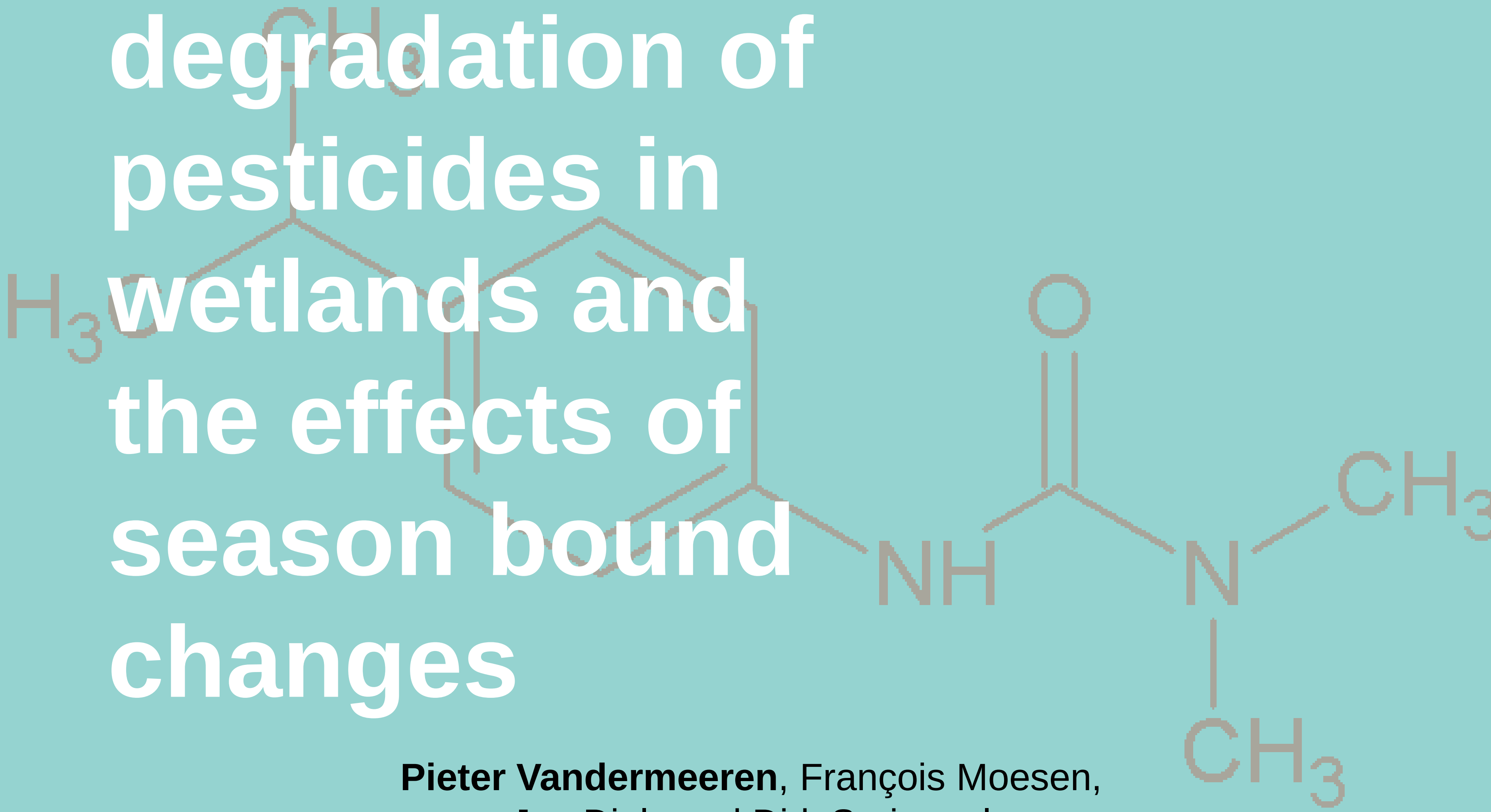
Microbial degradation of pesticides in wetlands and the effects of season bound changes



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Jan Diels and Dirk Springael

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Fate of pesticides in the environment

Melsterbeek
agriculture
0
20
km
N
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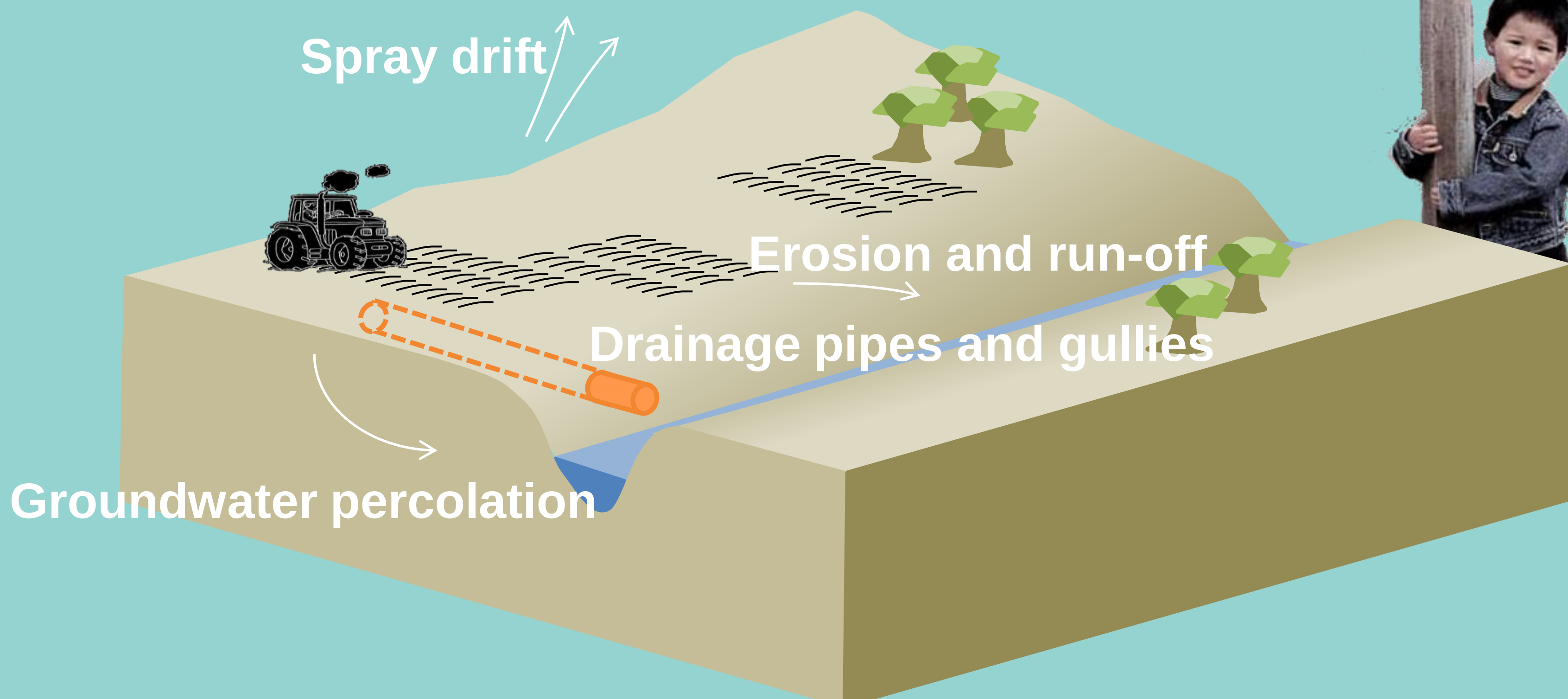
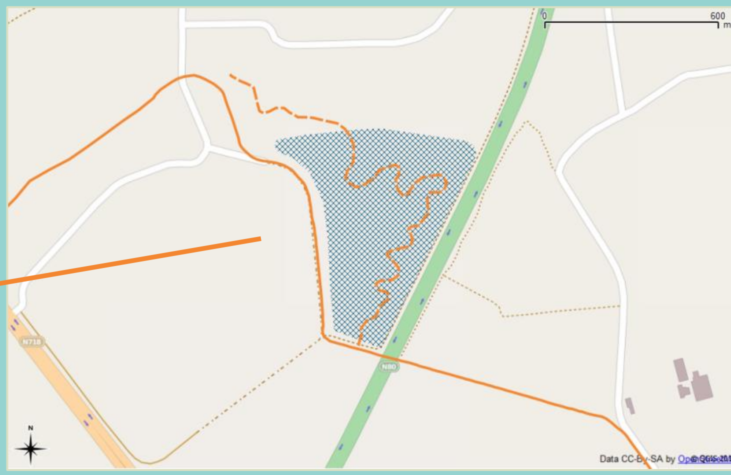
Inputs of pesticides into the environment





