



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

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

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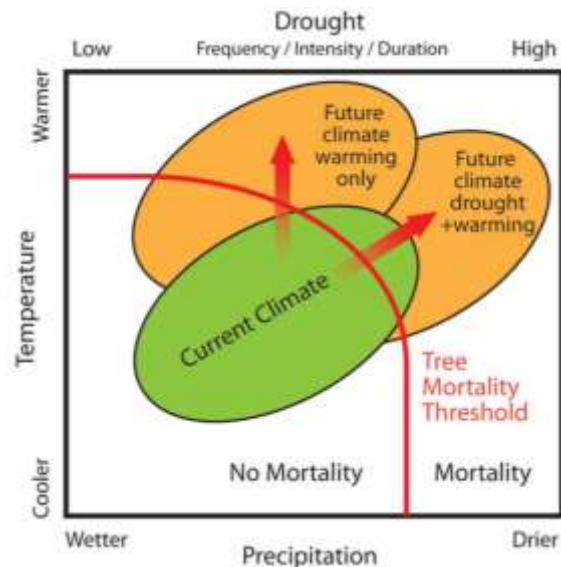
 CNRS

 IRBI

# Global changes and forestry management

## Global changes

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



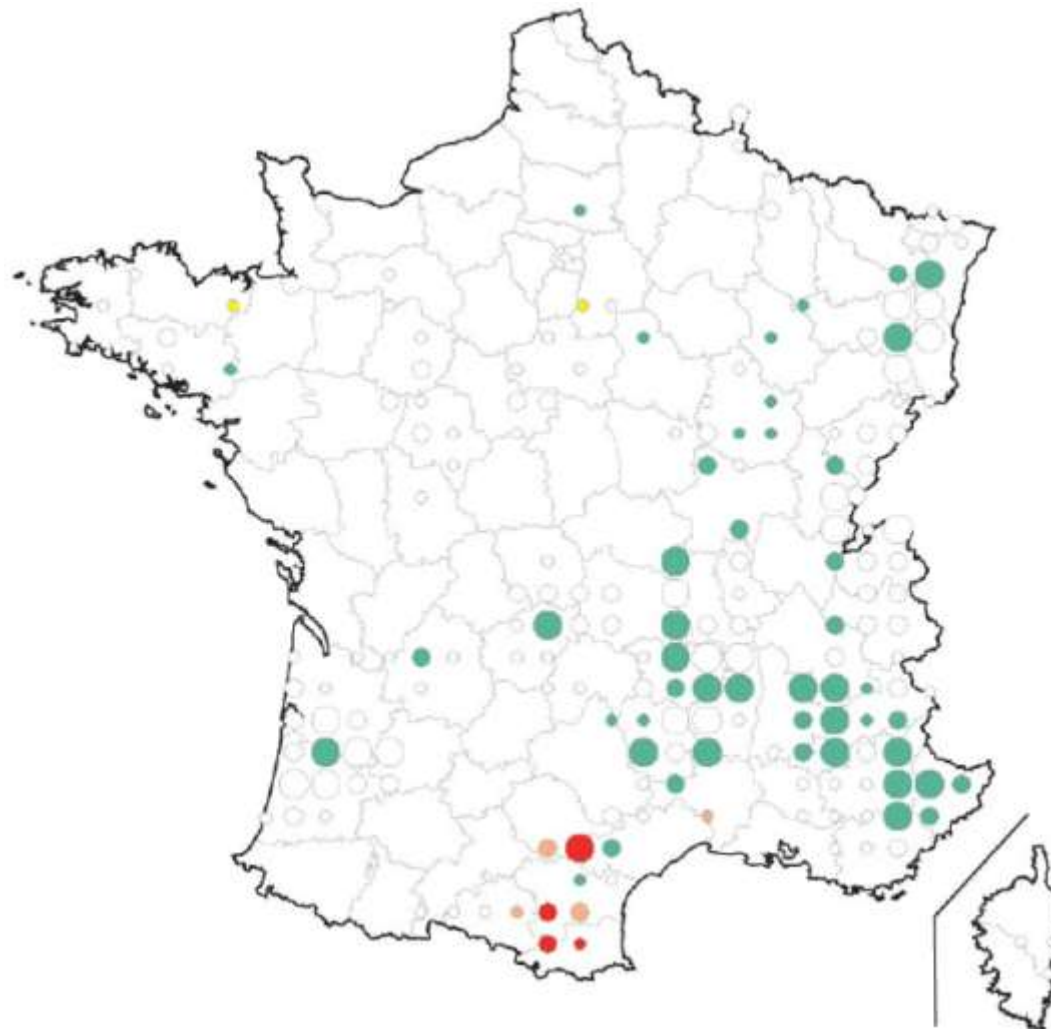
Allen *et al.*, 2015

