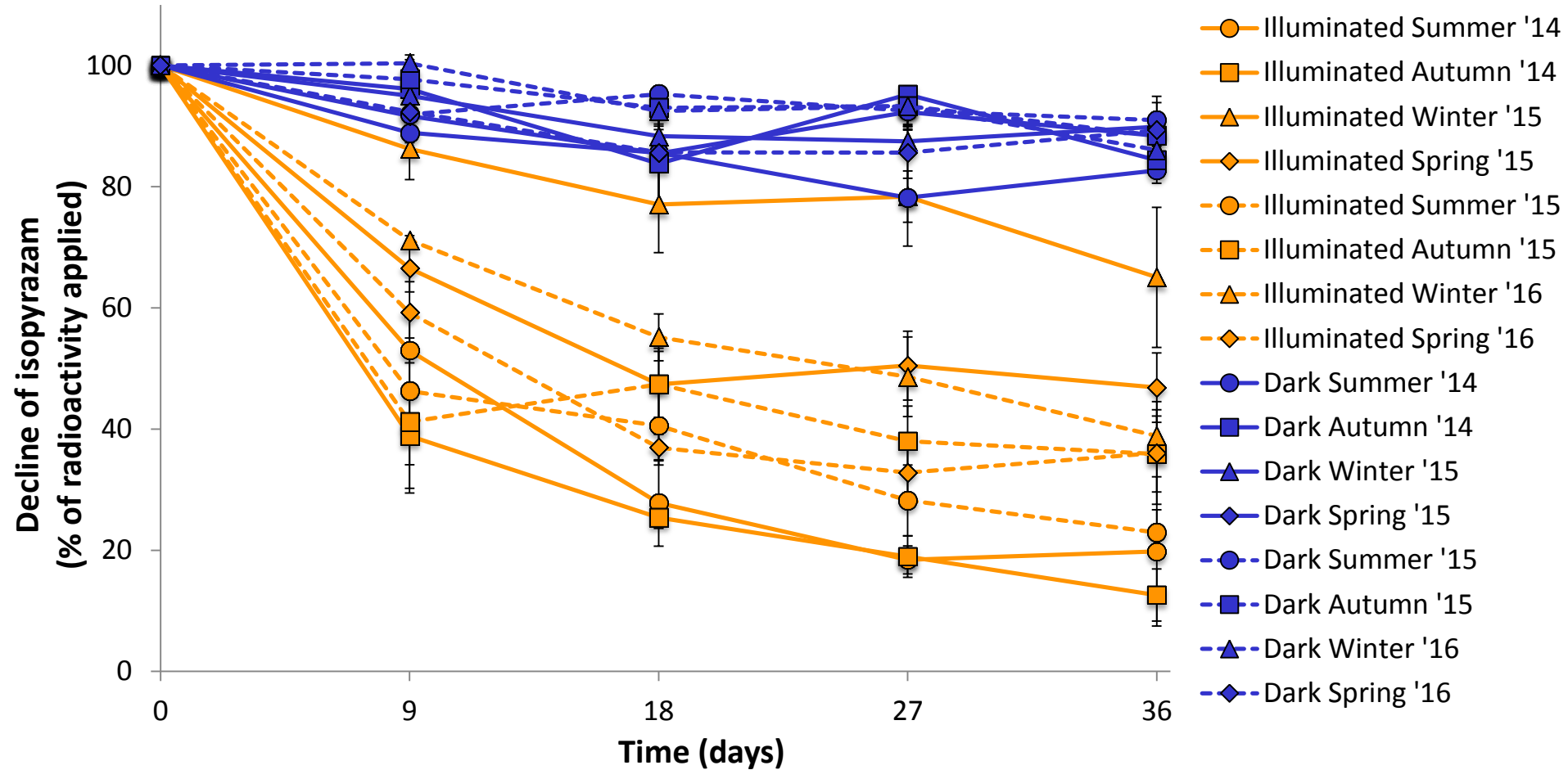




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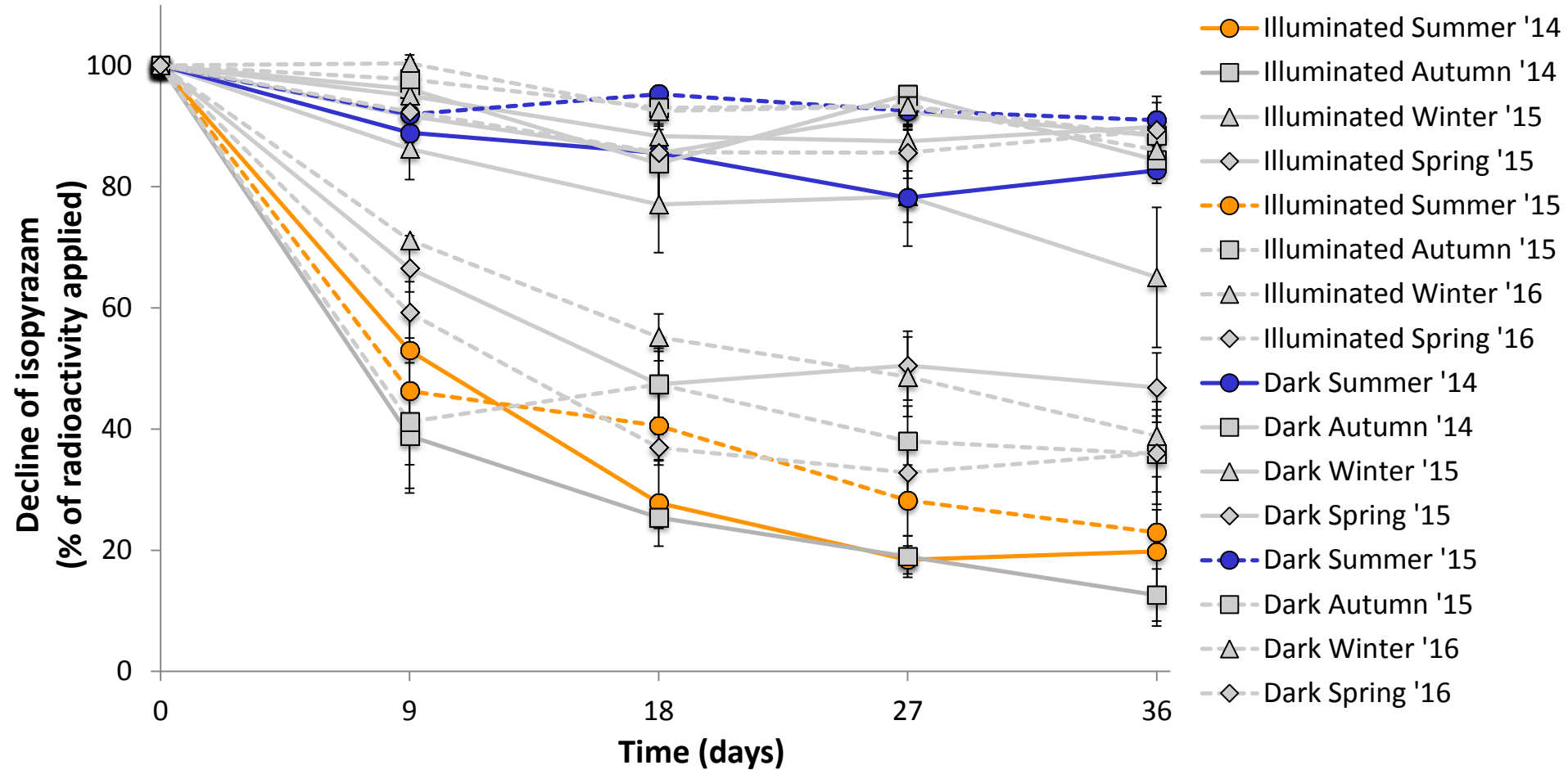
Results

Isopyrazam degradation



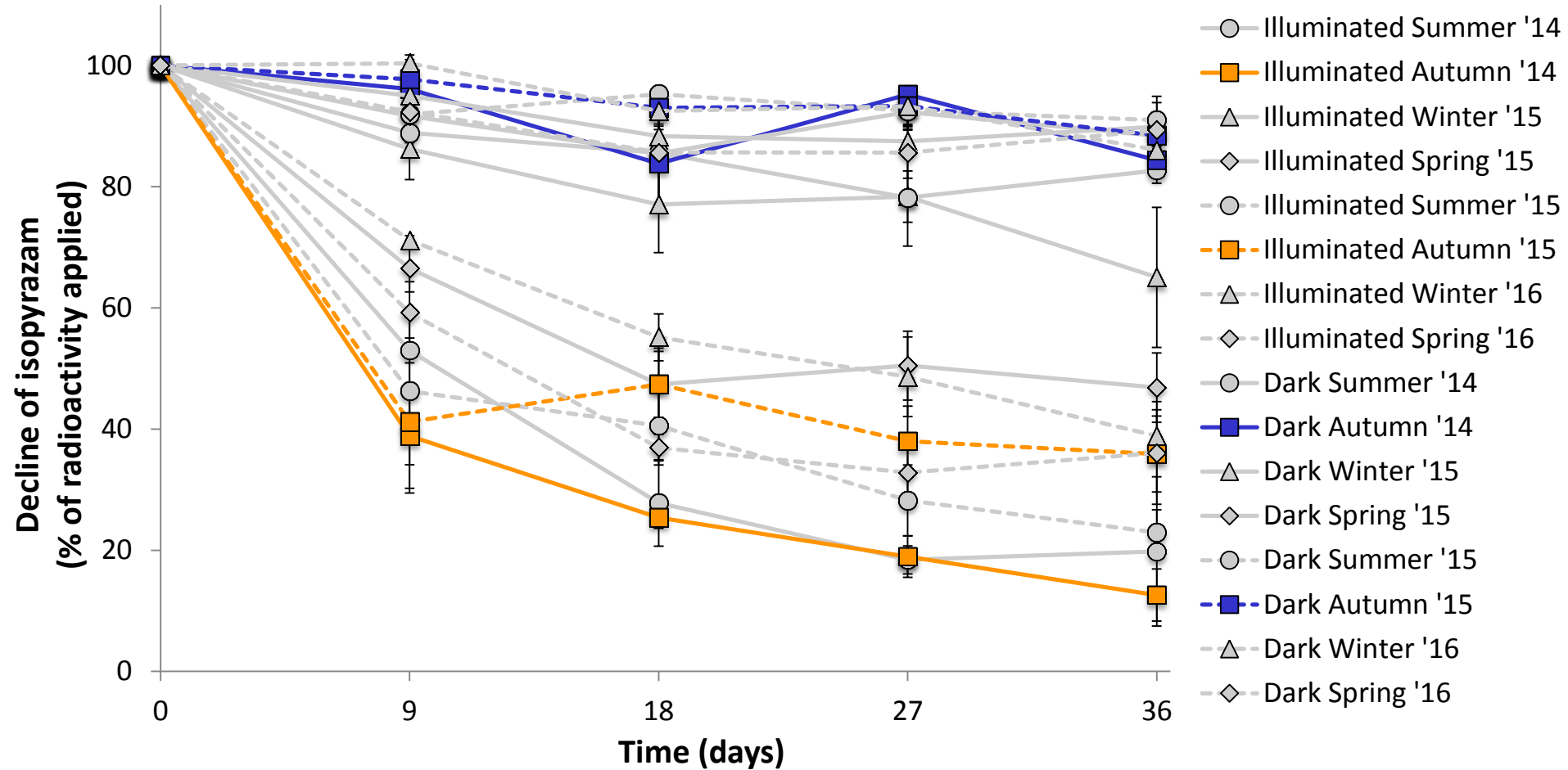
Error bars showing +/- standard deviation