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

Constructed and restored wetlands

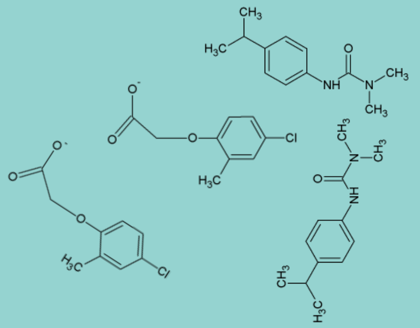
- First built for water retention and nature conservation

- Efficient retention of eroded soil, suspended matter, fertilizers and high sorbing pesticides
  (Shulz and Peall, Environmental science and technology, 2001)

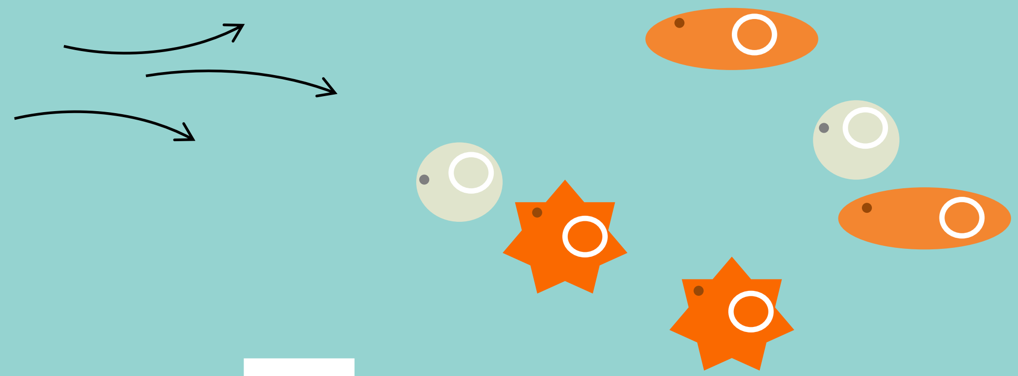
- Buffers for contaminants

- Few records for low sorbing compounds
  (Reichenberger *et al.*, Science of the Total Environment, 2007)

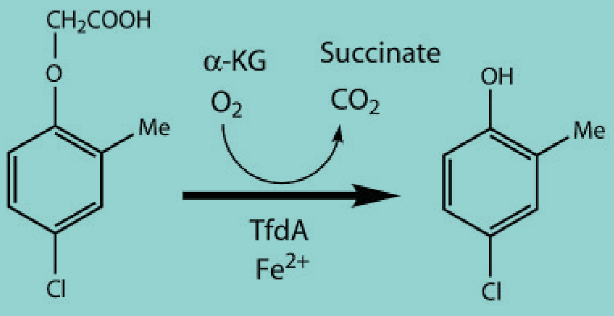
Microbial degradation of pesticides in wetlands



Soils that are regularly exposed to pesticides …



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

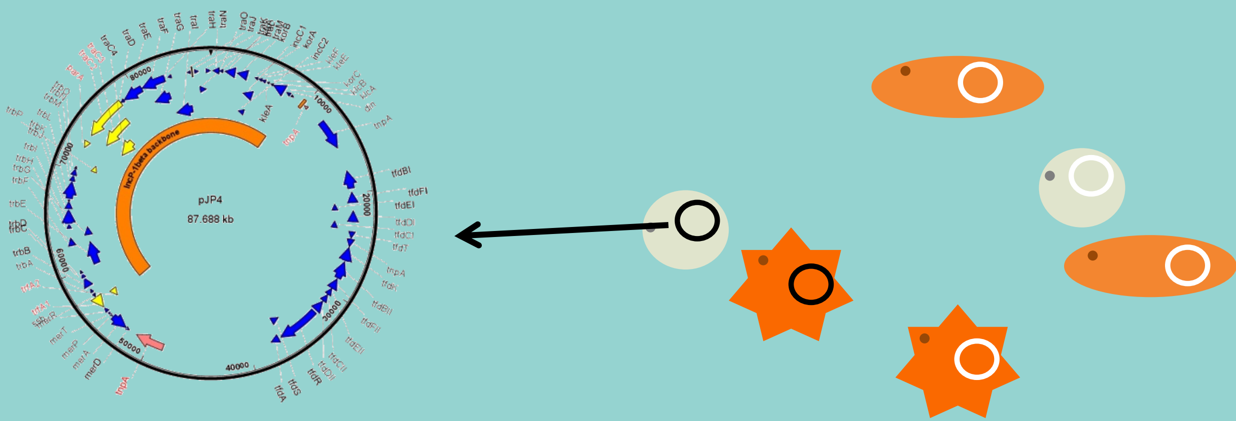


MCPA MCP

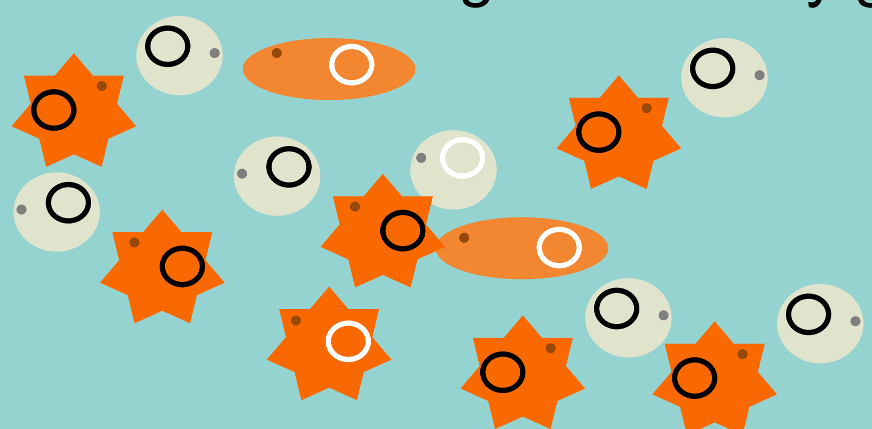
Image from Bælum *et al.* (Applied and Environmental Microbiology, 2006)

Microbial degradation of pesticides in wetlands

Genetic adaptation could have occurred …

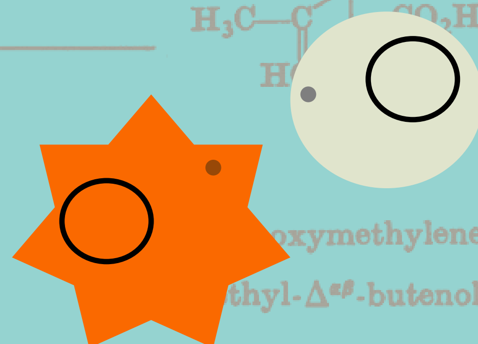


… and specialized microorganisms may grow/enrich.



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_2 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 phaze*.

