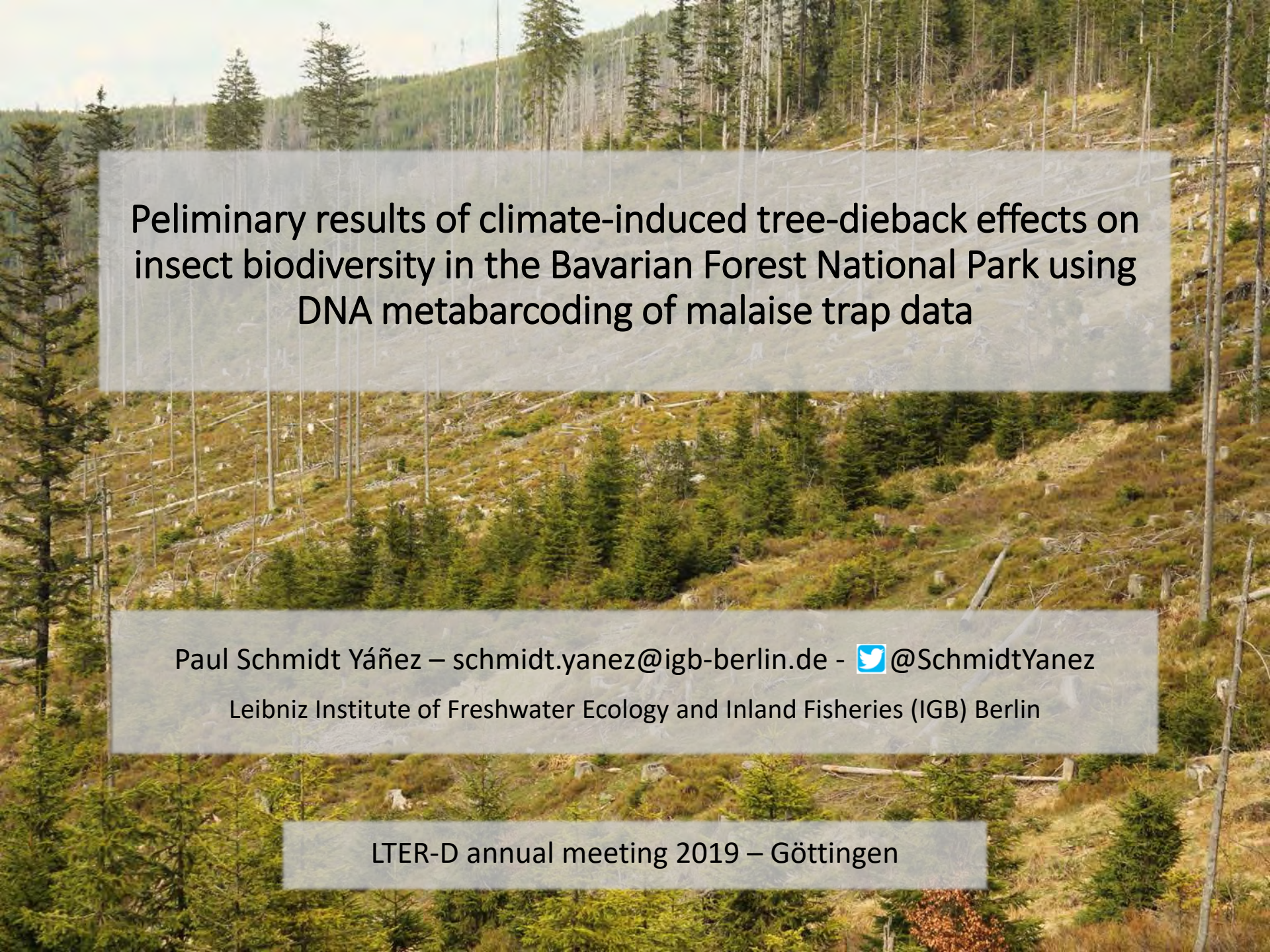


Peliminary results of climate-induced tree-dieback effects on insect biodiversity in the Bavarian Forest National Park using DNA metabarcoding of malaise trap data

Paul Schmidt Yáñez – schmidt.yanez@igb-berlin.de - @SchmidtYanez

Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) Berlin

LTER-D annual meeting 2019 – Göttingen

The CLIMTREE project

Produce cross-continental results of the impact of mountain forest die-offs on biodiversity and ecosystem services

