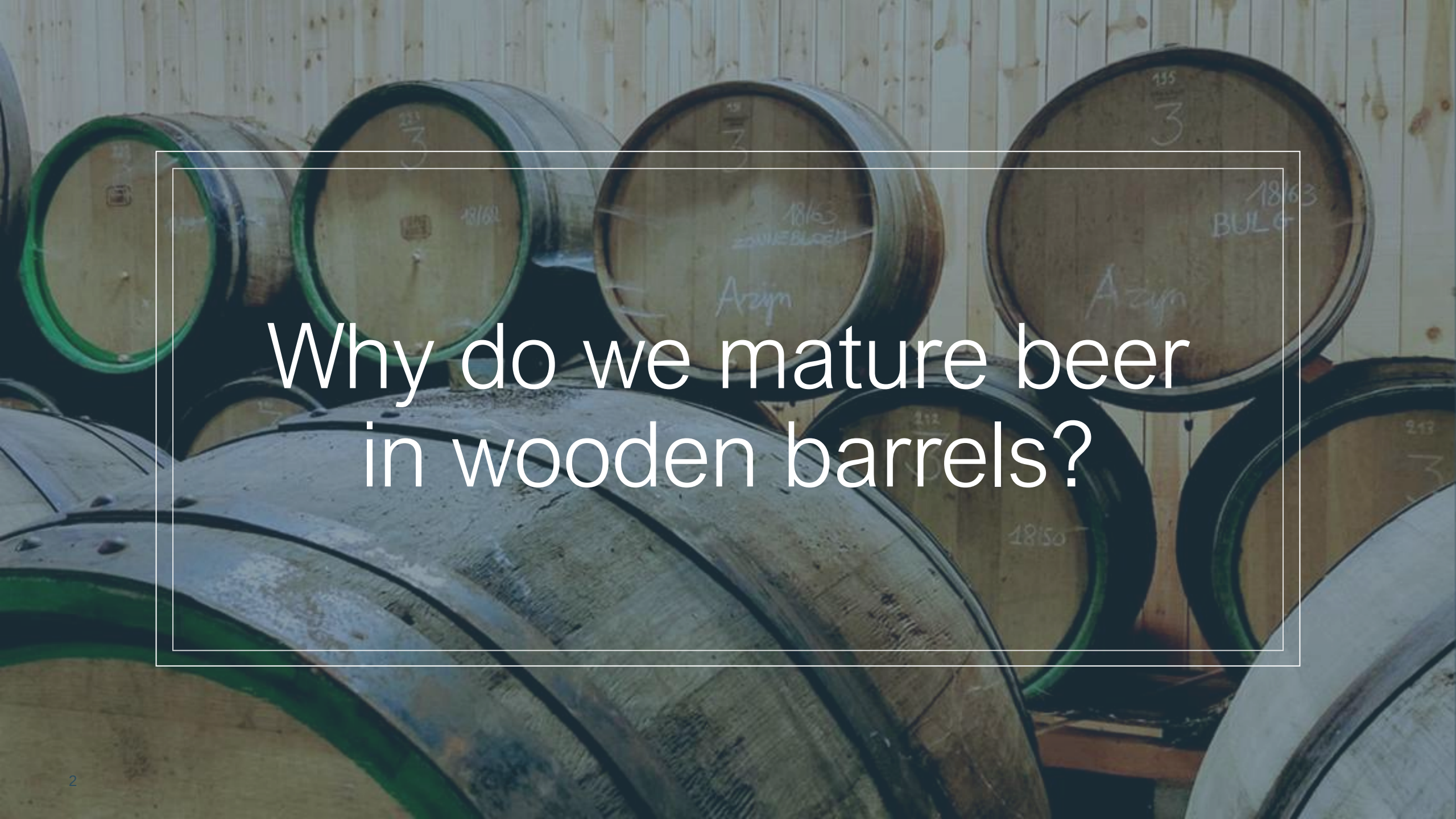
Does it matter who comes first?

