



# Biochar Affects Extracellular Enzymes, Microbes and Organic Matter Dynamics in Sediments

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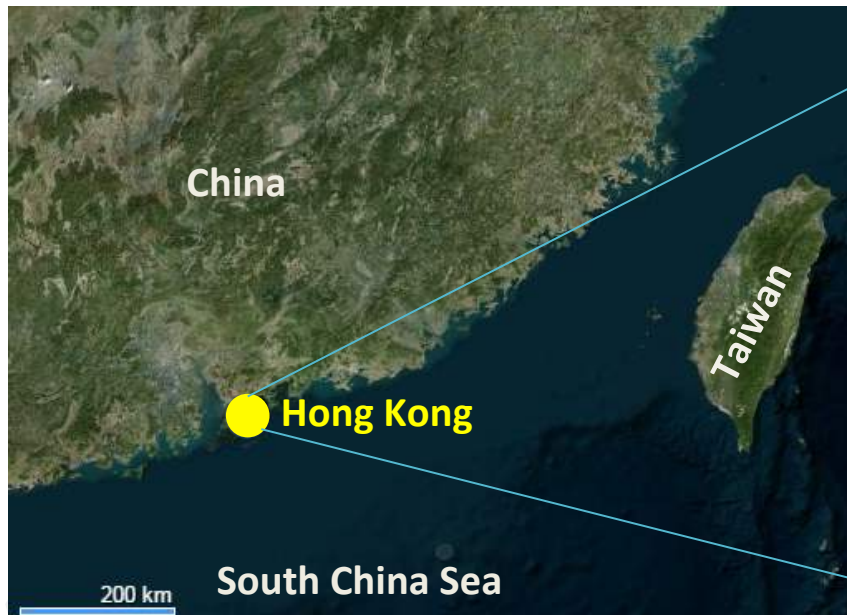
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# Sampling Sites and Samples

## Seasonal and spatial sampling



### Seasons (0-2 cm, surface)

- Dry season (March), recorded as **IZ1M**, **IZ2M**, **MG1M**, and **MG2M**.
- Wet season (November), recorded as **IZ1N**, **IZ2N**, **MG1N**, and **MG2N**.

### Depths

- Surface (0-2 cm) same as IZ1N, IZ2N, MG1N, and MG2N.
- Bottom (20-22 cm), recorded as **IZ1B**, **IZ2B**, **MG1B** and **MG2B**.



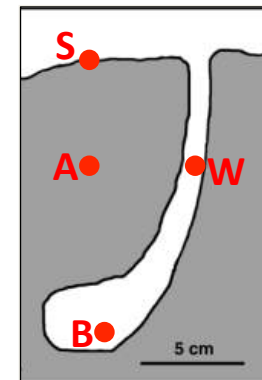
# Sampling Sites and Samples

## Burrows by epifauna



The shape of the burrow is like a 'J', and the depth to the bottom of burrow is usually 15-20 cm (*Kristensen 2008*).

- **Surface (S)**: Top 0-1 cm of sediment 5 cm away from any burrows,
- **Wall (W)**: Wall of burrow at a thickness of 0-0.5 cm,
- **Ambient (A)**: Depth of 5-6 cm under Surface, usually anoxic,
- **Bottom (B)**: The bottom of burrows.



# Methods

## Substrates and buffer used for enzyme assays (Dick 2011)

Enzyme	EC	Abbreviation	Substrate	Buffer
Phenol oxidase	1.14.18.1	PHO	L-3,4-dihydroxy phenylalanine(10 mM)	Acetate buffer (50 mM, pH 5.0)
$\beta$ -glucosidase	3.2.1.21	GLU	<i>p</i> -nitrophenyl- $\beta$ -D-glucoside (50 mM)	MUB, pH 6.0
<i>N</i> -acetyl- $\beta$ -glucosaminidase	3.2.1.14	NAG	<i>p</i> -nitrophenyl- <i>N</i> -acetyl- $\beta$ -D-glucopyranoside (10 mM)	Acetate buffer (100 mM, pH 5.5)
Acid phosphatase	3.1.3.2	ACP	<i>p</i> -nitrophenyl phosphate (50 mM)	MUB, pH 6.5

**PHO: one of the few enzymes able to attack phenolics**

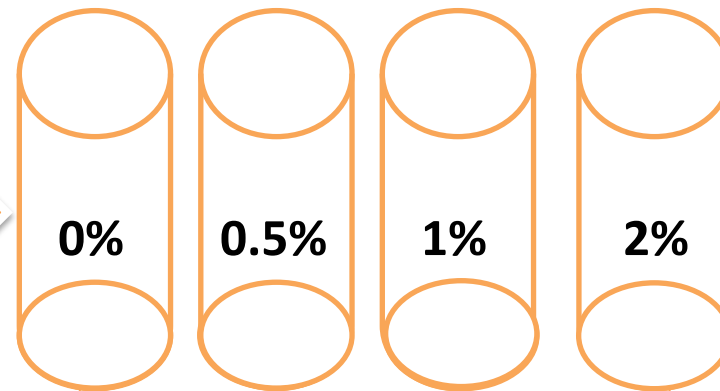
**GLU: catalyzes the final step in the breakdown of cellulose**