France

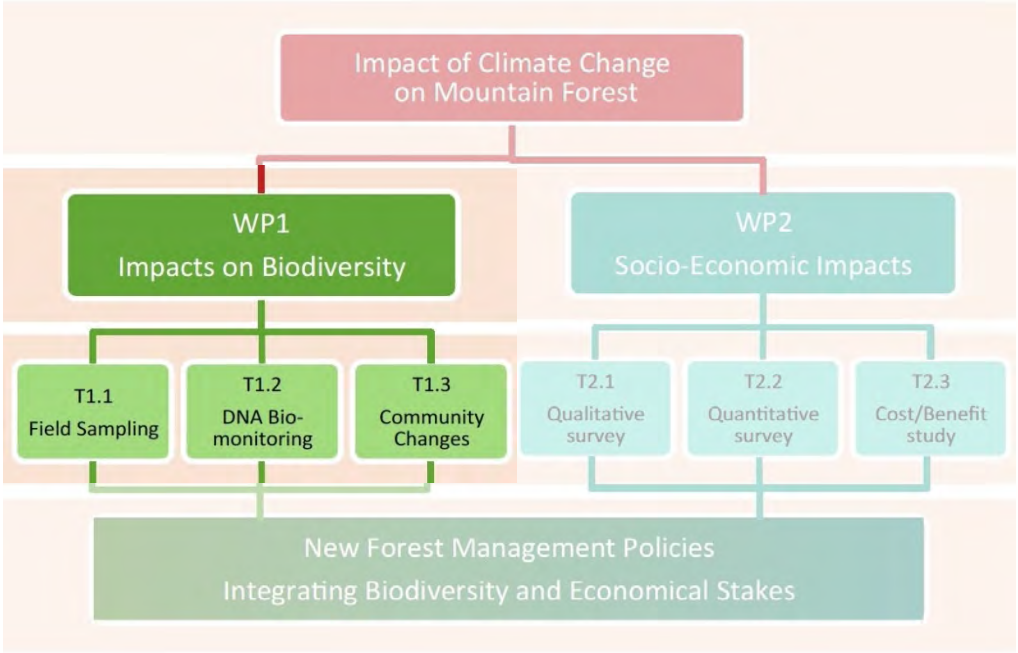
INRA, IRSTEA, IRBI
MNHN, Dynafor

IRSTEA

Germany

BFNP; IGB

Greifswald Uni



China

KIZ

KIB

Italy

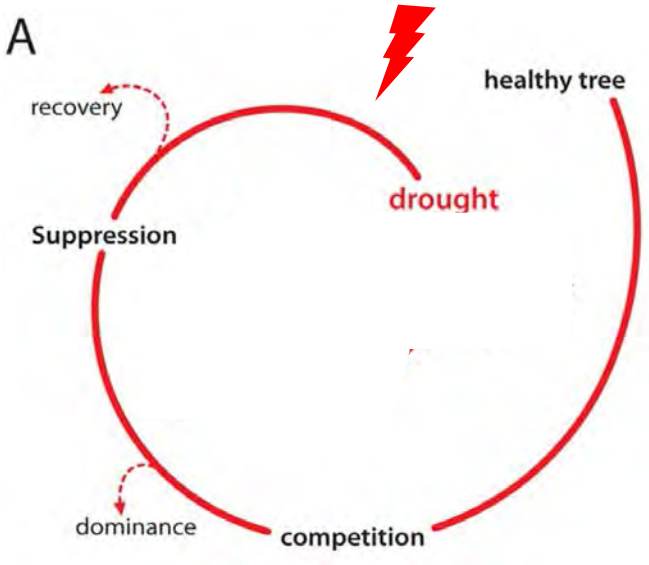
CNR-ISE

Padova Uni

WP1:
- Implement new biomonitoring pipeline (DNA metabarcoding)
- Relevant Taxa: fungi, soil meiofauna, **terrestrial & aquatic invertebrates**

Consequences of climate change

- Increasing stress (drought, fires, storms)



Modified after Allen et al 2016 - Ecography

Consequences of climate change

- Increasing stress (drought, fires, storms)
- Susceptibility to pathogens and pest insects increases

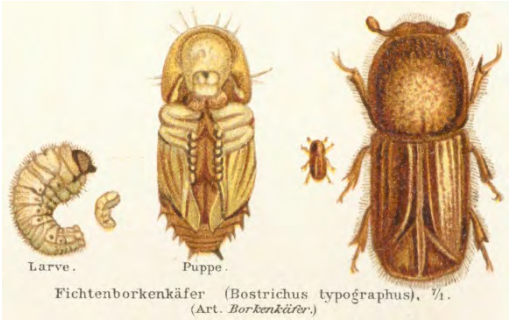
Modified after Allen et al 2016 - Ecography

Consequences of climate change

- Increasing stress (drought, fires, storms)
- Susceptibility to pathogens and pest insects increases
- More severe bark beetle outbreaks

➢ Changes on landscape level

Modified after Allen et al 2016 - Ecography

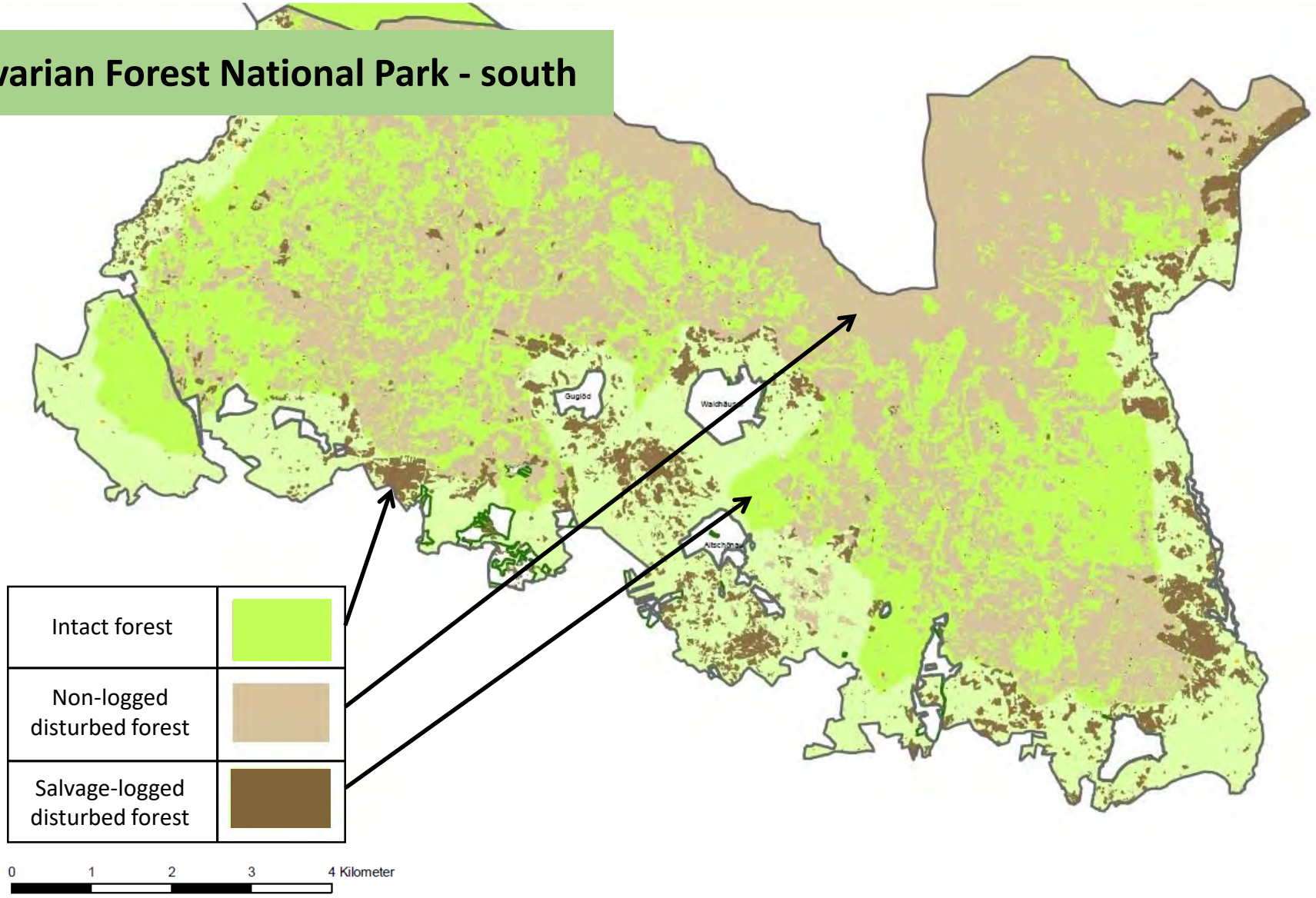


Ips typographus

Large scale landscape changes

Spruce stands in the Bavarian Forest NP, view from Rachel spring 2017

Bavarian Forest National Park - south



Question

How are terrestrial and aquatic invertebrate species composition influenced by tree die-back and management?

