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▶ **To cite this version:**

Paul Schmidt Yanez, Rodolphe Rougerie, Annie Bézier, Béatrice Courtial, Christophe Bouget, et al.. DNA Metabarcoding to quantify the ecological impact of climate-induced forest diebacks in the Pyrenees. 8. International Barcode of Life Conference (iBOL), Jun 2019, Trondheim, Norway. 16 p. hal-02787654

**HAL Id: hal-02787654**
**https://hal.inrae.fr/hal-02787654**

Submitted on 5 Jun 2020

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# DNA Metabarcoding to quantify the ecological impact of climate induced forest diebacks in the Pyrenees

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iBOL 2019 - Trondheim

Insect Biology Research Institute

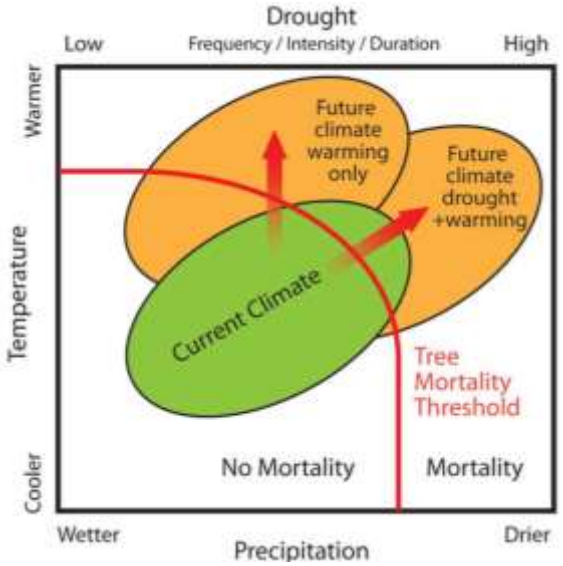
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# Global changes and forestry management