- Release of nutrients from sediment may *stimulate growth*

Goal

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

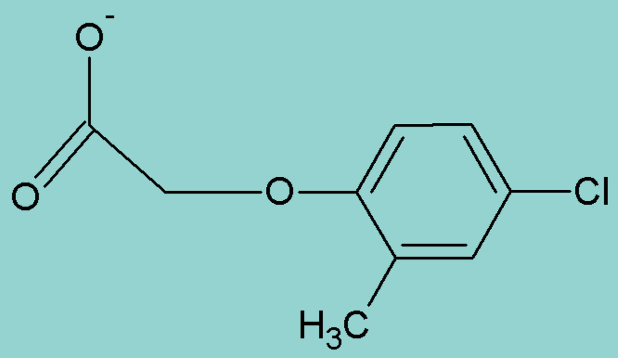
Objectives

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

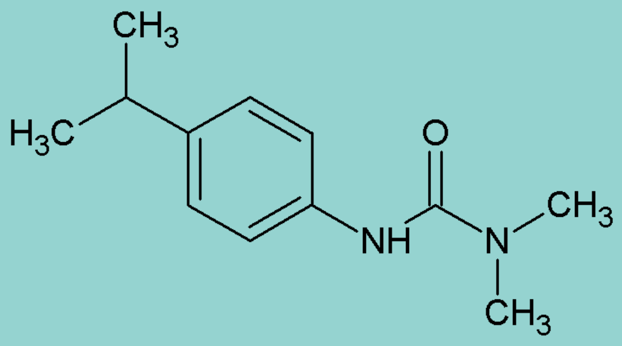


Herbicide to control annual and perennial broad-leaved weeds

Used for protection of
fruit
wheat, barley, …
lawn and grass courts
floriculture

Fast degradation in soil

IPU

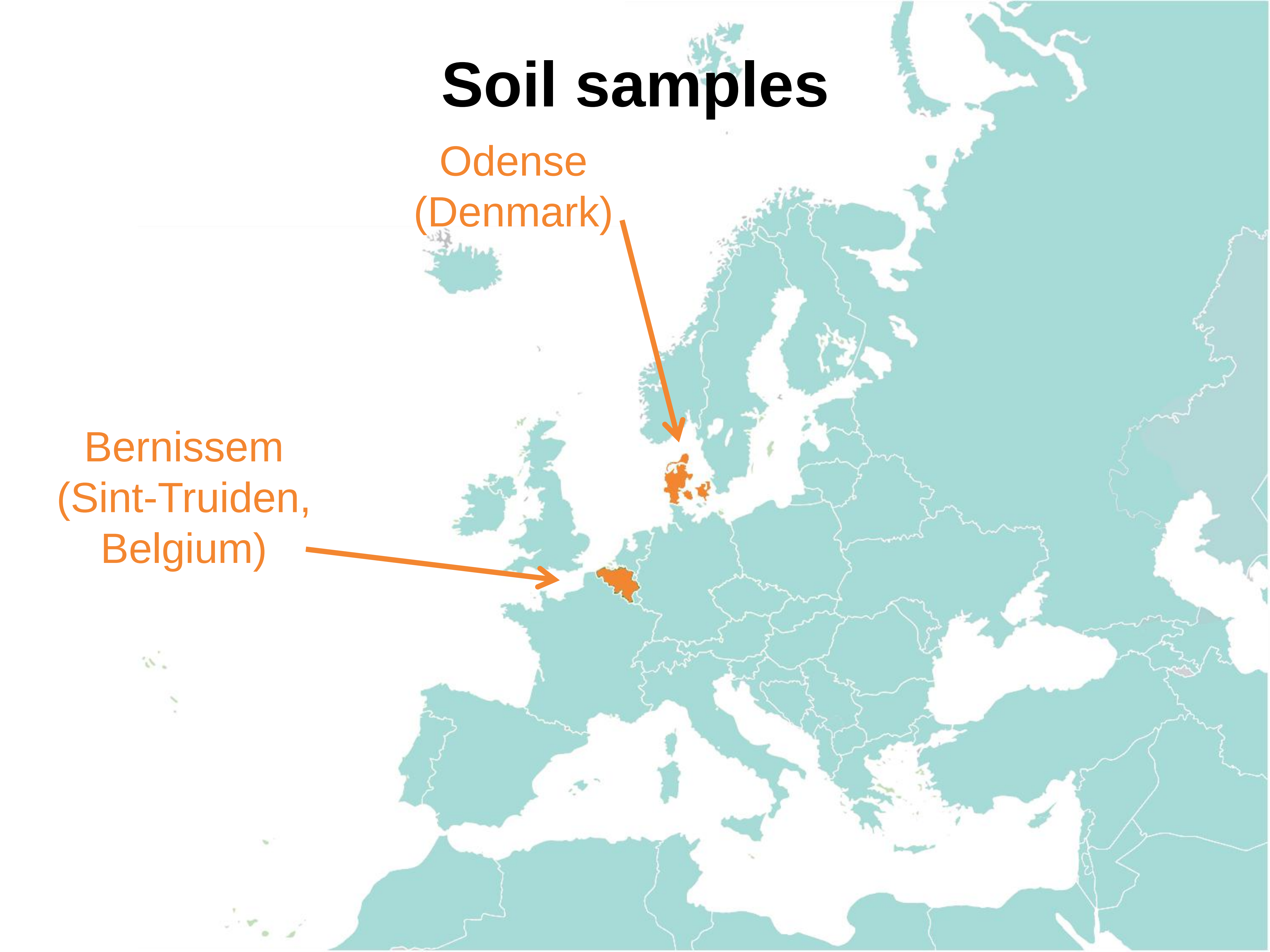


Herbicide to control annual grasses and many broad-leaved weeds

Used for protection of
wheat, barley, rye, …

Slow degradation

Soil samples



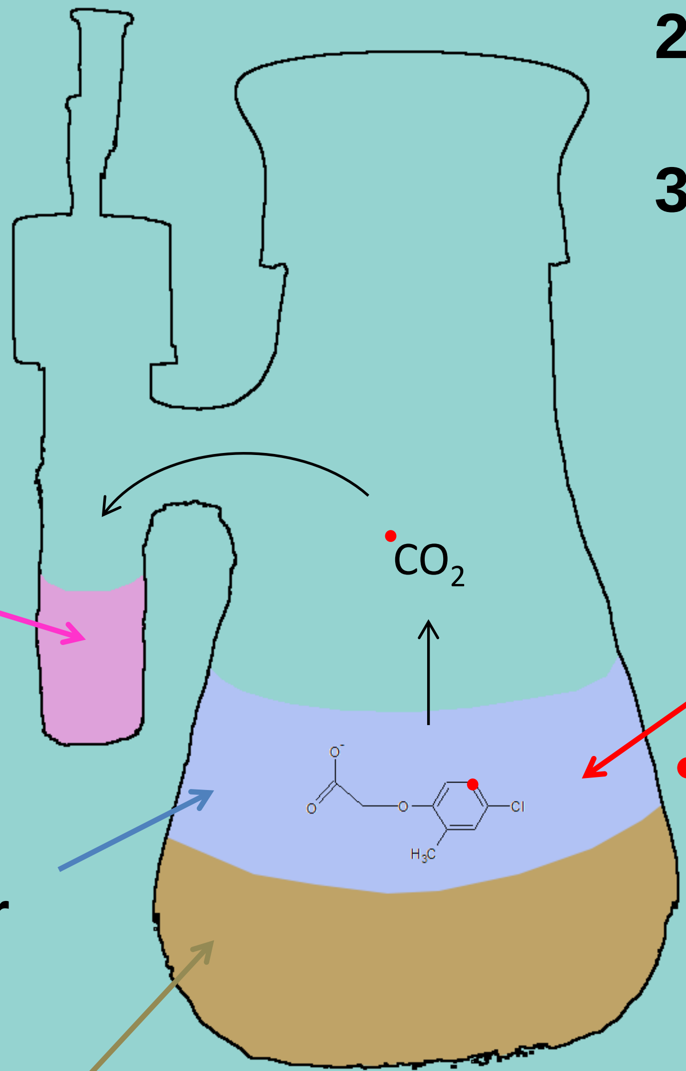
1

experiment

Lab microcosm wetland

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

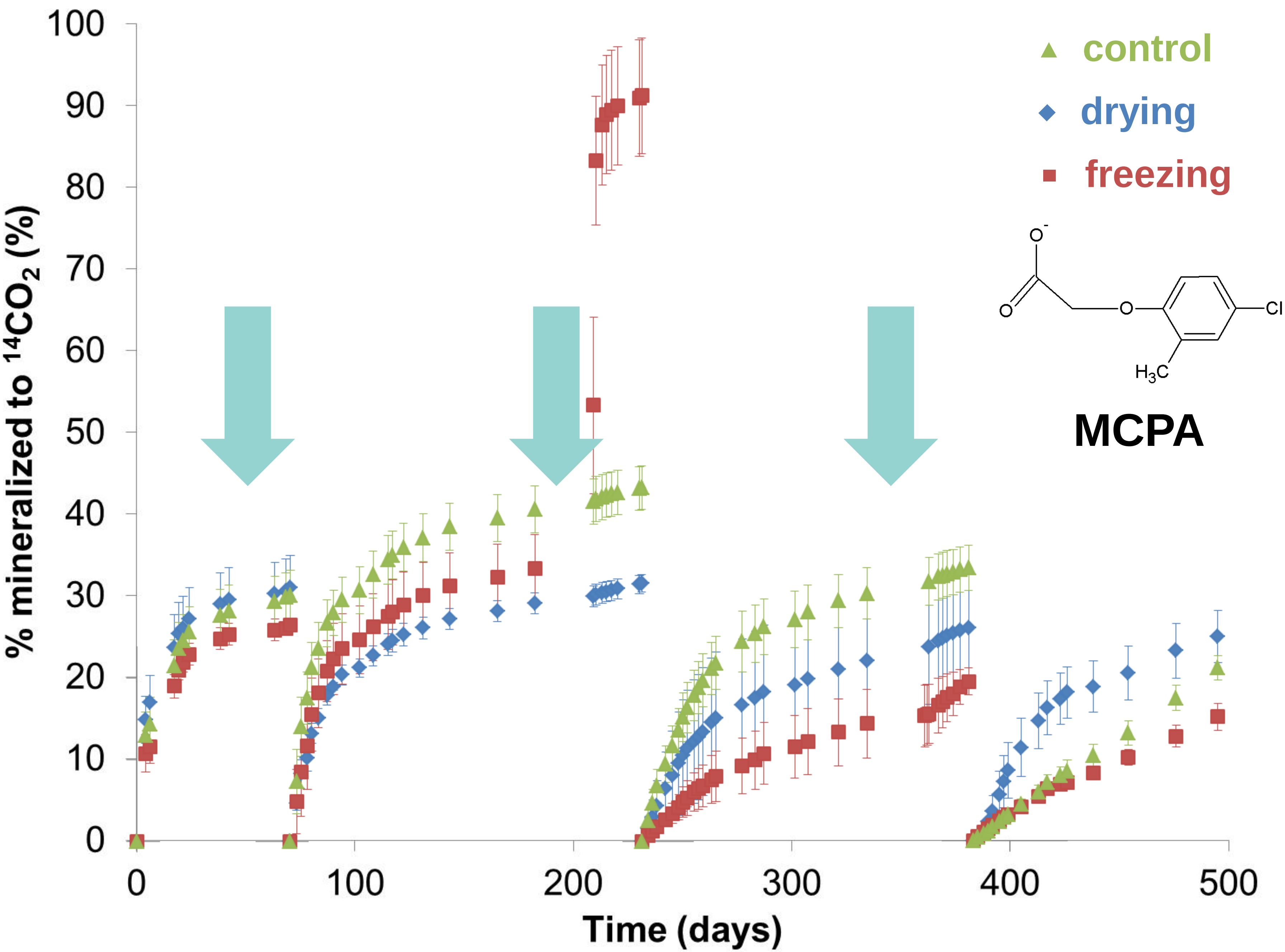
$^{14}CO_2$ recovery by NaOH and analysis with liquid scintillation counting (LSC)