## 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**

# Studied areas



$N_{\text{total}} = 56 \text{ plots}$

## 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



## Micro Dendro-habitats



*Larrieu et al., 2018*

## Canopy openness



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

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

$N_{\text{total}} = \mathbf{224 \text{ samples}}$

$n_{\text{ecological variables}} = \mathbf{84+}$

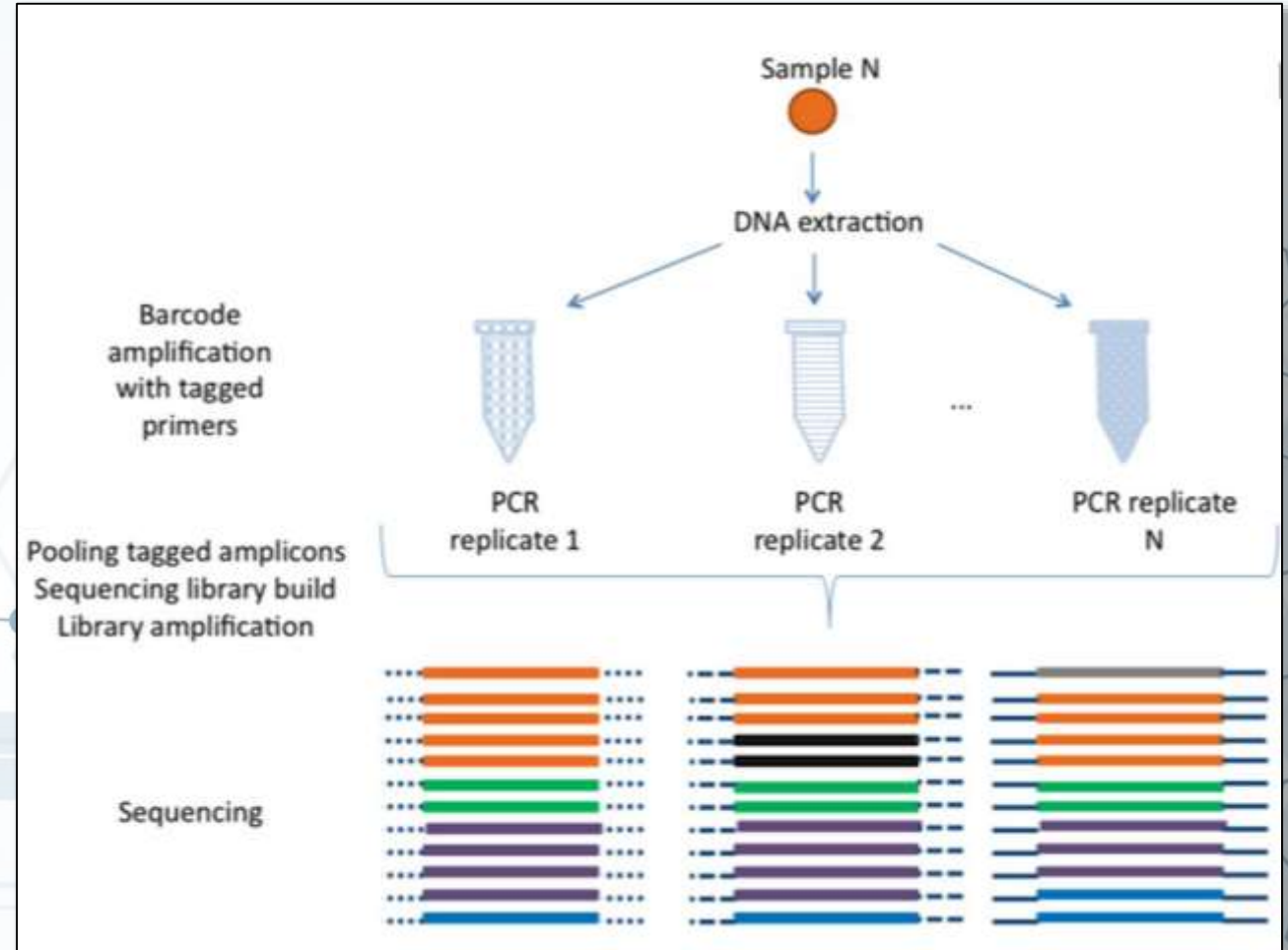
# Sample preparation for metabarcoding



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

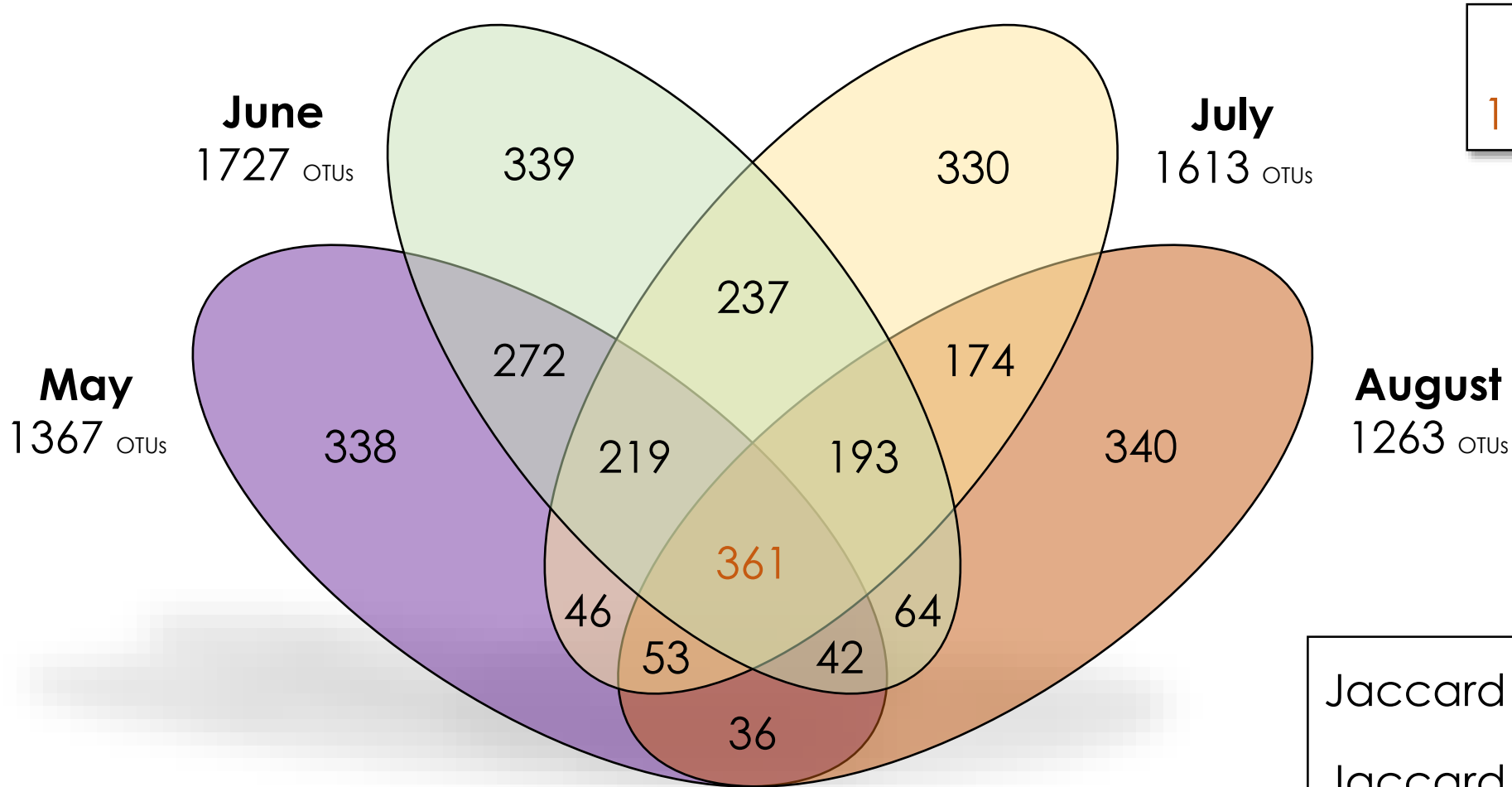
# Metabarcoding pipeline

- ❖ **COI** marker (313bp)  
*Leray et al., 2013*  
*Geller et al., 2013*
- ❖ PCR triplicates
- ❖ Illumina MiSeq V3 paired-end
- ❖ OTU clustering **97%** (sumacrust)
- ❖ **3 PCRs & 3 reads** minimum
- ❖ Taxonomic assignement using **BOLD**