## Global changes

*"Climate change, insect infestations, drought…"*




Allen *et al.*, 2015

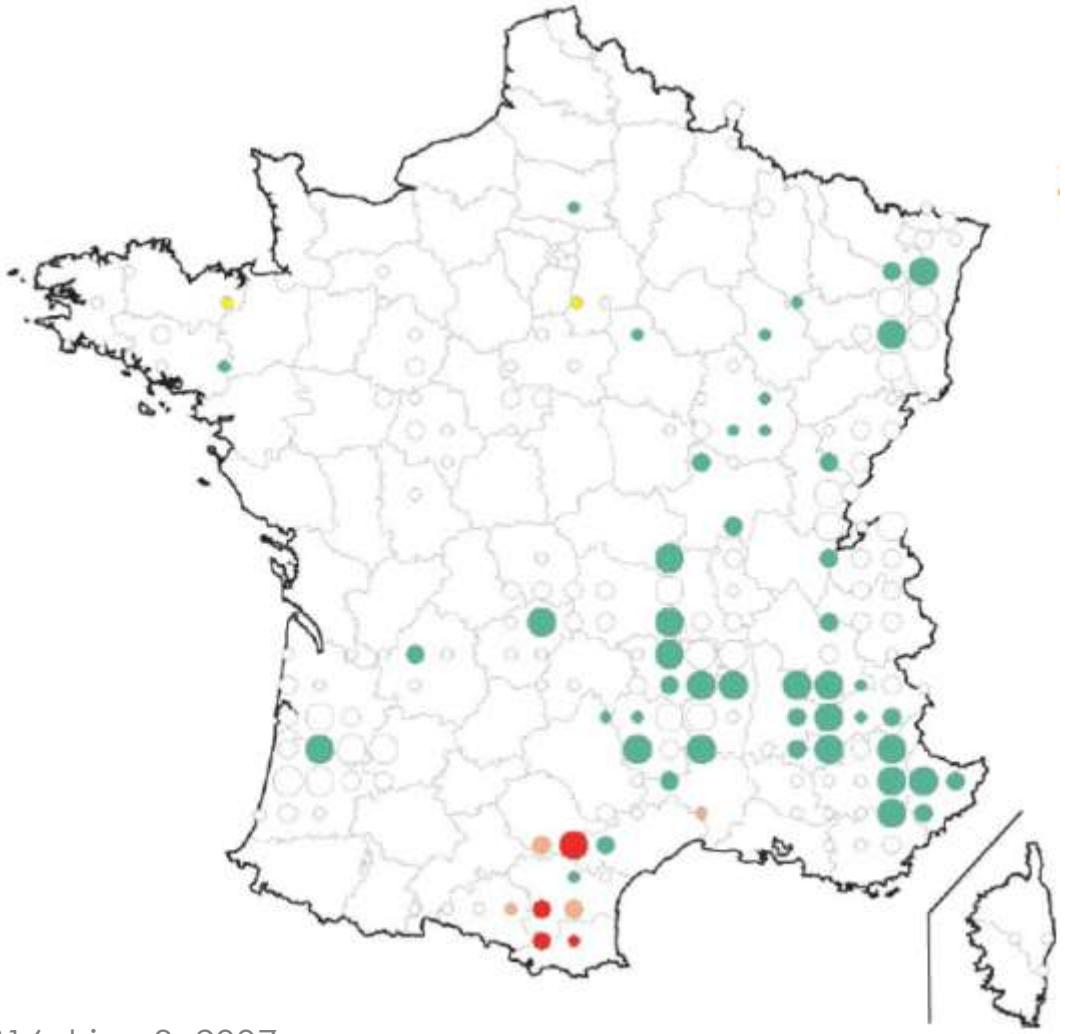
## Salvage logging

*"To harvest damaged trees in order to recover economic value otherwise lost. "*



# Droughts induce silver fir (*Abies alba*) die-off in Pyrenees





**Proportion of dominant trees with more than 25% of dead branches**

- More than **35%**
- From **20** to **35%**
- From **10** to **20%**
- Less than **10%**
- No tree in that case

**Number of living and dominant trees**

- More than **100**
- From **50** to **100**
- From **25** to **50**

Flot *et al*. IF n°16, trim. 3, 2007

# Studied areas







$N_{total}$ = **56 plots**

**2 valleys**

Vallée d'Aure
Pays de Sault

~190km distance