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

50 ml water

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



control
drying
freezing
MCPA
O-
O
O
Cl
H_3C
% mineralized to $^{14}CO_2$ (%)
Time (days)
0
10
20
30
40
50
60
70
80
90
100
0
100
200
300
400
500

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 $^{14}CO_2$ 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

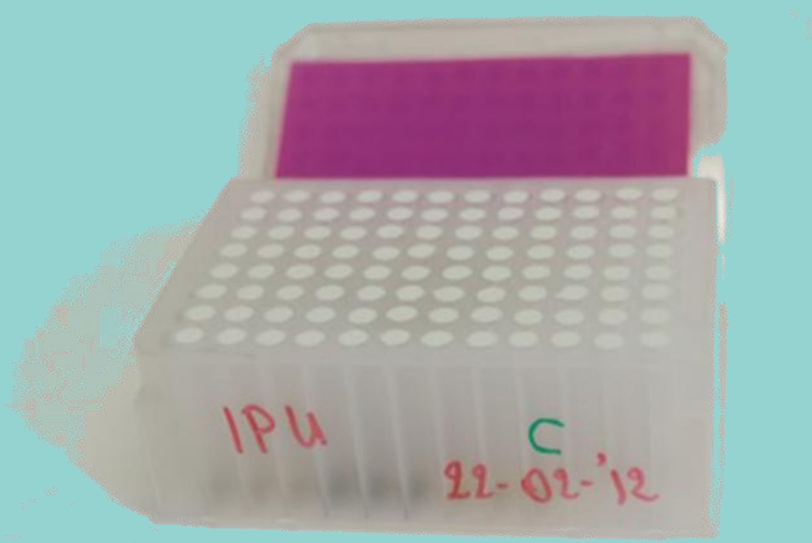
experiment

2

Soil samples were taken at 6 moments

August | December | January | February | March | May

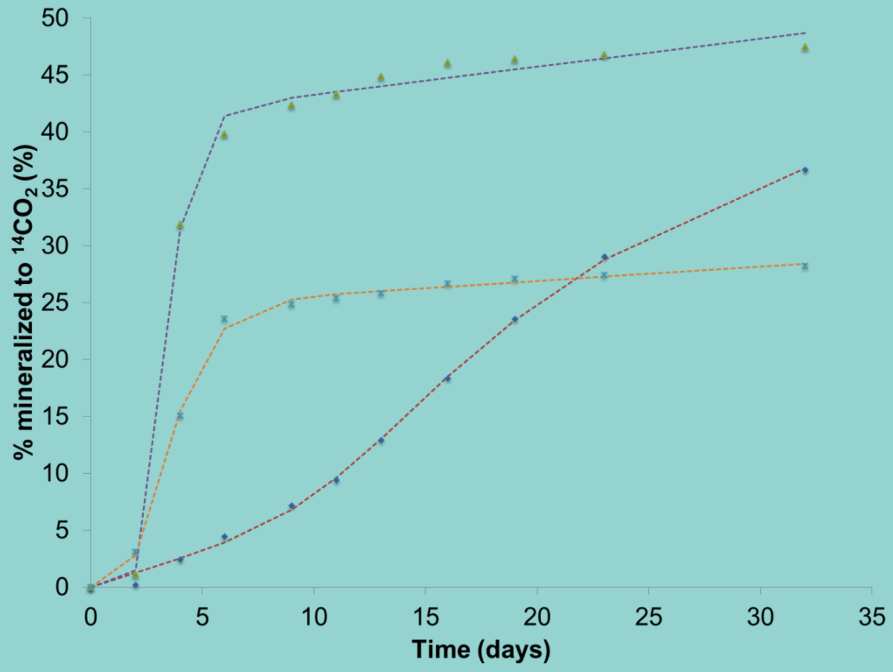
30 samples (n = 30) were taken within the wetland with 3 replicates per location
(within radius of 50 cm)



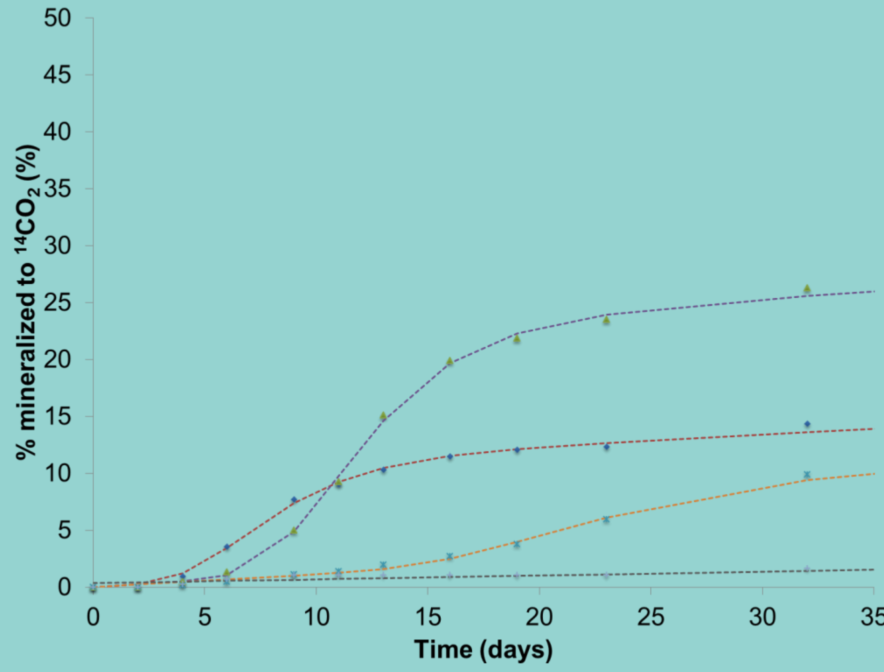
Johnsen *et al.*, Journal of Microbiological Methods, 2009

- 5 g sample was suspended in 5 ml minimal media (MMO)
- Suspensions were shaken head-over-end overnight
- 3 aliquots of 100 µl were transferred to microplate
- 60 Bq of ^{14}C-labelled MCPA or IPU were added
- Incubated @ 20 °C
- $Ca(OH)_2$-coated seals to capture $^{14}CO_2$