Exploring how microbial community is established in barrel-aged sour beers

Tin Kocijan, KU Leuven

With brewres, for brewers (S pivarima, za pivare) 27th March 2026

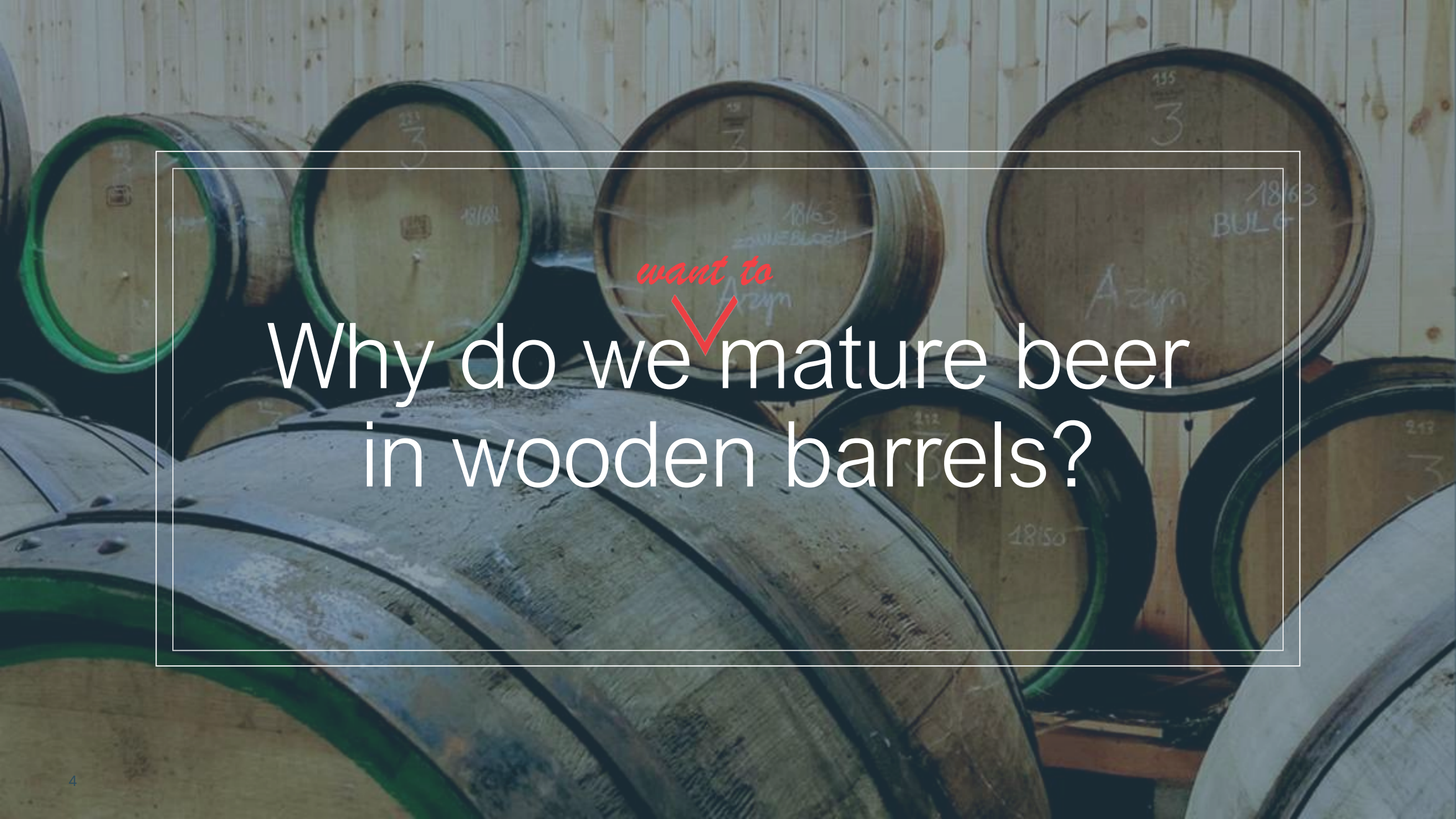


Why do we mature beer in wooden barrels?

Because we **have** to

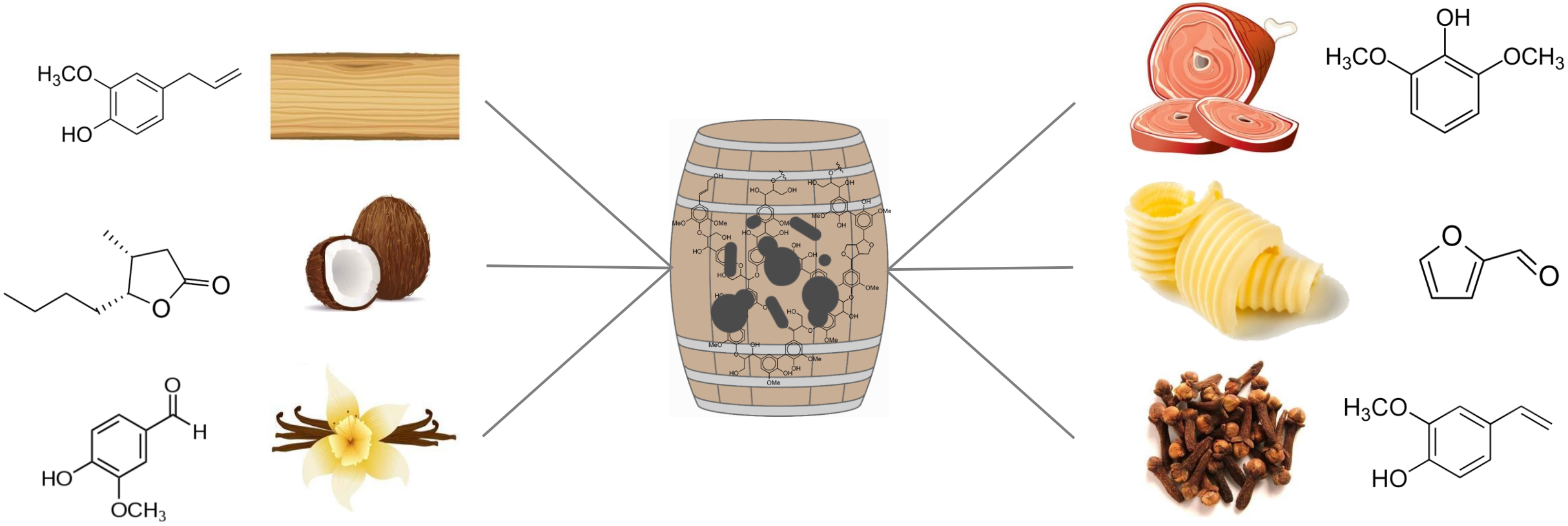
Barrels depicted on Trajan’s column (113 AD)

Because we **want** to



Why do we mature beer in wooden barrels?

