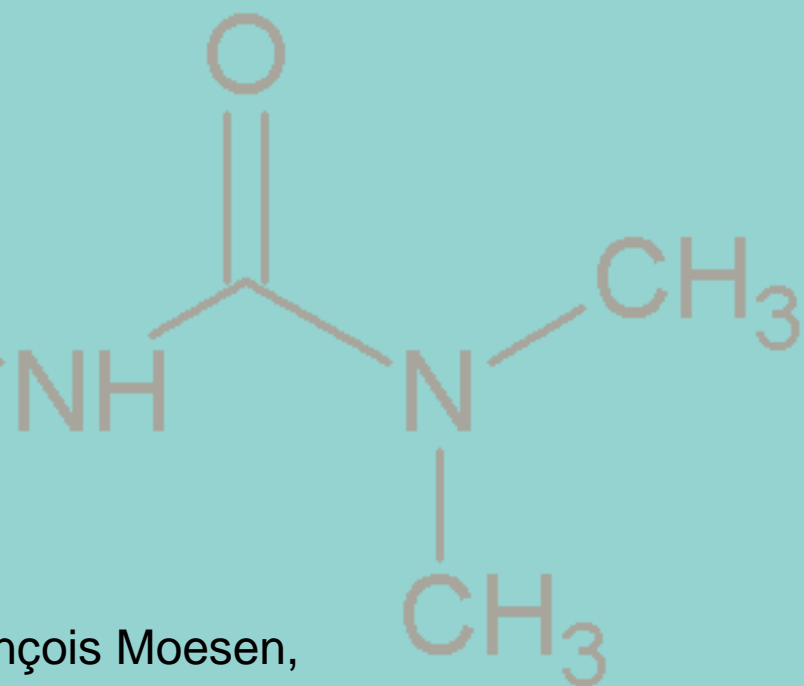


# Microbial degradation of pesticides in wetlands and the effects of season bound changes

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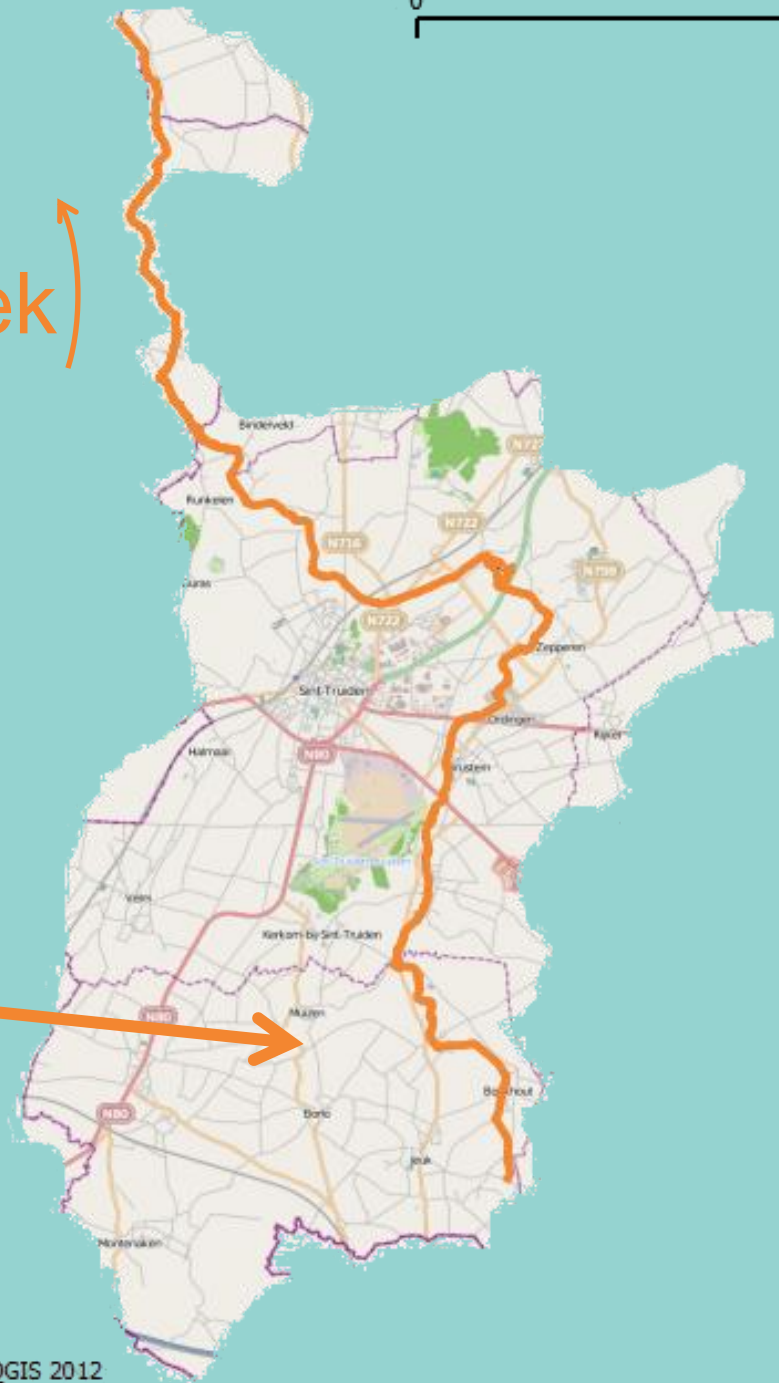