MCPA



IPU



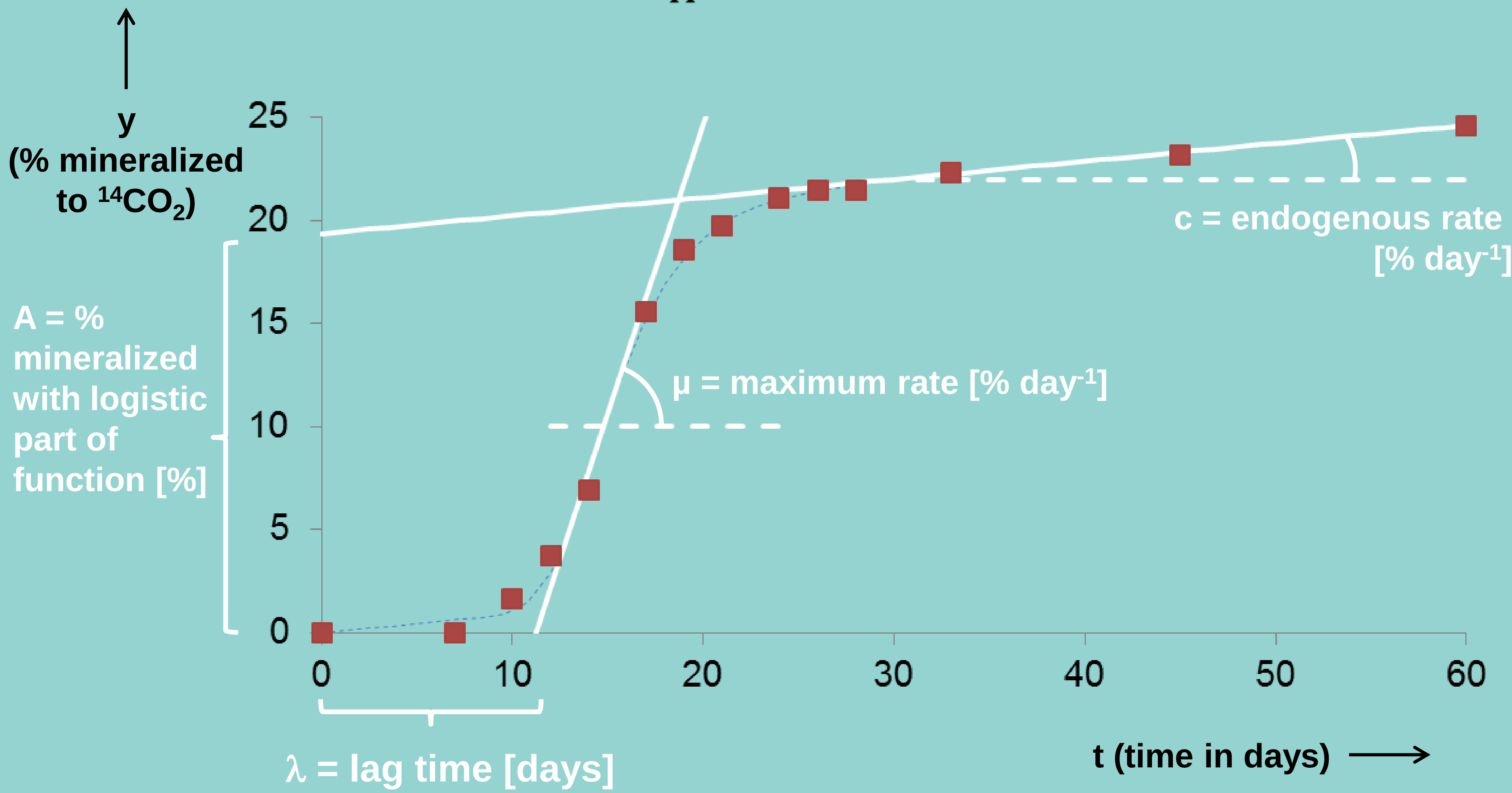
- Mineralization everywhere
- High mineralization rates
- High cumulative mineralization (up to 50 %)

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

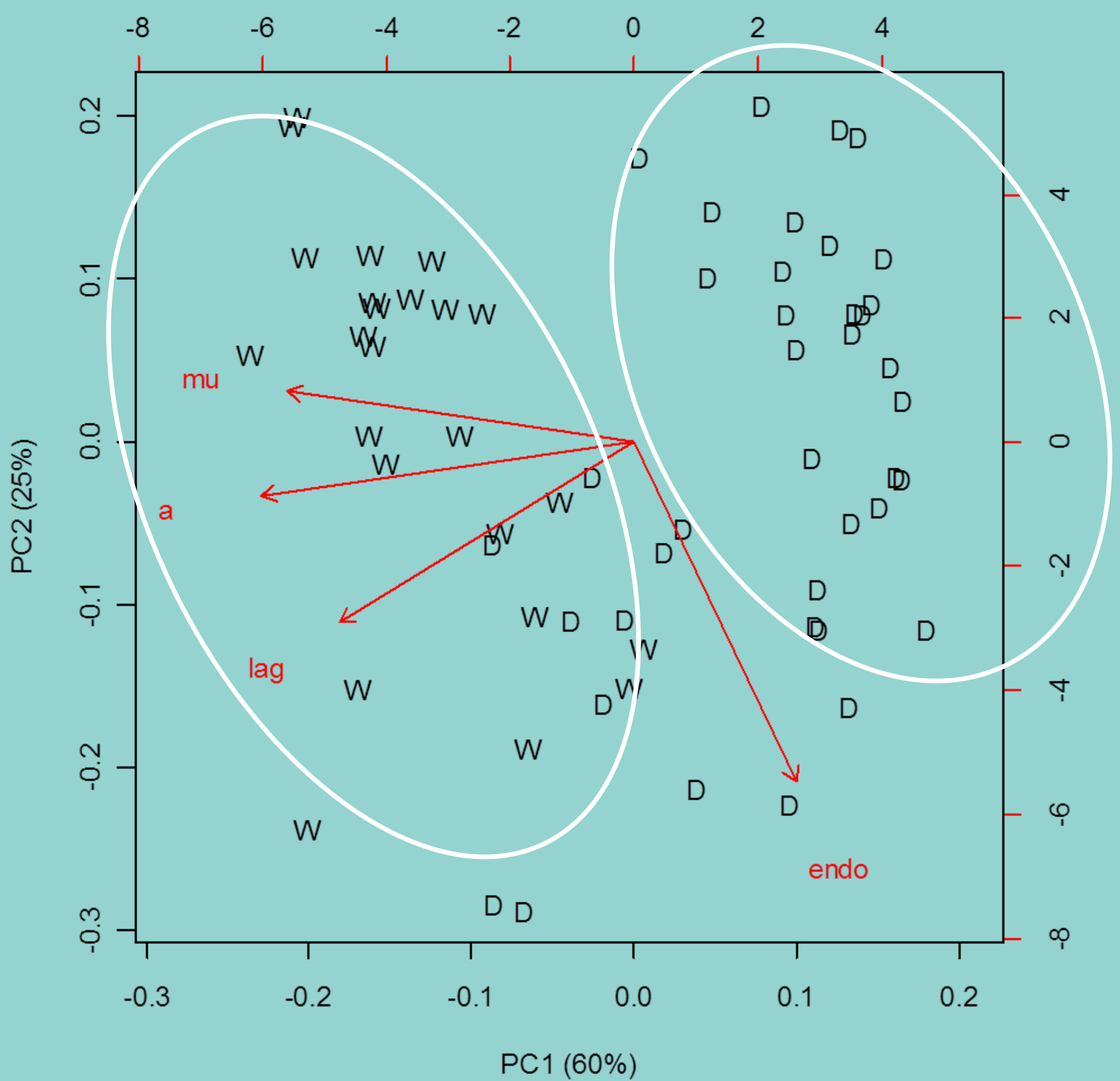
Modified Gompertz model

Modified from Zwietering *et al.*, AEM, 1990

$$y = A\,exp\left\{-exp\left[\frac{e}{A}(\mu\lambda-(\mu-c)t)+1\right]\right\}+ct$$

