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

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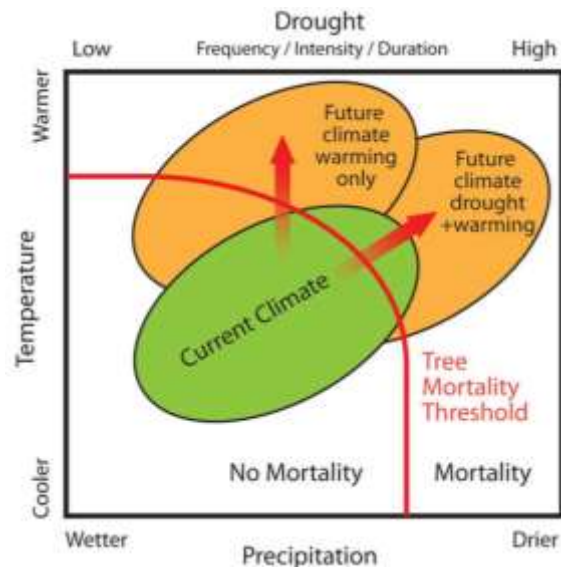
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Global changes and forestry management

Global changes

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



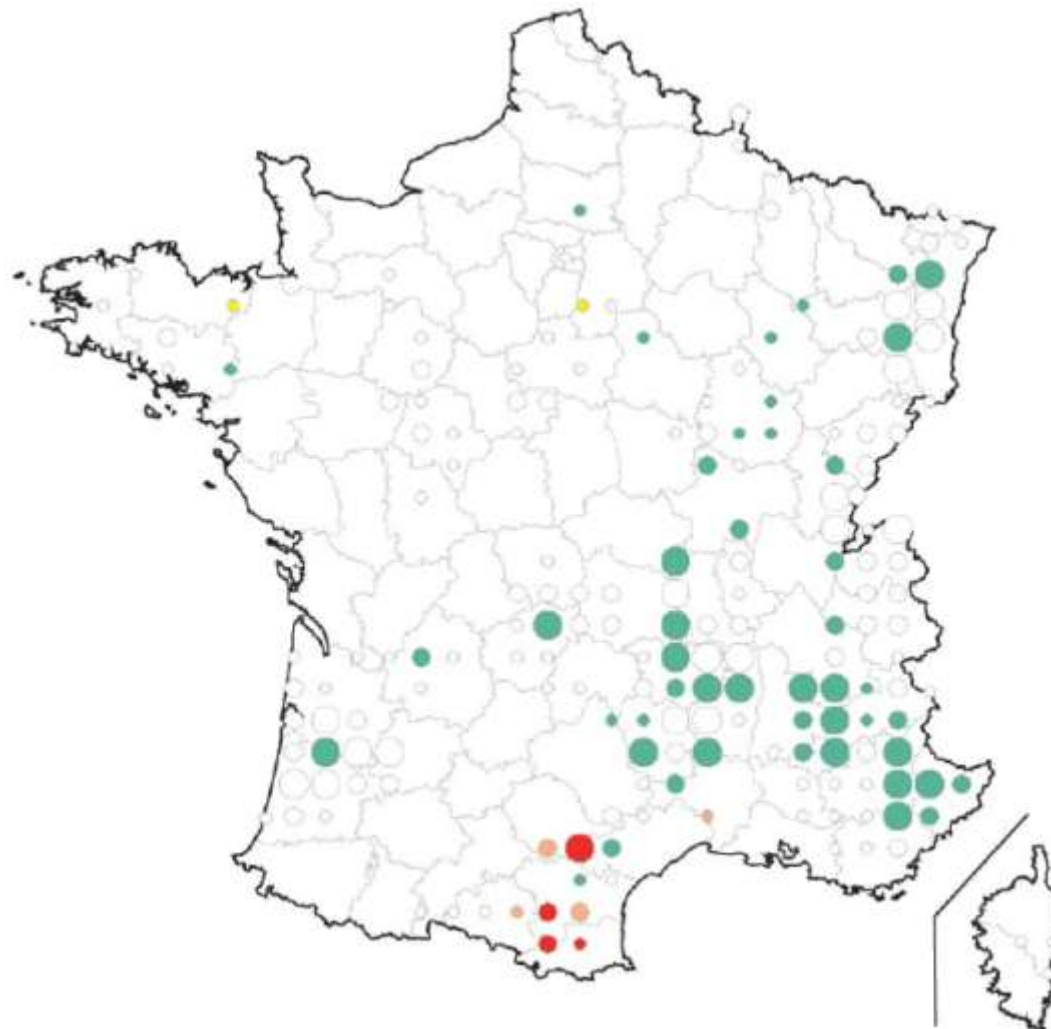
Allen *et al.*, 2015

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**

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



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



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

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

Sample preparation for metabarcoding



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

Metabarcoding pipeline

- ❖ **COI** marker (313bp)