*Zepeda-Mendoza et al., 2016*

# High general temporal turnover



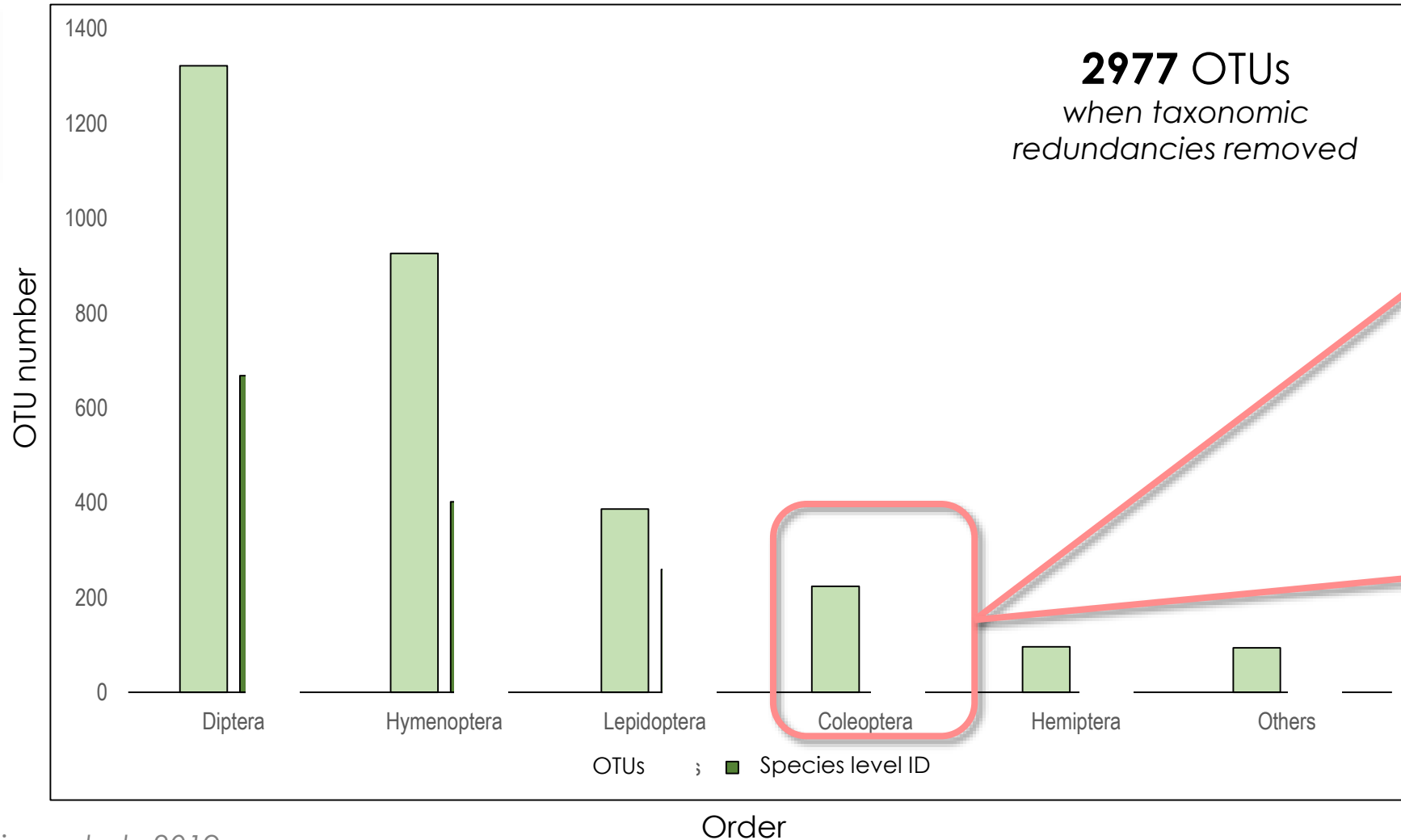