Isopyrazam degradation



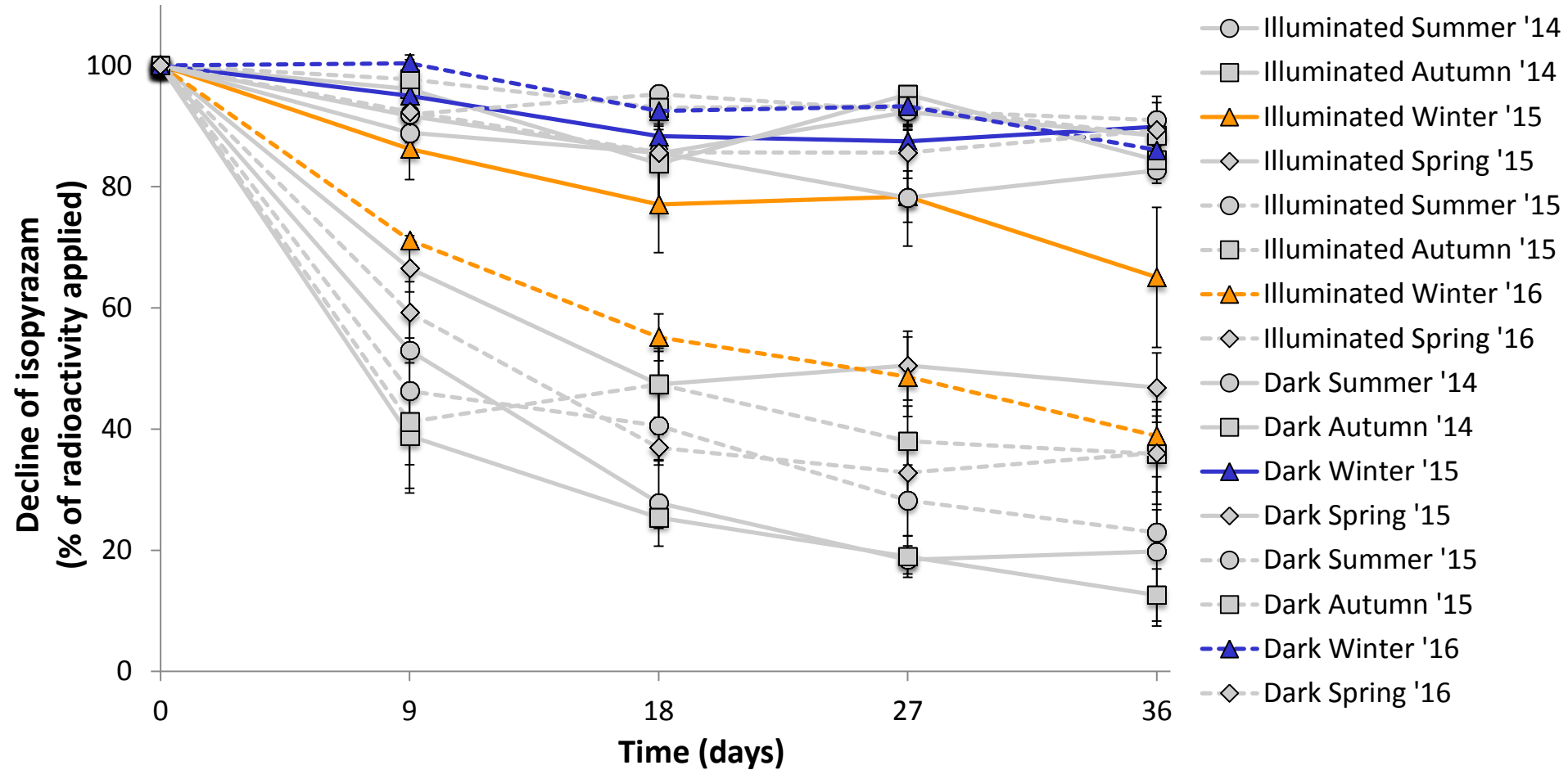
Error bars showing +/- standard deviation

Isopyrazam degradation



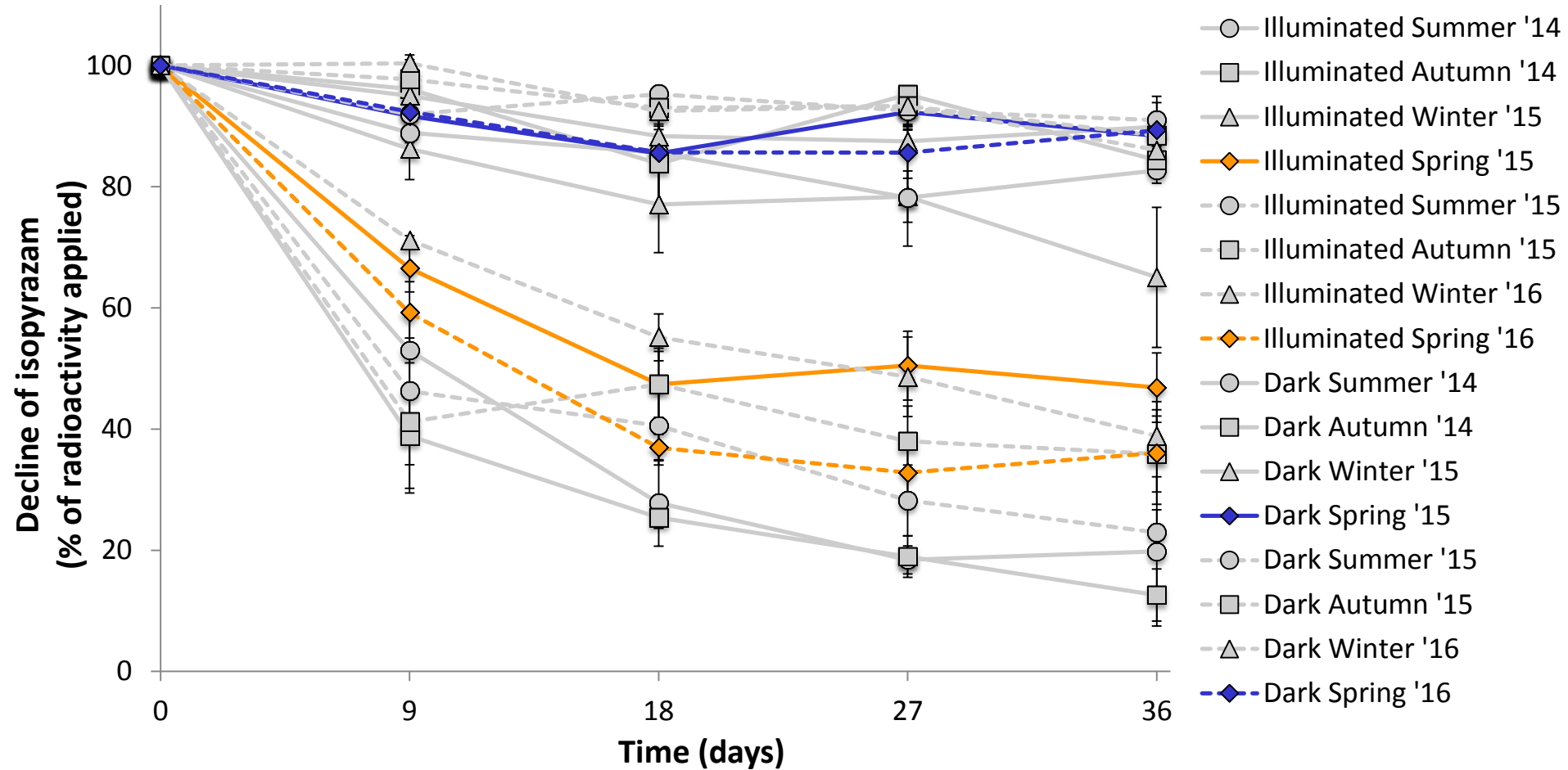
Error bars showing +/- standard deviation