**3 dieback level conditions**

- **Healthy** *(20 plots)*
- **Low dieback level** *(12 plots)*
- **High dieback level** *(24 plots)*

**2 salvage logging conditions within high dieback level**

- **Not harvested** *(12 plots)*
- **Harvested** *(12 plots)*

# Characteristics of each plot











**1 Malaise trap** in the center of **each plot**

Sampled **4 times** (each month) from **May** to **September**

## Micro Dendro-habitats



*Larrieu et al., 2018*

## Canopy openness



$N_{total}$ = **224 samples**

$n_{ecological\ variables}$ = **84+**

# Sample preparation for metabarcoding









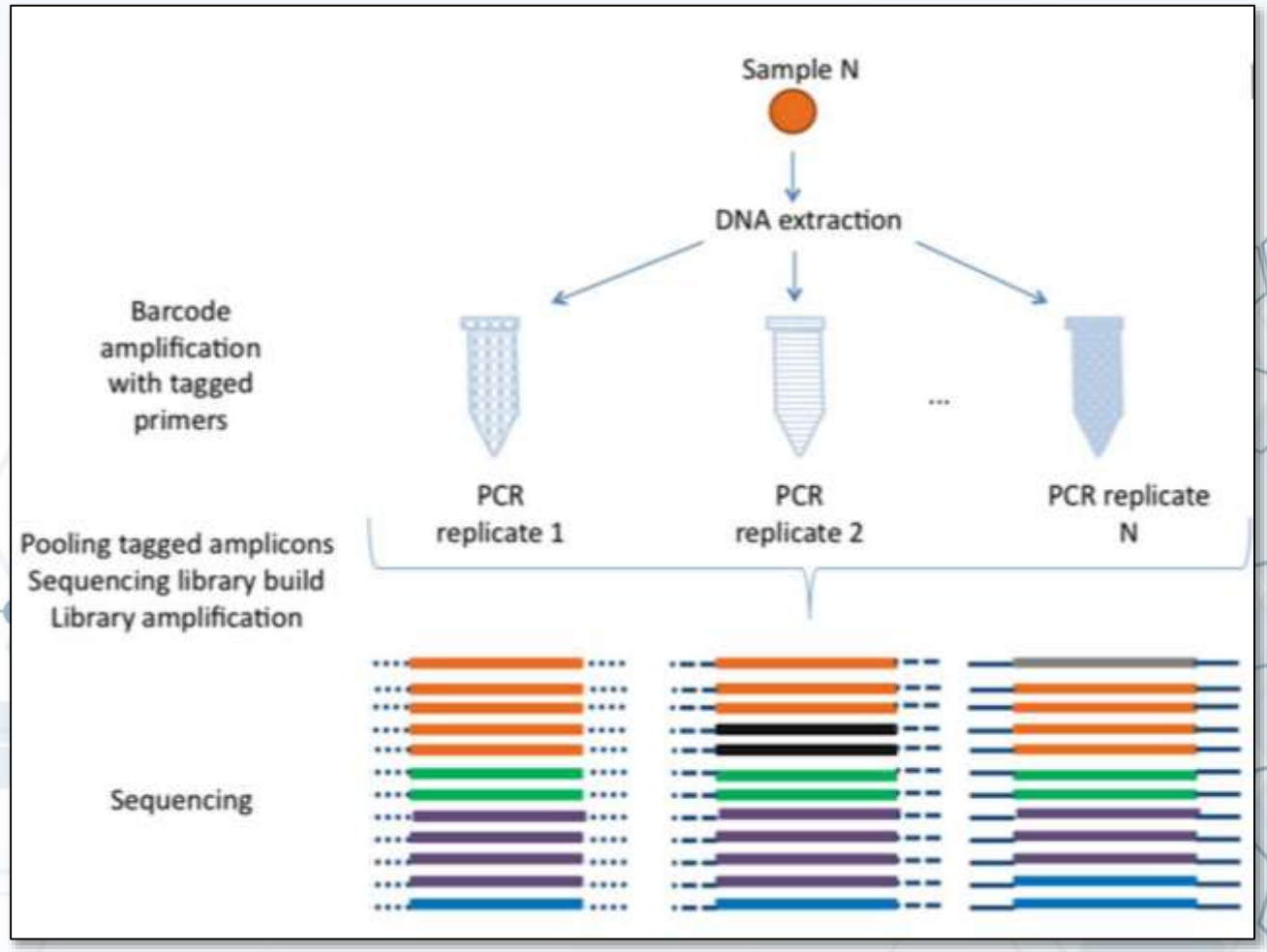
- Processed sample randomization
  *Bálint et al., 2018*
- Filtration & Rincing
- Drying overnight
- Homogeneization
  *Elbrecht et al., 2017*
- Grinding