**3041** OTUs

**11.9%** shared by all

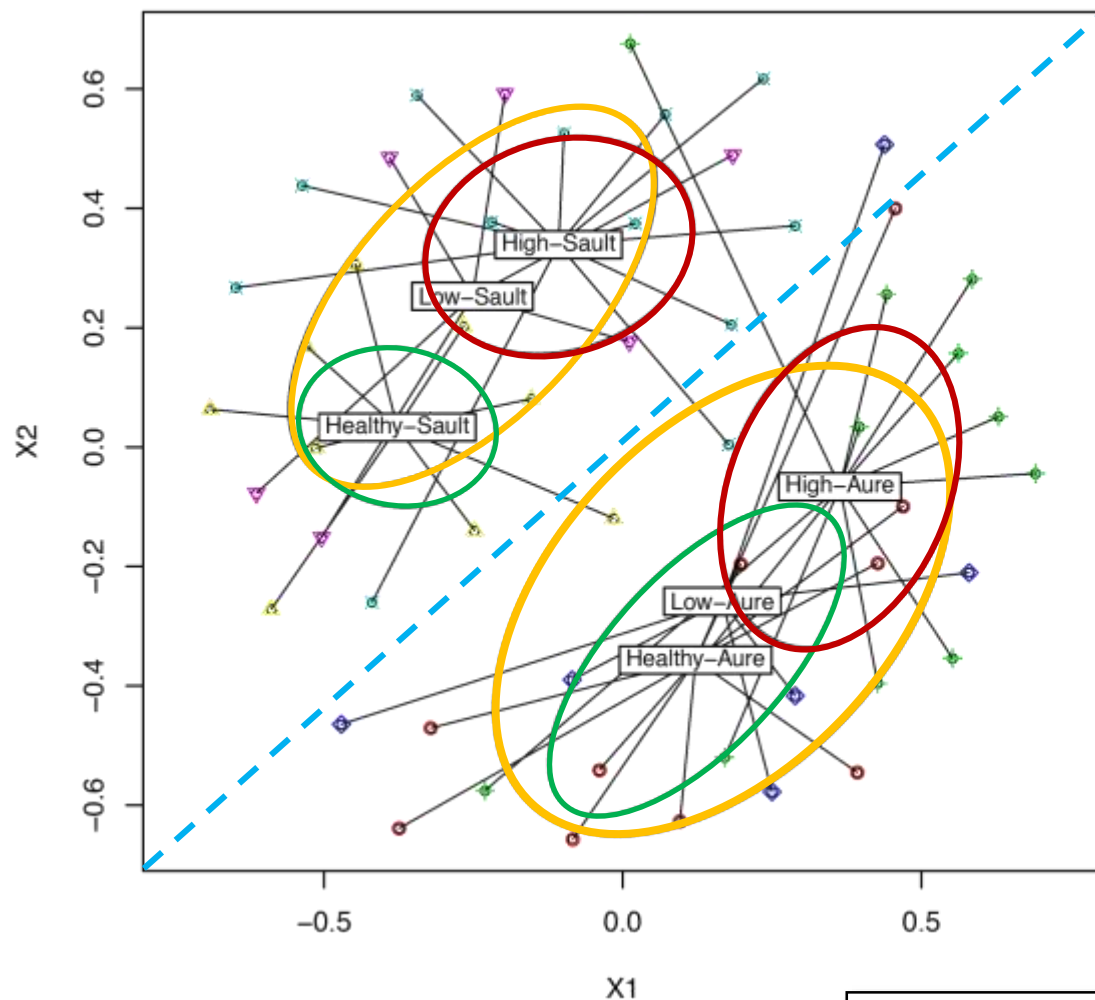
Jaccard Index :  $J_{\text{May} / \text{August}} = 0.23$