Malaise trap sampling

Intact spruce forest

Disturbed forest with die-off

Salvage - logged forests

Malaise trap sampling

240 malaise trap samples (3 habitats x 10 replicates x 8 time periods) – May-Sept 2017
Environmental parameters through the BIOKLIM project

Intact spruce forest

Disturbed forest with die-off

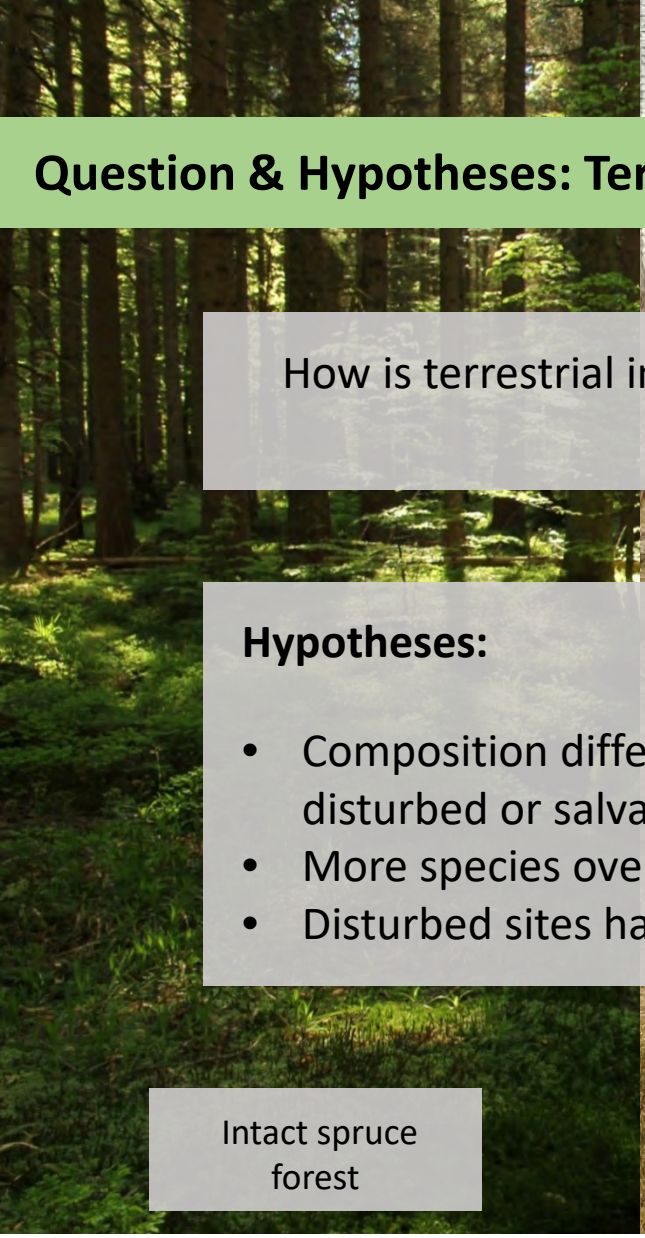
Salvage - logged forests

Question & Hypotheses: Terrestrial

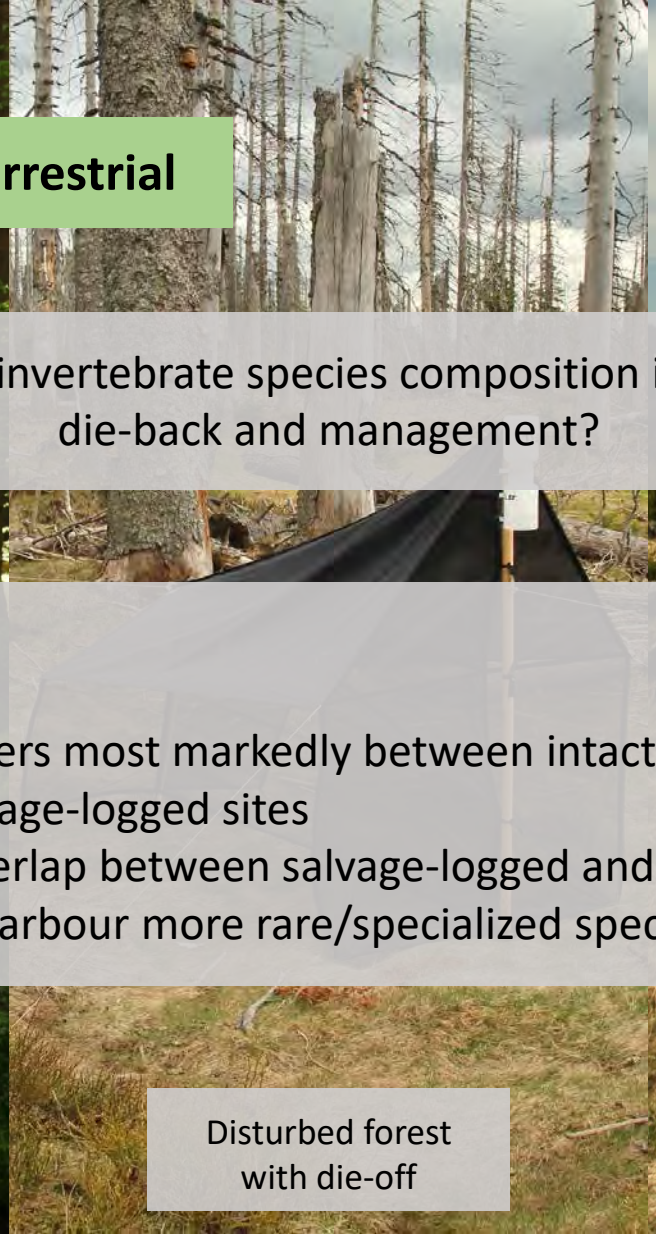
How is terrestrial invertebrate species composition influenced by tree die-back and management?

