



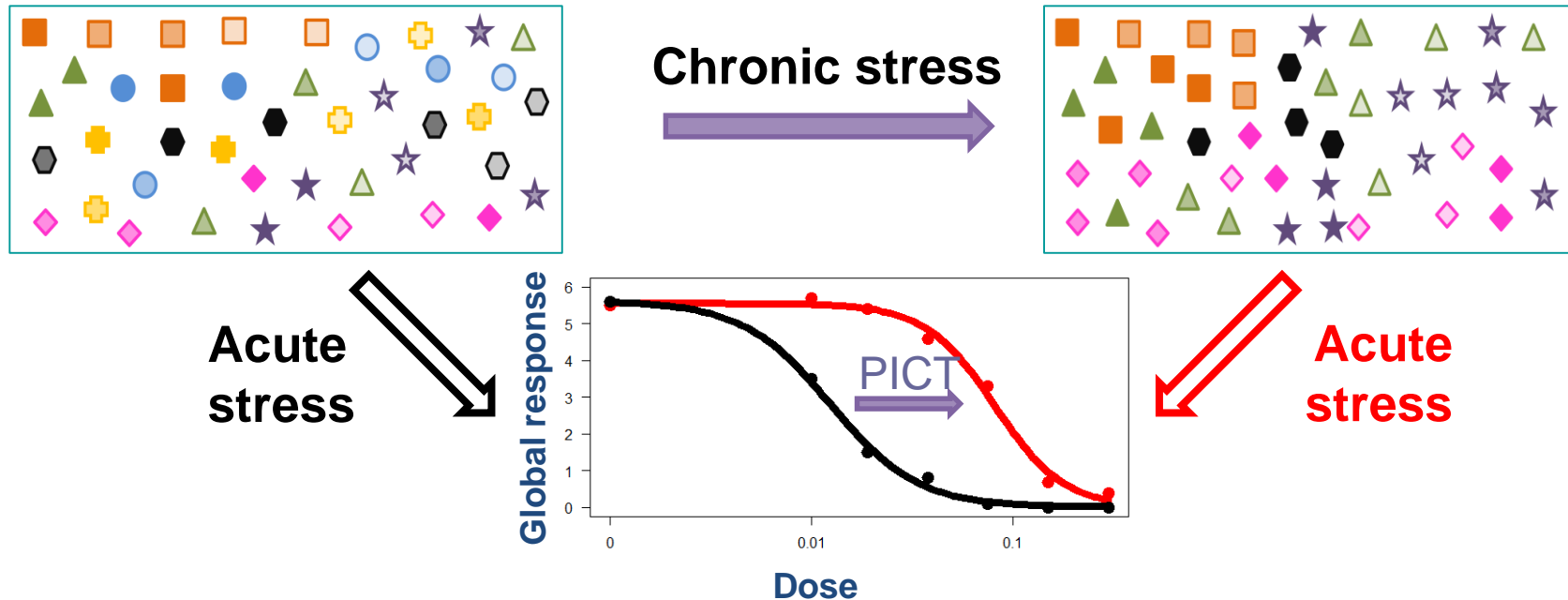
# Meta-transcriptomics reveals stress adaptation processes in microbial communities differing in exposure history

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# Context

- When microbial communities are chronically exposed to stress, adaptive mechanisms render them more tolerant
- Pollution-Induced Community Tolerance (PICT) concept



# Objectives

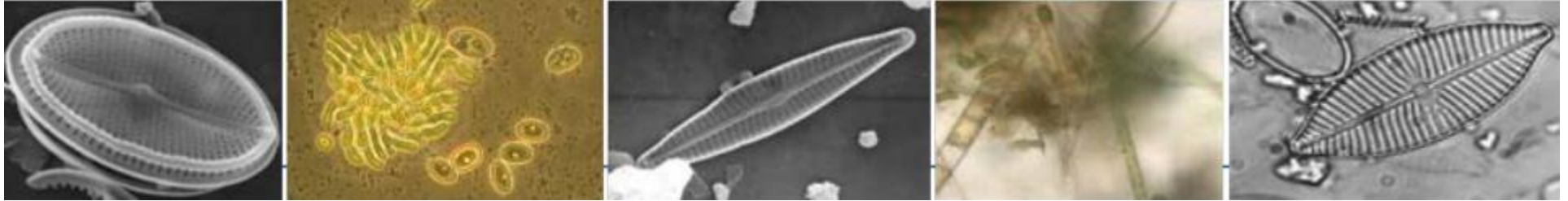
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- Community tolerance is usually related to changes in species composition
- **Aim:** Understanding of the **molecular mechanisms of stress adaptation** at the community level
- **Meta-transcriptomics** is a relevant methodology to tackle this challenge because it provides **comprehensive functional information** at molecular level
- **Hypotheses:**
  - Untargeted meta-transcriptomics (RNA-seq) combined with dose-response modelling allows to identify sensitive pathways responsive to acute stress
  - Comparison of gene expression profiles of communities differing in exposure history unravels molecular mechanisms of stress adaptation and community tolerance induction