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

# Incomplete reference libraries & taxonomic assignment



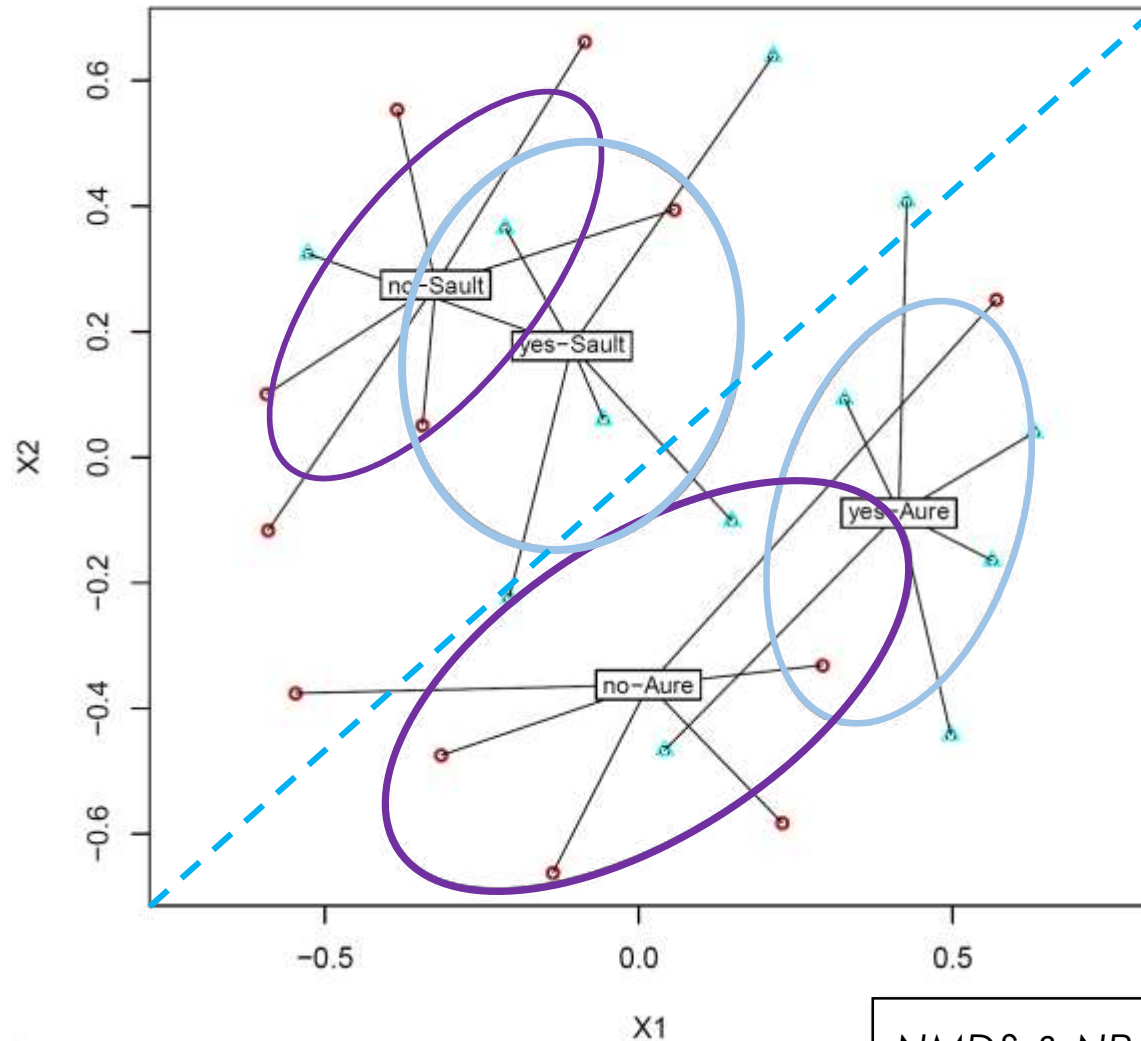
# Insect composition varies with dieback level