Hypotheses:

- Composition differs most markedly between intact forest and disturbed or salvage-logged sites
- More species overlap between salvage-logged and disturbed sites
- Disturbed sites harbour more rare/specialized species



Intact spruce forest



Disturbed forest with die-off

Salvage - logged forests

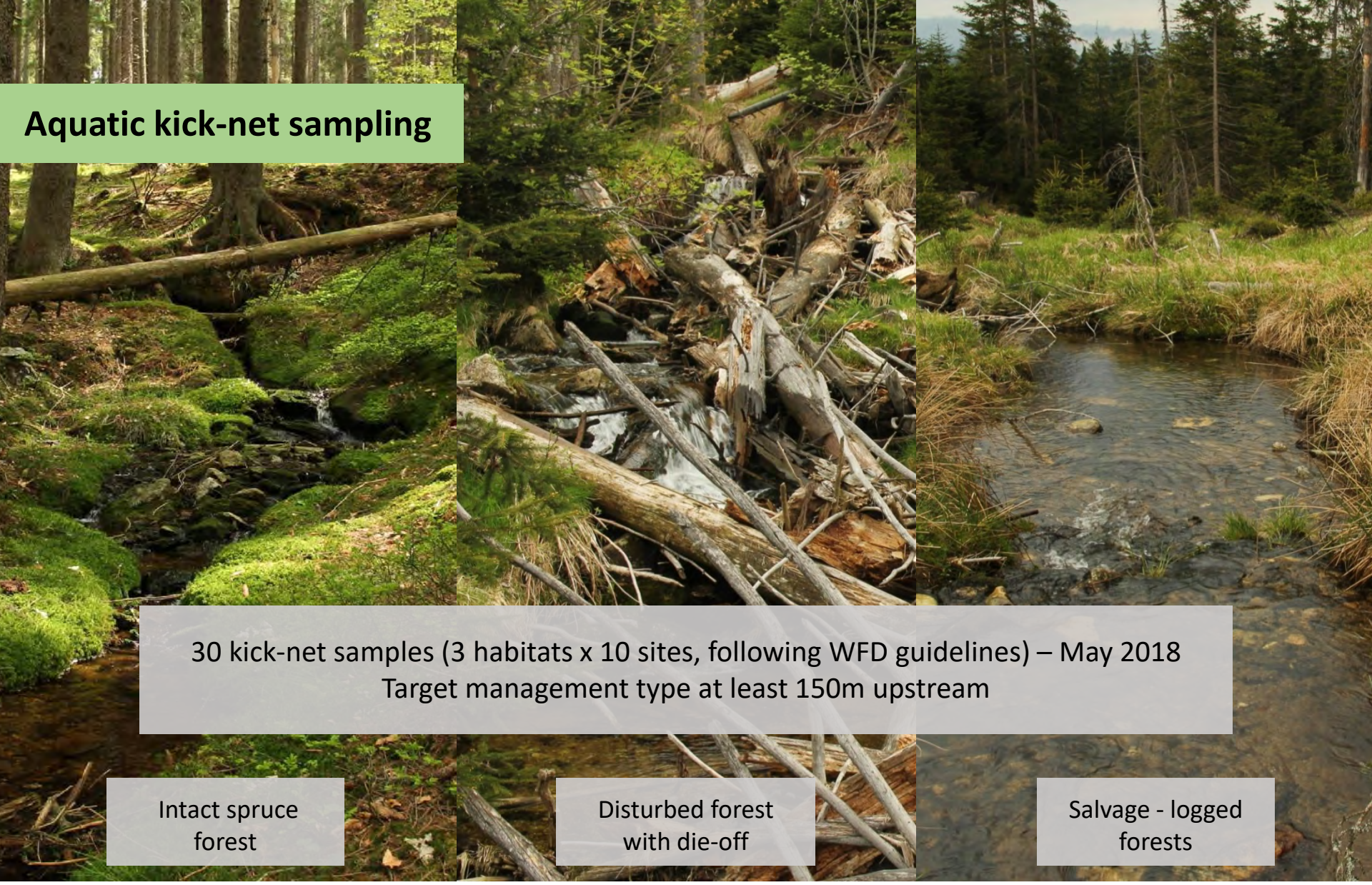
Aquatic kick-net sampling

Intact spruce forest

Disturbed forest with die-off

Salvage - logged forests

Aquatic kick-net sampling



30 kick-net samples (3 habitats x 10 sites, following WFD guidelines) – May 2018
Target management type at least 150m upstream

Intact spruce forest

Disturbed forest with die-off

Salvage - logged forests

Question & Hypotheses: Aquatic

How is aquatic invertebrate species composition influenced by tree die-back and management?

Hypotheses:

- More shredders + filter feeders in intact forests areas (**more organic matter input**)
- More grazers, shift towards autochthonous food sources in disturbed and salvage-logged areas (**more light -> algal growth**)

Intact spruce forest

Disturbed forest with die-off

Salvage - logged forests

Bulk sample processing

Filtering and weighing

* © Paul Schmidt Yanez

Bulk sample processing

Filtering and weighing

Sample storage

* © Paul Schmidt Yanez

Bulk sample processing

Filtering and weighing

Sample storage

Dried arthropods

* © Paul Schmidt Yanez

Bulk sample processing

Filtering and weighing

Sample storage

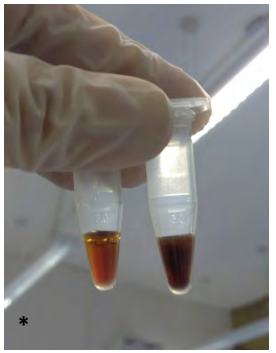
Dried arthropods

Sample homogenization

* © Paul Schmidt Yanez

Bulk sample processing

Filtering and weighing



DNA extraction

Sample storage

Dried arthropods

© IKA

Sample homogenization

* © Paul Schmidt Yanez

Laboratory peculiarities

(Simplified) laboratory setup:

- Bulk metabarcoding using:
  - Leray - Geller COI-primer (313 bp)
  - Non-native mock community (positive control)
  - Extraction and negative controls
- Three PCR replicates (DAMe pipeline)

Bioinformatics workflow

Bioinformatics pipeline:

Very conservative to avoid false positives!

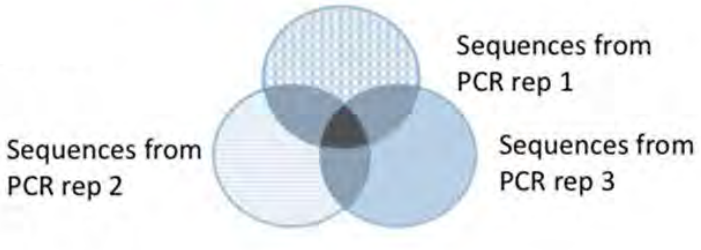
- Quality check and multiple error corrections

Bioinformatics workflow

Bioinformatics pipeline:

Very conservative to avoid false positives!