**NAG: one of the *N*-targeting hydrolytic enzymes**

**ACP: hydrolyzes phosphomonoesters and phosphodiester to release inorganic phosphate**

# Incubation Experiments

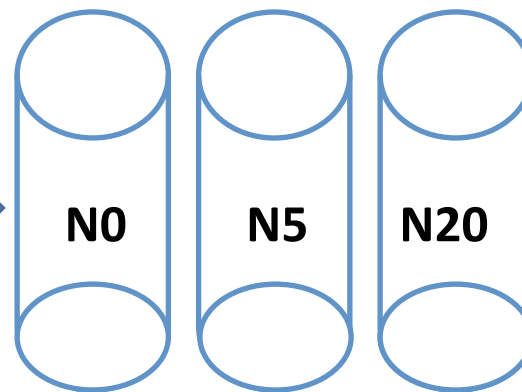
**Biochar  
Addition**



25 °C for 90 days.

The surface area of the biochar:  $332 \text{ m}^2 \text{ g}^{-1}$ , and the content of C and N: 67.48% and 0.77%, respectively (Xu *et al.* 2012).

**N  
Addition**



25 °C for 45 days

N0: 0  $\mu\text{g/g}$  Sediment  
N5: 5  $\mu\text{g/g}$  Sediment  
N20: 20  $\mu\text{g/g}$  Sediment

# Materials and Methods

## Measured parameters

- ✚ Sediment organic C (SOC), and total N and P, and soluble phenolics
- ✚ PHO, peroxidase (POD), GLU, NAG and ACP activity assayed spectrophotometrically
- ✚ Microbial (bacterial and fungal) abundance estimated by qPCR
- ✚ Microbial community by NGS

## Calculations

● **Equation 1:**  $\Delta C (\%) = [(C_{\text{treatment}} - C_{\text{control}}) / C_{\text{control}}] \times 100,$

$\Delta C$ : sediment organic C (SOC),  $C_{\text{treatment}}$ : C content in treated sediment,  $C_{\text{control}}$ : C of biochar added in sediment.

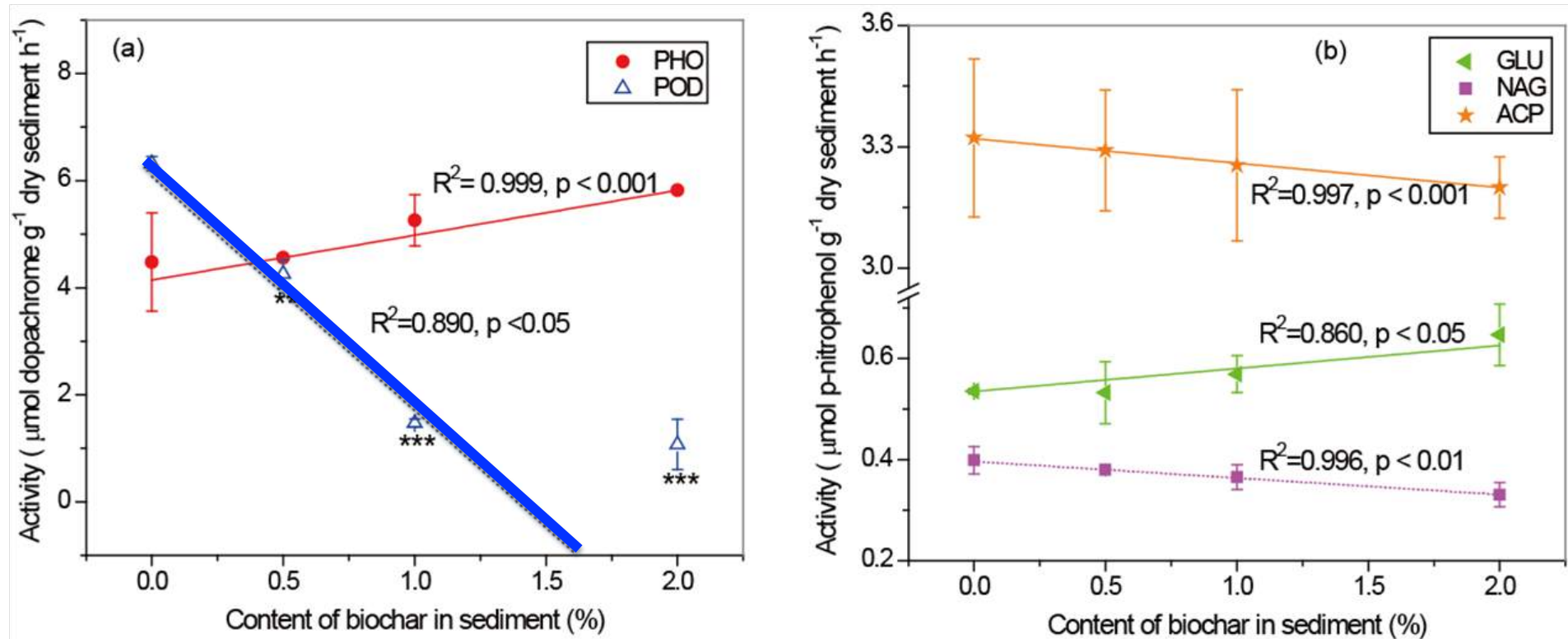
● **Equation 2:**  $\Delta EA (\%) = [(EA_{\text{treatment}} - EA_{\text{control}}) / EA_{\text{control}}] \times 100,$