Leray *et al.*, 2013

Geller *et al.*, 2013

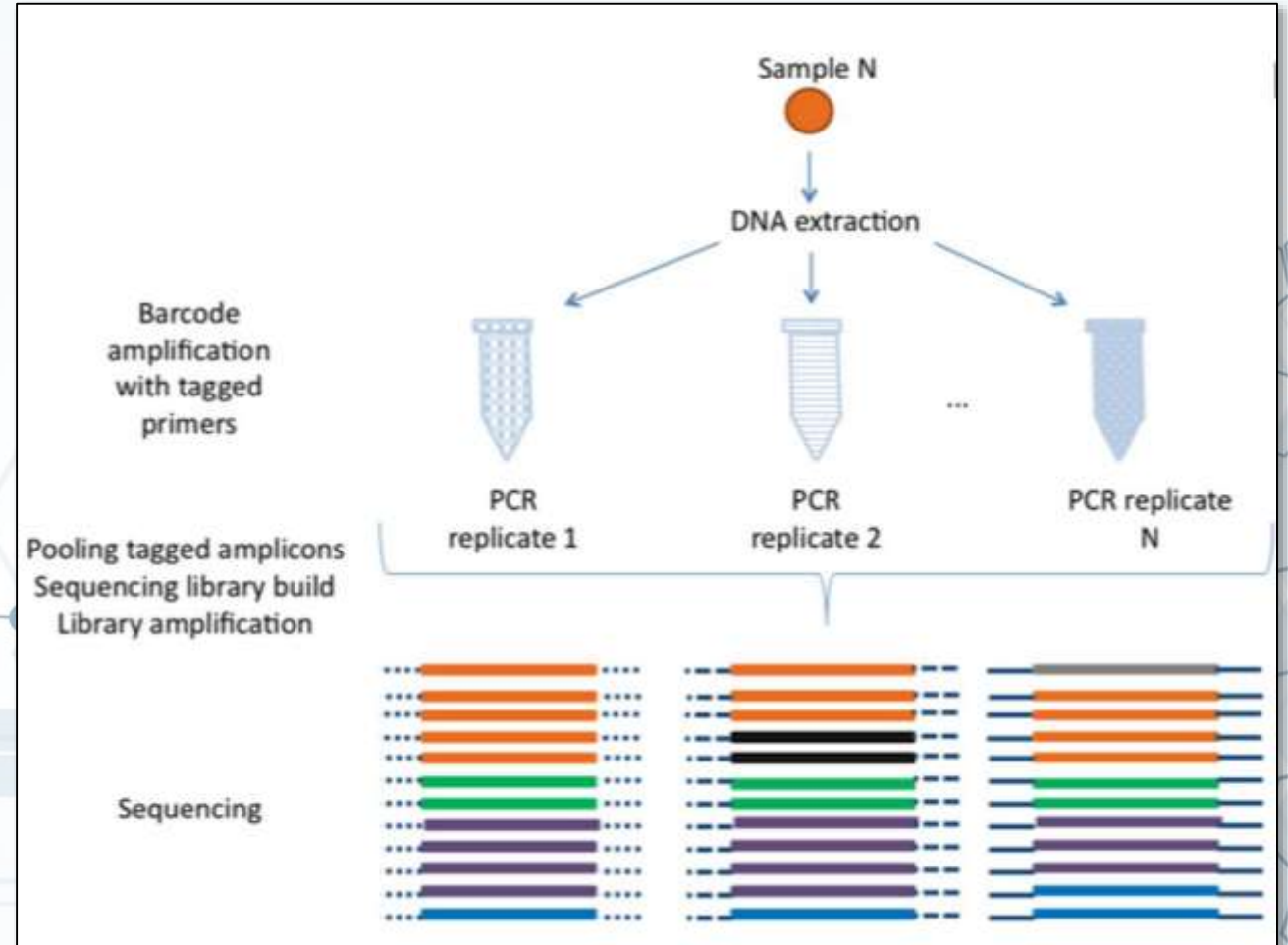
- ❖ PCR triplicates

- ❖ Illumina MiSeq V3 paired-end

- ❖ OTU clustering **97%** (sumacrust)