- Quality check and multiple error corrections
- Stringent filtering (min 3 PCRs & 3 reads each)



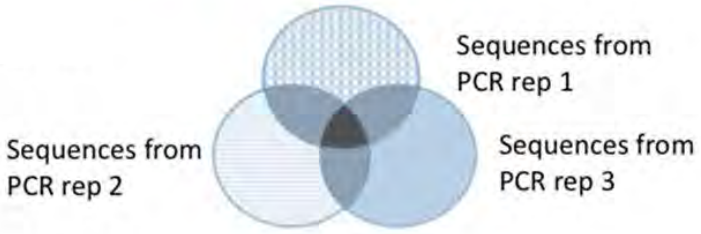
Modified after Zepeda-Mendoza et al. 2016 - BMC

Bioinformatics workflow

Bioinformatics pipeline:

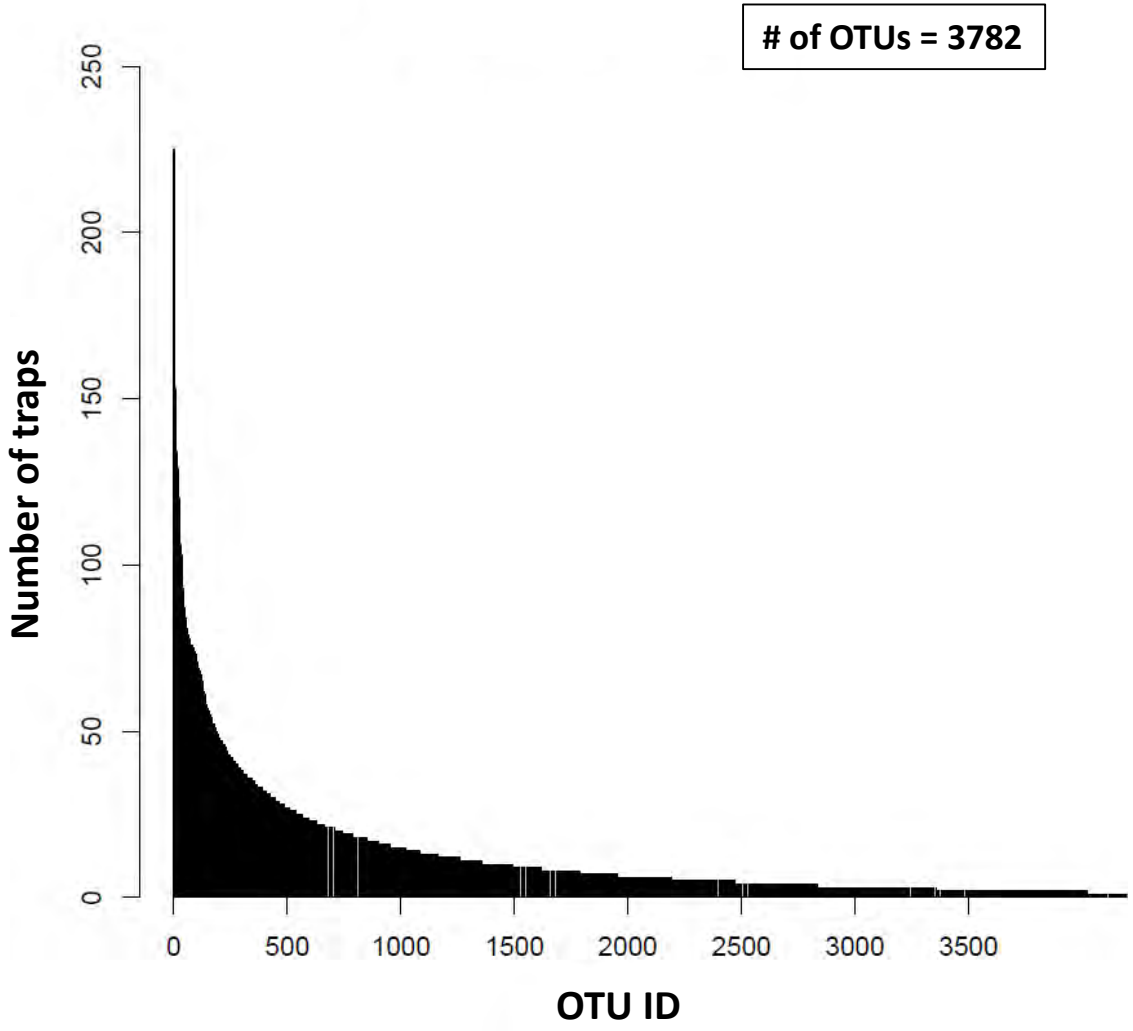
Very conservative to avoid false positives!

- Quality check and multiple error corrections
- Stringent filtering (min 3 PCRs & 3 reads each)
- Clustering & OTU assignment (RDP Midori classifier – NCBI based)



Modified after Zepeda-Mendoza et al. 2016 - BMC

OTU distribution across traps



OTU assignment

10 most common OTUs

# of traps	Midori_species_ID	species_prob
125	Ichneumon discoensis	0,5
103	Dyscritomyia lucilioides	0,11
202	Botanophila brunneilinea	0
135	Formica fusca	0,9
150	Dolichopus stenhammari	0,34
91	Myrmica ruginoidis	0,98
143	Clusiodes clandestinus	0,29
120	Spilogona magnipunctata	0,16
75	Drosophila cauverii	0,02
33	Erynnia tortricis	0,23

OTU assignment

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Formica fusca

Myrmica ruginoidis

OTU assignment

10 most common OTUs

# of traps	Midori_species_ID	species_prob	Midori_family_ID	family_prob
125	Ichneumon discoensis	0,5	Ichneumonidae	1
103	Dyscritomyia lucilioides	0,11	Calliphoridae	0,15
202	Botanophila brunneilinea	0	Anthomyiidae	0,01
135	Formica fusca	0,9	Formicidae	1
150	Dolichopus stenhammari	0,34	Dolichopodidae	0,48
91	Myrmica ruginoidis	0,98	Formicidae	1
143	Clusiodes clandestinus	0,29	Clusiidae	0,3
120	Spilogona magnipunctata	0,16	Muscidae	0,65
75	Drosophila cauverii	0,02	Drosophilidae	0,27
33	Erynnia tortricis	0,23	Tachinidae	0,36