Wood ageing yields complex flavours and aromas to beers



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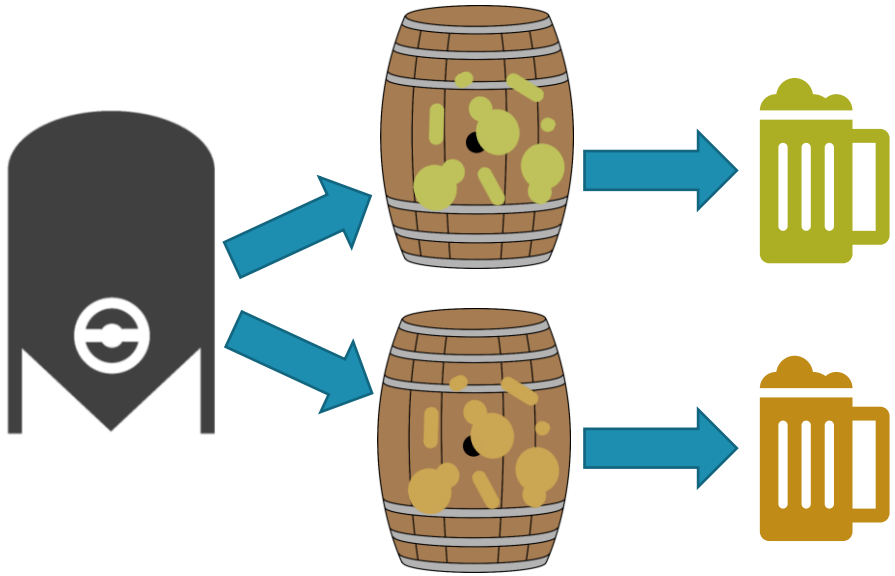
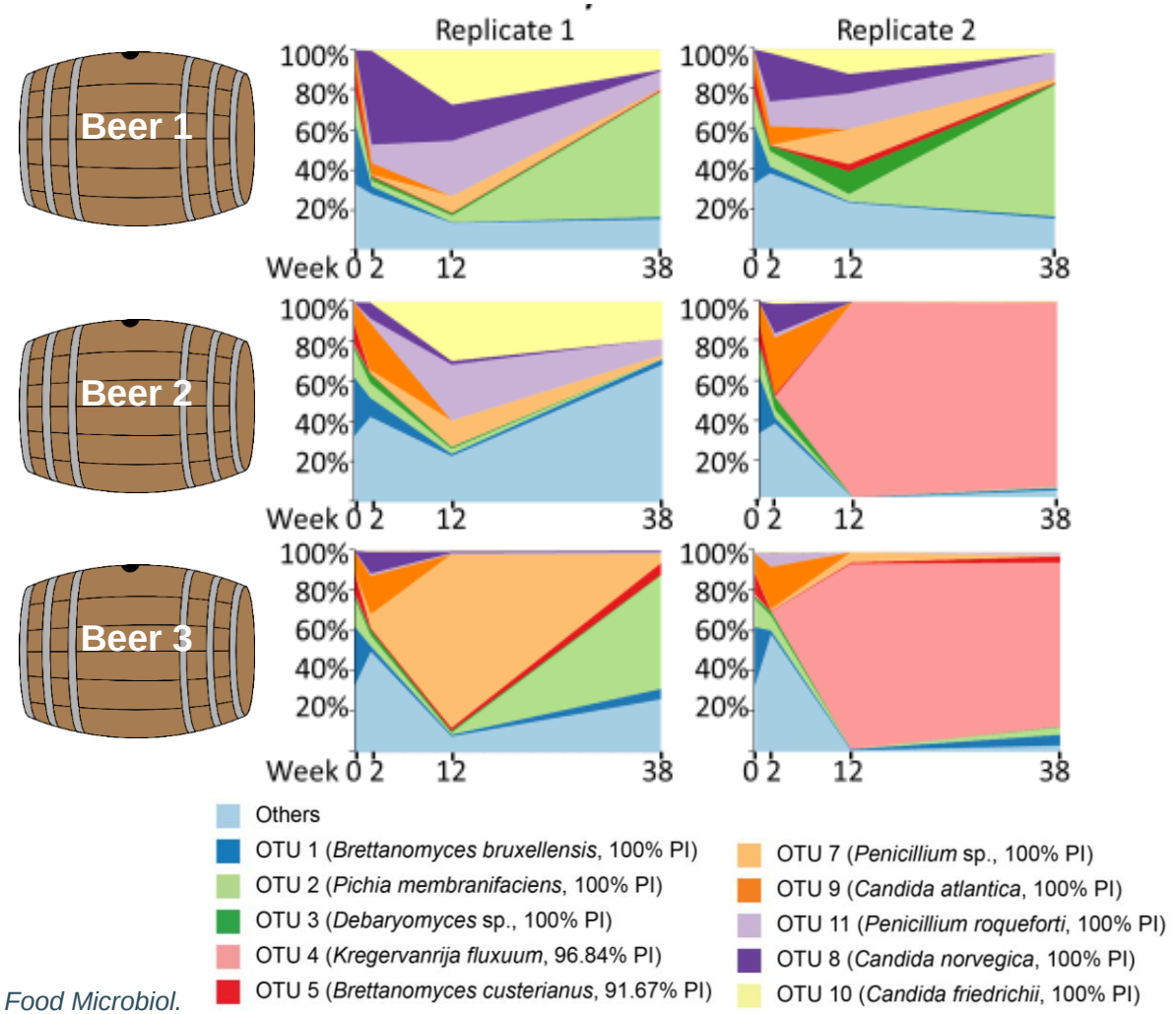
Problem: Lack of consistency and predictability

Different microbial communities are developing in the **same beer**!