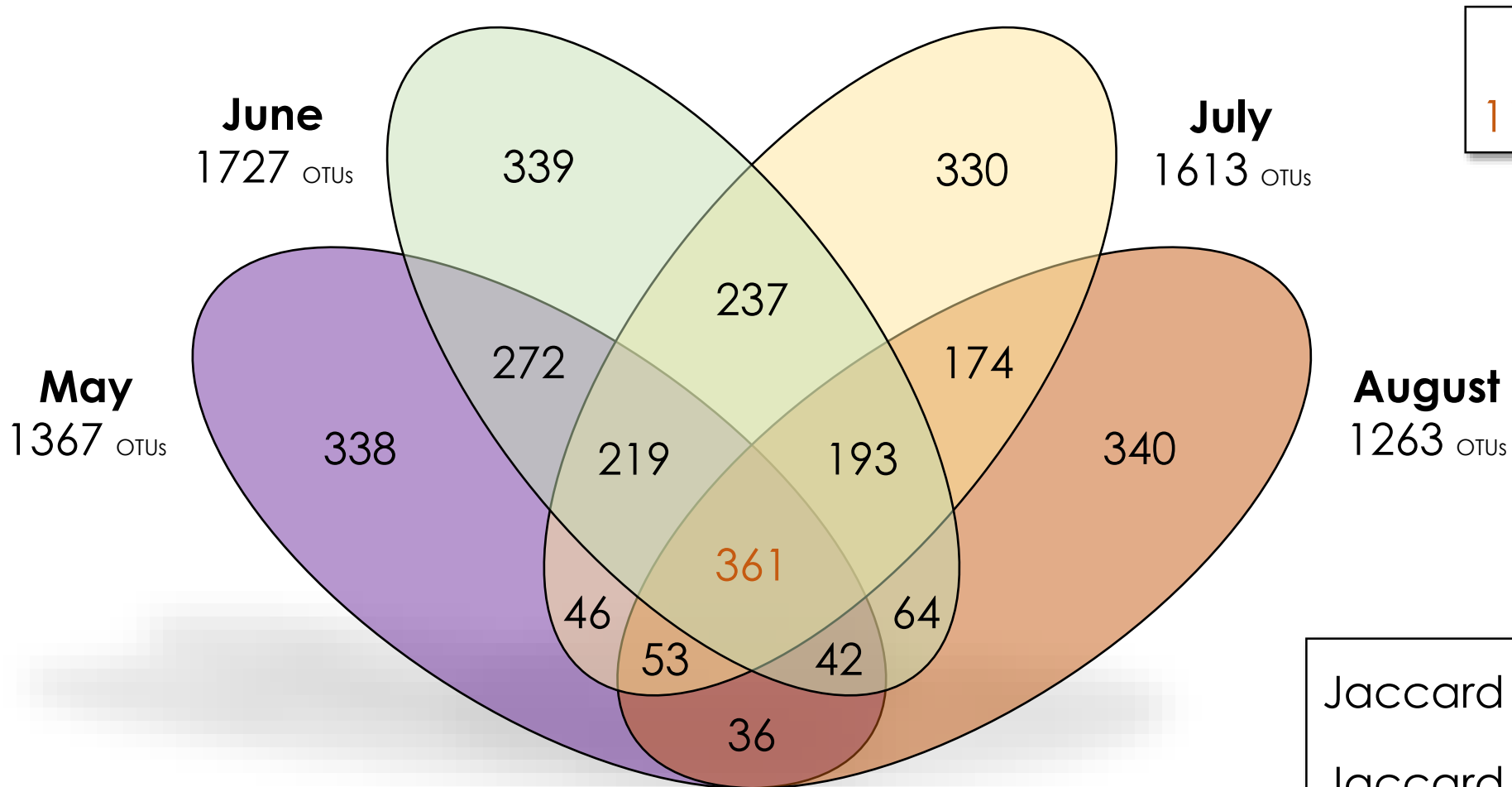
- ❖ **3 PCRs** & **3 reads** minimum

- ❖ Taxonomic assignement using **BOLD**