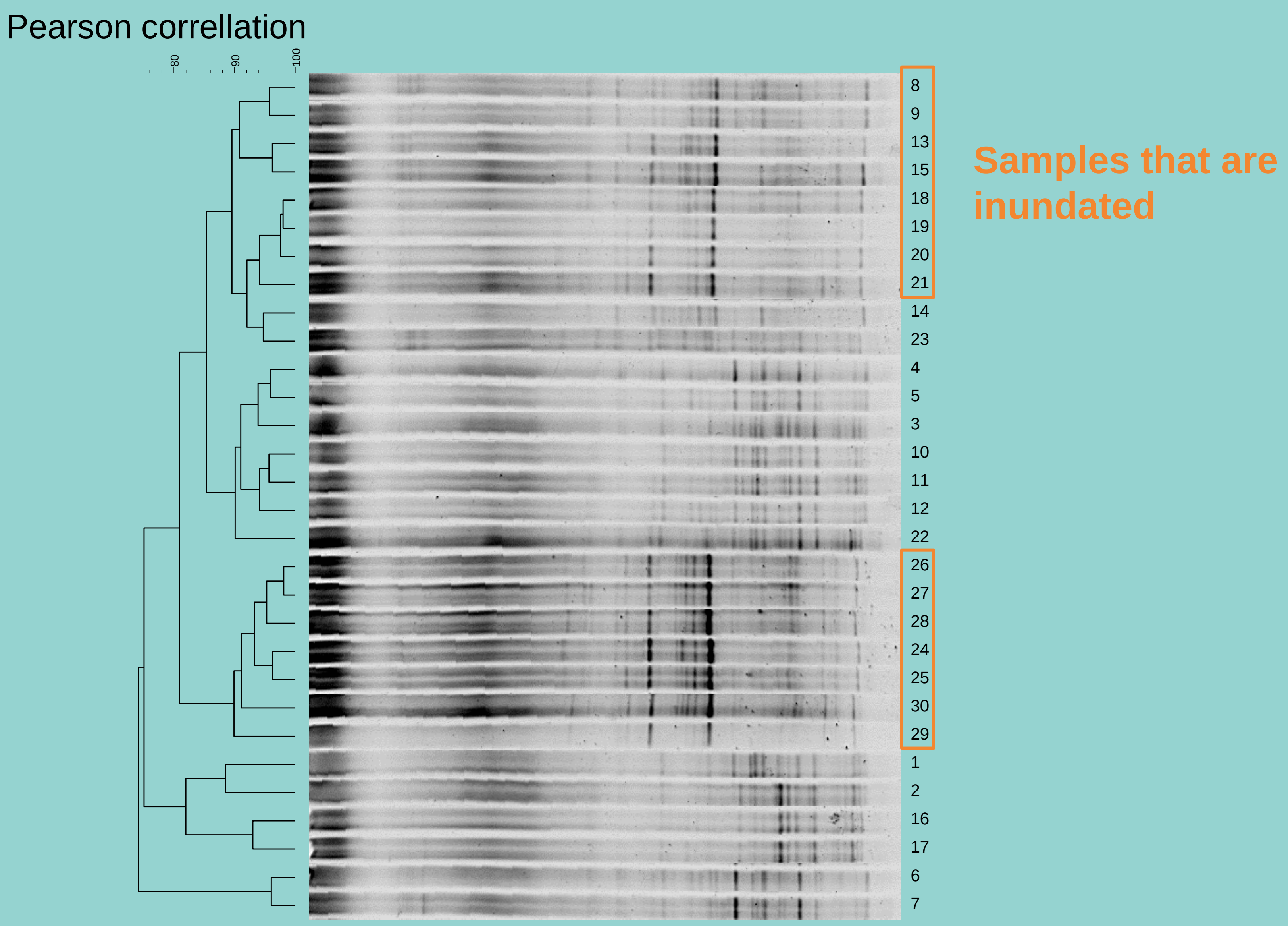


MCPA (January)



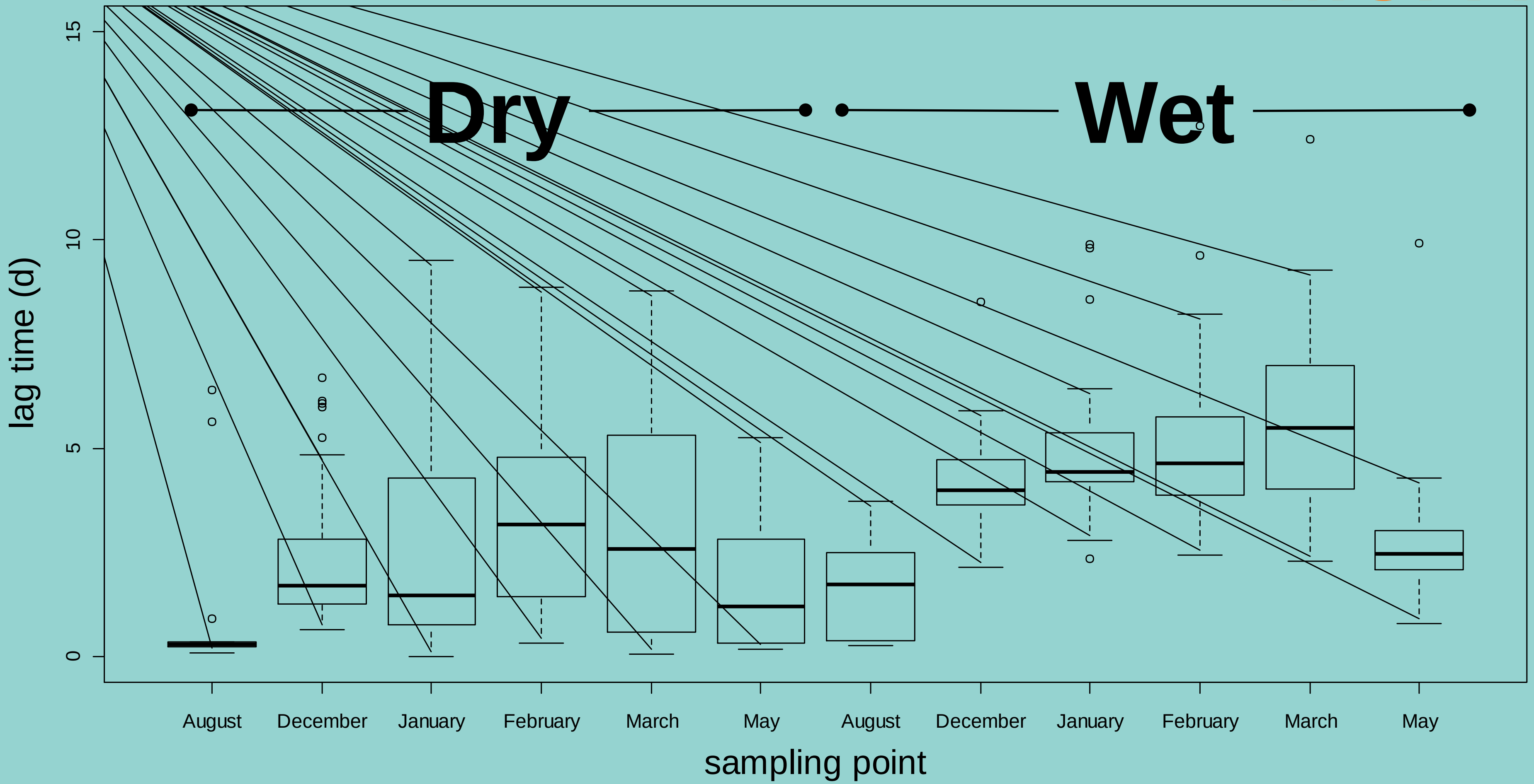
DGGE betaproteobacteria (January)

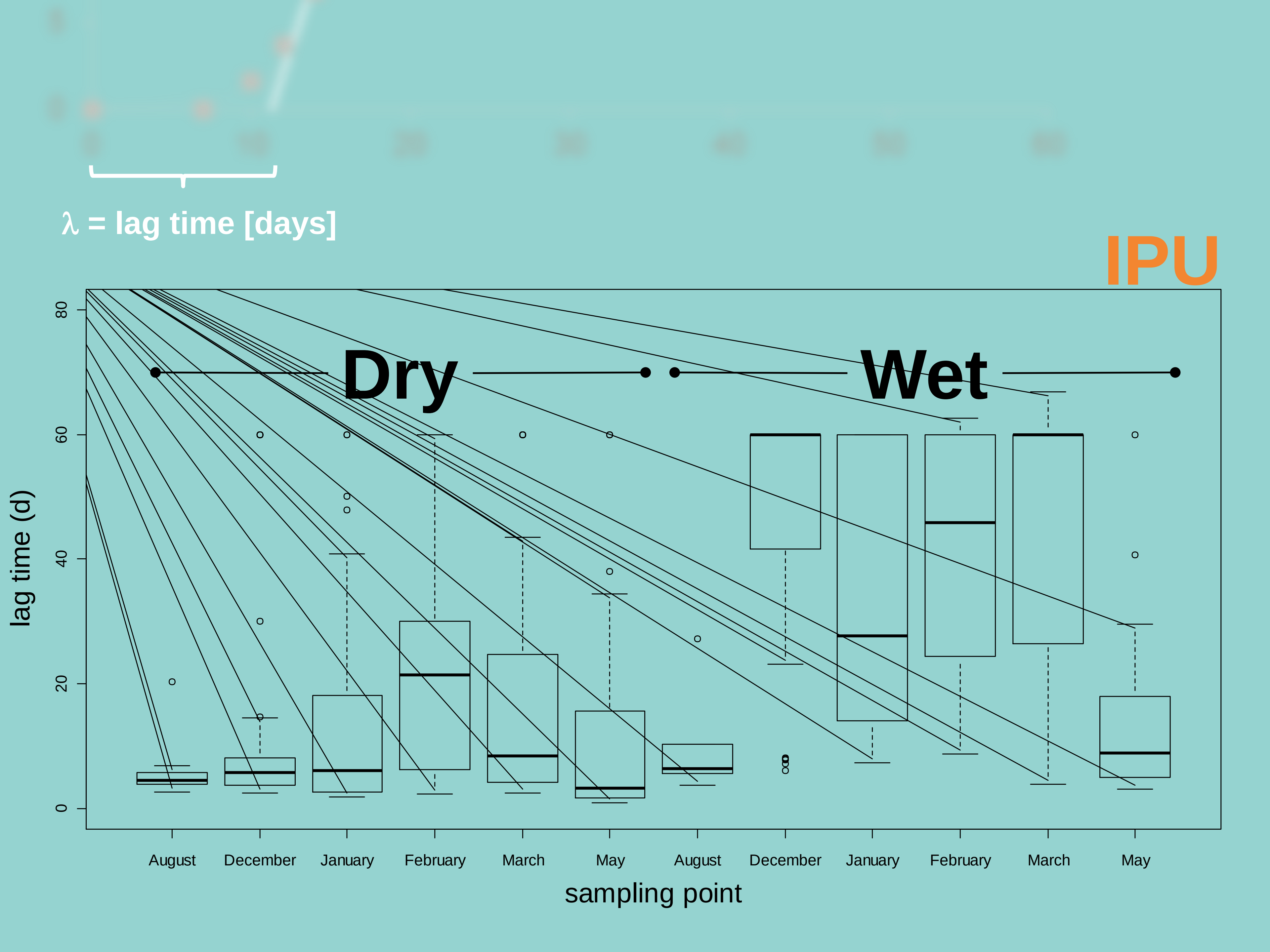
F948ß & R1492 and F984GC & R1378 (nested)