# Metabarcoding pipeline

- **COI** marker (313bp)

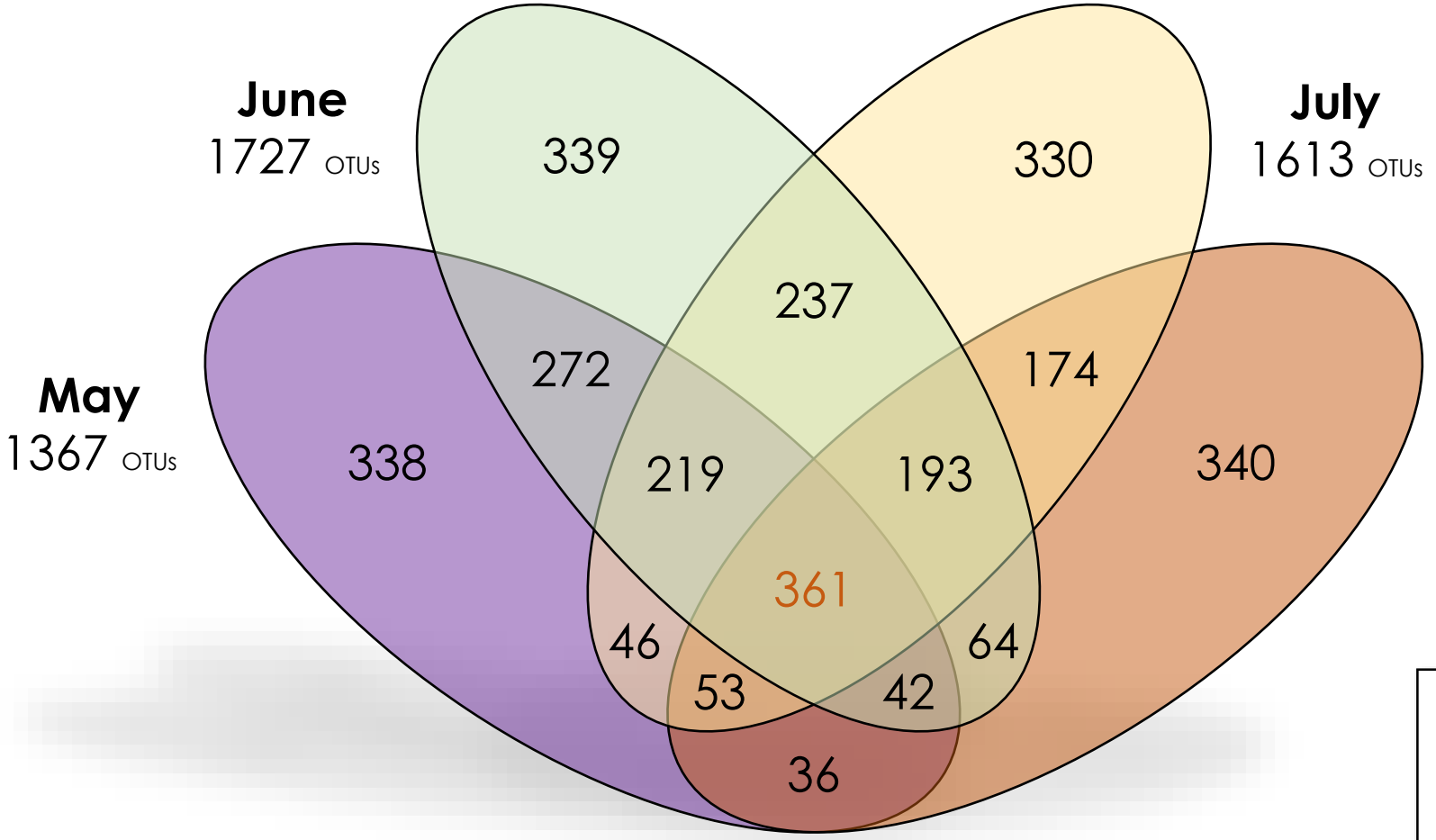
  Leray *et al.*, 2013

  Geller *et al.*, 2013
- PCR triplicates
- Illumina MiSeq V3 paired-end
- OTU clustering **97%** (sumaclust)
- **3 PCRs** & **3 reads** minimum
- Taxonomic assignement using **BOLD**