Zepeda-Mendoza *et al.*, 2016

High general temporal turnover



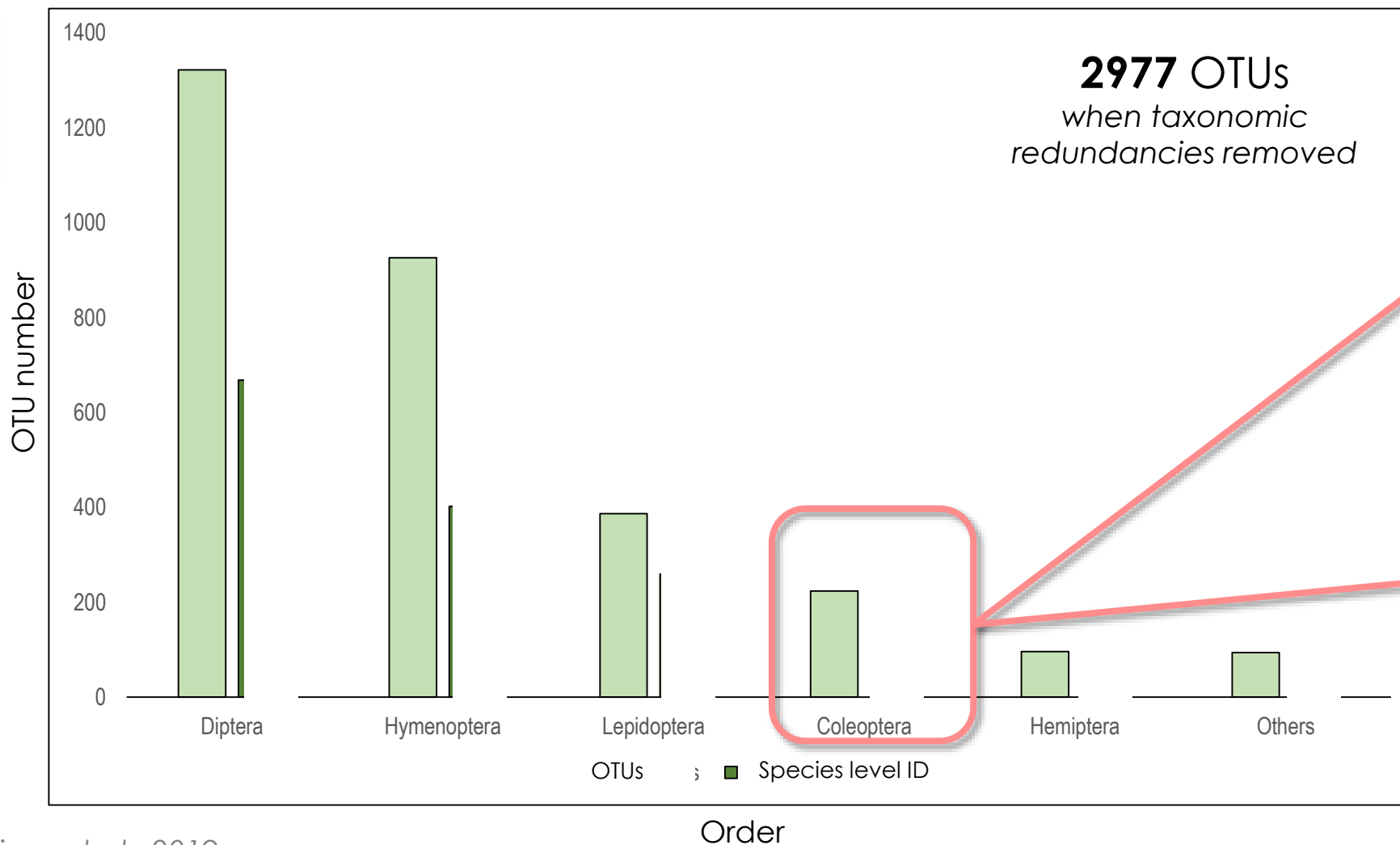
3041 OTUs

11.9% shared by all