Isopyrazam degradation



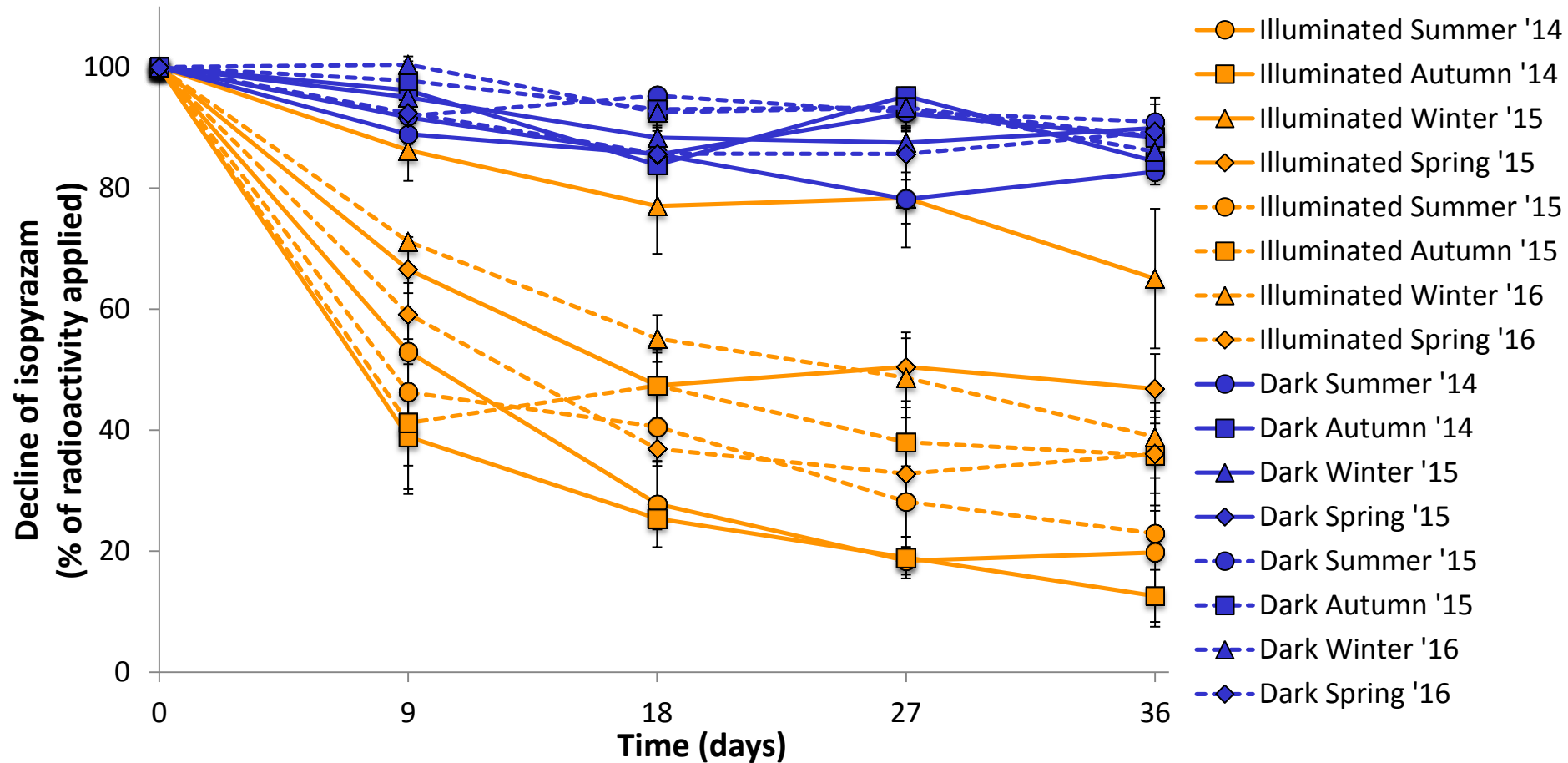
Error bars showing +/- standard deviation

Isopyrazam degradation



Error bars showing +/- standard deviation

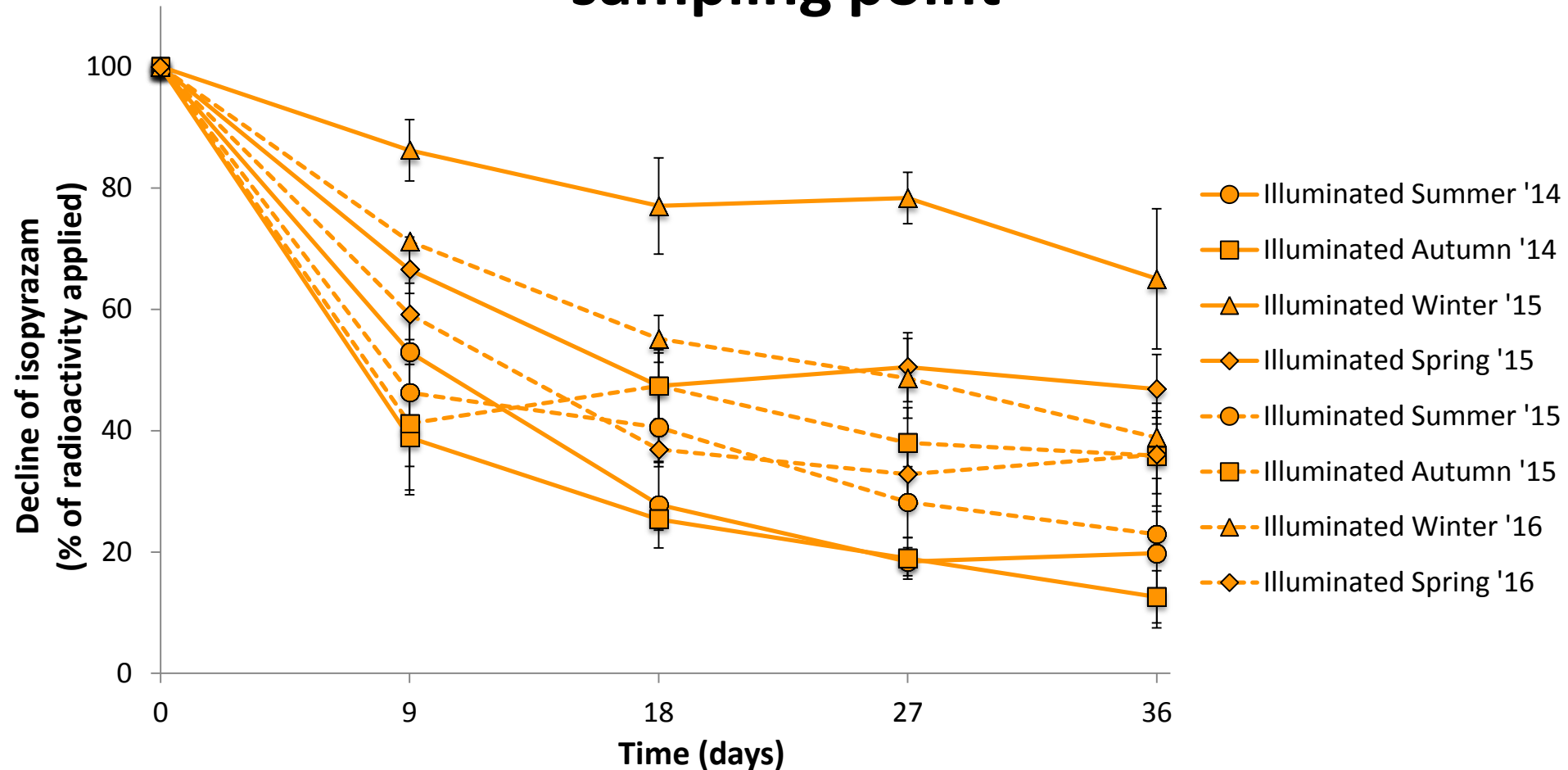
Isopyrazam degradation between light treatment



Significantly **faster** degradation in **illuminated** systems compared to dark
No difference in degradation between sampling points in the **dark** microcosms

Error bars showing +/- standard deviation

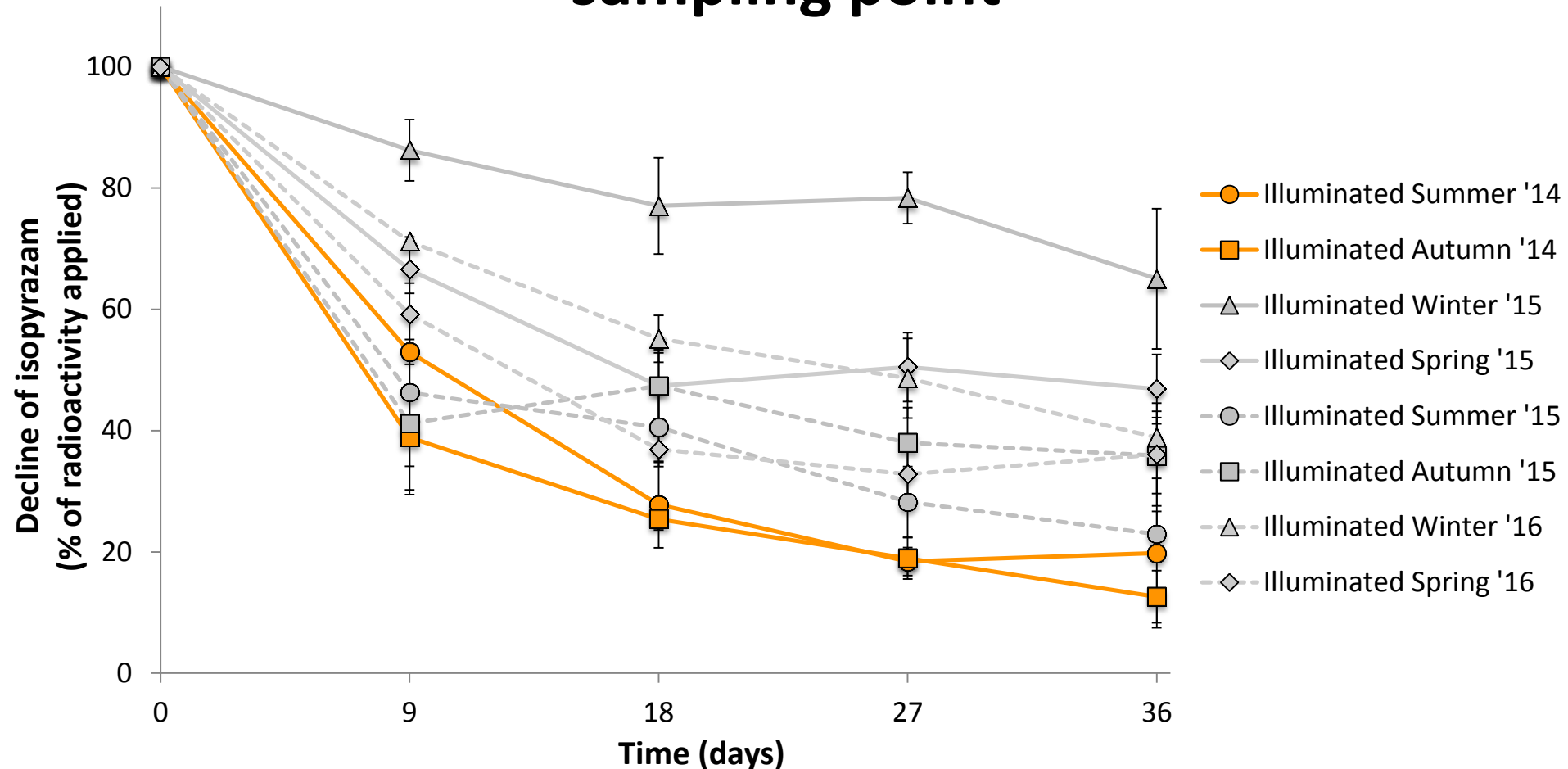
Isopyrazam illuminated degradation between sampling point