λ = lag time [days]

MCPA

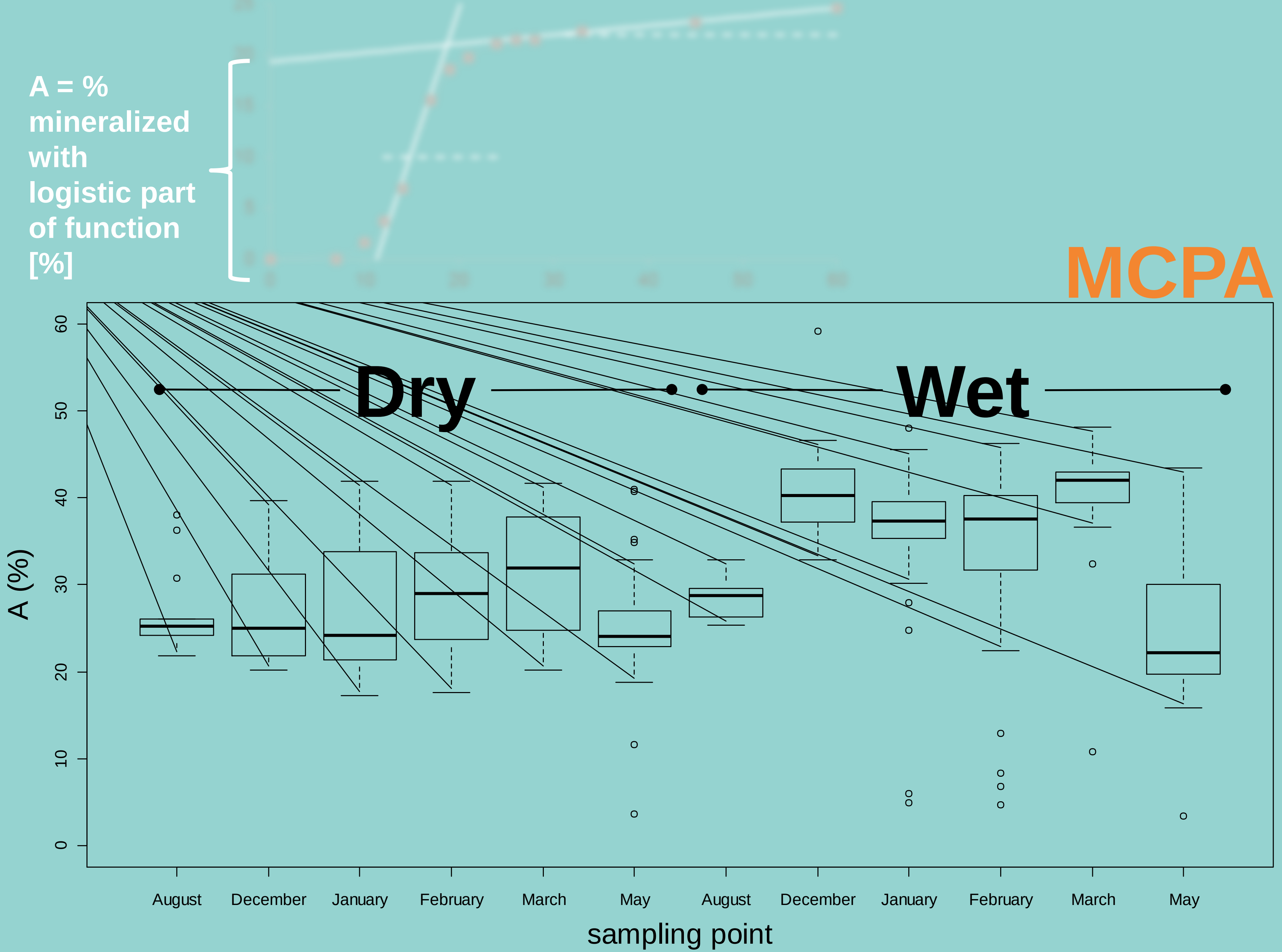




λ = lag time [days]
IPU
Dry
Wet
lag time (d)
0
20
40
60
80
August
December
January
February
March
May
August
December
January
February
March
May
sampling point

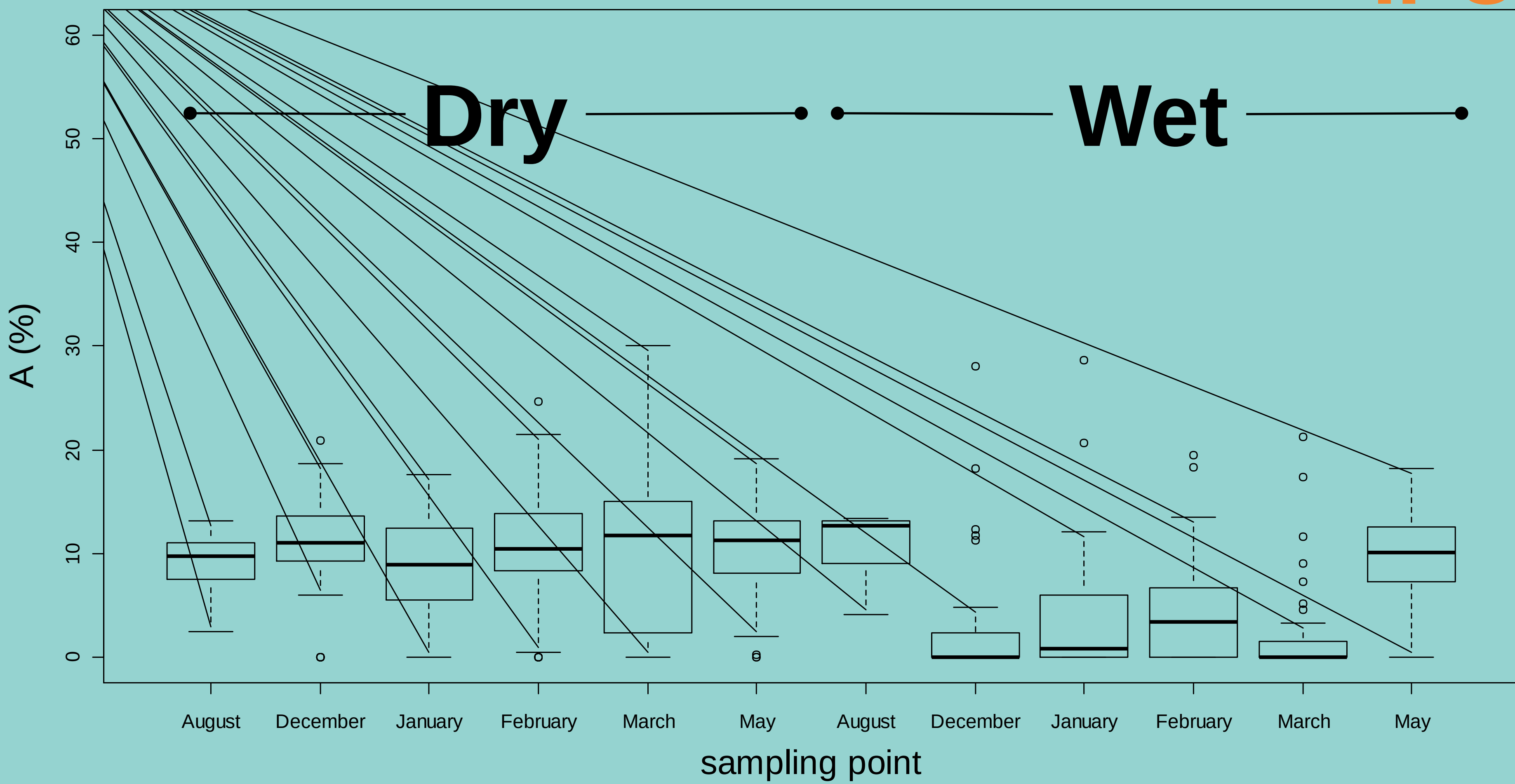
A = % mineralized with logistic part of function [%]