Different end product and **profit losses**





Bossaert *et al.*, 2020, *Int. J. Food Microbiol.*

Solution: Develop process control strategy

Focus on **controlling the microbiological aspect** of the wood maturation of beer

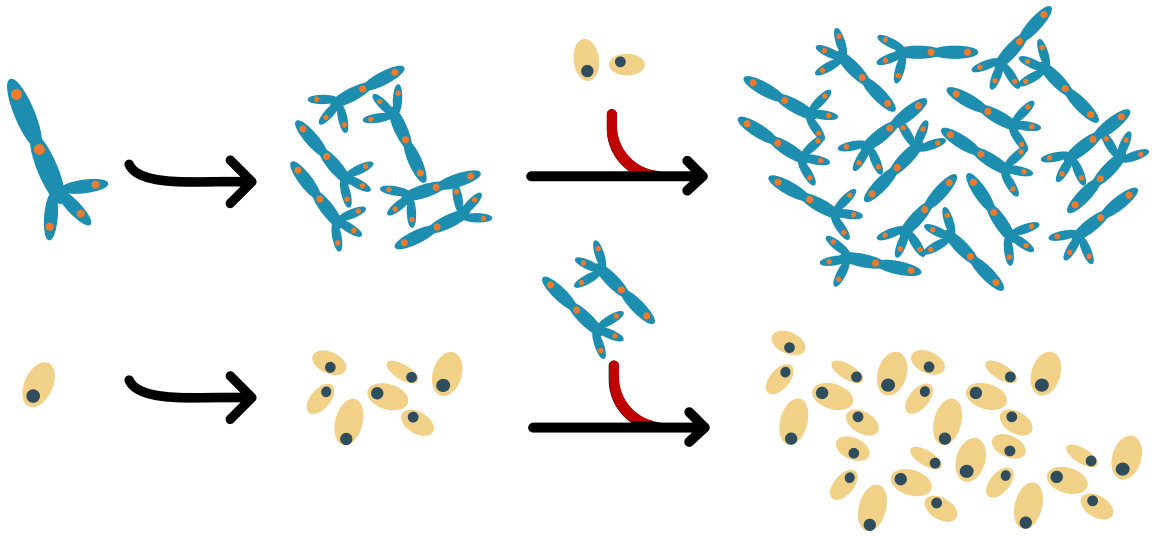
How are the microbial communities **established?**
What are the **driving factors?**

Priority effects are driving microbial community establishment

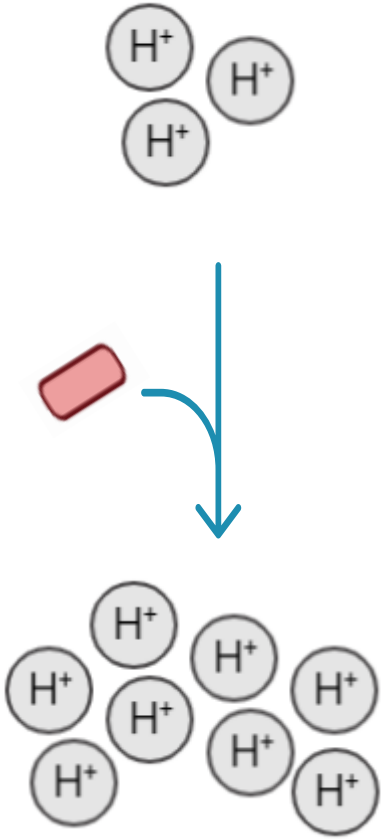
The **arrival timing** of species into the niche will influence what **community composition** will look like in the future.

Chapell and Fukami, 2018, *Yeast*
Chapell et al., 2022, *eLife*

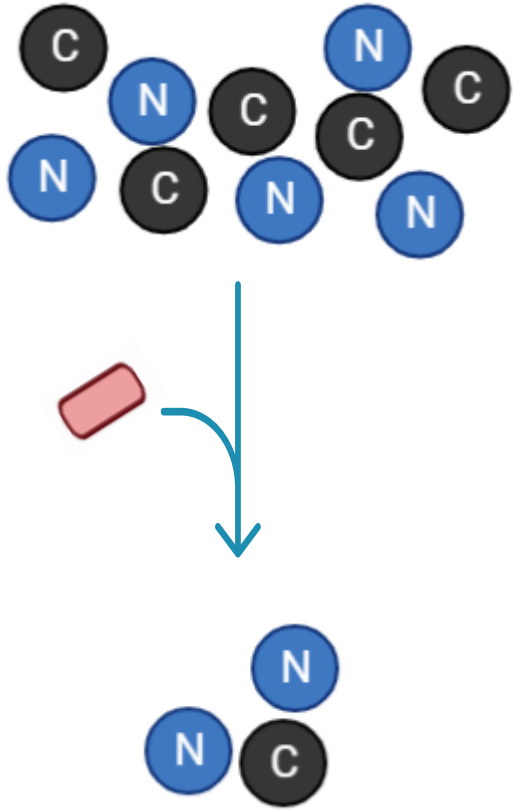
Priority effects are driving microbial community establishment



Niche **modification**



Resource **consumption**

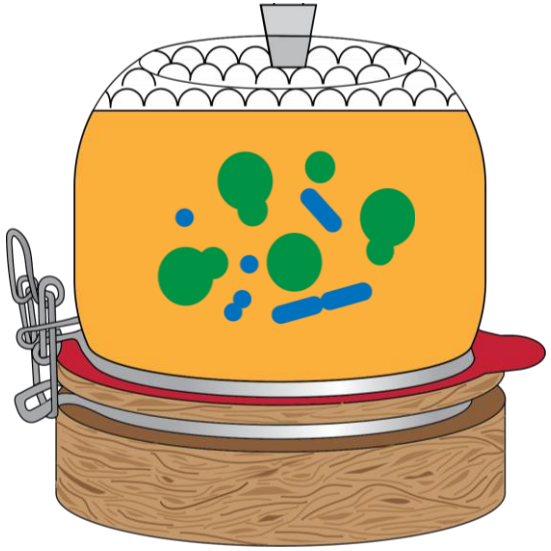


Chapell and Fukami, 2018, *Yeast*
Chapell et al., 2022, *eLife*

Hypothesis

Order of microbial colonization plays a key role in the microbial community establishment during wood maturation of beer