Variable degradation in the illuminated treatments between sampling points

Error bars showing +/- standard deviation

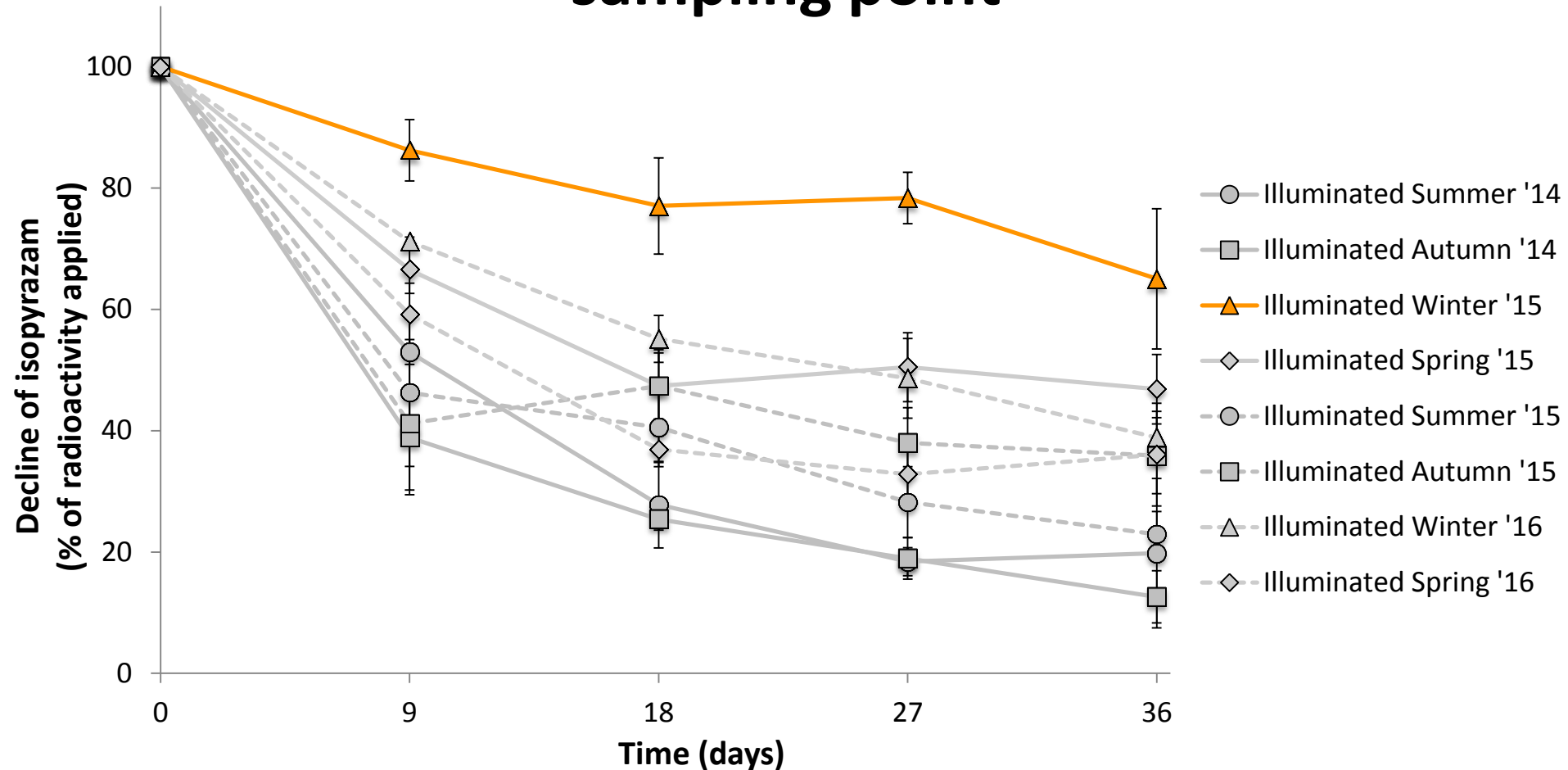
Isopyrazam illuminated degradation between sampling point



Summer and autumn 2014 significantly quicker compared to winter and spring points

Error bars showing +/- standard deviation

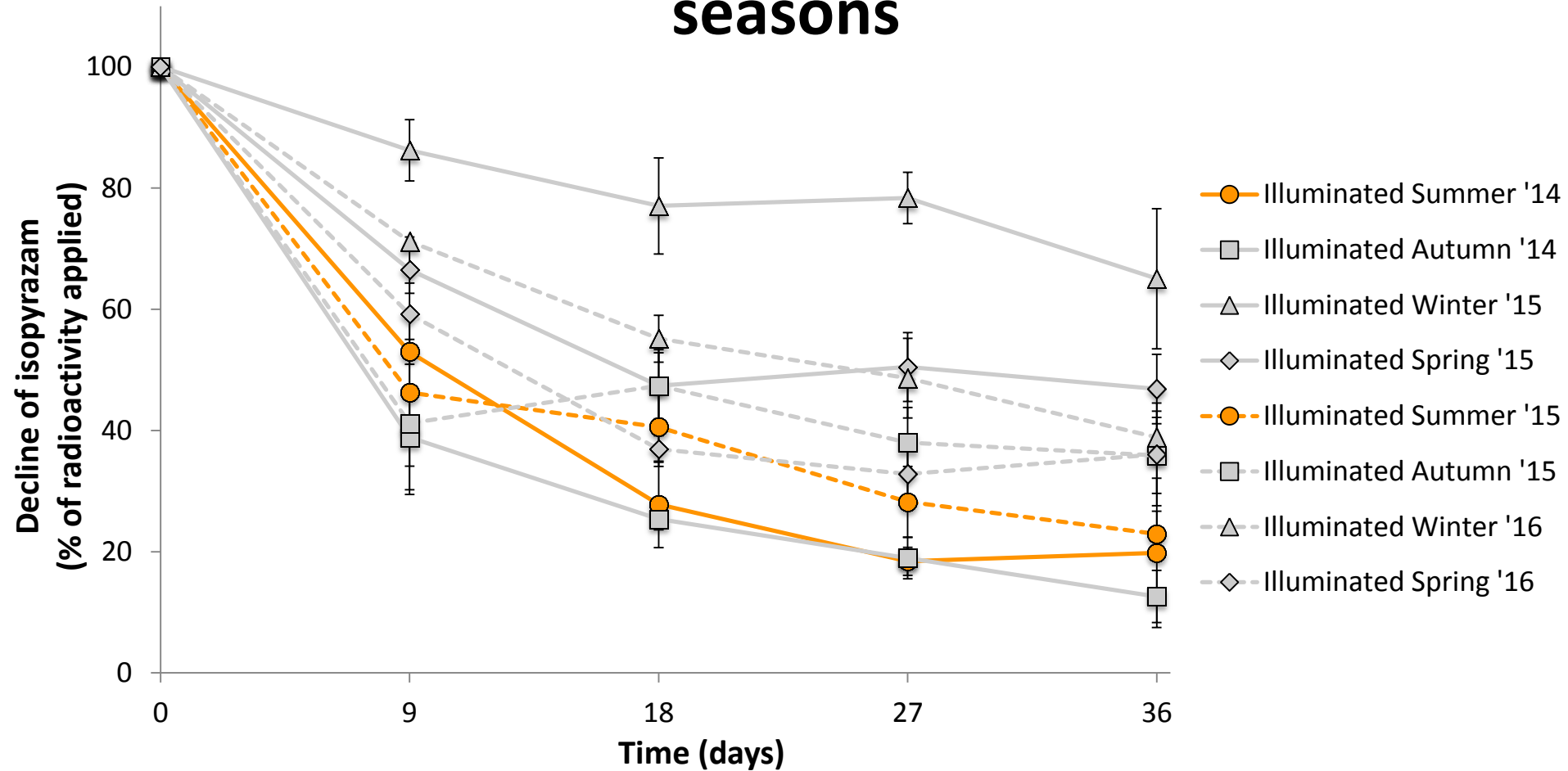
Isopyrazam illuminated degradation between sampling point



Winter 2015 significantly **slower** compared to all other sampling points

Error bars showing +/- standard deviation

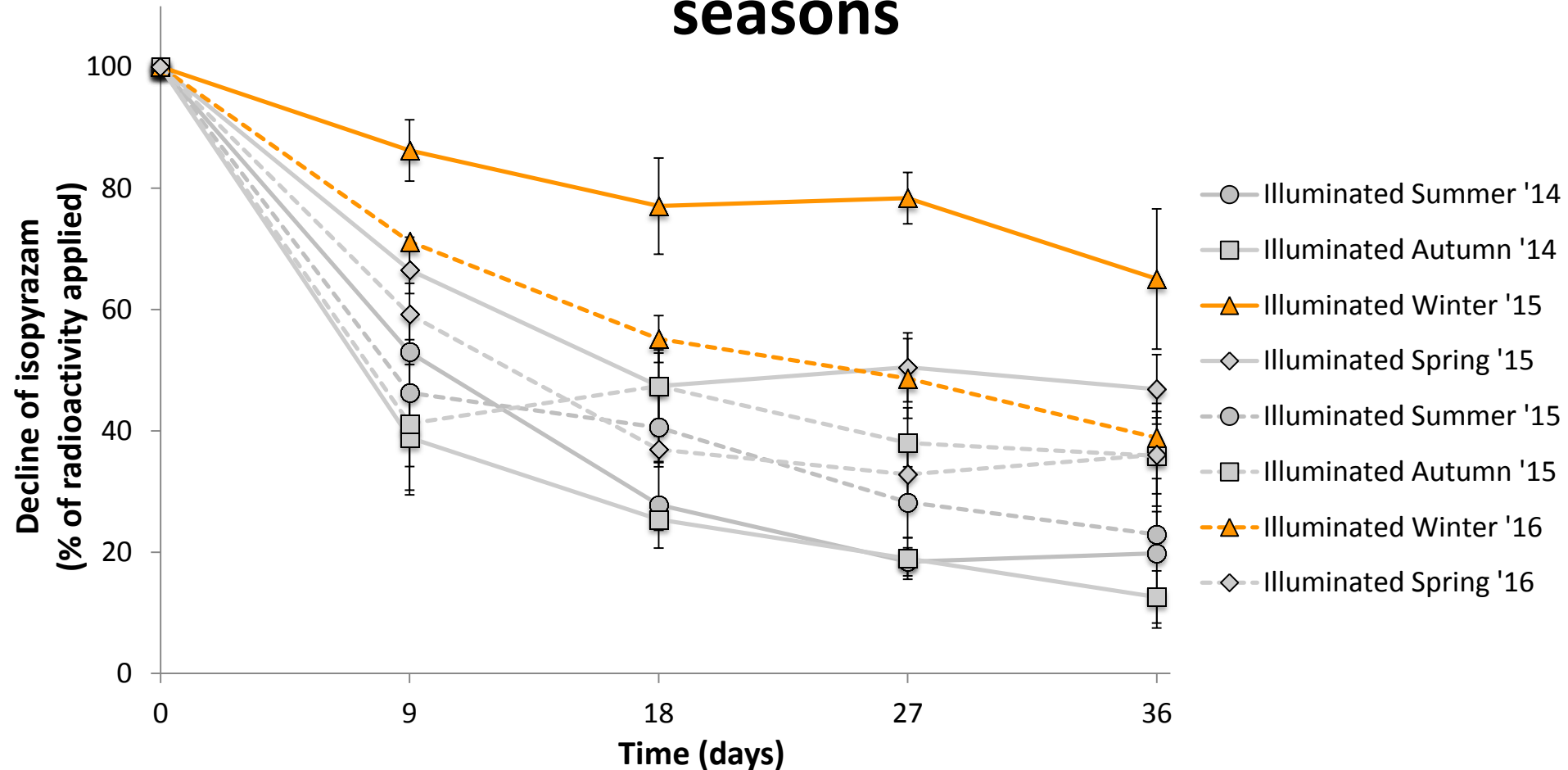
Isopyrazam illuminated degradation between seasons