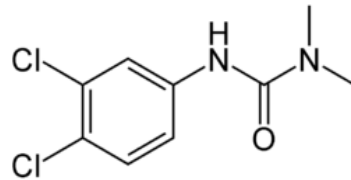
# Case study

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- Natural aquatic microbial communities, biofilm sampled in the Parthe river (Germany)



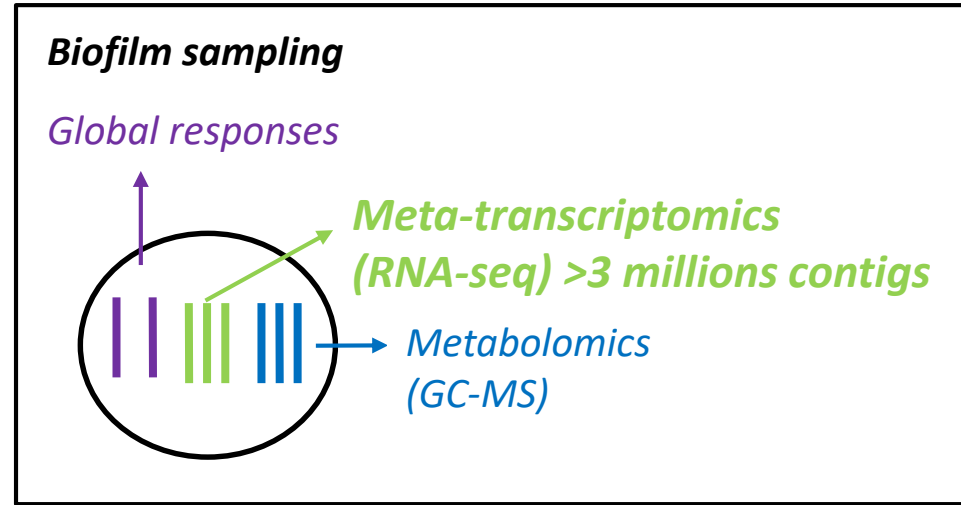
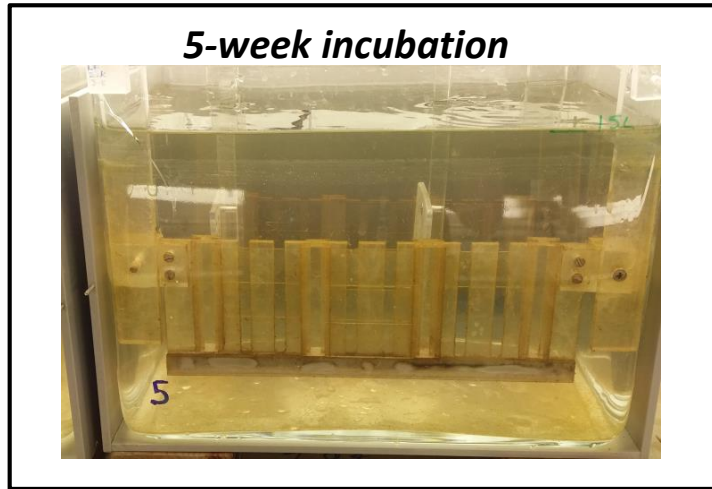
- Herbicide diuron as model compound, photosynthesis inhibitor