Development of tractable *in vitro* ecosystem



Controllability

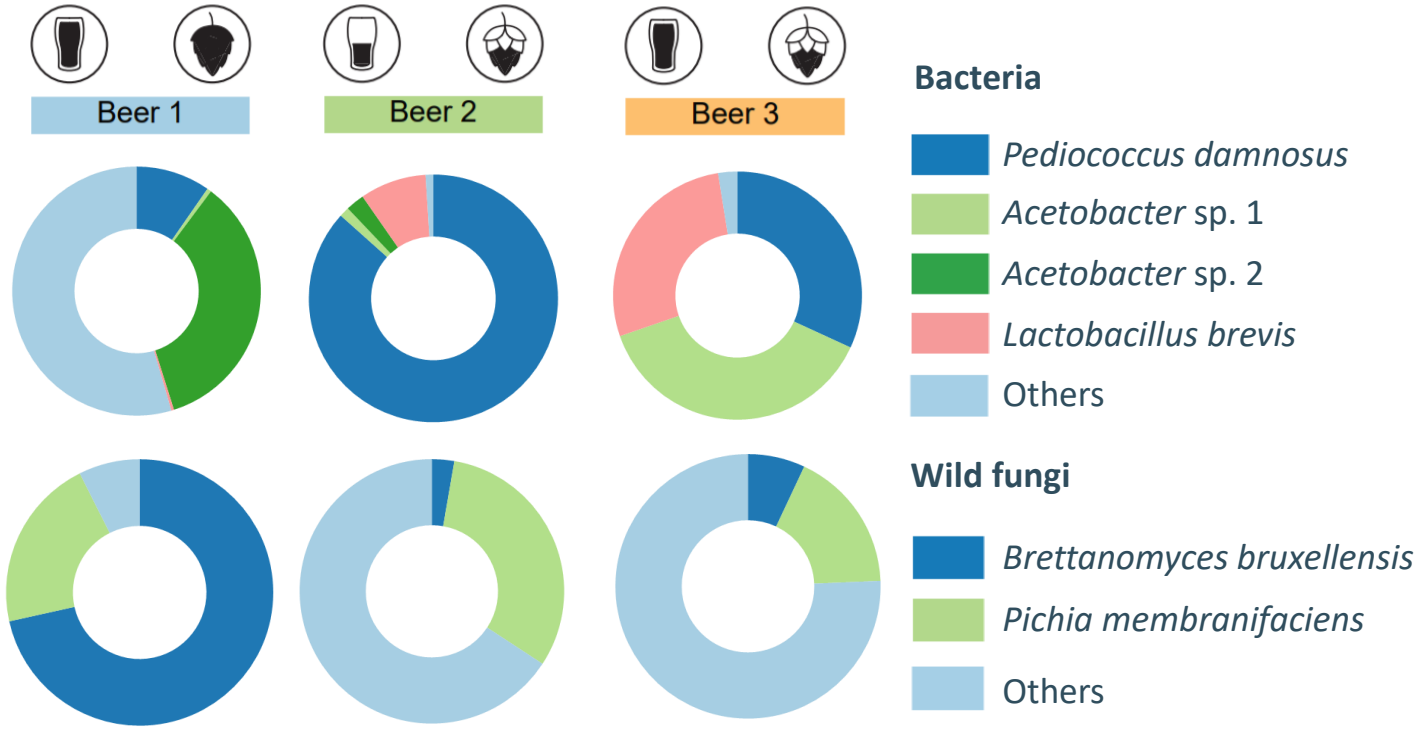
Modularity

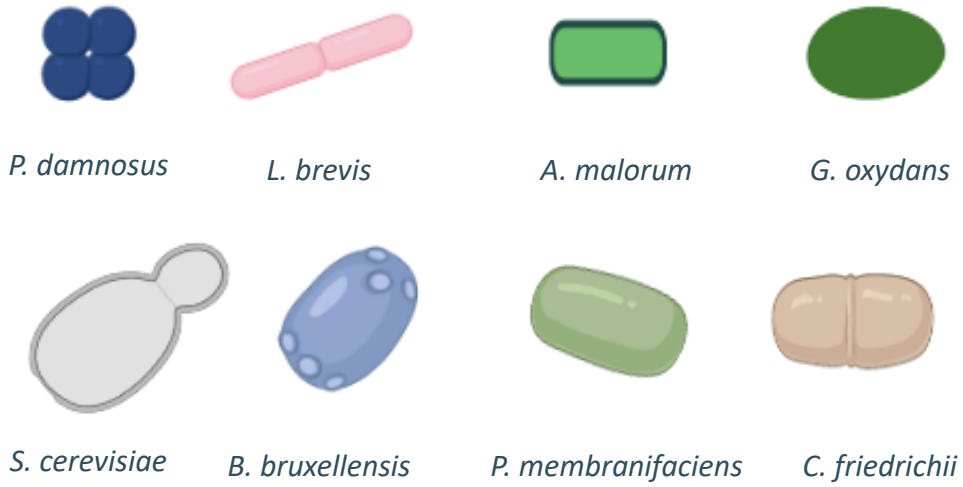
Reproducibility

Bossaert *et al*., 2023, *BrewingScience*

Wolfe and Dutton, 2015, *Cell*

Microbial community in the *in vitro* ecosystem





Bossaert et al., *IJFM*, 2020

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THE 8TH FILM BY Leuven Beer Research Institute productions
Pichy mem
Acetto
P-dio
Bretty brett
Sacchy sacch
Candy
Goxy
Lacty B
THE HATEFUL EIGHT
IN labs nearby JANUARY 8

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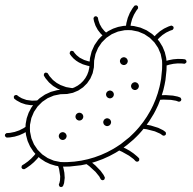
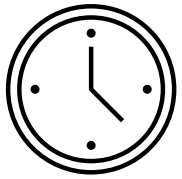
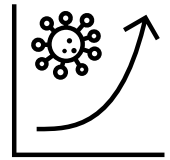
Which microbes will be tested for their priority effect?



How long should be the **priority effect window**?



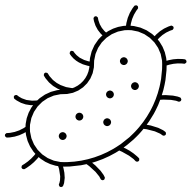
What should be the **size** of the **cell inoculum**?