Significant difference of decline **between** different **season years**, except in summer
Temporal variation in degradation, but **not** a seasonal effect

Error bars showing +/- standard deviation

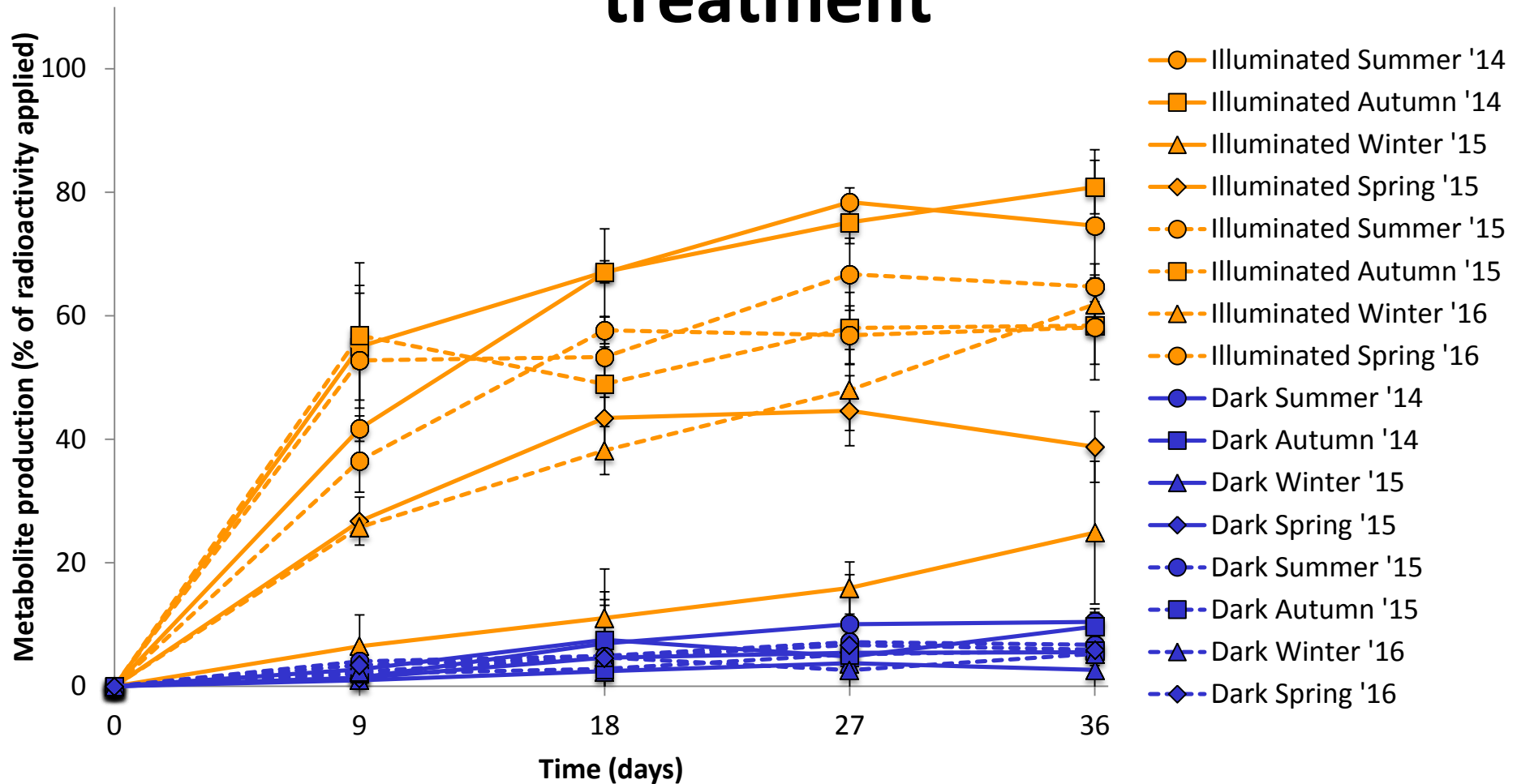
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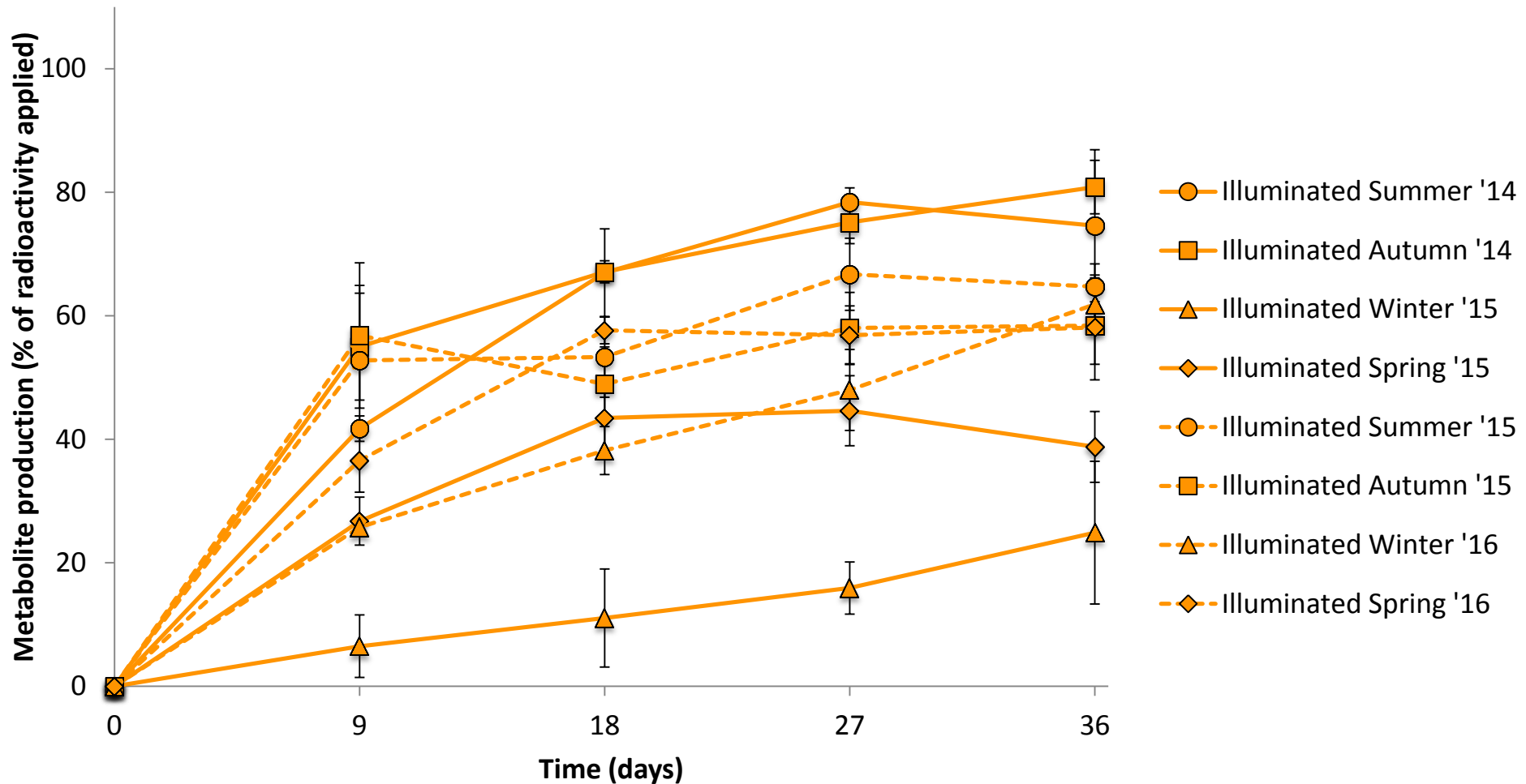
Metabolite generation between light treatment