OTU assignment

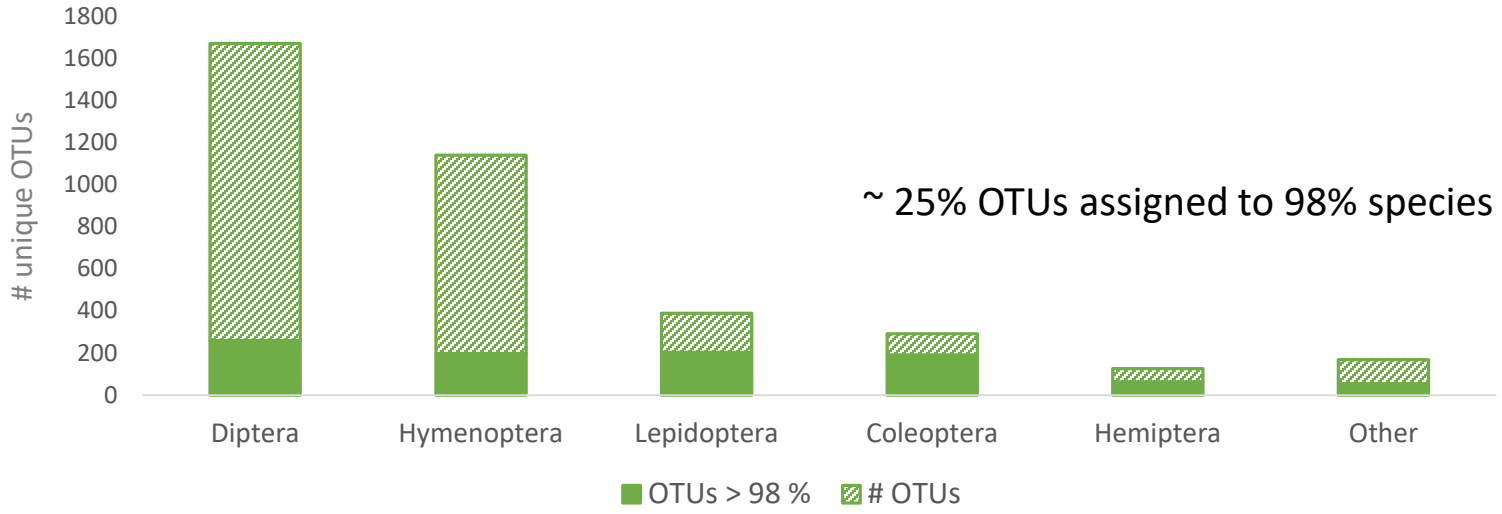
# of traps	Midori_species_ID	species_prob	BOLD_ID	similarity
125	Ichneumon discoensis	0,5	Ichneumonis gracilentus	100
103	Dyscritomyia lucilioides	0,11	Rhagio conspicuus	100
202	Botanophila brunneilinea	0	Megaselia nigriceps	100
135	Formica fusca	0,9	Formica fusca	100
150	Dolichopus stenhammari	0,34	Dolichopus discifer	100
91	Myrmica ruginoidis	0,98	Myrmica ruginoidis	100
143	Clusiodes clandestinus	0,29	Acnemia nitidicollis	100
120	Spilogona magnipunctata	0,16	Coenosia means	100
75	Drosophila cauverii	0,02	Neurigona quadrifasciata	100
33	Erynnia tortricis	0,23	Ocytata pallipes	100