*Zepeda-Mendoza et al., 2016*

# High general temporal turnover




**3041** OTUs

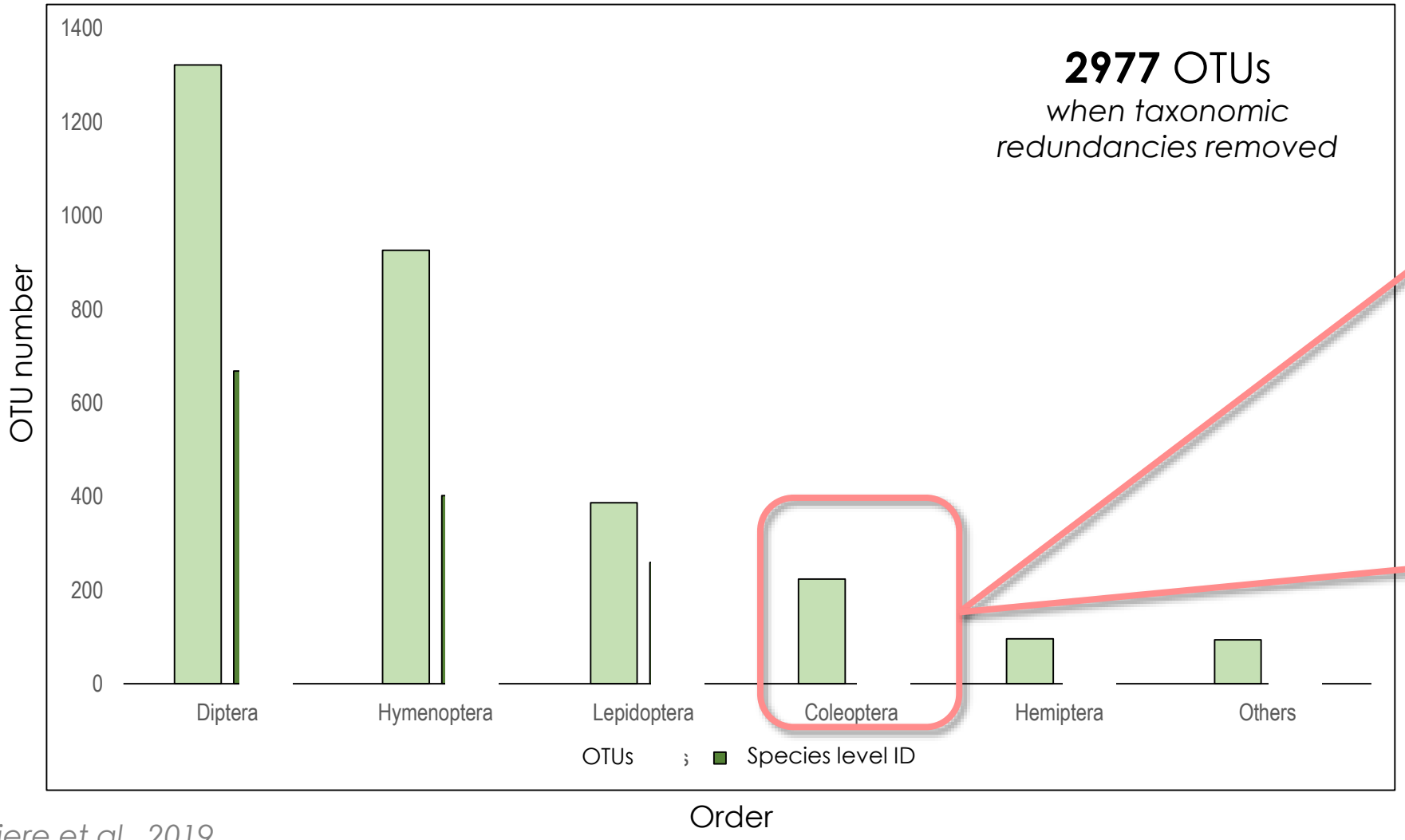
11.9% shared by all

Jaccard Index : $J_{May / August} = 0.23$

Jaccard Index : $J_{June / July} = 0.43$

# Incomplete reference libraries & taxonomic assignment



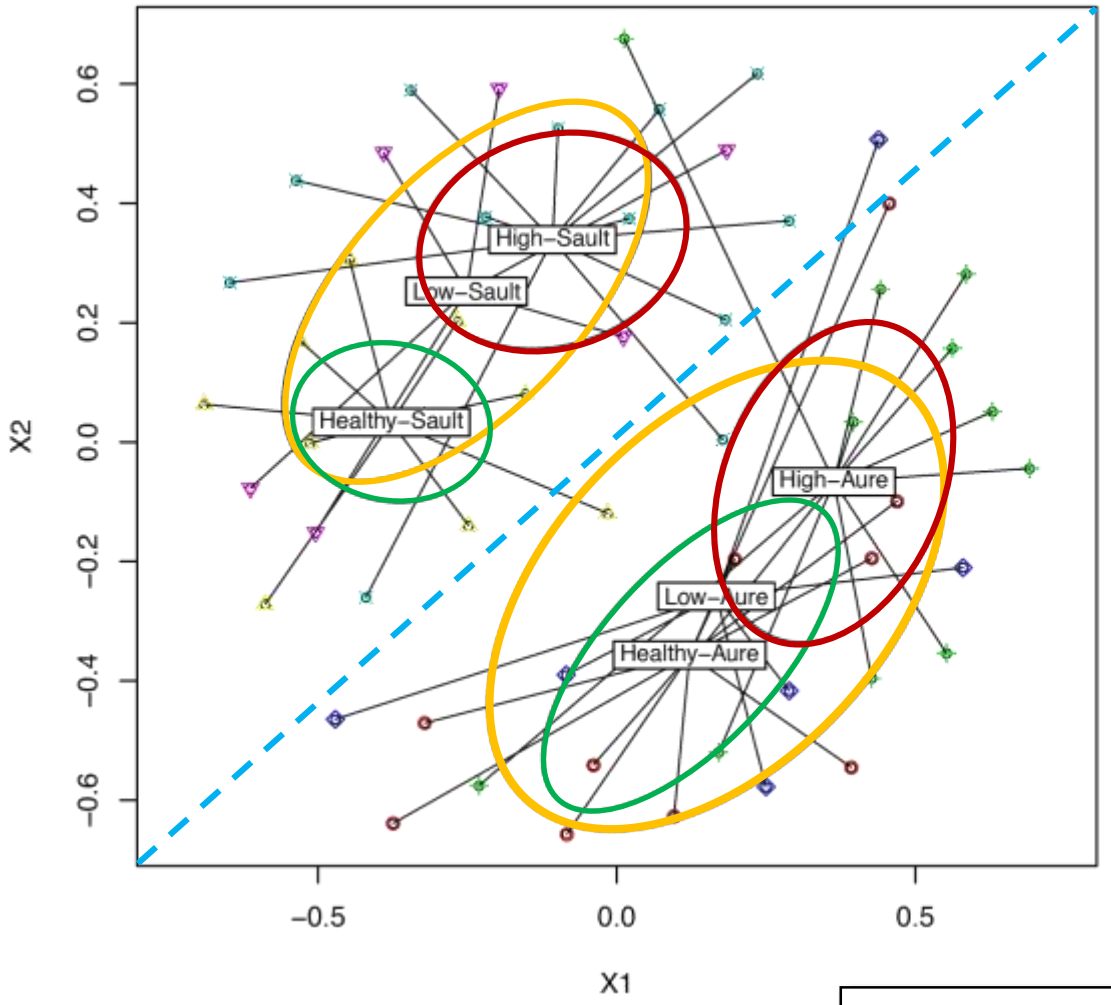