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

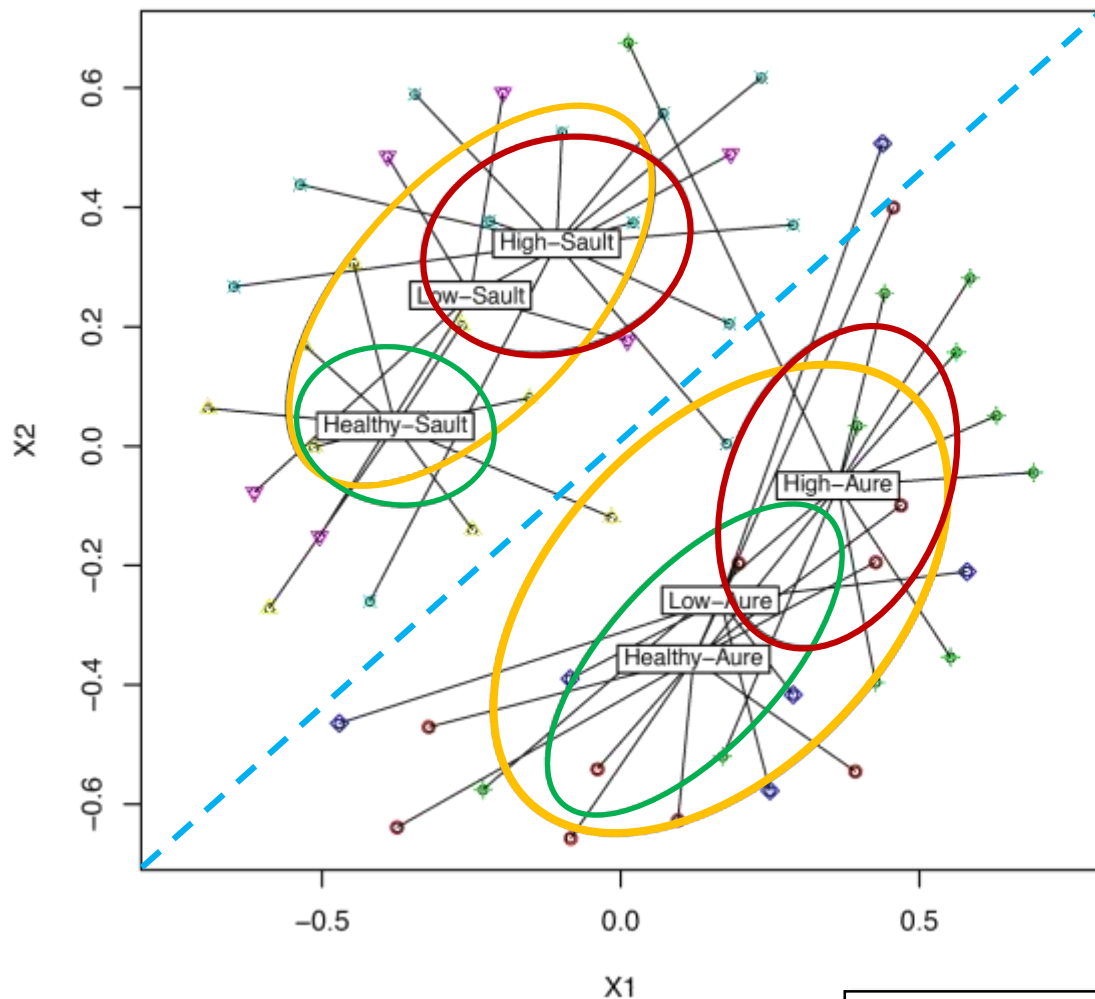
Jaccard Index : $J_{\text{June} / \text{July}} = 0.43$