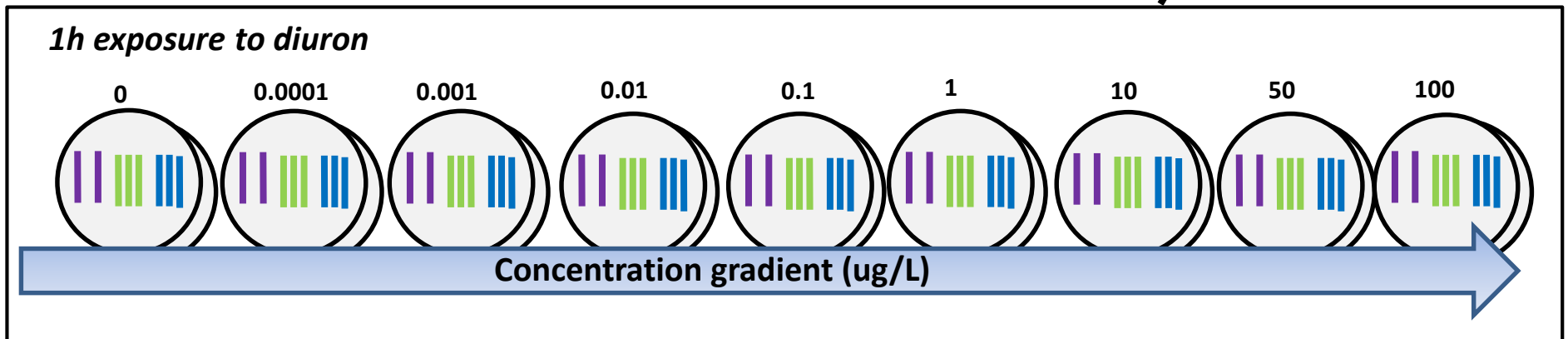
# Experiment

Chronic stress (4  $\mu\text{g/L}$  diuron) or not (control)

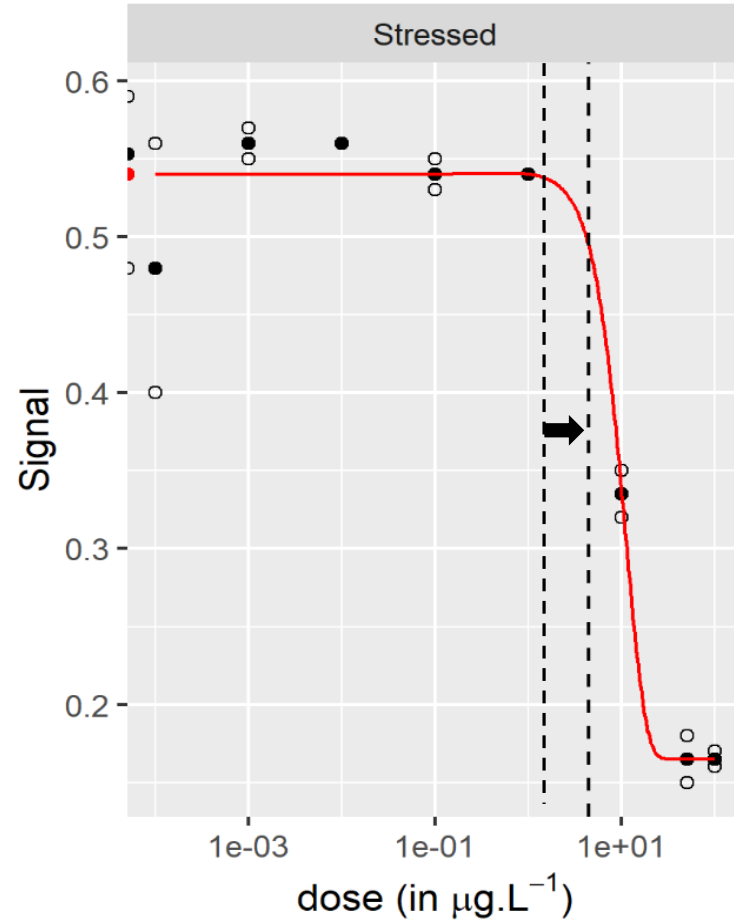
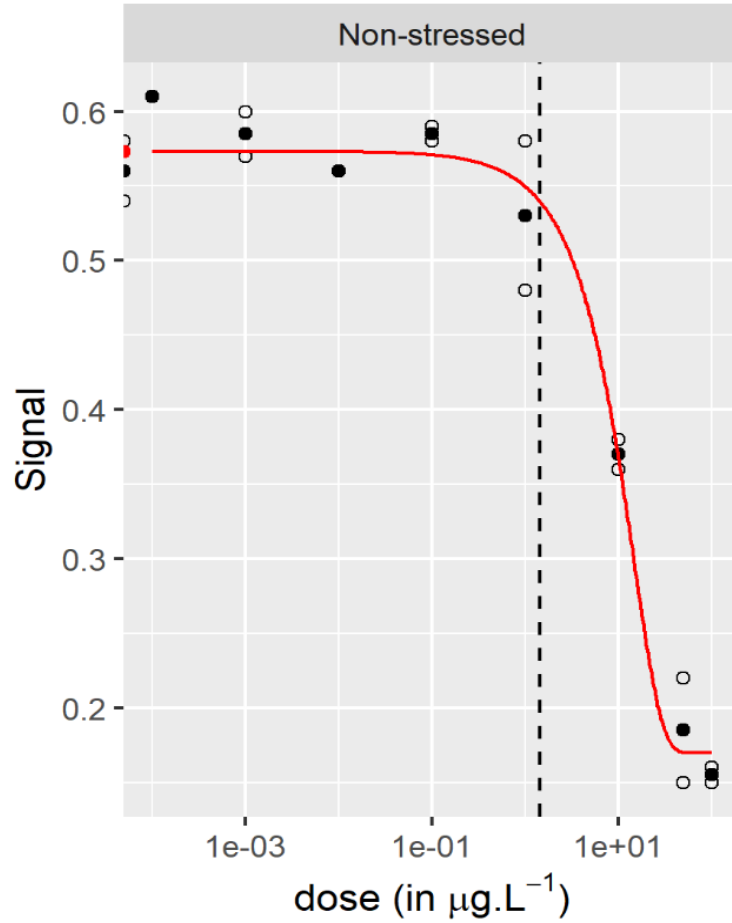
« Stressed community » « Non-stressed community »