Which microbes will be tested for their priority effect?

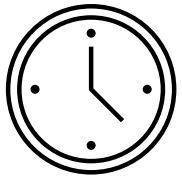
Acetobacter malorum

Brettanomyces bruxellensis



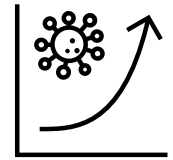
How long should be the **priority effect window**?

2 weeks



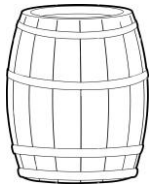
What should be the **size** of the **cell inoculum**?

100 cells/mL



Belgian blond
6.5% ABV x 29.3 IBU

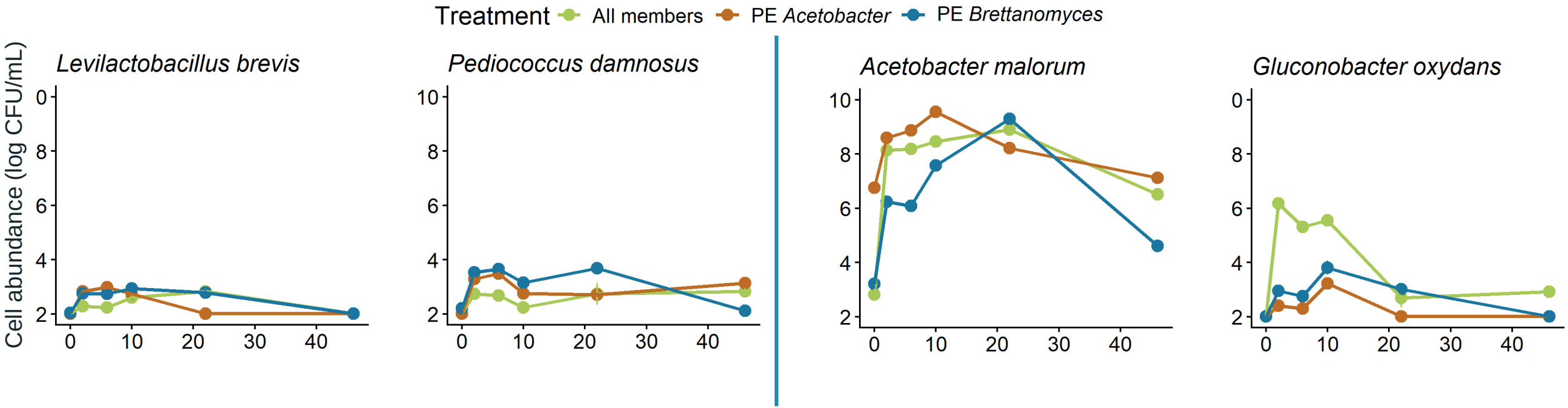
21°C



New European oak

qPCR

0, 2, 6,10, 22, 46 weeks



Priority effects exerted by *Acetobacter and Brettanomyces* have overall **facilitative effect** on LAB in the first ten weeks

After ten weeks priority effect of *Acetobacter* has inhibitory effect for on *Levilactobacillus*, but no effect on *Pediococcus*.

Priority effect exerted by *Brettanomyces* has **inhibitory effect** on acetic acid bacteria

Priority effect exerted by *Acetobacter* and *Brettanomyces* has **inhibitory effect** on *Gluconobacter*.

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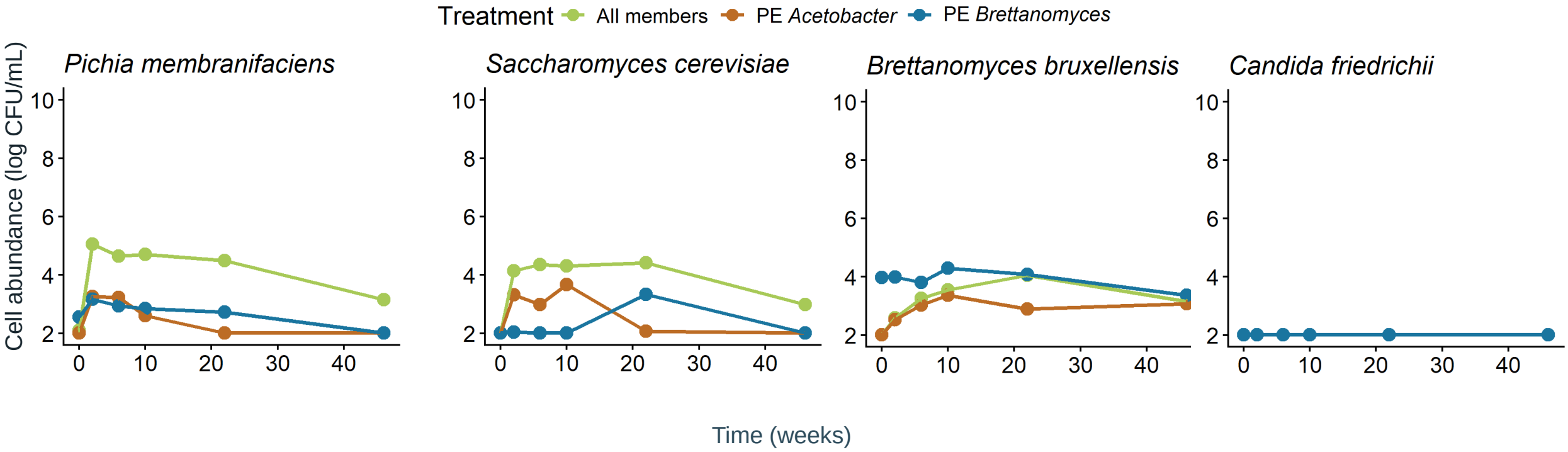


Priority effects exerted by *Acetobacter* and *Brettanomyces* have **inhibitory effect** on *Saccharomyces* and *Pichia*.