Incomplete reference libraries & taxonomic assignment



Moriniere et al., 2019

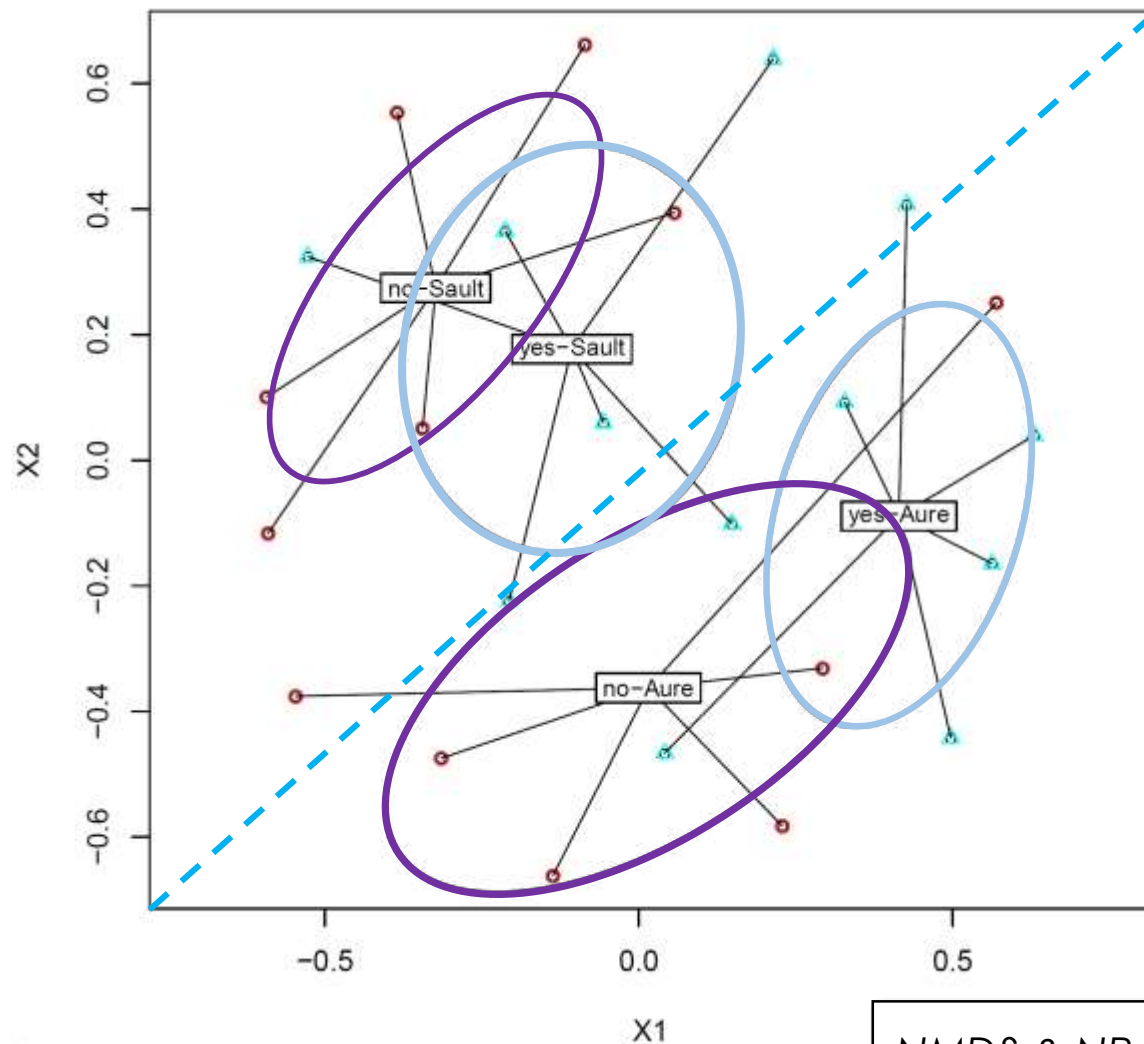
Insect composition varies with dieback level