Significantly more metabolite production in illuminated systems

Error bars showing +/- standard deviation

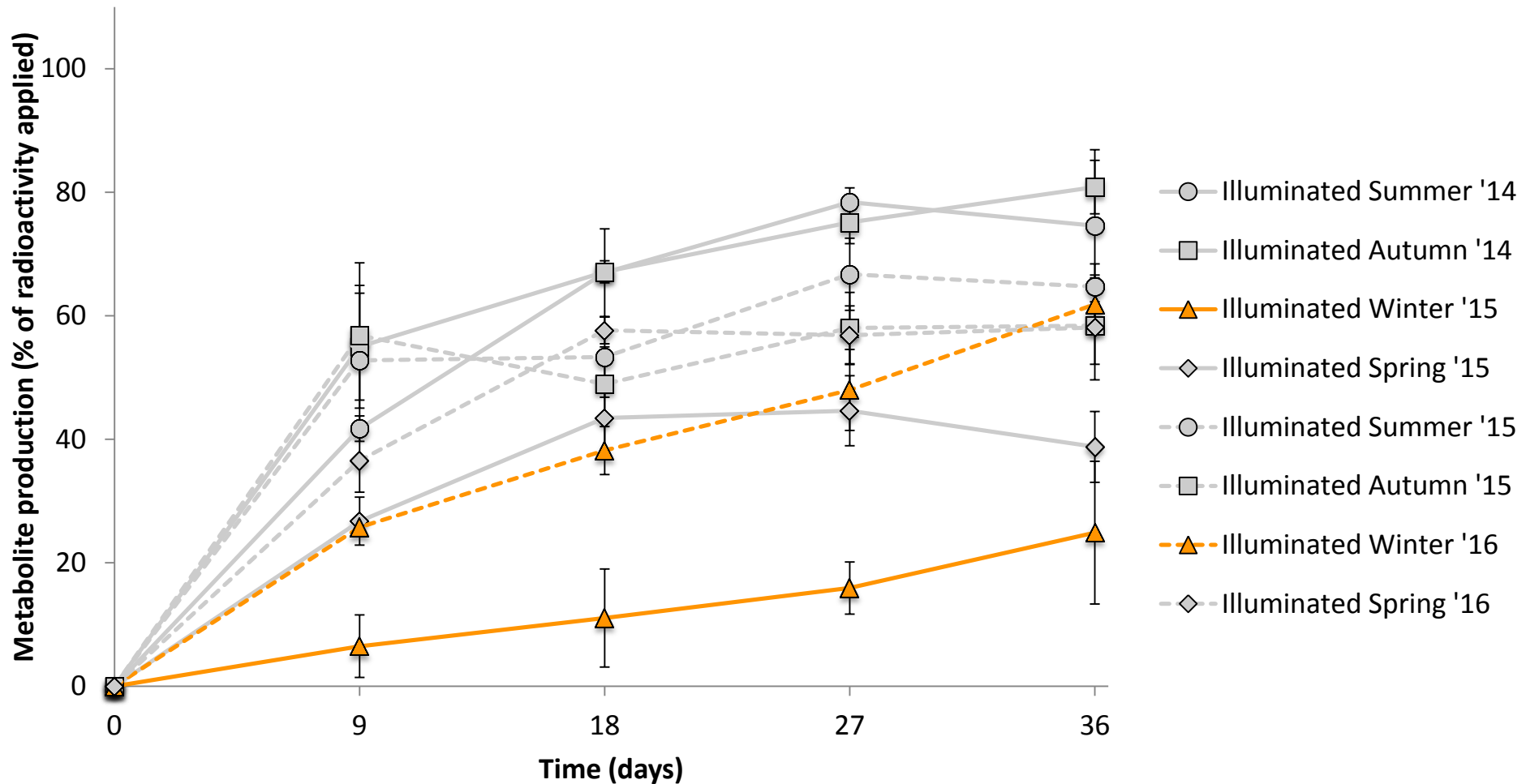
Illuminated metabolite generation



Variable metabolite generation in illuminated treatments between sampling points

Error bars showing +/- standard deviation

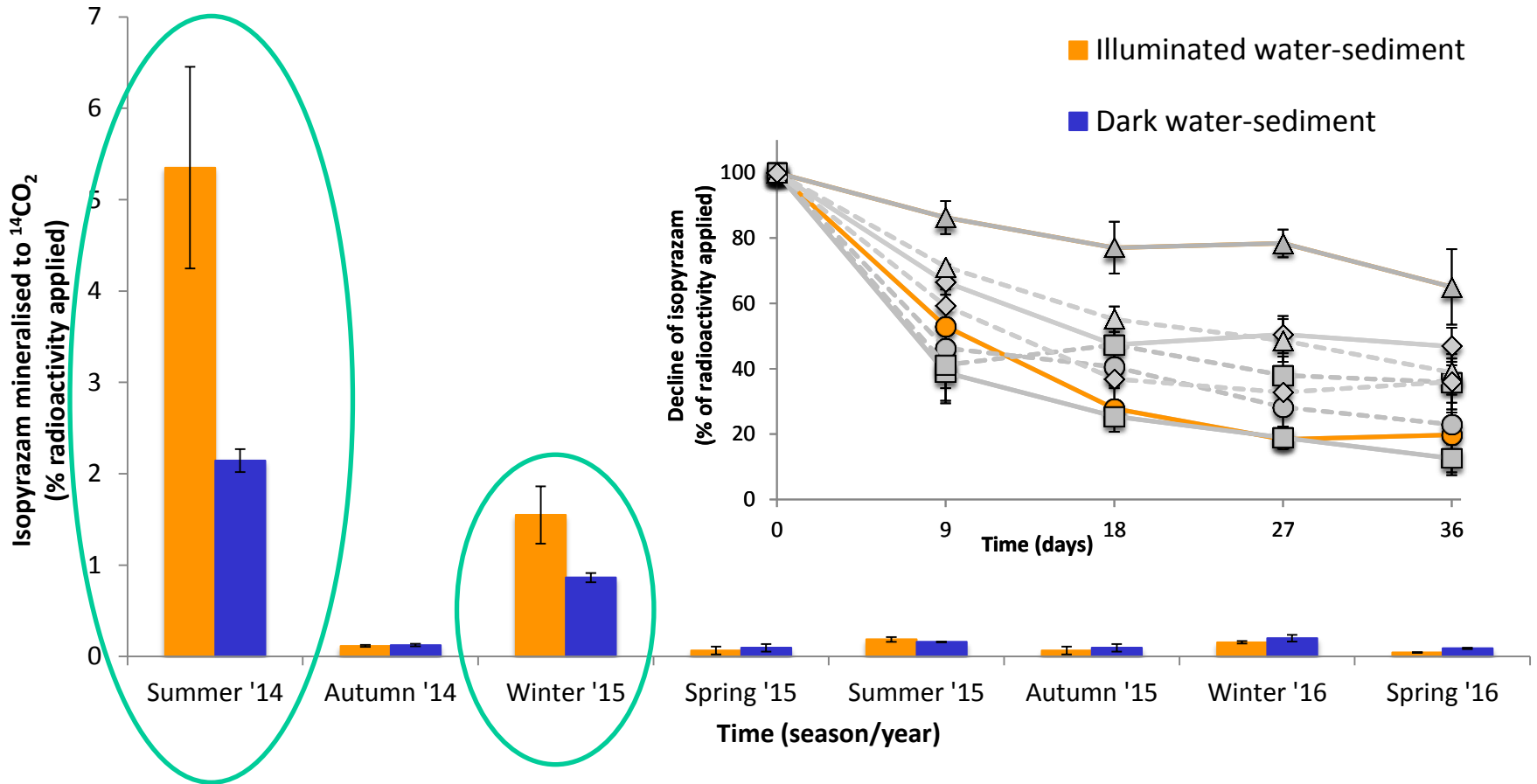
Illuminated metabolite generation



Significantly more metabolite production in **winter 2016** compared to winter 2015