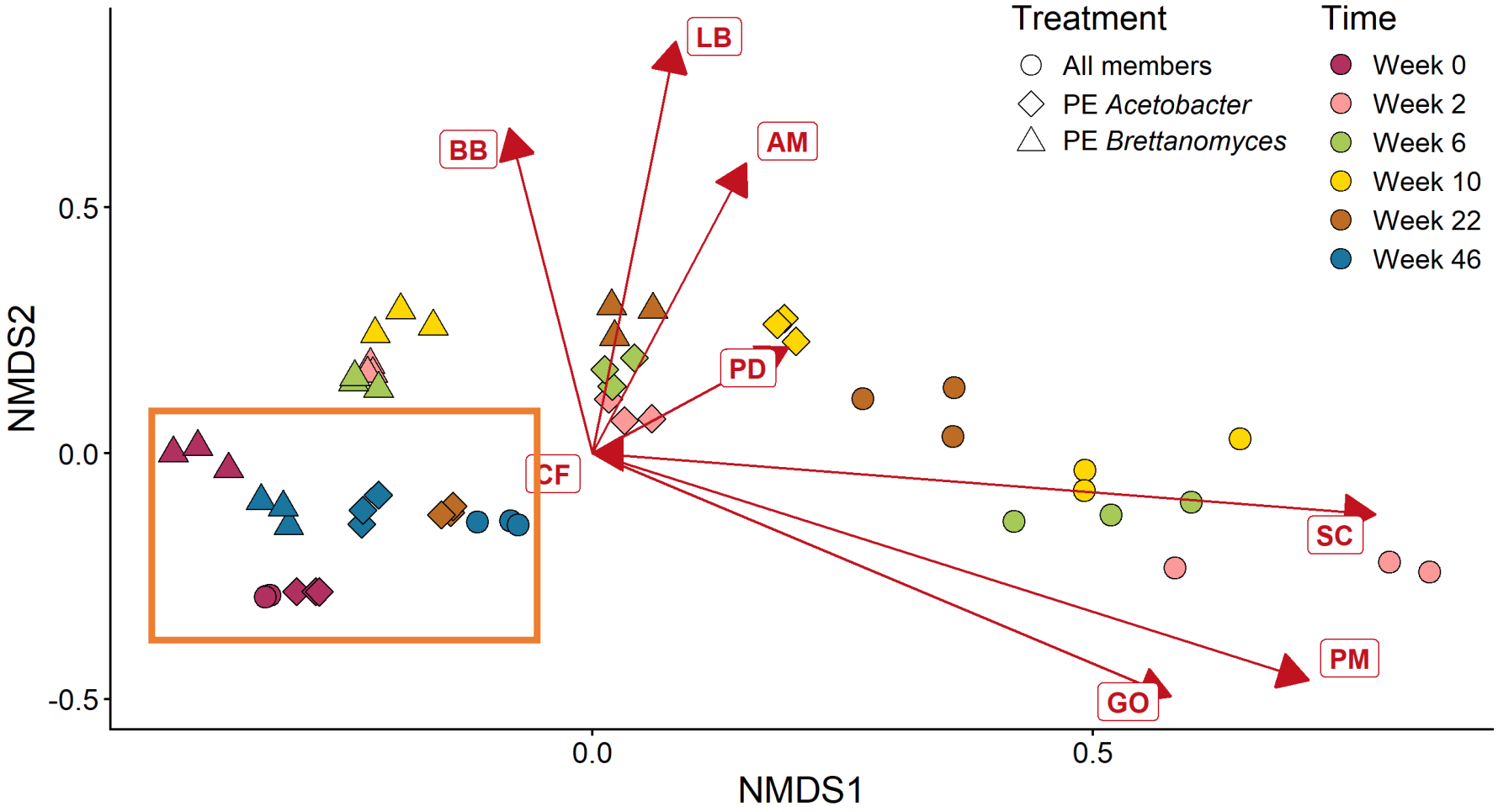
Priority effect exerted on *Pichia* is stronger than on *Saccharomyces*.

Priority effect exerted by *Acetobacter* has **inhibitory effect** on *Brettanomyces* after ten weeks.

Candida does not experience any growth.