- 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

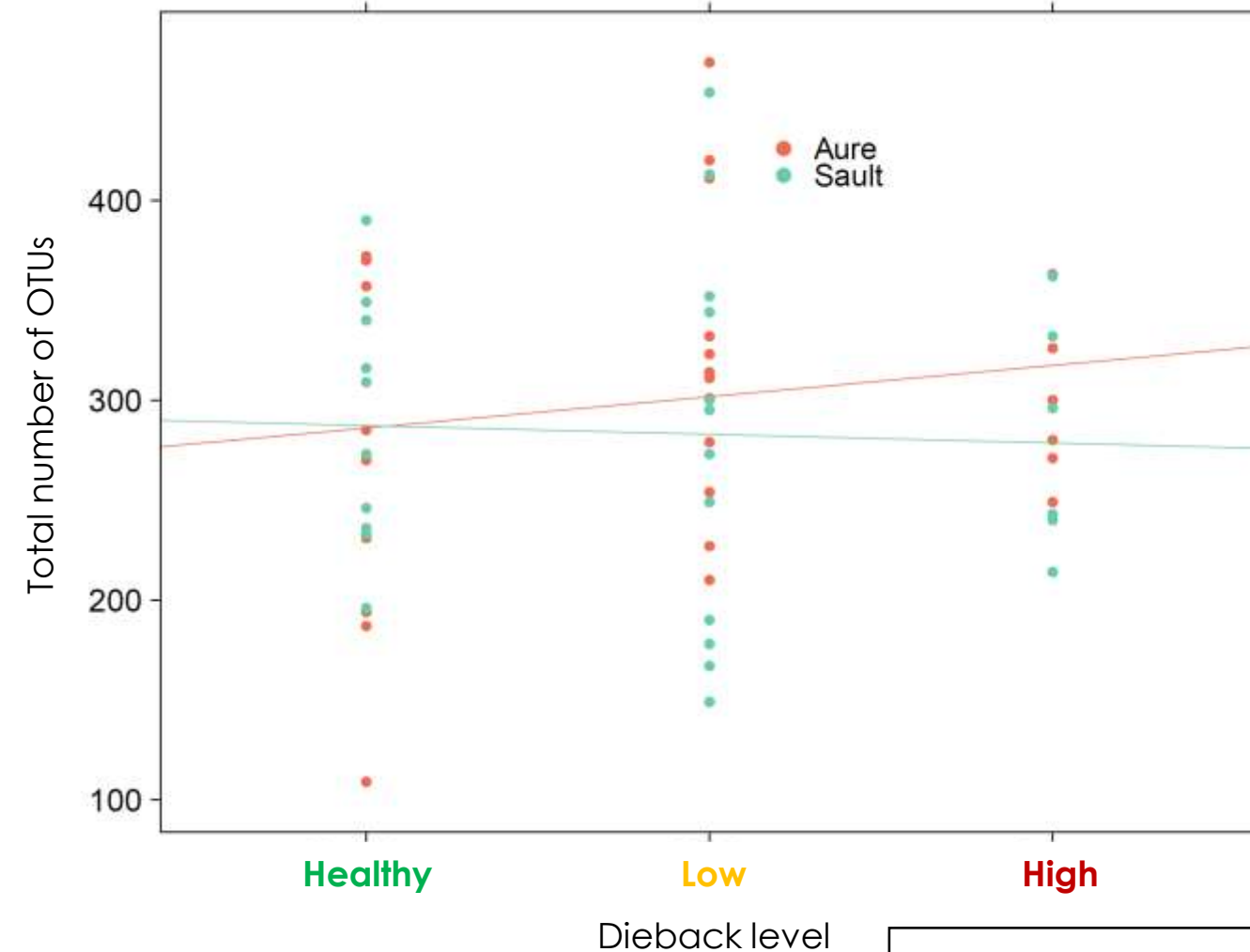


- Strong **regional effect**
- **No significant difference** between **not harvested** and **salvage logged**
- **No direct impact of salvage logging** on total insect composition **detected**



NMDS & NPmanova

# 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

*Leuchtman 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 !