# Fate of pesticides in the environment





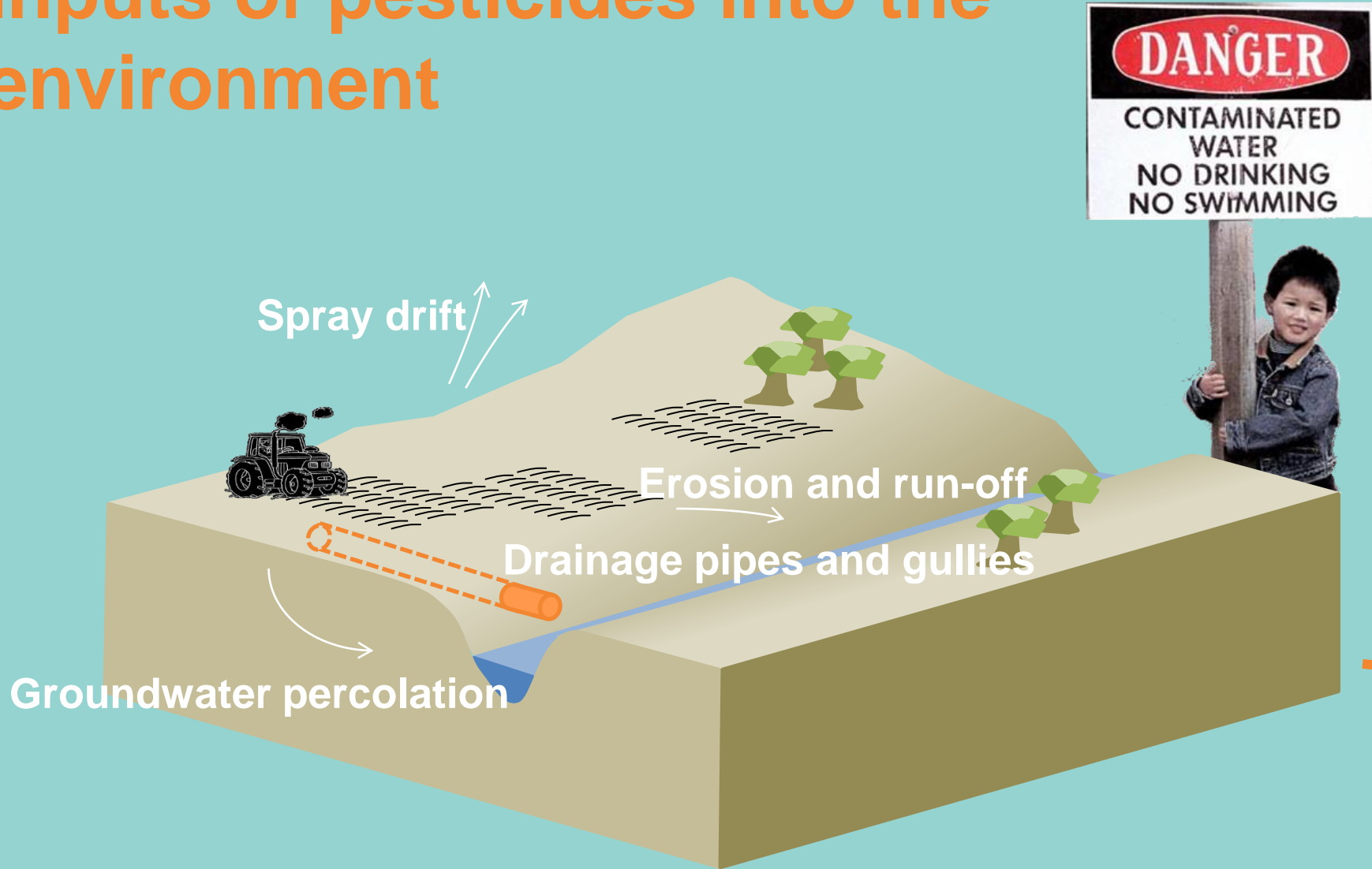
Melsterbeek



agriculture



# Inputs of pesticides into the environment

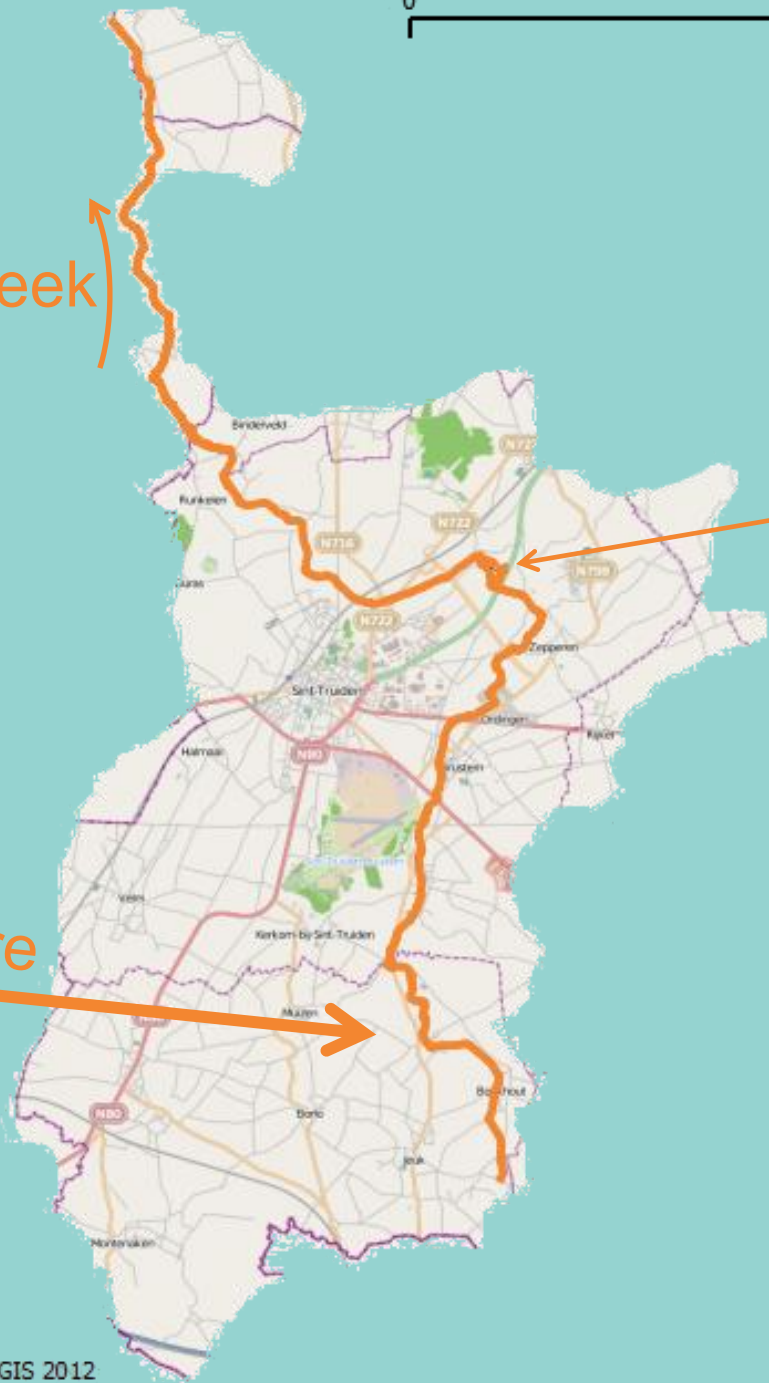




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Controlled flooding basin in Bernissem (Sint-Truiden, Belgium)



# Constructed and restored wetlands



- First built for water retention and nature conservation
- Efficient retention of eroded soil, suspended matter, fertilizers and high sorbing pesticides

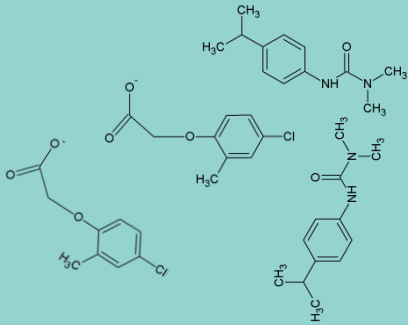
(Shulz and Peall, Environmental science and technology, 2001)



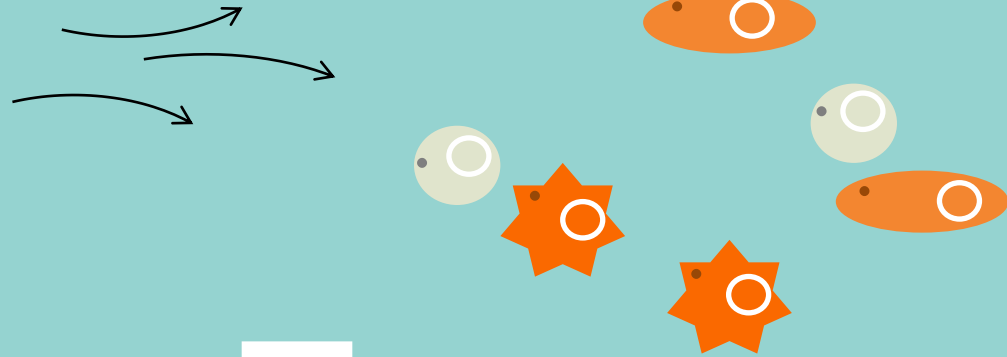
- Buffers for contaminants
- Few records for low sorbing compounds

(Reichenberger *et al.*, Science of the Total Environment, 2007)

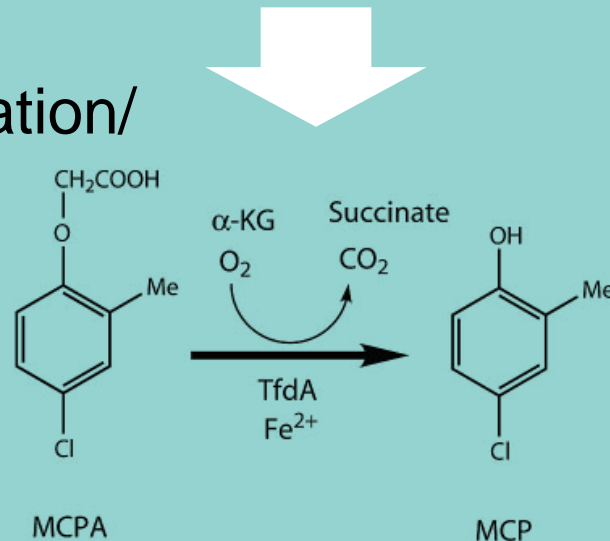
# Microbial degradation of pesticides in wetlands



Soils that are regularly exposed to pesticides ...