*Moriniere et al., 2019*

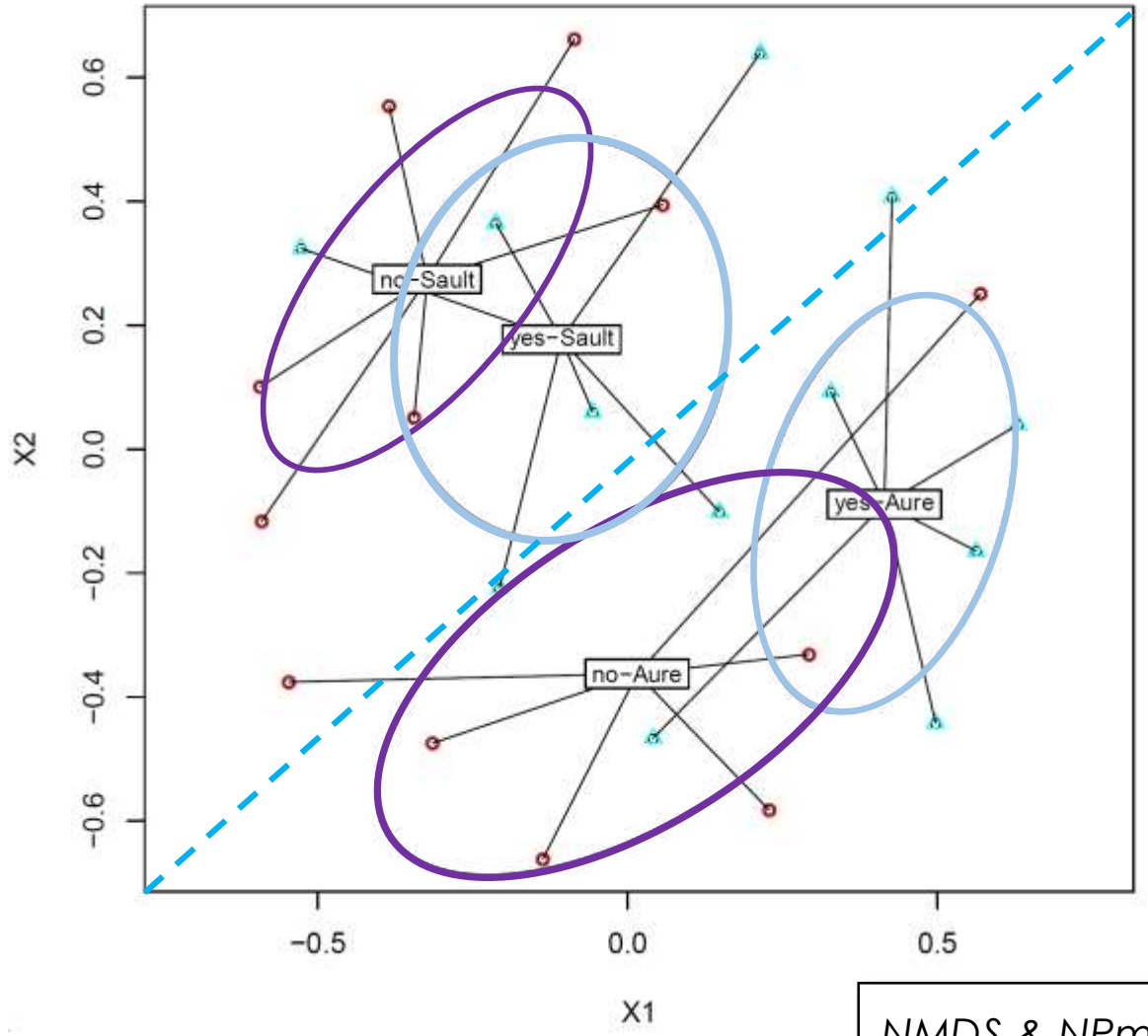
# Insect composition varies with dieback level




- Strong **difference** in insect composition **between both valleys**
- **Highly significant difference** (***) between **healthy** & **high level of dieback**
- **Low level of dieback** shows **no difference** in insect composition