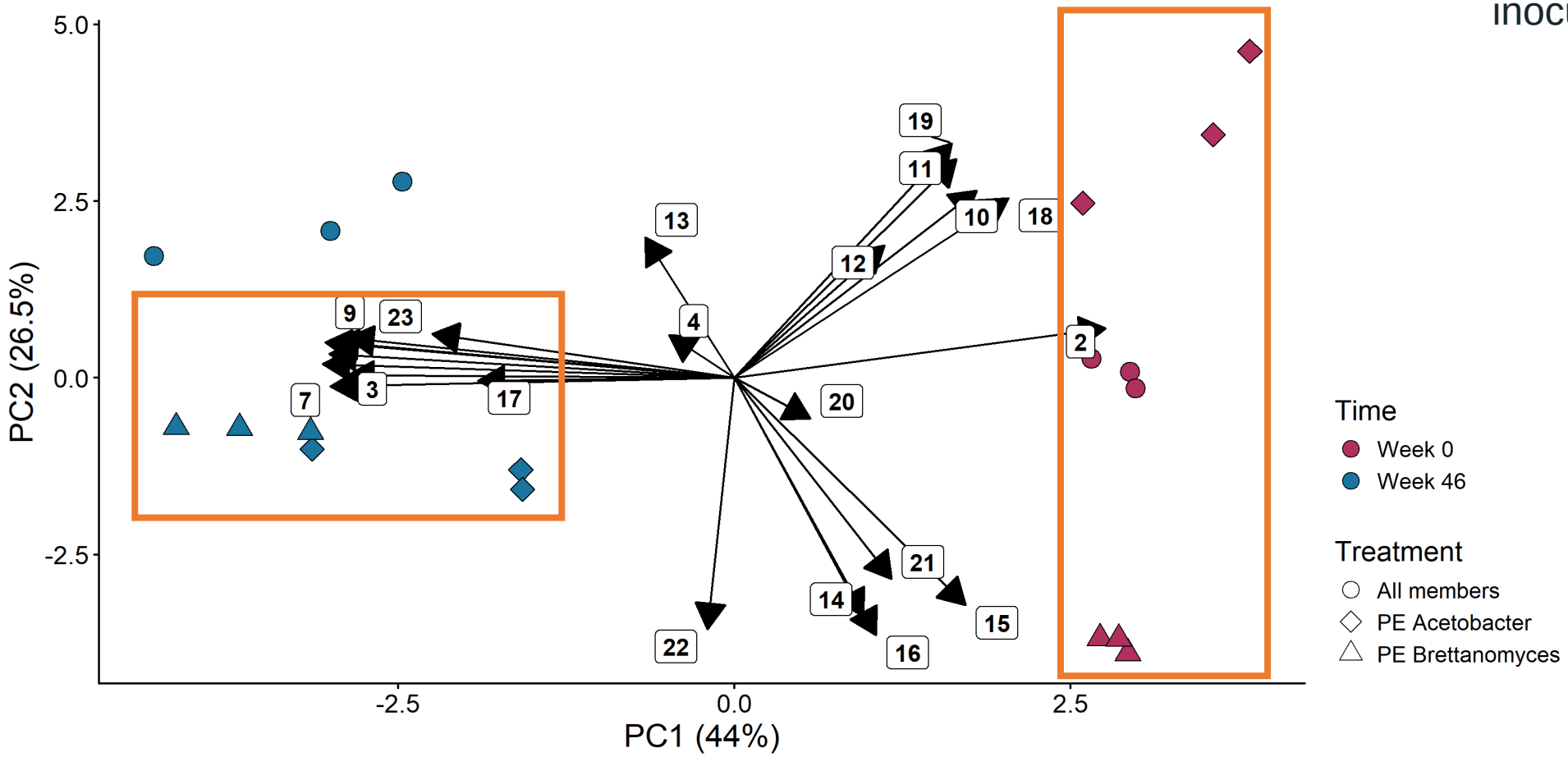
Priority effects are **diminished** on microbiological level after 46 weeks of maturation.

Microbial community composition in week 46 is **similar** to the community at the beginning of maturation.



Acetobacter and *Brettanomyces* priority effects **persist on chemical level** through 46 weeks.

Acetobacter and *Brettanomyces* differently **change starting beer chemistry** in two weeks of prior inoculation.



Does it matter who comes first?

Acetobacter malorum		*Brettanomyces bruxellensis*
Pediococcus *Levilactobacillus*	+	*Pediococcus* *Levilactobacillus*
Brettanomyces *Gluconobacter* *Pichia* *Saccharomyces*	−	*Acetobacter* *Gluconobacter* *Pichia* *Saccharomyces*

It does… but it depends on the context

Microbiological level

Priority effects on microbial community are **diminished** after 46 weeks of maturation

Chemical level

Priority effects on beer chemistry **persist** through 46 weeks of maturation

Future perspectives

- Further evaluate how *Acetobacter* and *Brettanomyces* exert priority effects
  - niche modification (pH reduction) and/or resource consumption (DP >3 sugar analysis)
- Dissect pairwise microbiological interactions in the in vitro ecosystem
- Whole genome sequencing of community members
  - Observed effects have a **genetic basis** through which involved species can exert a significant impact on other community members

Prof. Sam Crauwels

Prof. Bart Lievens

Prof. Kevin Verstrepen

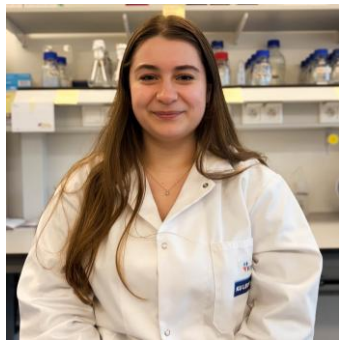
Prof. Tadashi Fukami

Dr. Lucas Nell

Dr. Sofie Bossaert

Nicolas Rojas-Preciado

Dr. Beatriz Herrera-Malav

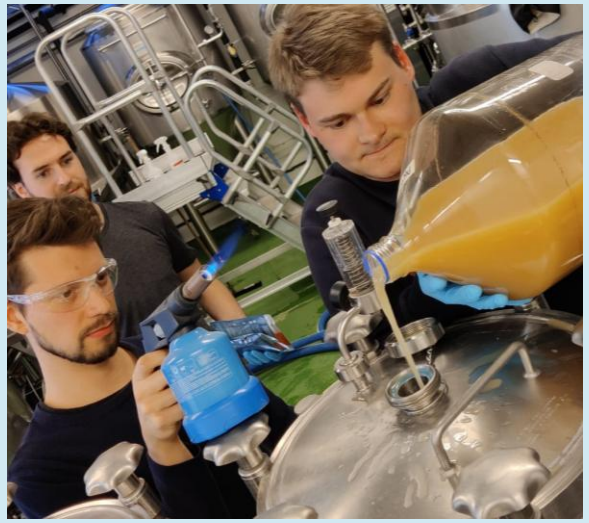


Lotte Van Landschoot

KU LEUVEN

Postgraduate Program
Malting and Brewing Sciences

Postgraduate Program Malting & Brewing Sciences



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- Get hands on **brewing experience** with our malting and brewing practical classes
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PROGRAM SUMMARY
Duration: September - June
Study points: 45 ECTS, full time
Location: Leuven, Belgium
Language: English

Questions?