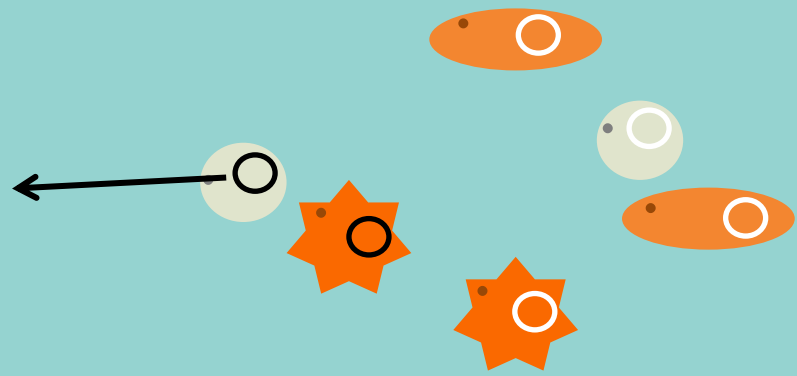
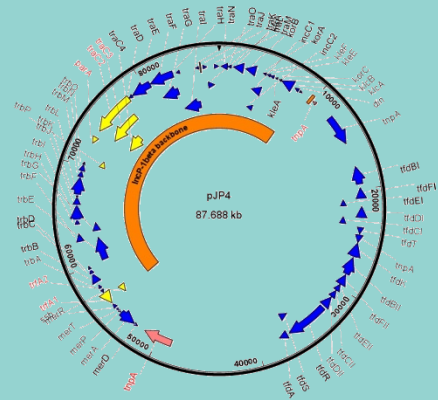


... start to show an accelerated mineralization/ degradation of pesticides.

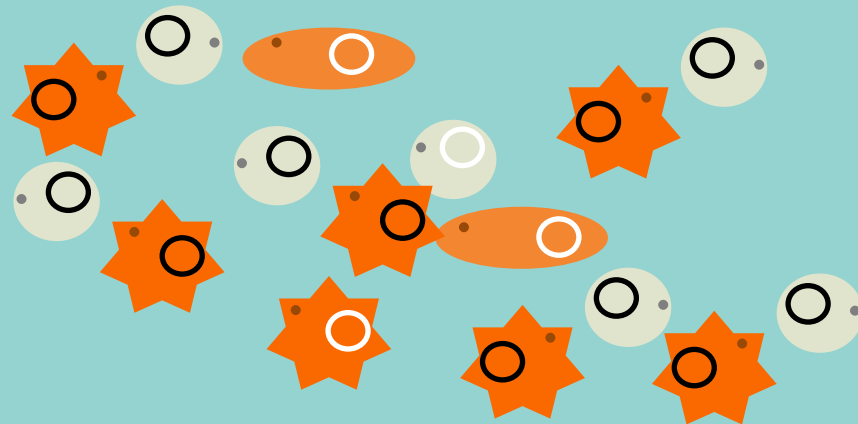


# Microbial degradation of pesticides in wetlands

Genetic adaptation could have occurred ...



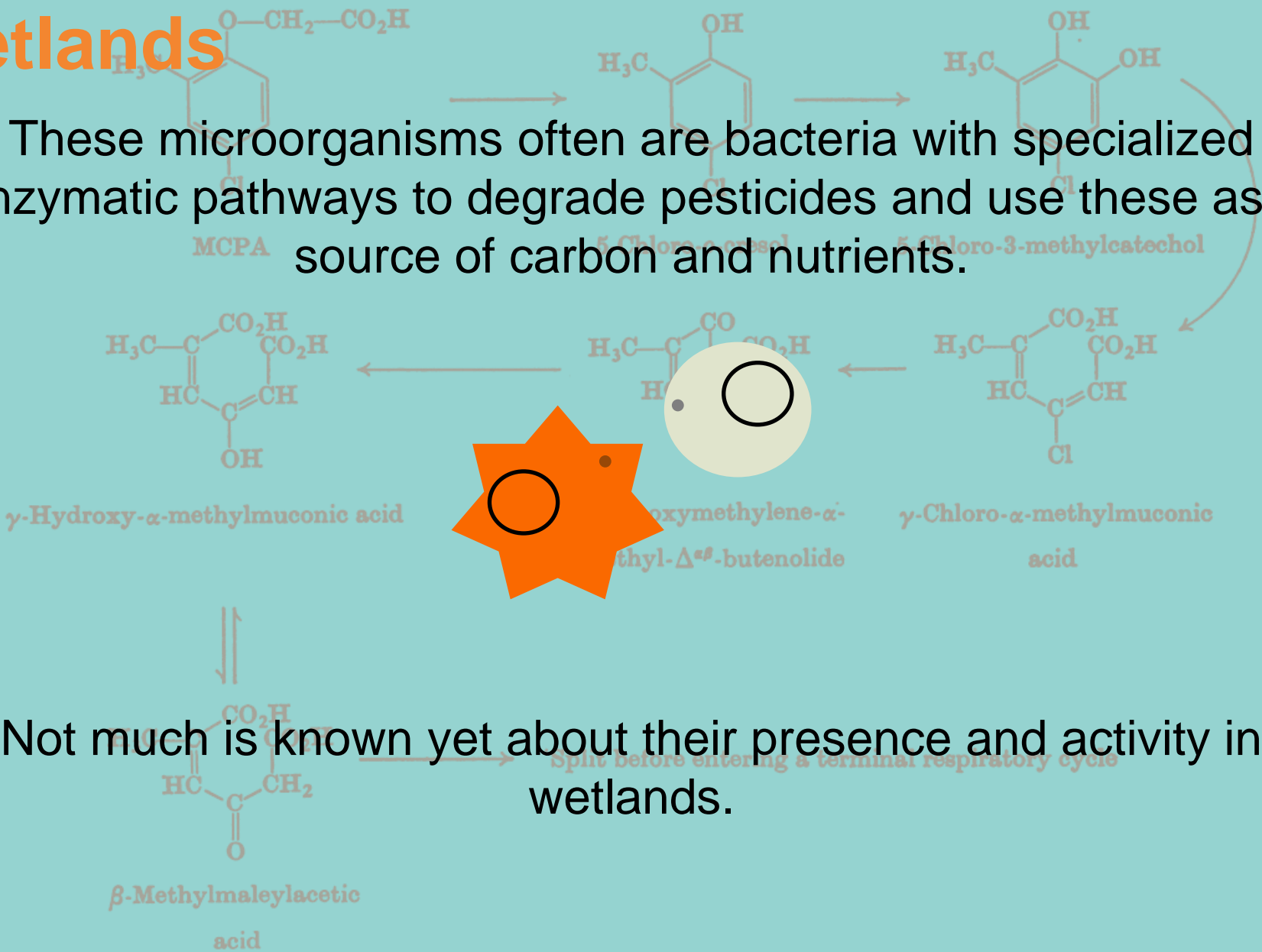
... and specialized microorganisms may grow/enrich.