*NMDS & NPmanova*

# Insect composition does not change with salvage logging

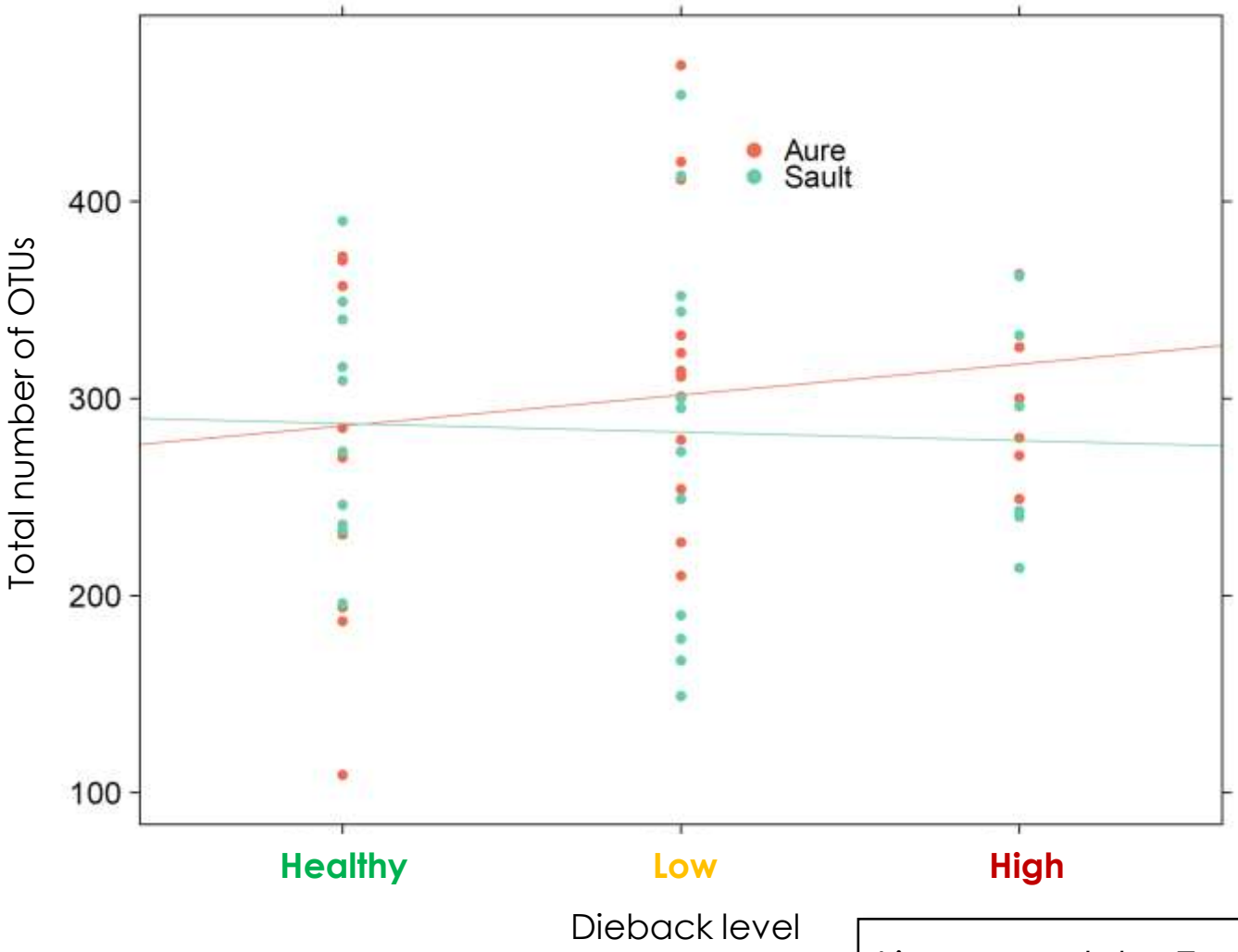



*NMDS & NPmanova*

- Strong **regional effect**
- **No significant difference** between **not harvested** and **salvage logged**
- **No direct impact** of **salvage logging** on total insect composition **detected**



# Species richness does not vary with dieback level




- **No valley difference**
- **No significant difference** between **Healthy**, **Low** and **High** **dieback levels** in terms of species richness **detected**



*Linear model & Type II Anova*

# Species associated with dieback levels

*Potential candidates for bio-indication*

## Healthy

12 specific OTUs



CBG Photography Group - CC

*Fannia abrupta*

Native from Nearctic
Apparently introduced in China (2016)
No information about France

## Low dieback level

21 specific OTUs



*Pungeleria capreolaria*

Feed on *Picea abies* and *Abies alba*
Fly from mid June to September

## High dieback level

10 specific OTUs



Marko Mutanen - CC

*Botanophila brunneilinea*

Larval stage on *Centaurea sp.* plants

*Leuchtmann et al., 2015*

*Package R Indicspecies*

*OTUs found at least in 10 plots*

# To sum up before flying away!

- Usefulness of **metabarcoding on Malaise** trap samples for **bio-monitoring**
- Strong **temporal** and **regional effects**
- **Need to complete reference libraries** for European **Diptera** and **Hymenoptera** in BOLD
- **Direct impact** of **high dieback level** on insect composition
- **No direct impact** of salvage logging
- Potential **bio-indicator candidates** for **dieback levels**