- 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

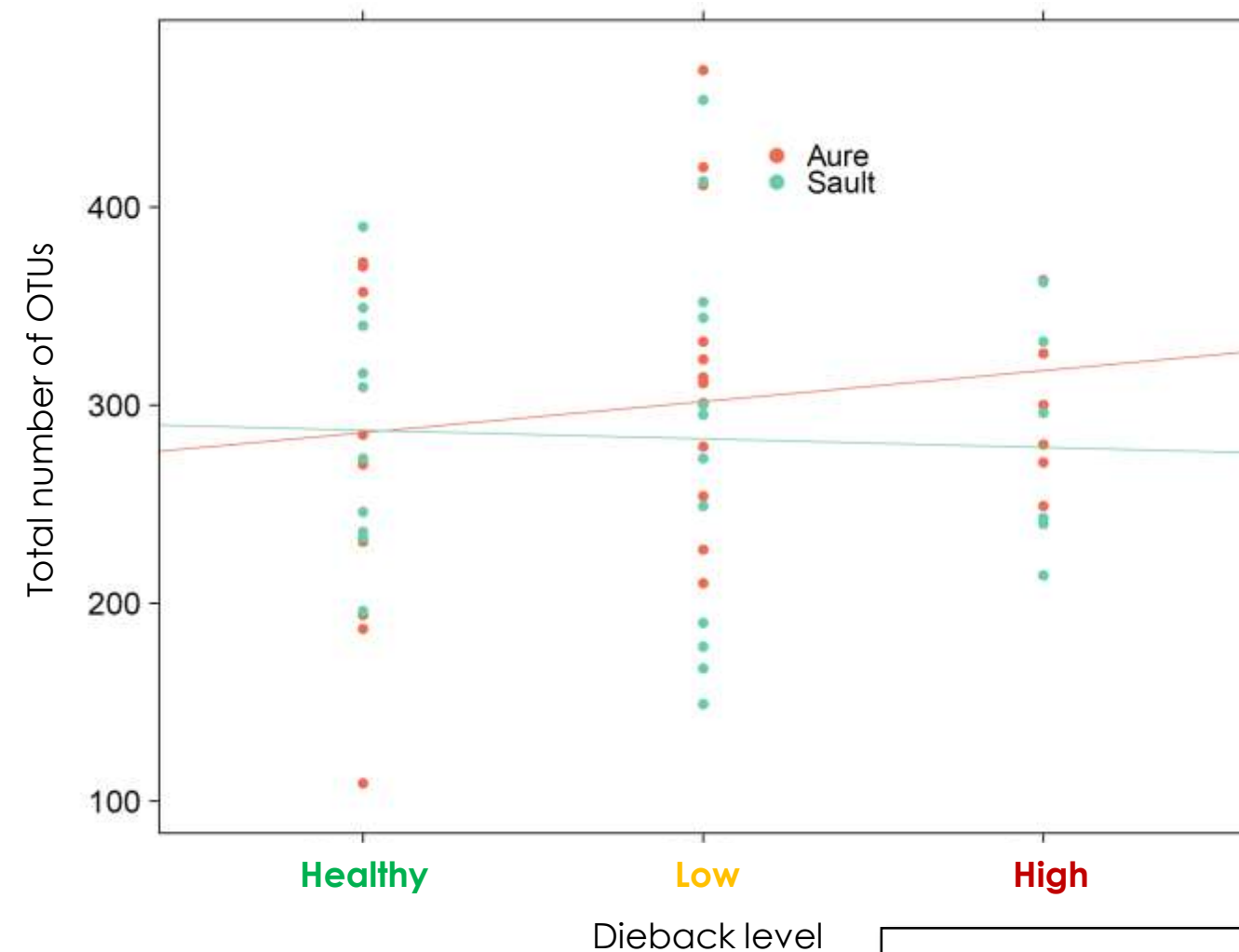


- Strong **regional effect**
- **No significant difference** between **not harvested** and **salvage logged**
- **No direct impact of salvage logging** on total insect composition **detected**