Error bars showing +/- standard deviation

Mineralisation

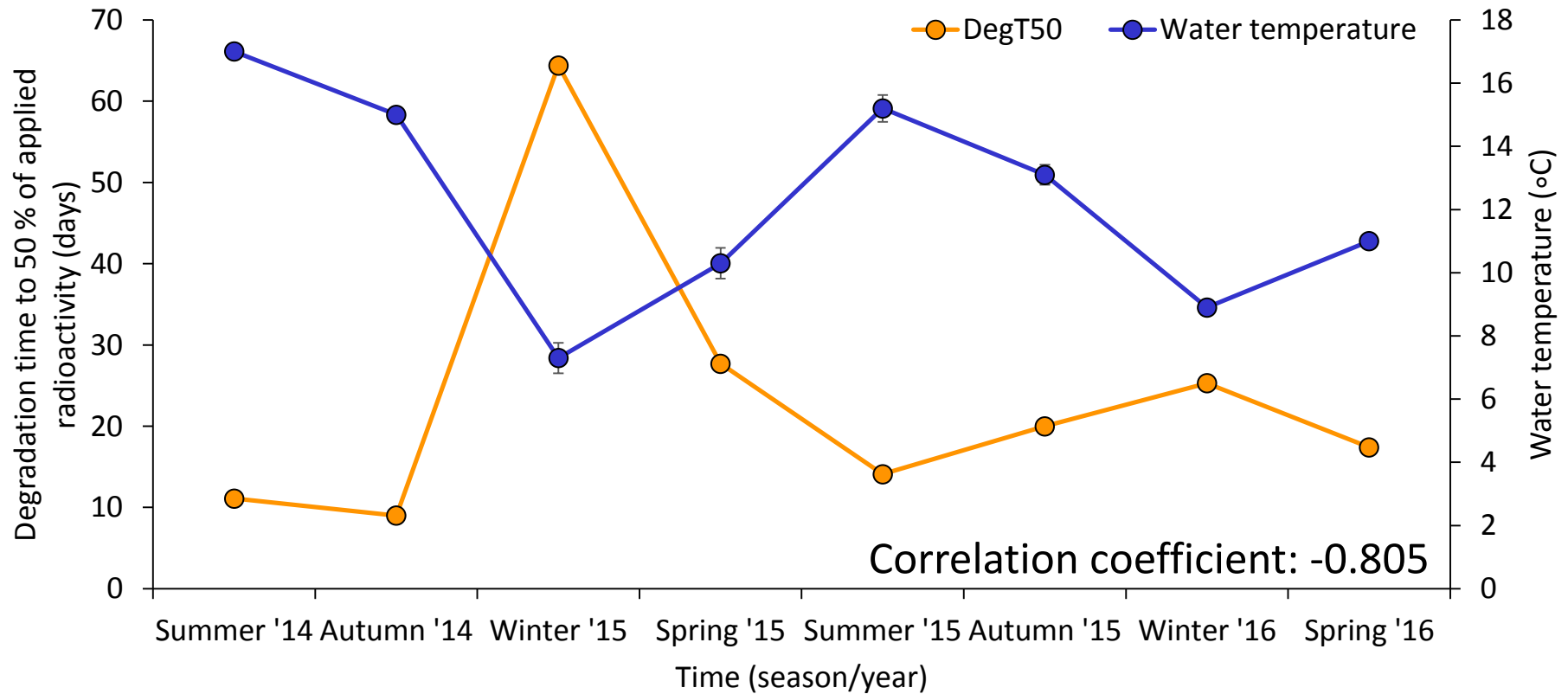


Increased mineralisation in **summer 2014**

Increased mineralisation in **winter 2015** despite **slower degradation**

Error bars showing +/- standard deviation

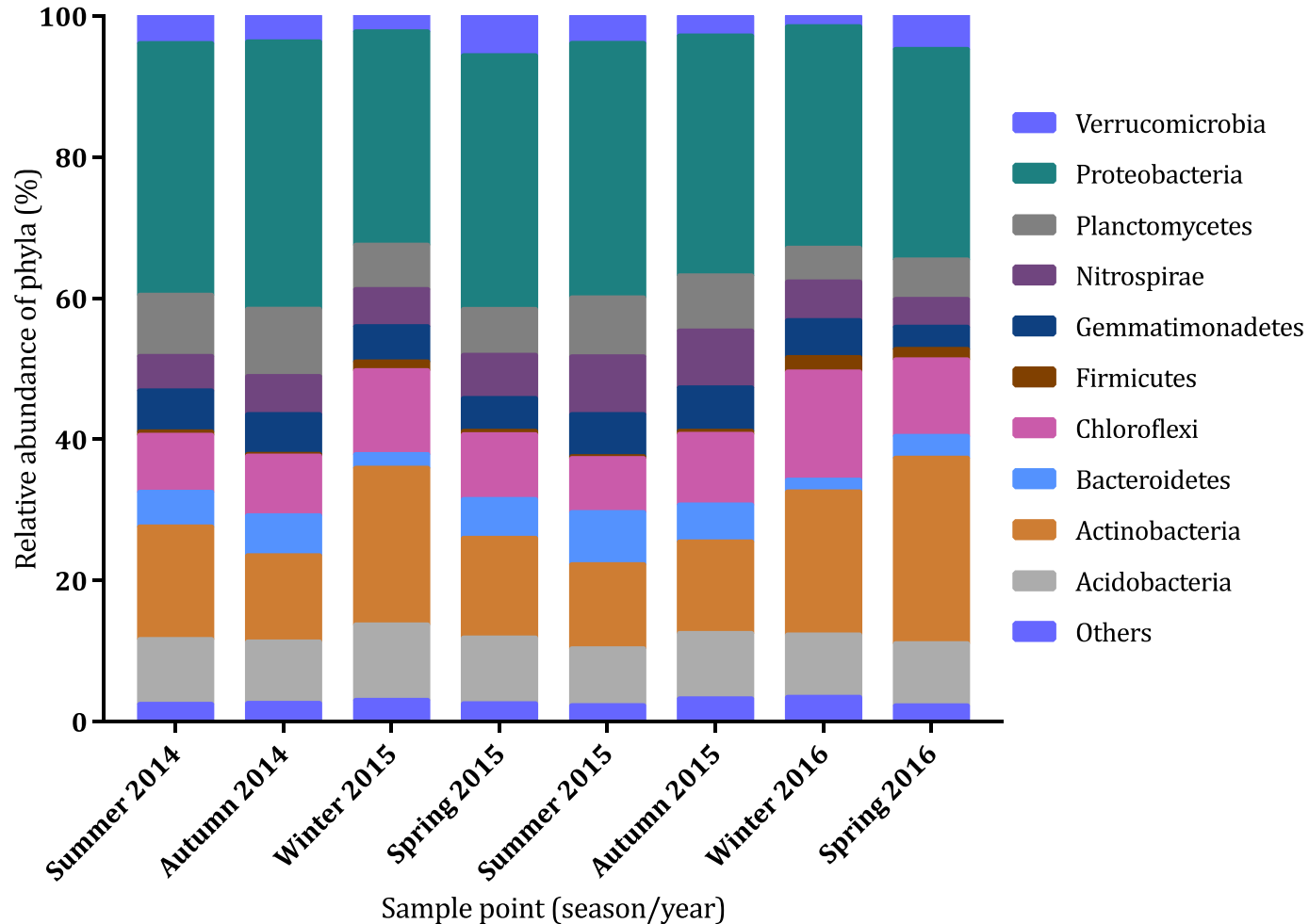
Sample site water temperature vs. Illuminated DegT50



When ambient water temperature at the sample site is **colder**, it takes **longer** for isopyrazam to degrade by 50 %

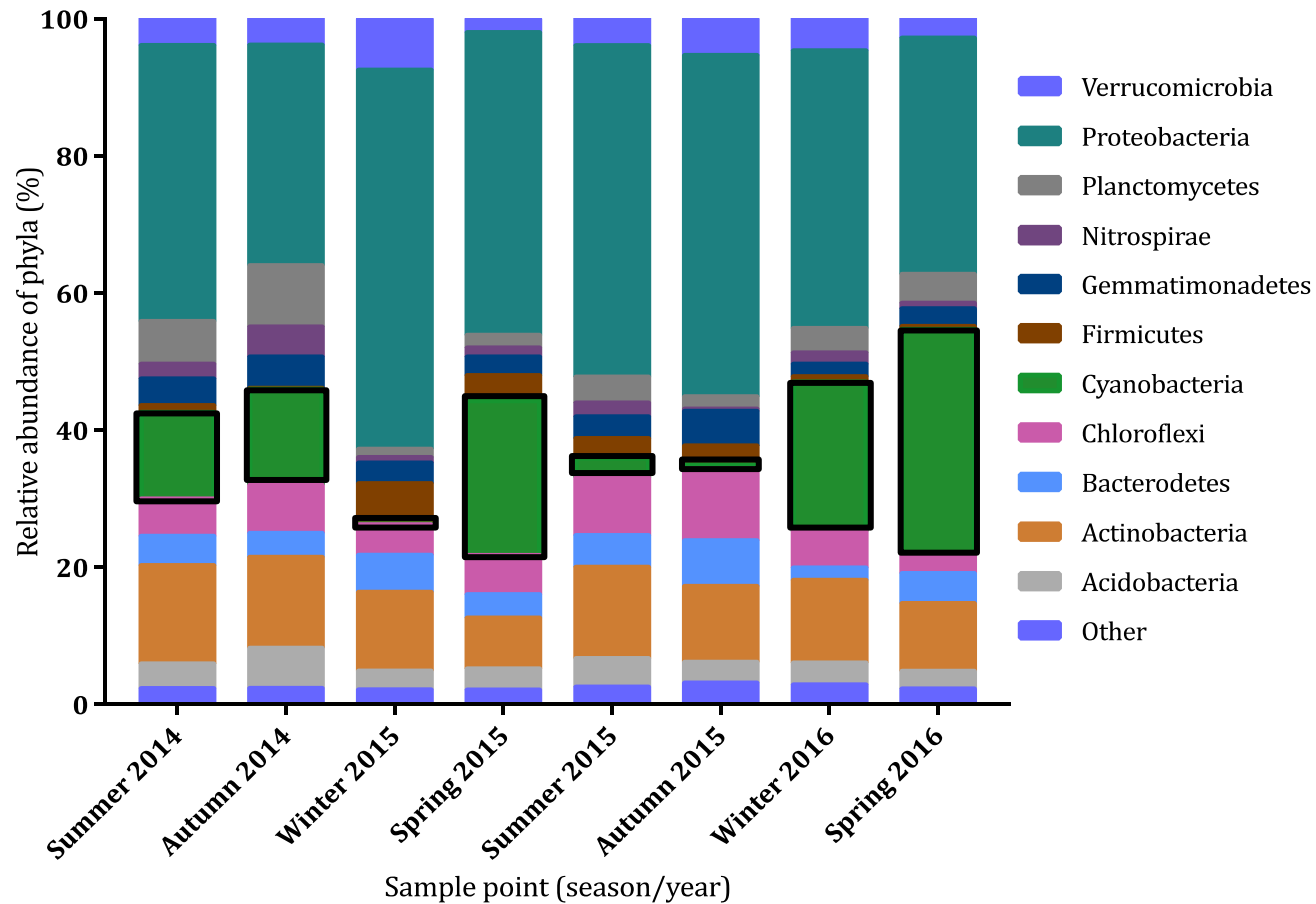
Error bars showing +/- standard deviation

Sample site sediment bacterial community structure



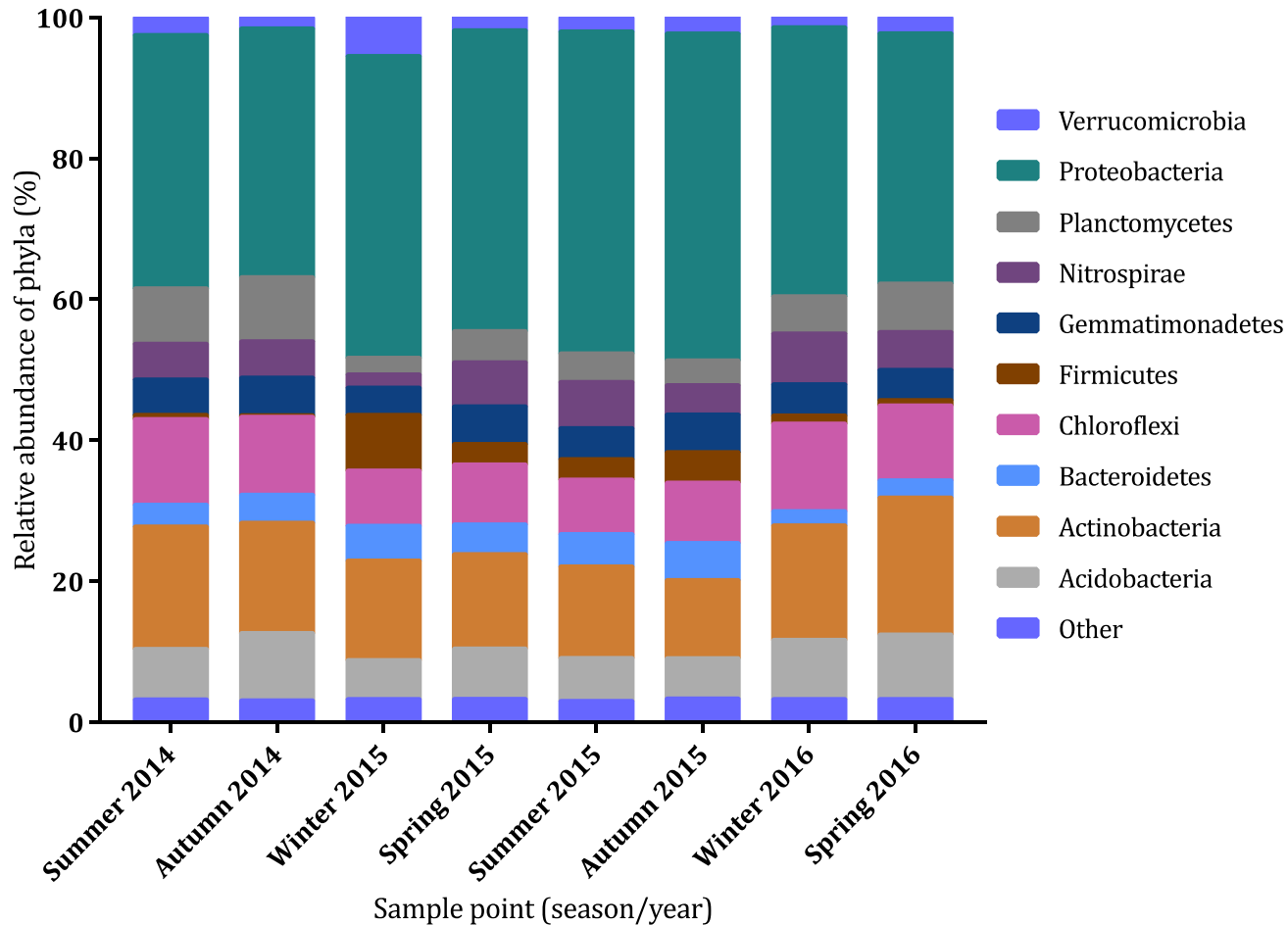
Sediment at the sample site is **not very variable** over sampling points