# Microbial degradation of pesticides in wetlands

These microorganisms often are bacteria with specialized enzymatic pathways to degrade pesticides and use these as a source of carbon and nutrients.



Not much is known yet about their presence and activity in wetlands.

# Vulnerability of soil microorganisms to seasonal changes



- Soil microorganisms have protection mechanism against decreasing water potential (Kieft *et al.*, Soil Biology and Biochemistry, 1987)
- Cell lysis when rapid rewetting due to osmotic shock
- Increased respiratory burst after rewetting, but slower growth due to recovery of dormant cells (Lovieno and Bååth, FEMS Microbiology Ecology, 2008)
- Adaptation of microorganism to osmotic shock
- Growth at low temperatures by psychrotrophic bacteria (Russel *et al.*, Philosophical Transactions of the Royal Society B: Biological Sciences, 1990)
- Reduced metabolic activity at low temperatures
- Cell lysis: intracellular crystals and osmotic shock due to extracellular crystals concentrating soil solutes (Walker *et al.*, Applied and Environmental Microbiology, 2006)
- Moderate lethal effect on bacteria leads to less significant CO<sub>2</sub> bursts

# Vulnerability of soil microorganisms to seasonal changes



## Possible effects:

- Vulnerable populations may decay among which pesticide degraders. Recovery of the pesticide degradation capacity can result in *lag phase*.
- Release of nutrients from sediment may *stimulate growth*

## Goal



**To study the microbial degradation of moderately sorbing pesticides in riparian wetlands**

## Objectives

**ONE**

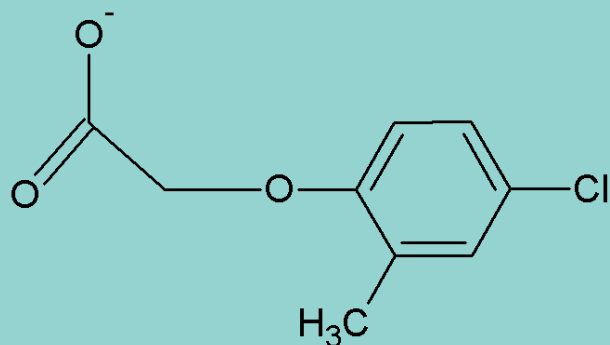
Is the capacity for mineralization of pesticides present in wetlands?

**TWO**

Are there any effects of seasonal changes on the capacity and kinetics of mineralization of pesticides in wetlands?

# Model compounds

## MCPA



Herbicide to control annual and perennial broad-leaved weeds

Used for protection of fruit

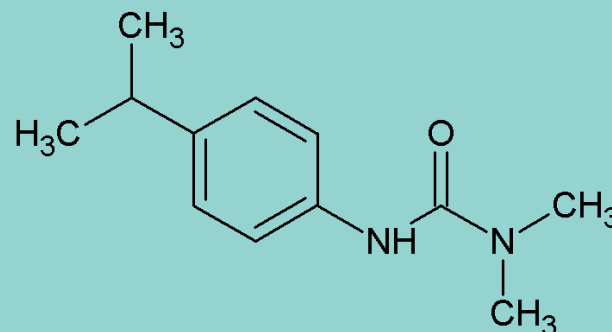
wheat, barley, ...

lawn and grass courts

floriculture

Fast degradation in soil

## IPU



Herbicide to control annual grasses and many broad-leaved weeds

Used for protection of wheat, barley, rye, ...

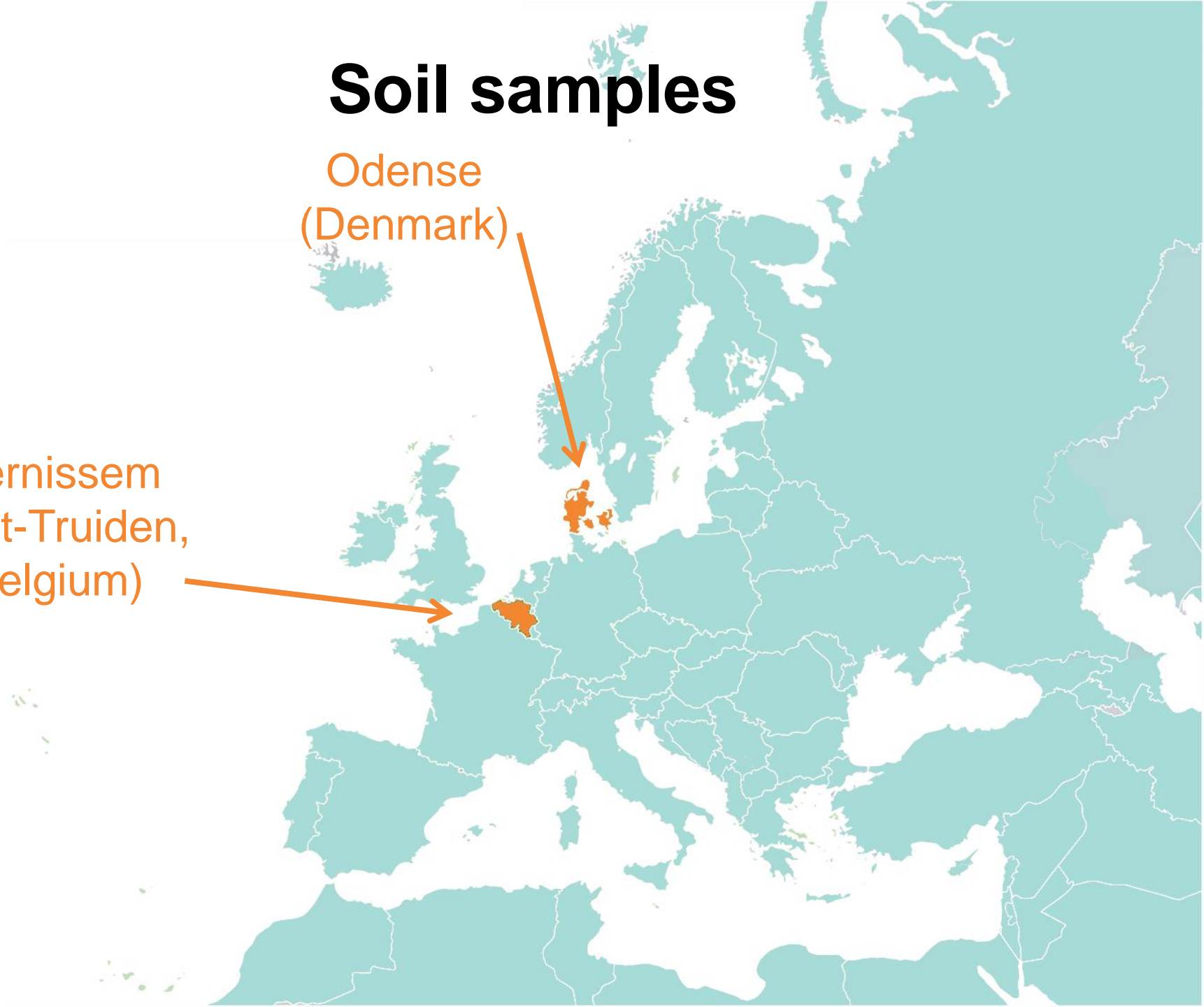
Slow degradation

# Soil samples

Odense  
(Denmark)