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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Berlin Center
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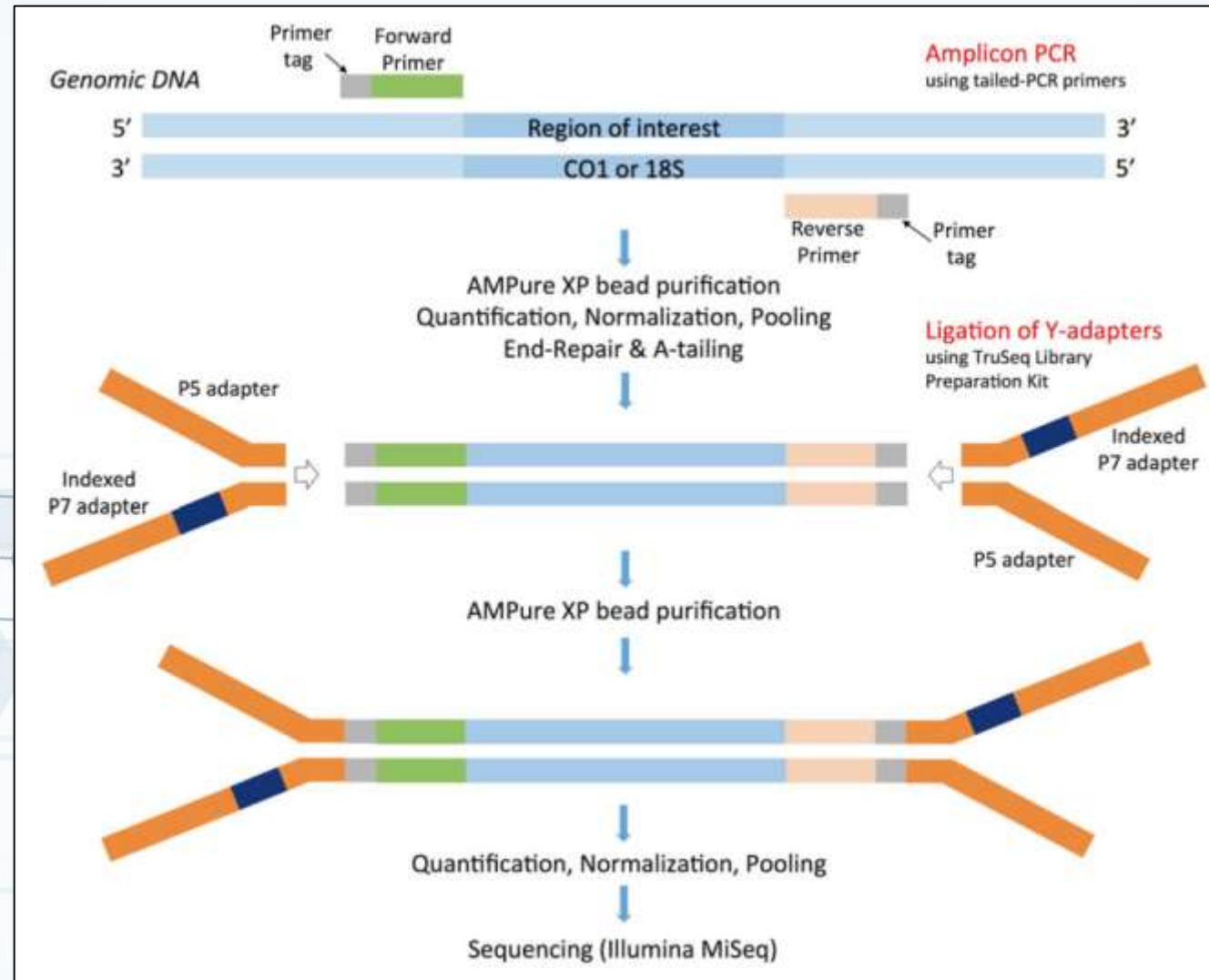
ECOLE DOCTORALE **SSBCV**



Deutscher Akademischer Austauschdienst
German Academic Exchange Service



Twin-tagging – Dual Indexing PCRs



Leray et al., 2016 (chap. 14)