Leibniz-Institut für  
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ECOLE DOCTORALE **SSBCV**



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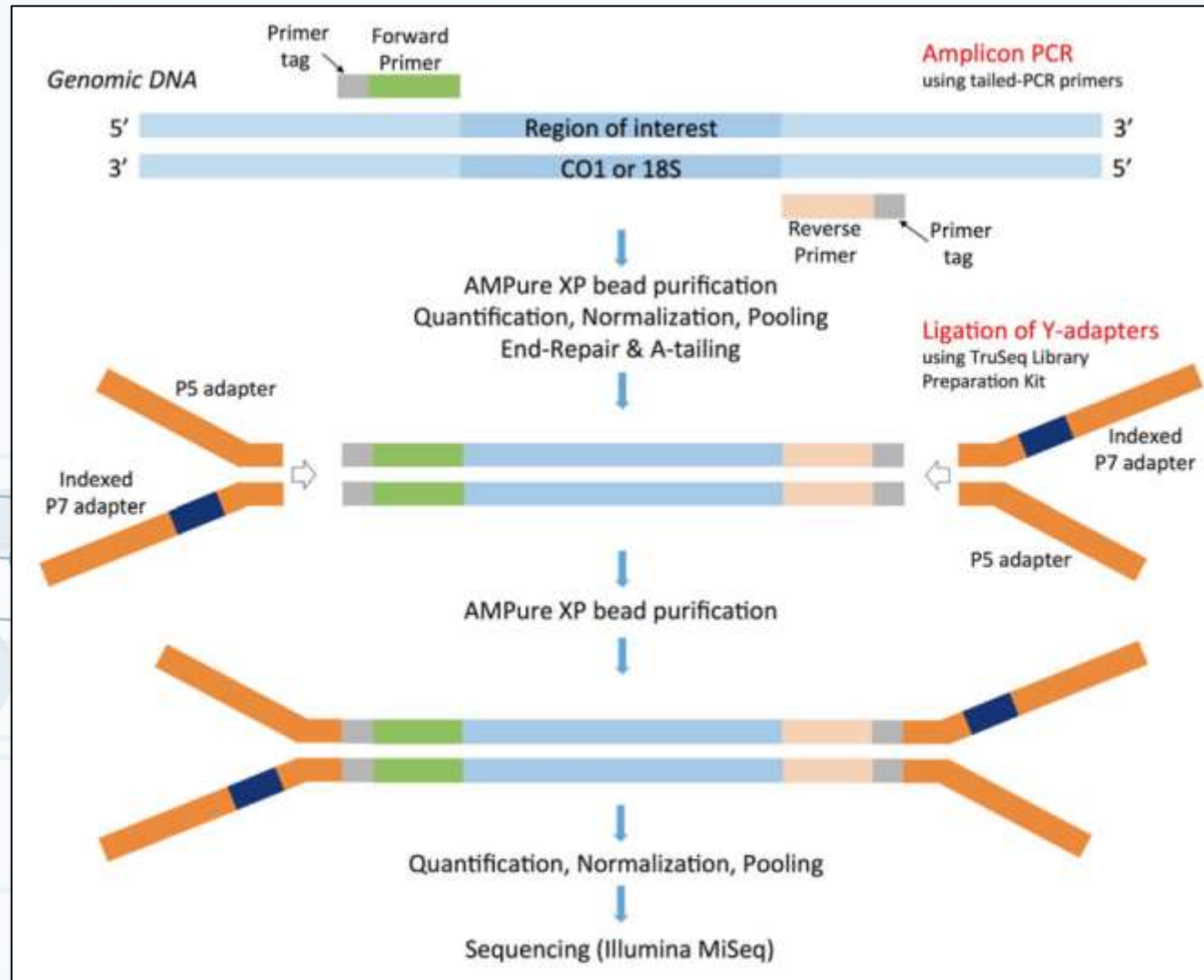
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# Twin-tagging – Dual Indexing PCRs



Leray et al., 2016 (chap. 14)