Bernissem  
(Sint-Truiden,  
Belgium)





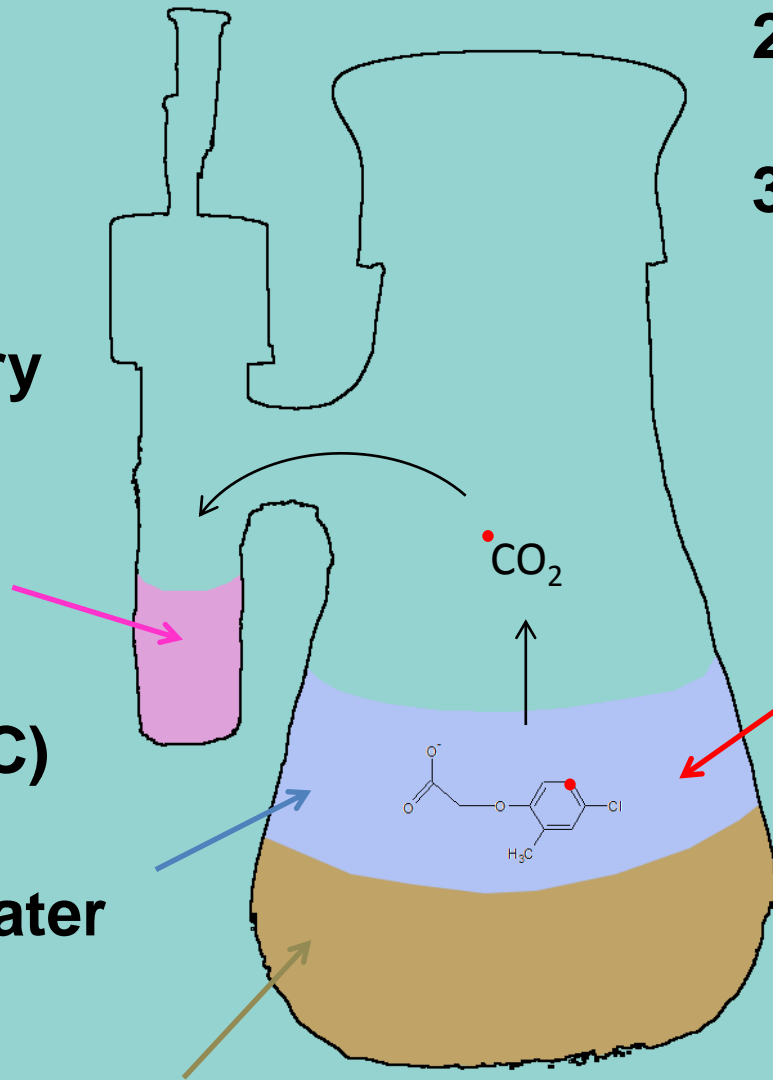
# Lab microcosm wetland





1. Controls
2. Dry/wet: 3 weeks at 30°C after drainage
3. Freeze/thaw: 3 weeks at -18°C

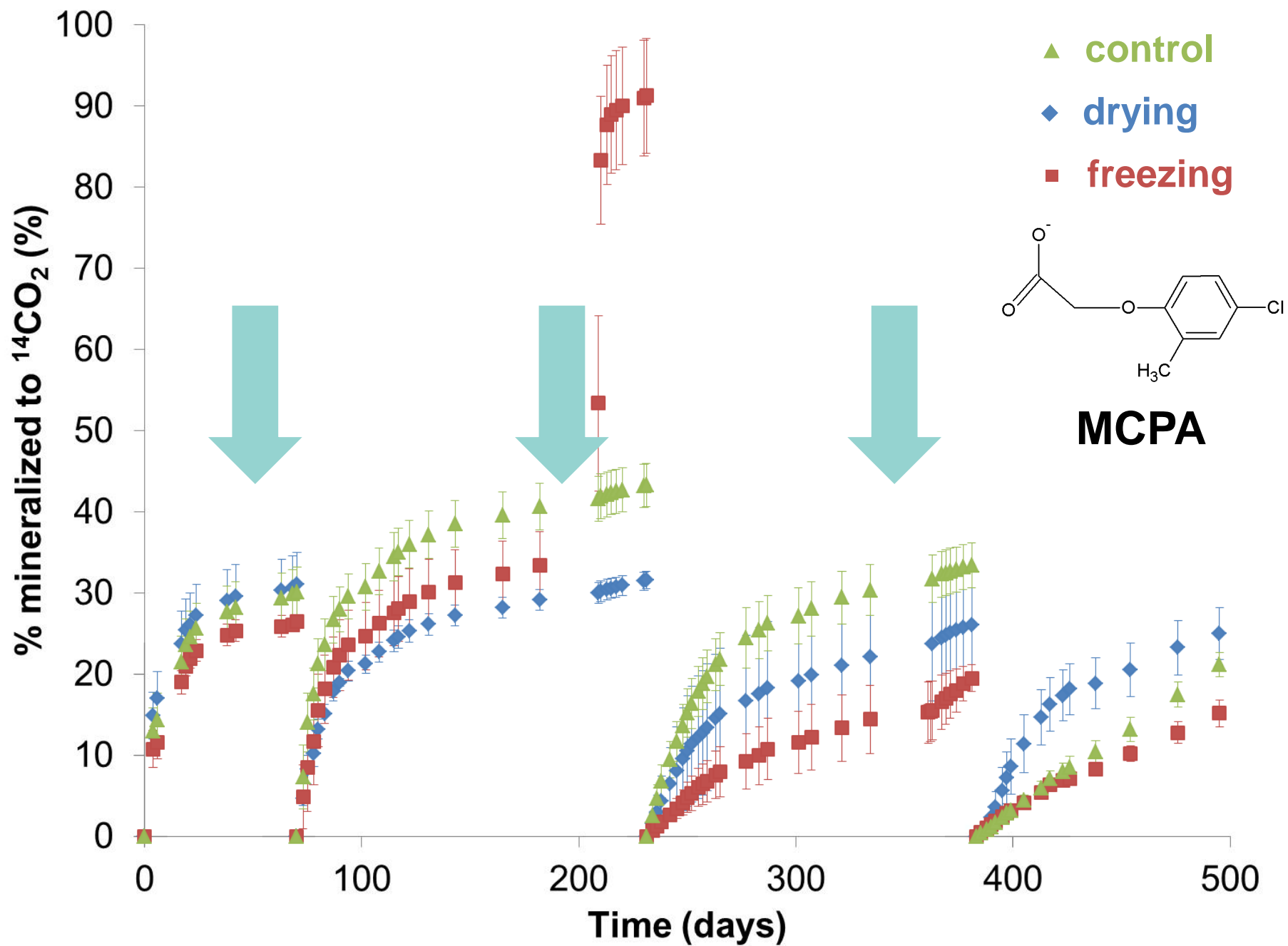
$^{14}\text{CO}_2$  recovery by **NaOH** and analysis with liquid scintillation counting (LSC)



Surface water spiked with 2 µg/l  $^{14}\text{C}$ -ring labelled  $\bullet$ MCPA or  $\bullet$ IPU

50 ml water

50 g of wetland soil from Denmark (MCPA) or Belgium (MCPA and IPU)



# Conclusions

- Mineralization of MCPA and IPU was observed under flooded conditions
- Mineralization of IPU was much slower and to a lower extent
- First order recovery of  $^{14}\text{CO}_2$  without lag time
- The mineralization under flooded conditions was affected by drying and freezing, but system was resilient
- Stimulated mineralization after 3 drying periods
- Diffusion and sorption in the sediment