Acute stress



# Photosynthesis



# Transcriptomics data analysis

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- Meta-transcriptome analysis handled by the DRomics R package



- A workflow dedicated to omics data obtained using a typical dose-response design
  - 1. Import and transformation / normalization of data
  - 2. Selection of responsive contigs
  - 3. Dose-Response modelling of selected contigs → DR trend (inc, dec, U, bell)
  - 4. Derivation of a sensitivity value → Benchmark Dose (BMD)
- Functional annotation (GO terms for biological processes)

# Transcriptomics data analysis

Non-stressed community

3,502,153 contigs

Stressed community

**1. DATA NORMALIZATION (rlog)**

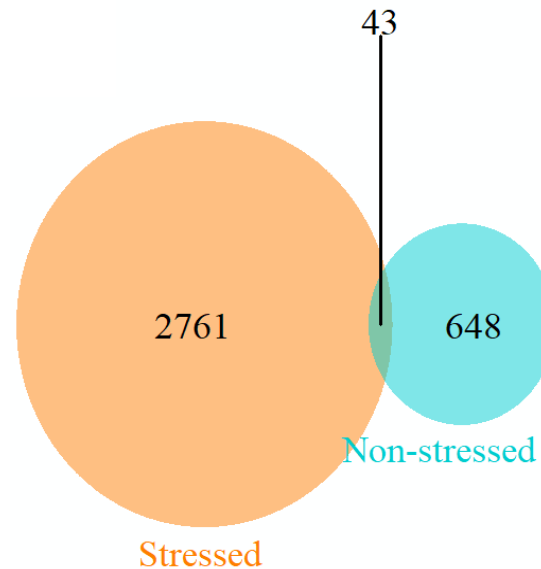
**2. RESPONDING ITEM SELECTION (quadratic trend test)**



2804 contigs



691 contigs





# Transcriptomics data analysis

Non-stressed community

3,502,153 contigs

Stressed community

## 1. DATA NORMALIZATION (rlog)

## 2. RESPONDING ITEM SELECTION (quadratic trend test)



2804 contigs



691 contigs

## 3. DOSE-RESPONSE MODELLING



2372 contigs



559 contigs

	Non-stressed community	Stressed community
<b>Increasing</b>	27 % (n = 149)	71 % (n = 1687)
<b>Decreasing</b>	43 % (n = 242)	9% (n = 220)
<b>U-shape</b>	15% (n = 83)	11% (n = 255)
<b>Bell-shape</b>	15 % (n = 85)	9 % (n = 210)