# Thank you for your attention !



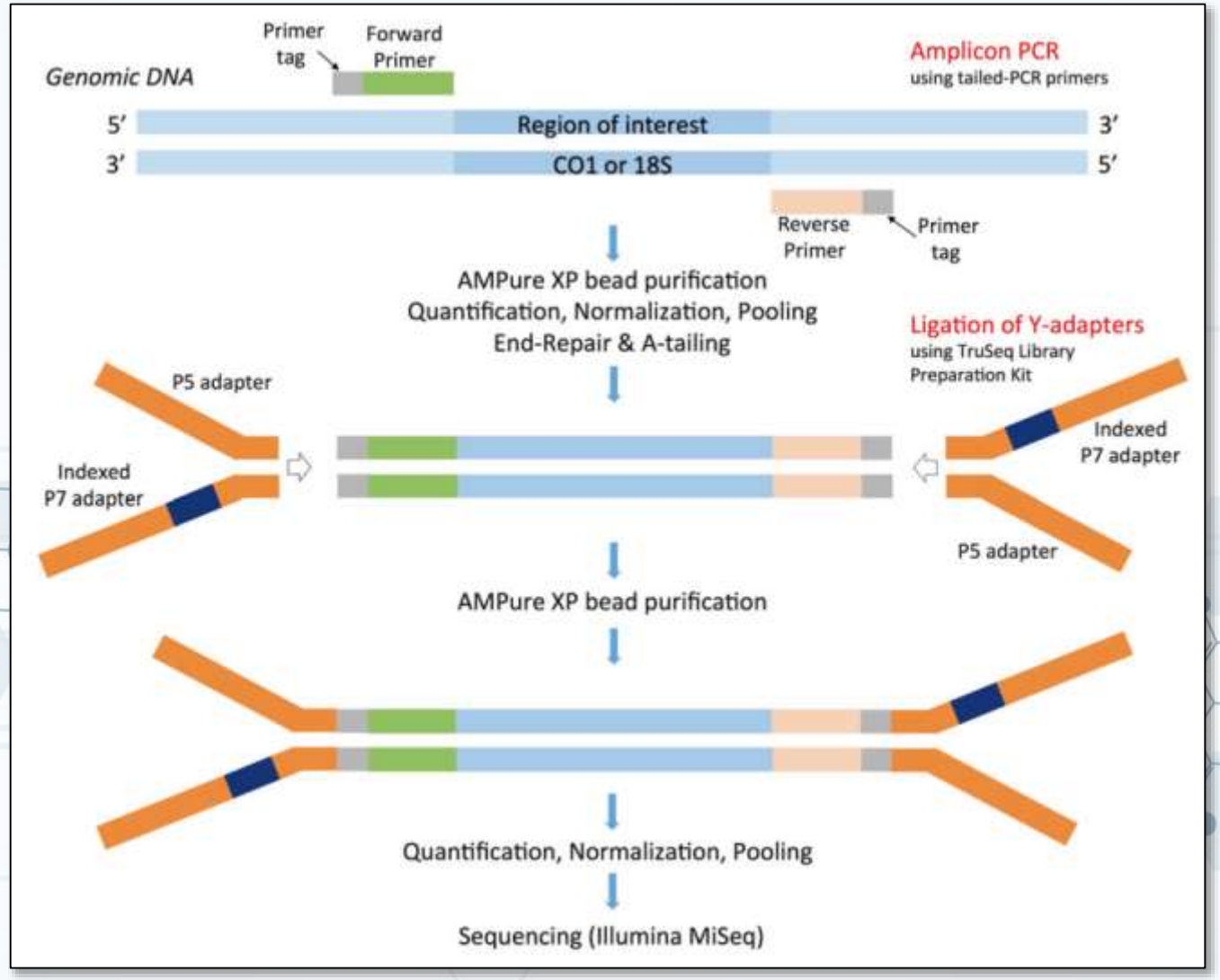




Thank you to:

- Kévin Speder
- Joël Meunier
- Cai Wang
- Yang Chunyan
- Susan Mbedi
- Laurent Burnel
- Jérôme Molina
- Carl Moliard

# Twin-tagging – Dual Indexing PCRs




*Leray et al., 2016 (chap. 14)*