2

experiment



# Soil samples were taken at 5 moments

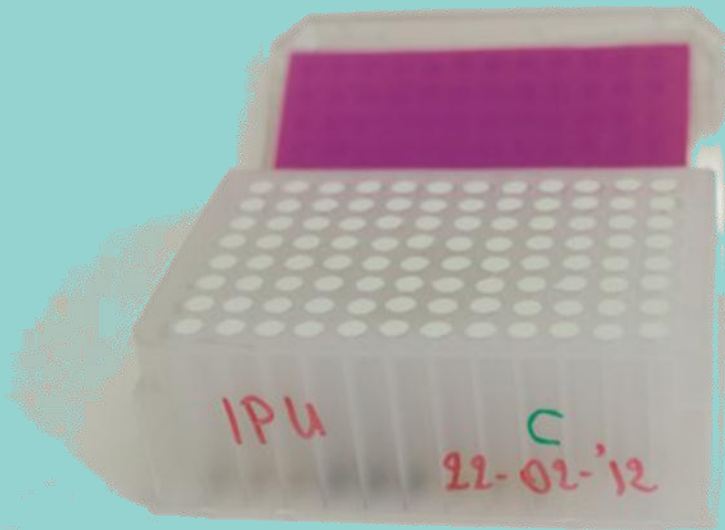


30 samples (n = 30) were taken within the wetland with 3 replicates per location (within radius of 50 cm)



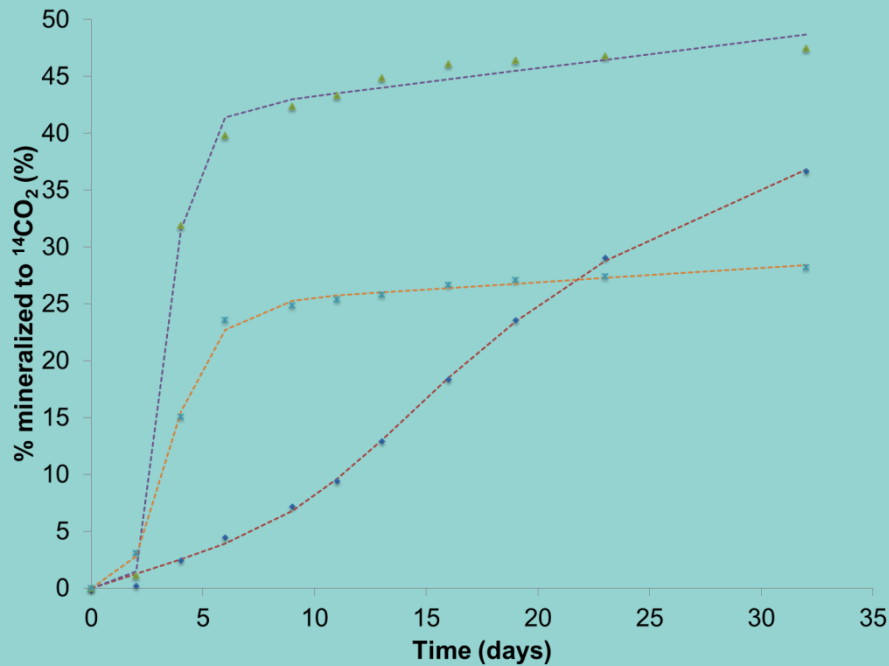


- 5 g sample was suspended in 5 ml minimal media (MMO)
- Suspensions were shaken head-over-end overnight
- 3 aliquots of 100  $\mu$ l were transferred to microplate
- 60 Bq of  $^{14}\text{C}$ -labelled MCPA or IPU were added
- Incubated @ 20  $^{\circ}\text{C}$
- $\text{Ca}(\text{OH})_2$ -coated seals to capture  $^{14}\text{CO}_2$



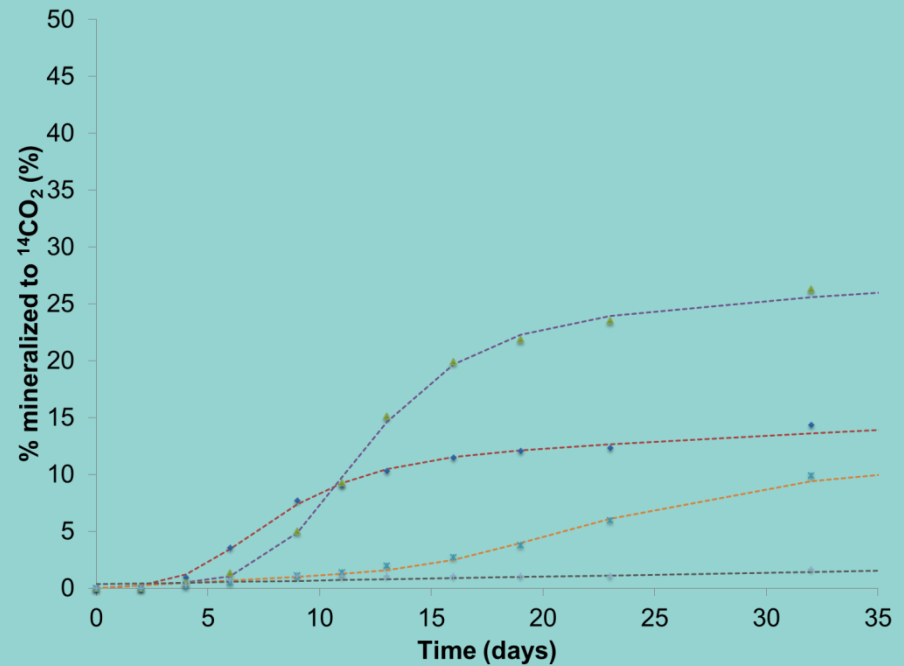


# MCPA

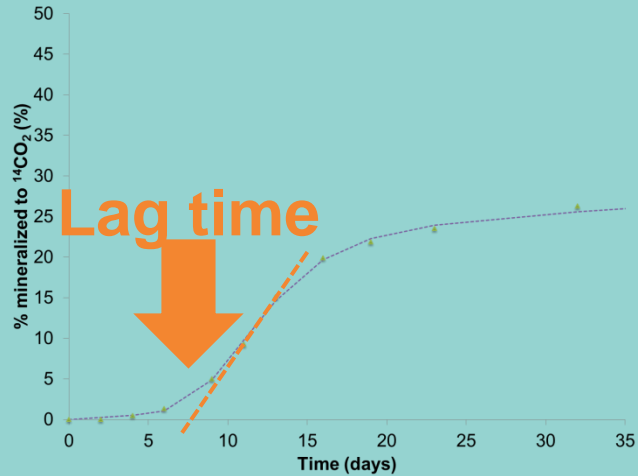


- Mineralization everywhere
- High mineralization rates
- High cumulative mineralization (up to 50 %)