# Transcriptomics data analysis

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Non-stressed community

3,502,153 contigs

Stressed community

## 1. DATA NORMALIZATION (rlog)

## 2. RESPONDING ITEM SELECTION (quadratic trend test)



2804 contigs



691 contigs

## 3. DOSE-RESPONSE MODELLING



2372 contigs



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## 4. DERIVATION OF SENSITIVITY VALUE (BMD)

# Transcriptomics data analysis

Non-stressed community

3,502,153 contigs

Stressed community

## 1. DATA NORMALIZATION (rlog)

## 2. RESPONDING ITEM SELECTION (quadratic trend test)



691 contigs



2804 contigs

## 3. DOSE-RESPONSE MODELLING



559 contigs



2372 contigs

## 4. DERIVATION OF SENSITIVITY VALUE (BMD)

## FUNCTIONAL ANNOTATION (GO biological processes)



216 contigs

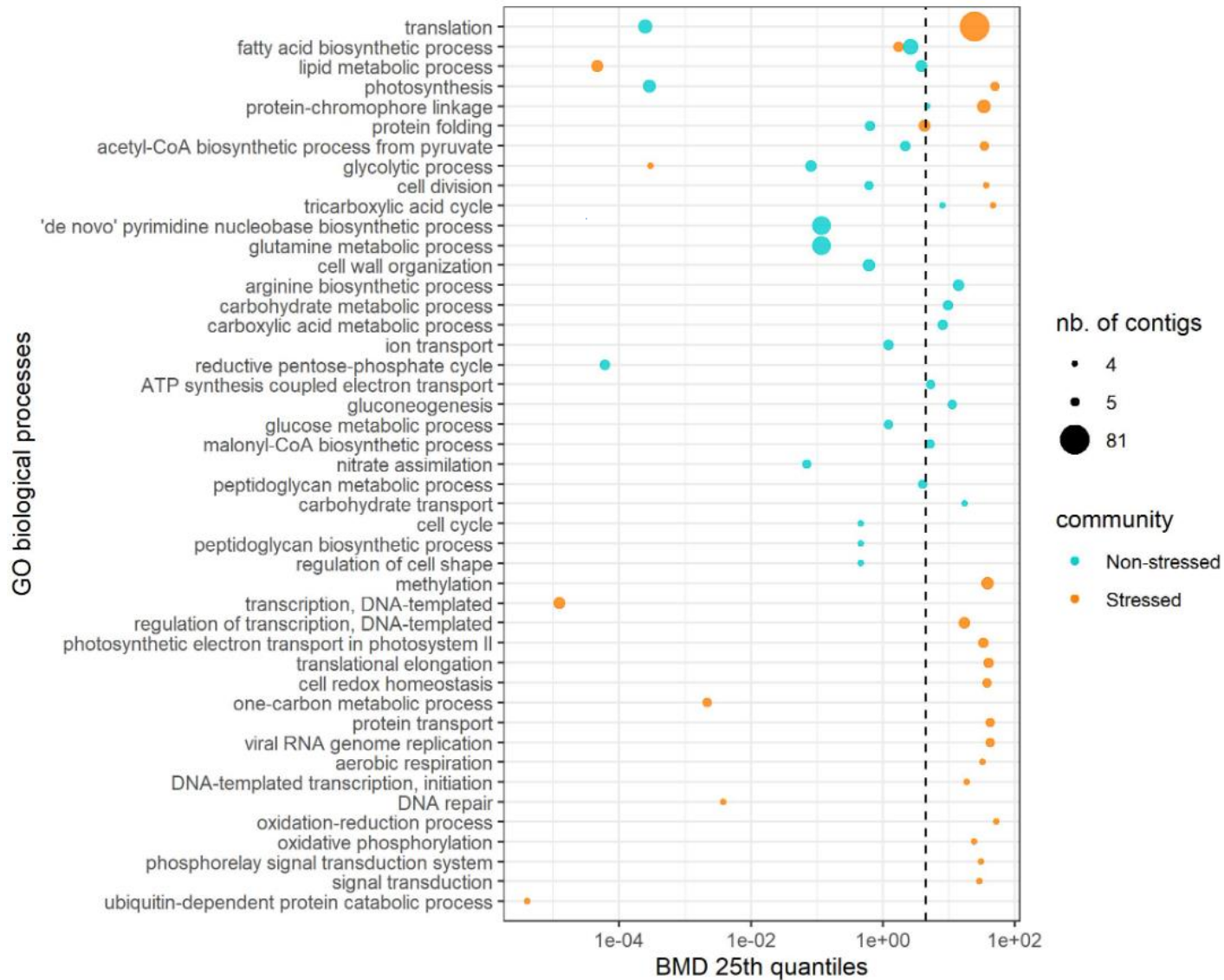


343 contigs

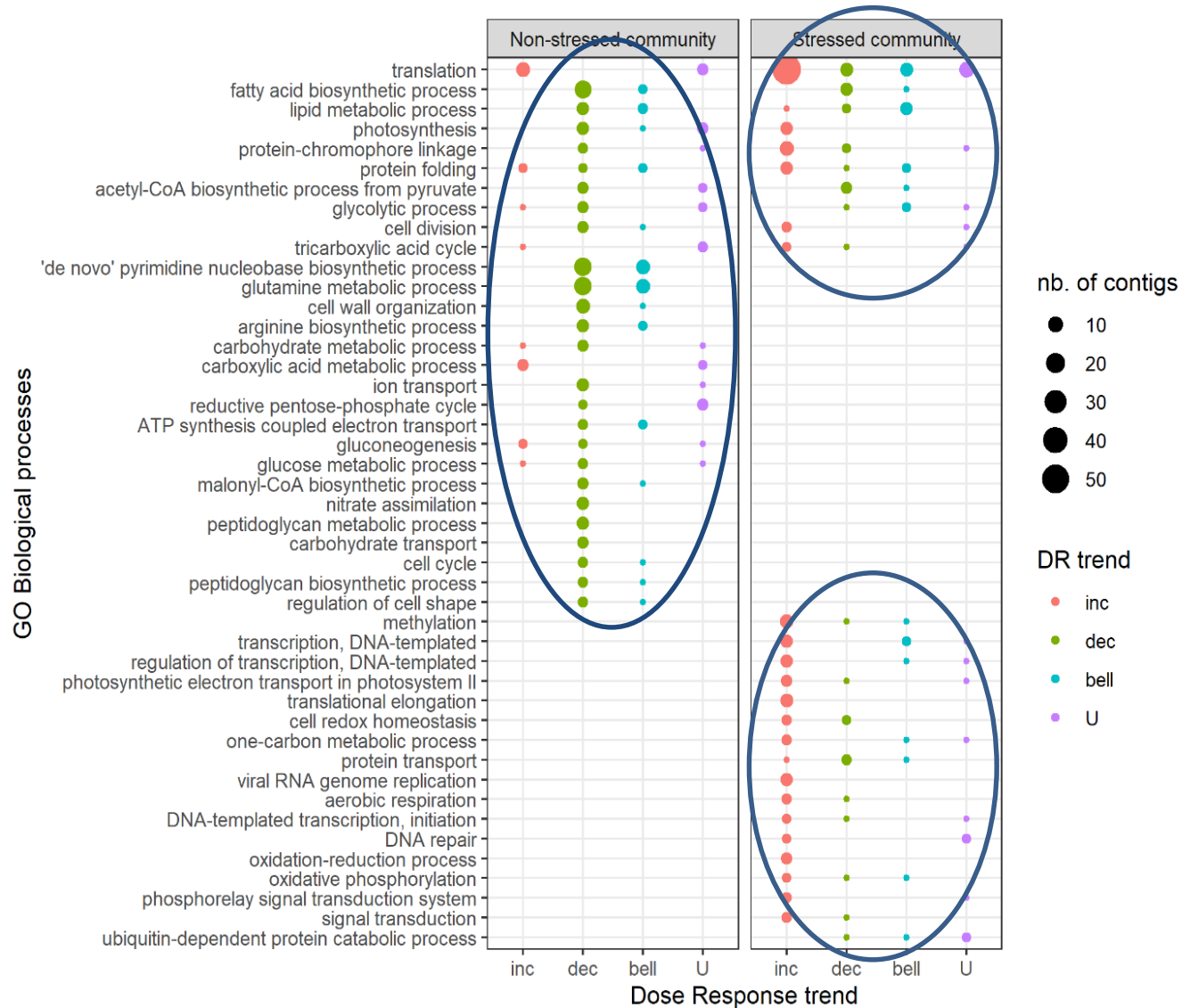
111 distinct regulated processes

209 distinct regulated processes

# Sensitivity of GO biological processes

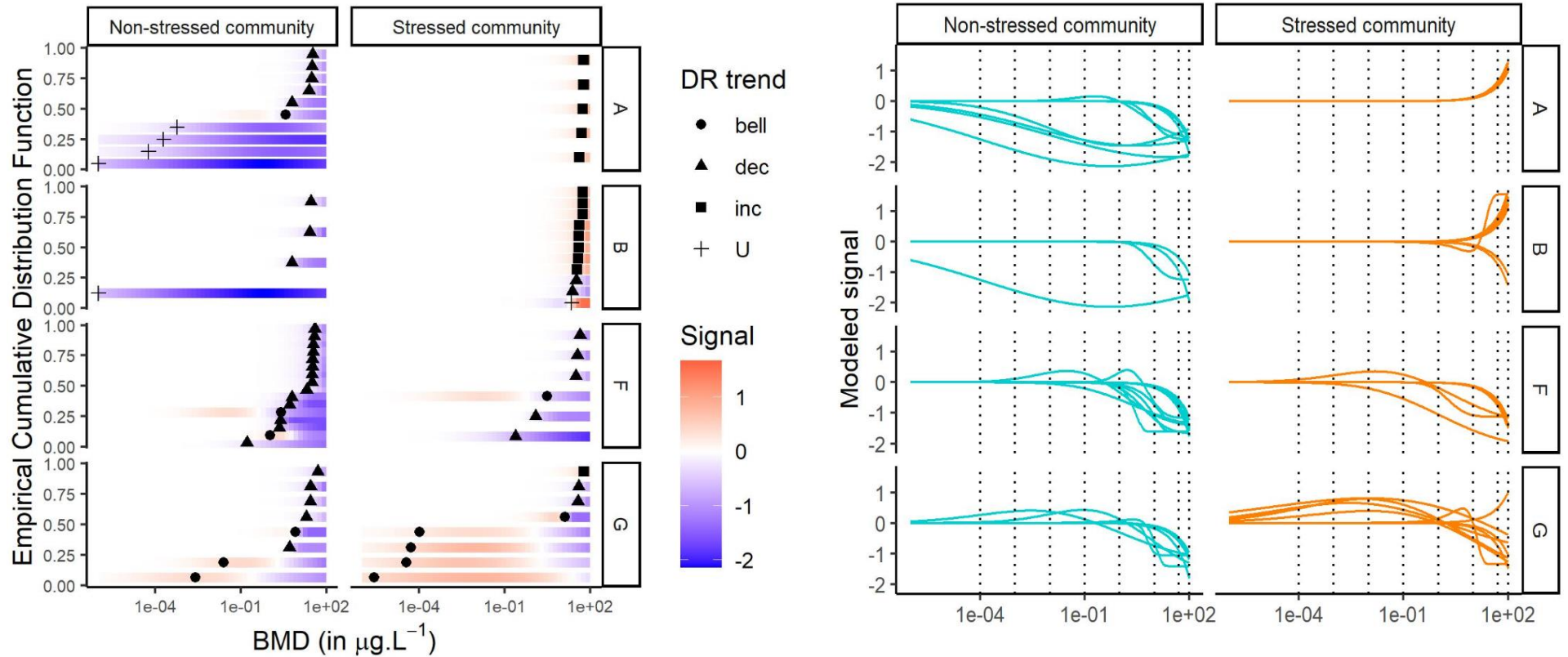


# Trends of dose-response curves





# Sensitivity of some GO biological processes



Two pathways closely **related to the diuron mode of action**

(A) Photosynthesis (B) Protein-chromophore linkage

Two pathways **sensitive in both communities**

(F) Fatty acid biosynthetic process (G) Lipid metabolic process

# Conclusion and significance

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- This study highlights substantial changes in the molecular stress responses of microbial communities after chronic exposure.
- Stressed and non-stressed communities differed in their
  - processes activated during consecutive short-term acute exposure
  - DR trends and sensitivity of these processes
- Differences in regulation specifically in photosynthesis
- Combining untargeted omics technologies and a dose-response experimental design is a powerful approach to understand molecular mechanisms in ecotoxicology
- The approach could be successful to tackle stress responses of other organisms





# Thank you !

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