MCPA

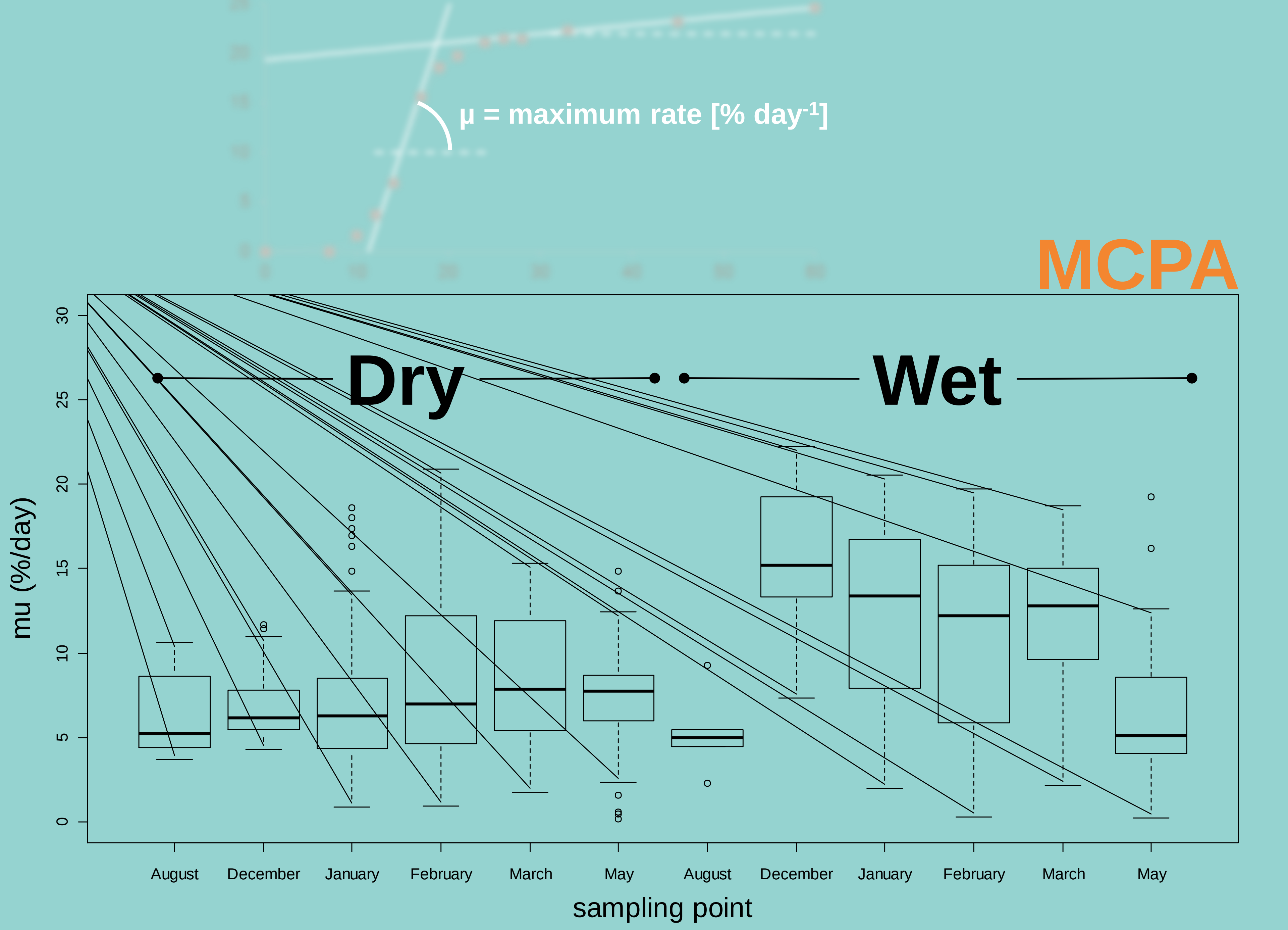


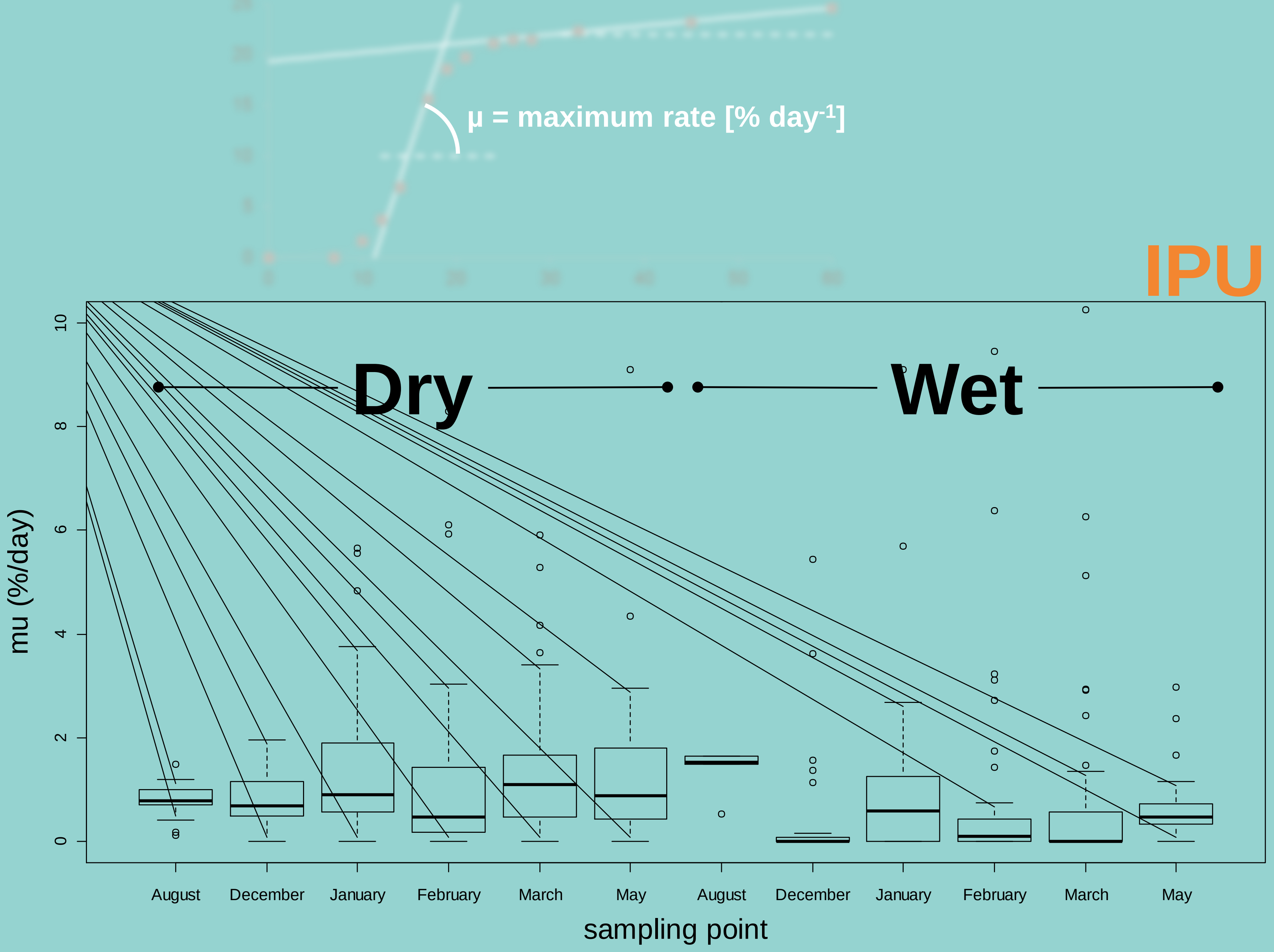
A = % mineralized with logistic part of function [%]

IPU



MCPA


μ = maximum rate [% day-1]
Dry
Wet
mu (%/day)
0
5
10
15
20
25
30
August
December
January
February
March
May
August
December
January
February
March
May
sampling point



μ = maximum rate [% day-1]
IPU
Dry
Wet
mu (%/day)
0
2
4
6
8
10
August
December
January
February
March
May
August
December
January
February
March
May
sampling point

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 rates were higher and relatively more MCPA was converted to CO_2
- No clear effect of a freezing period *in situ* on the mineralization in the lab
- In May, the discrepancy between the inundated and dry samples disappeared, for both MCPA and IPU

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yes…

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

yes…

Many thanks to

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Prof. Jan Diels

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Watering van Sint-Truiden and IWT