Ichneumonis gracilentus

Rhagio conspicuus

Taxonomic coverage – RDP Midori

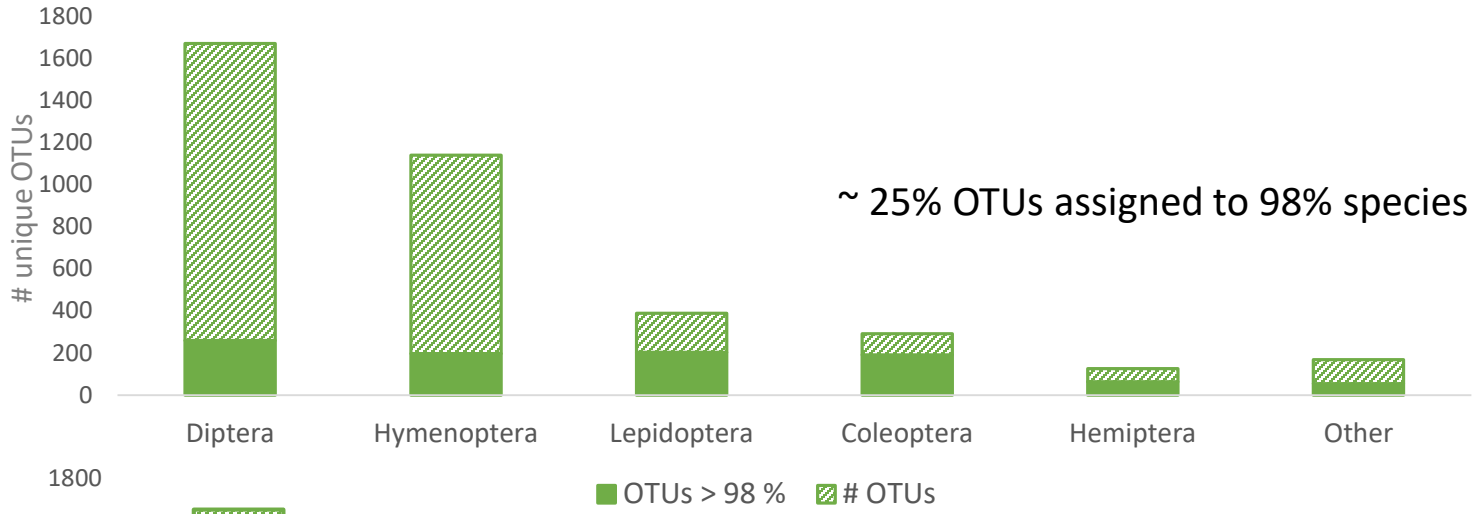
# of OTUs = 3782



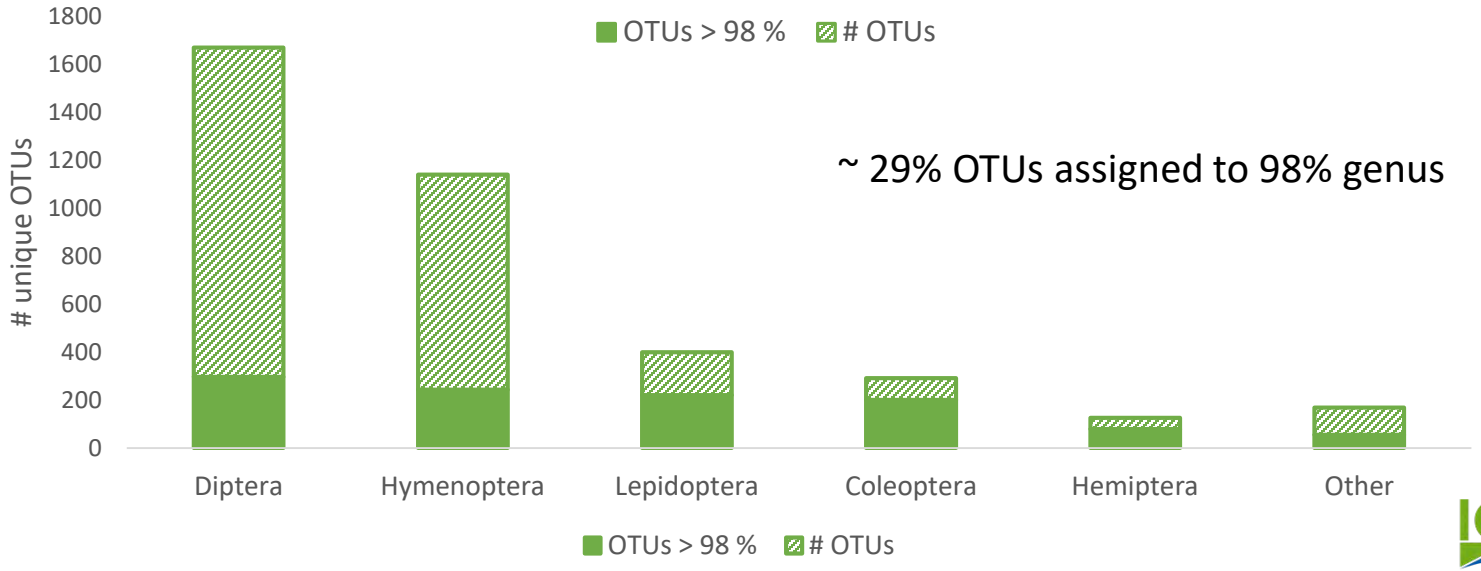
~ 25% OTUs assigned to 98% species

Taxonomic coverage – RDP Midori

# of OTUs = 3782



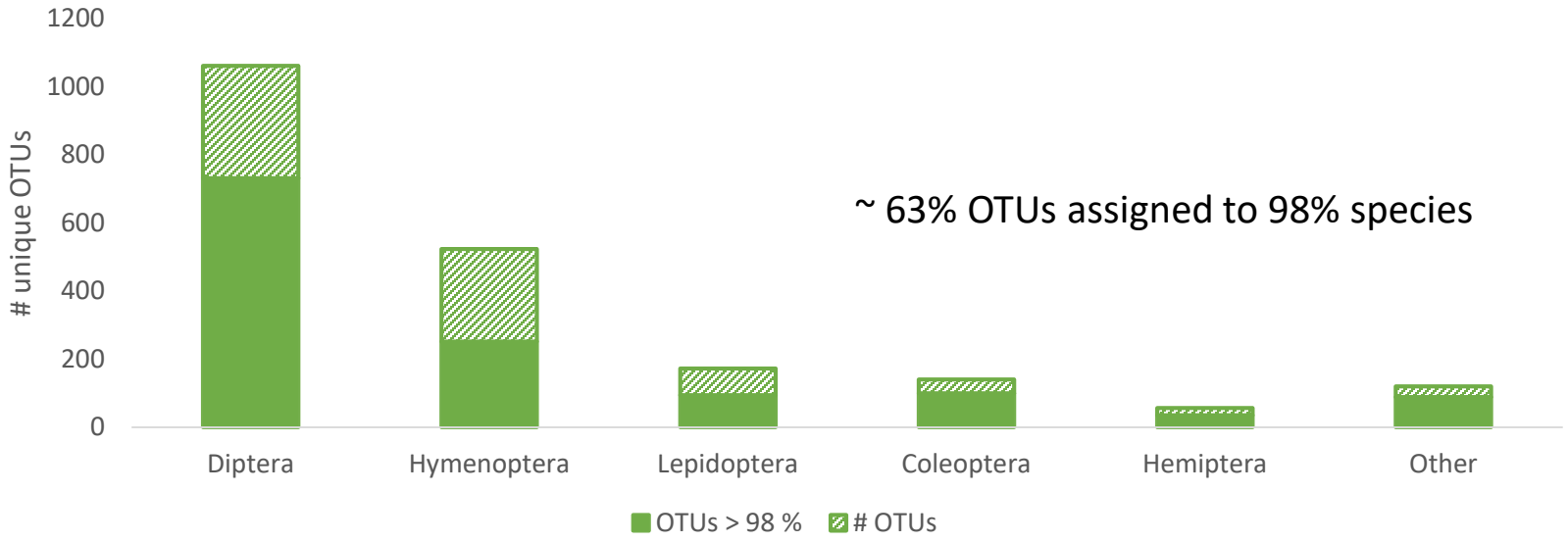
~ 25% OTUs assigned to 98% species



~ 29% OTUs assigned to 98% genus

Taxonomic coverage – BOLD

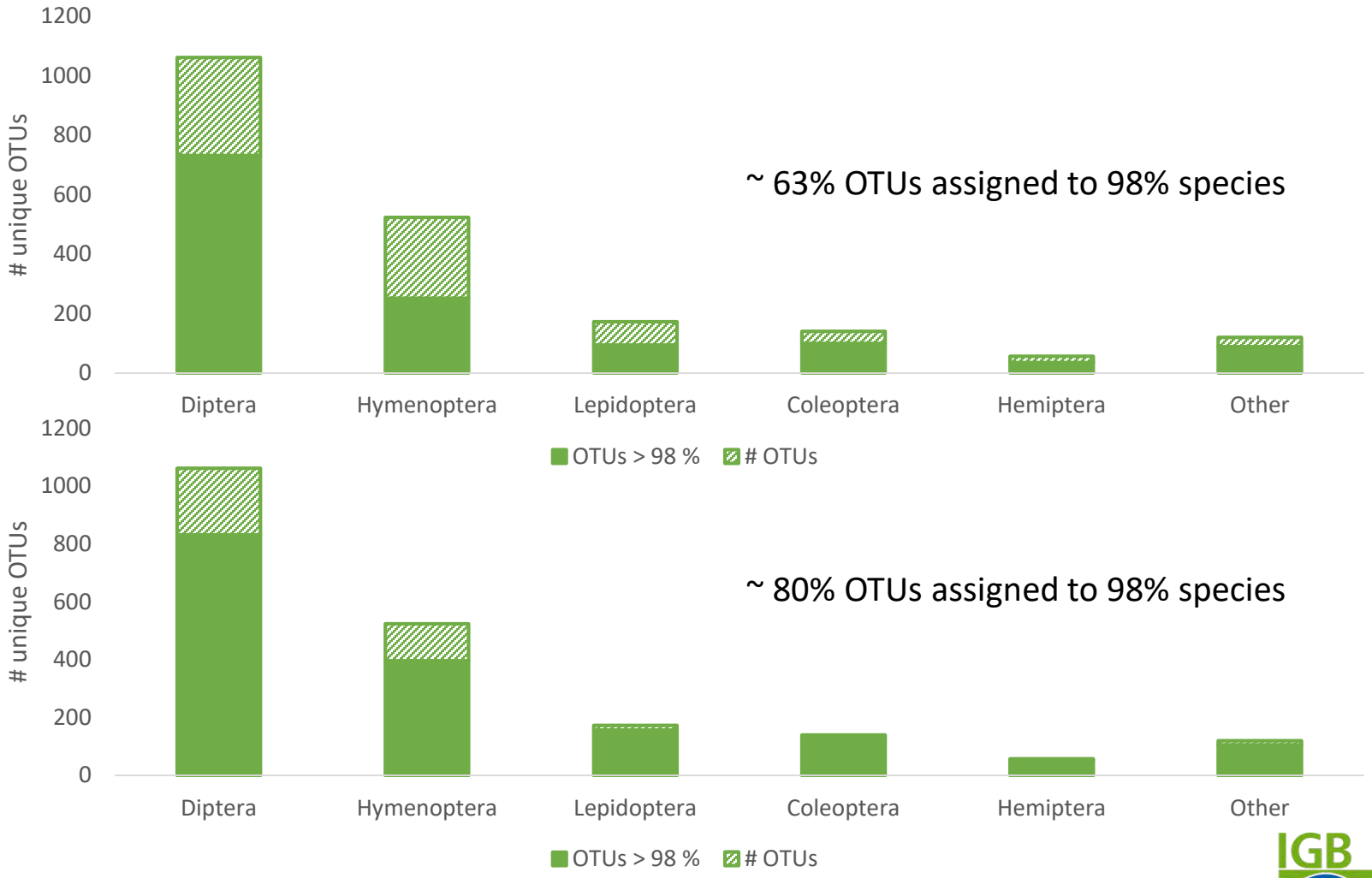
# of OTUs = 2076



~ 63% OTUs assigned to 98% species

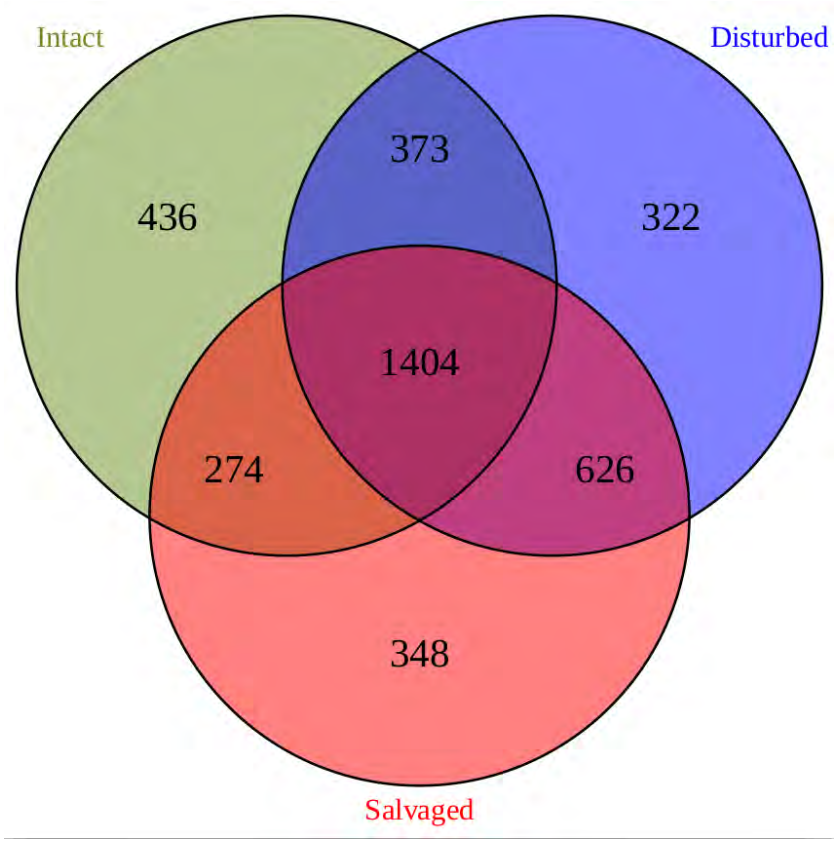
Taxonomic coverage – BOLD

# of OTUs = 2076



Habitat species turnover

# of OTUs = 3782



Jaccard dissimilarity index:

intact - disturbed	0.48
intact - salvaged	0.52
disturbed - salvaged	0.39

Discussion

Summary:

- OTUs differ markedly between habitats, but have large overlap
- More similar between disturbed and salvage-logged forests
- Intact forest has most unique OTUs
- (Rare species and specialists missing in salvage-logged forests)

Problems to resolve

Optimal filtering threshold

- No false positives
- Maximum number of control OTUs
- Sequence copy number threshold

Alternative taxonomic assignment methods

- ~~Bad Midori RDP classifier assignment of diverse taxa~~
- Switch to BOLD, most recent species incorporated

Relevance

- Mountain forest management
  (**national parks/forest owners/landscape managers/scientists**)
- Standardize biomonitoring
  (**affordable, reliable, repeatible & verifiable**)
- Improve metabarcoding methods & database
  (**global DNA barcoding campaigns**)

CLIMTREE

➢ Develop early warning signals to detect forest die-off

➢ Determine thresholds of forest loss for biodiversity collapse

Special thanks to:

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Doug W. Yu – University of East Anglia
& Kunming Institute of Zoology

Carlos Lopez-Vaamonde – IRBI Tours
& INRA Orléans

CLIMTREE

DFG Deutsche Forschungsgemeinschaft

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