# IPU



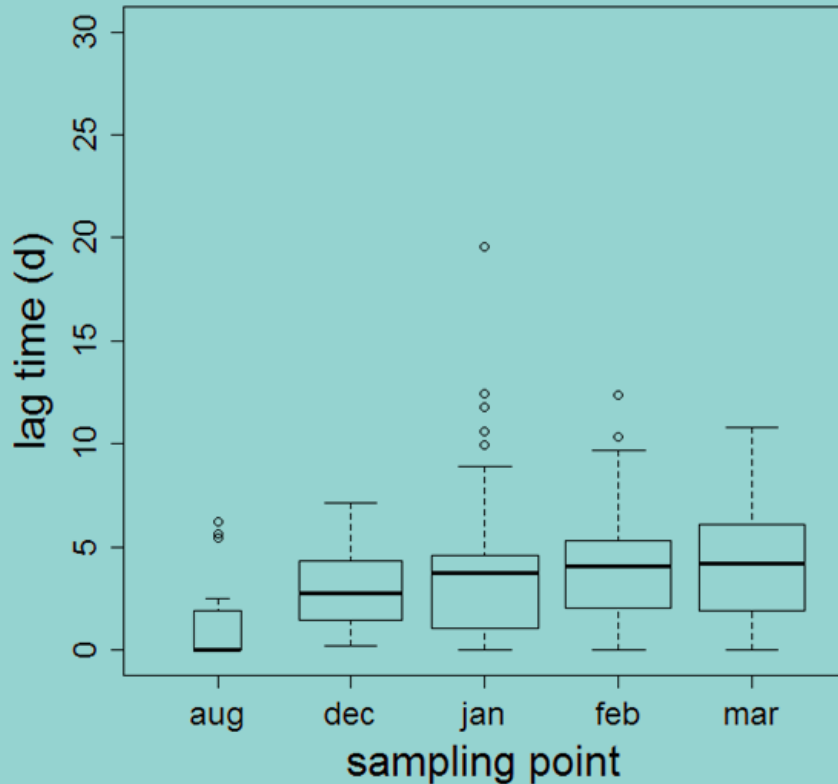
- Not all samples active
- Longer lag times
- Lower cumulative mineralization (up to 30 %)



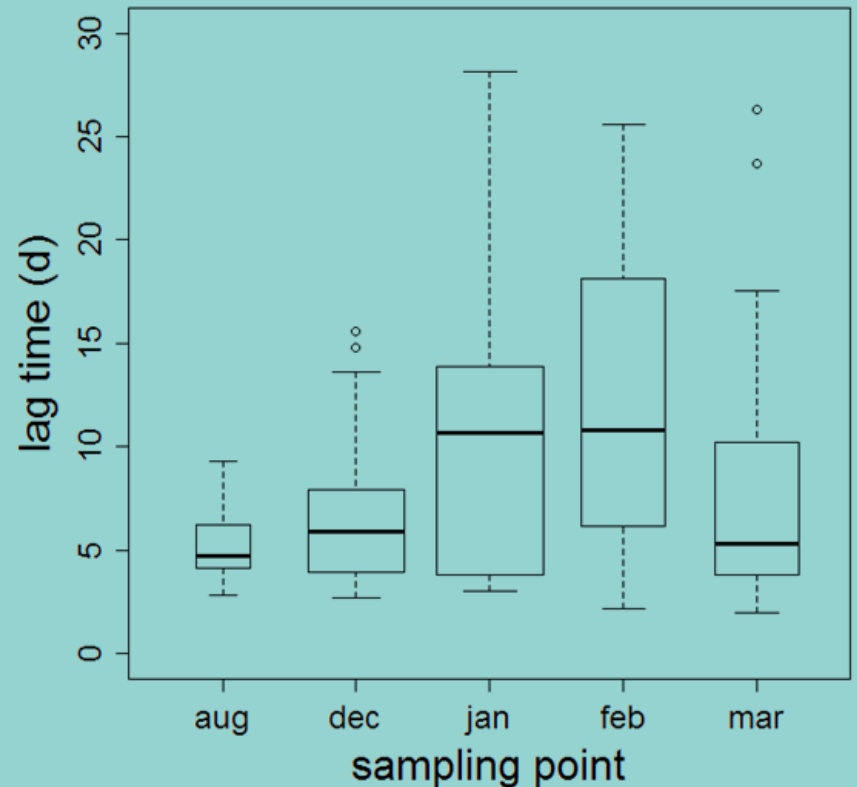
Lag times MCPA up to 20 days

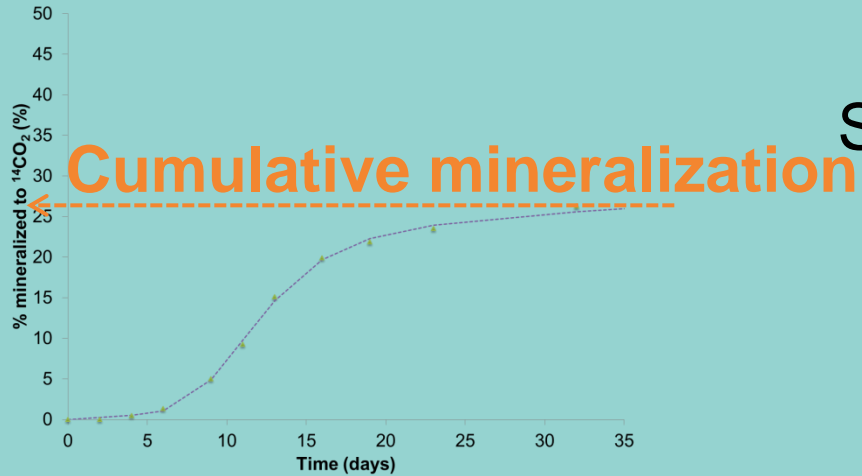
Median lag time IPU longer in January and February