Illuminated microcosm sediment bacterial community



Different bacterial communities over **time** – especially **cyanobacteria** abundance - even though incubated under the **same lab conditions**

Dark microcosm sediment bacterial community



Little cyanobacteria in dark systems – **bacteria present in the light aids degradation?**

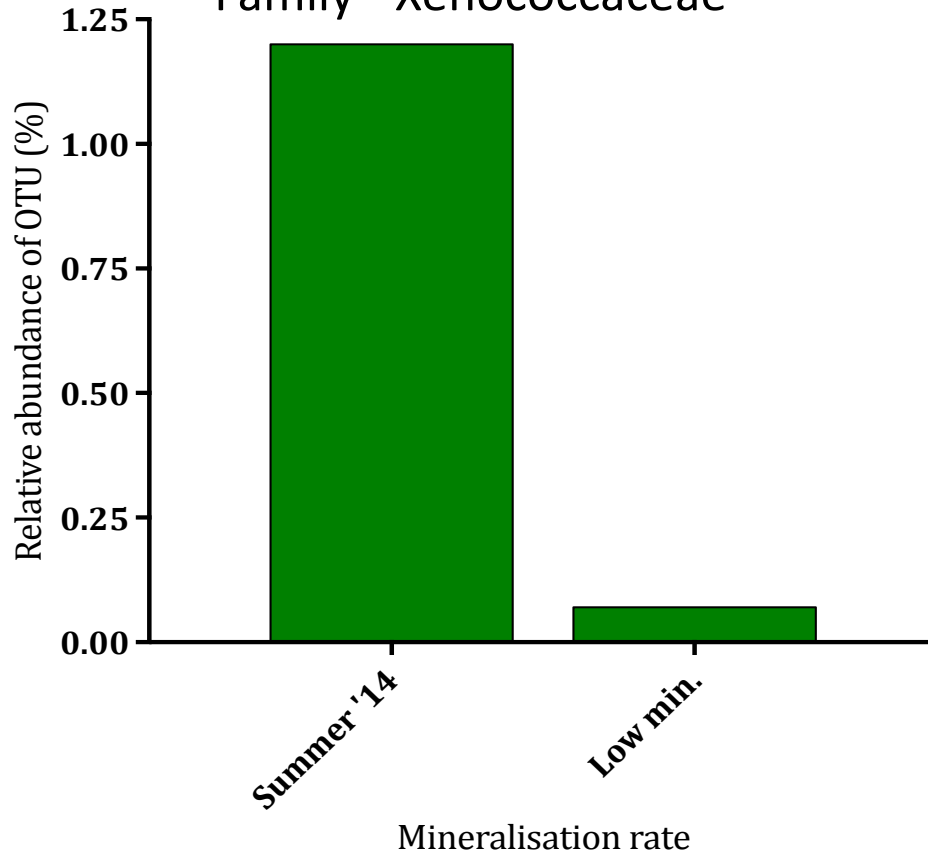
OTUs specific to transformation

- ▶ **No specific** phyla or taxa linked with **higher mineralisation** or **degradation rate**
- ▶ Further comparisons at the **O**perational **T**axonomic **U**nit level

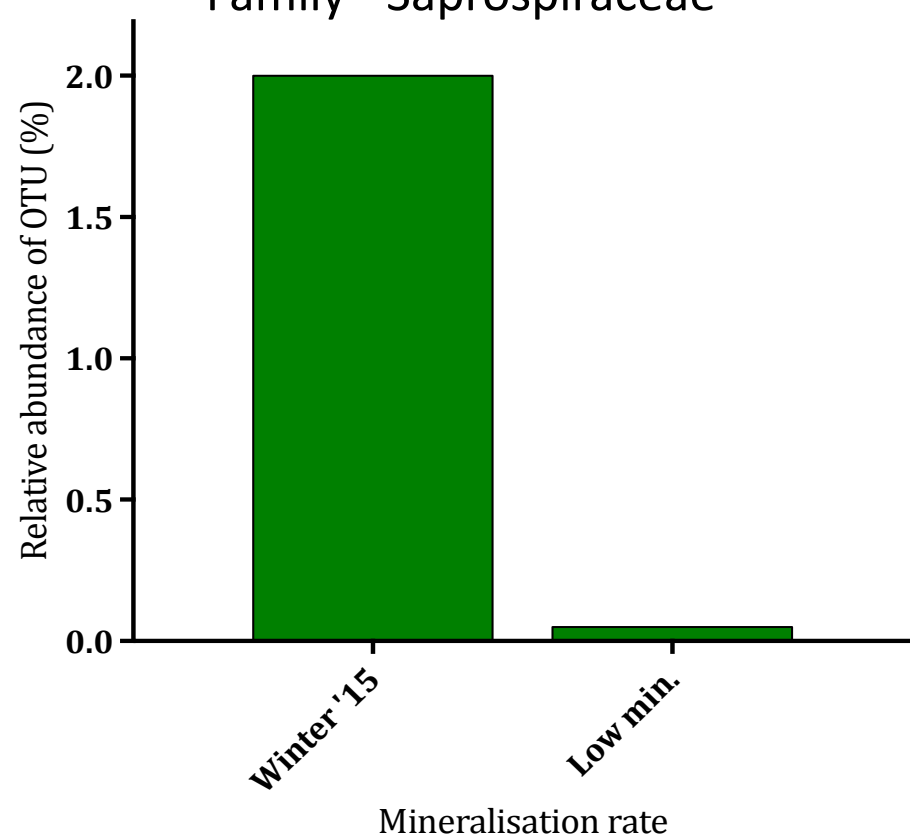


OTUs specific to mineralisation

Phyla - Cyanobacteria
Family - Xenococcaceae



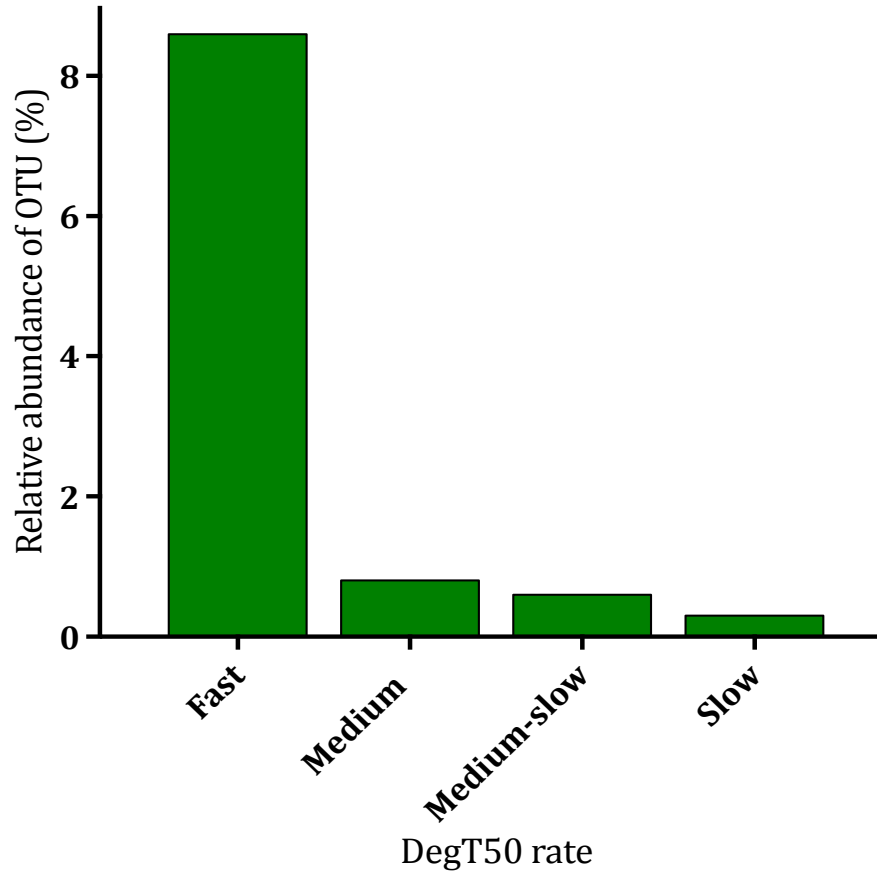
Phyla - Bacteroidetes
Family - Saprospiraceae



OTUs specific to DegT50

Phyla - Cyanobacteria

Family - Pseudanabaenaceae



Conclusions

1. How is **isopyrazam degradation** affected by;

- **Non-UV light?**
- **Temporal variation?**

- ▶ **Little degradation** in dark treatments regardless of sampling point
- ▶ **Increased isopyrazam degradation** with the addition of non-UV light at all times of year
- ▶ Degradation **varies** throughout sampling points but it is **not closely linked** to season – variable community **metabolic potential**
- ▶ Mineralisation is very **variable** between sampling points



Conclusions

2. What is the role of the **microbial community** in these interactions?
 - ▶ **Temporal shifts** in communities at the sample site but **no clear link** with **sampling point** or isopyrazam **degradation**
 - ▶ Bacterial community **changes** when incubated under **non-UV light** and **phototrophs**, e.g. cyanobacteria, can proliferate
 - ▶ Although **no clear links** between specific bacterial or phototrophic **groups** and isopyrazam degradation or mineralisation, certain **OTUs** are more abundant when mineralisation or degradation rates are **higher**
 - ▶ **Water temperature** at the time of sampling may play a role in **shaping the community** and ultimately what drives degradation



Acknowledgments



Long suffering river helpers

Helen Jones

Emily Stoakes

Chris Hale

Mark Day

Martin Holdworth

Harriet Moreland

Mansoor Saeed





Thanks for listening
Any questions?