Lag times MCPA



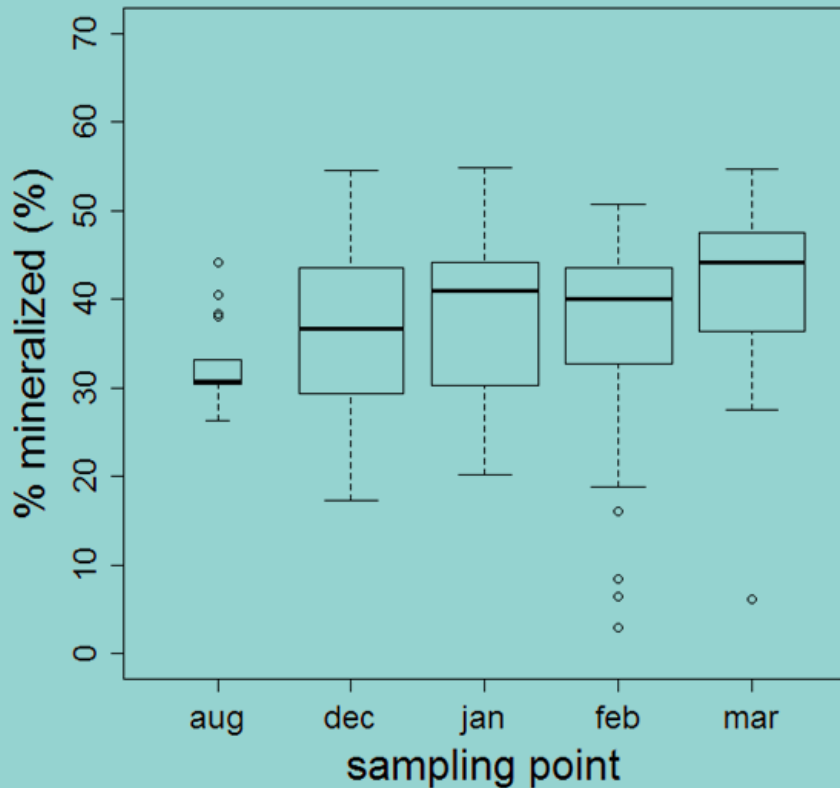
Lag times IPU



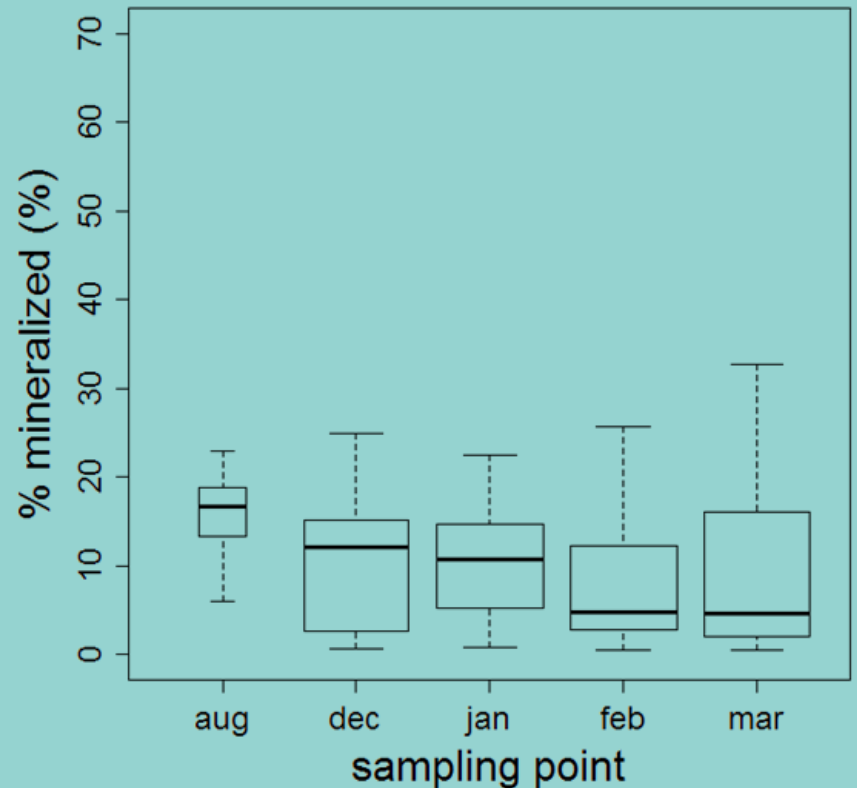


Samples reach a lower plateau in February compared to the other months → influence of freezing period

MCPA mineralization

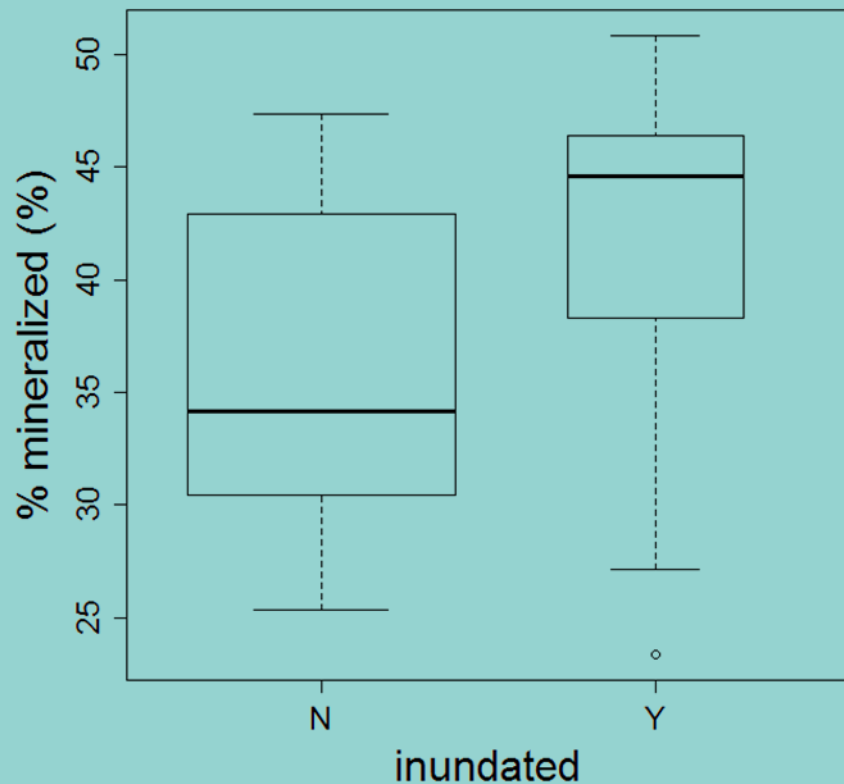


IPU mineralization

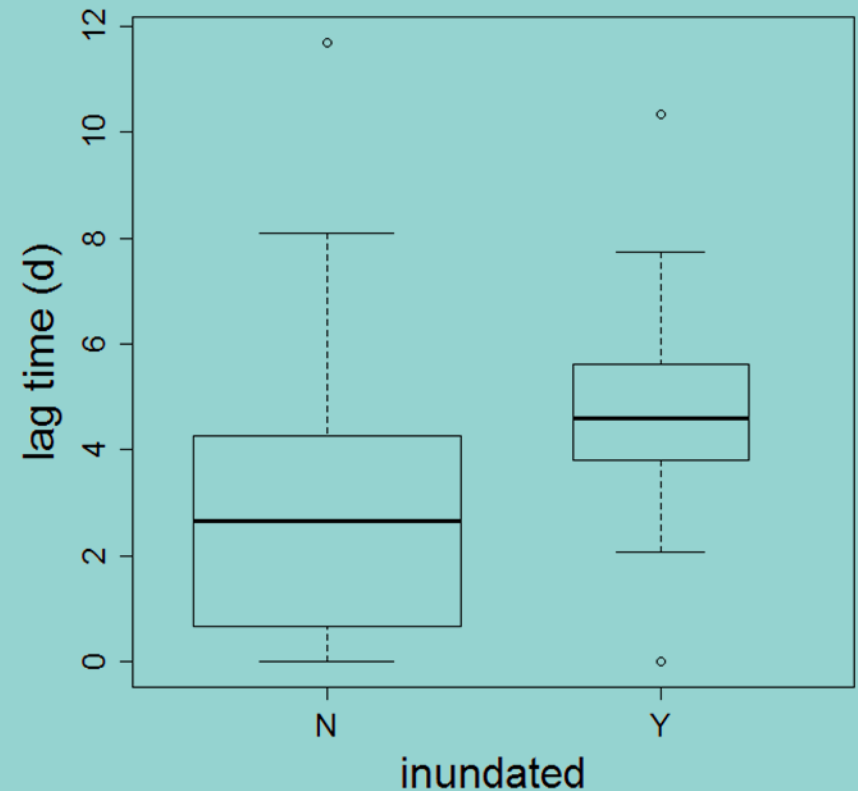


# Effect of inundation on mineralization of MCPA

## Cumulative mineralization



## Lag time



# Conclusions

- MCPA was mineralized throughout the wetland at every time point
- IPU was only mineralized in samples that were not inundated
- Inundated samples had longer lag times, but converted relatively more MCPA to CO<sub>2</sub>
- No clear effect of a freezing period *in situ* on the mineralization in the lab

## Goal

To study the microbial degradation of moderately sorbing pesticides in riparian wetlands

## Objectives

**ONE**

**yes** Is the capacity for mineralization of pesticides present in wetlands?

**TWO**

**Only in microcosms** Are there any effects of seasonal changes on the capacity and kinetics of mineralization of pesticides in wetlands?

# Many thanks to

Prof. Dirk Springael

Prof. Jan Diels

Inge Dehantschutter and François Moesen

Kenneth Simoens and my colleagues

Watering van Sint-Truiden and IWT