EA: Enzyme activity

● **Equation 3:**  $\Delta MA (\%) = [(MA_{\text{treatment}} - MA_{\text{control}}) / MA_{\text{control}}] \times 100,$

MA: Microbial abundance

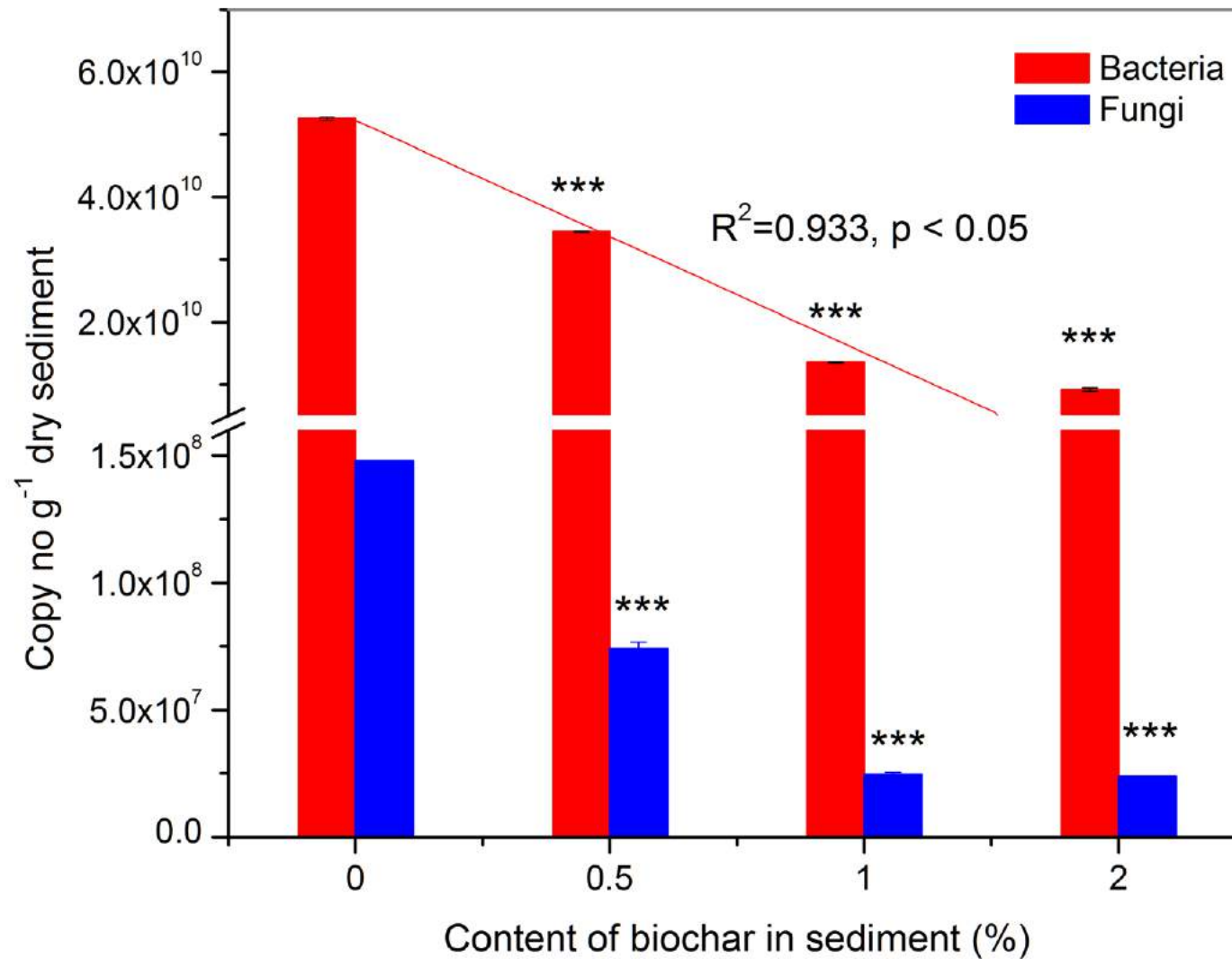
# Dynamics of enzyme activity after biochar addition



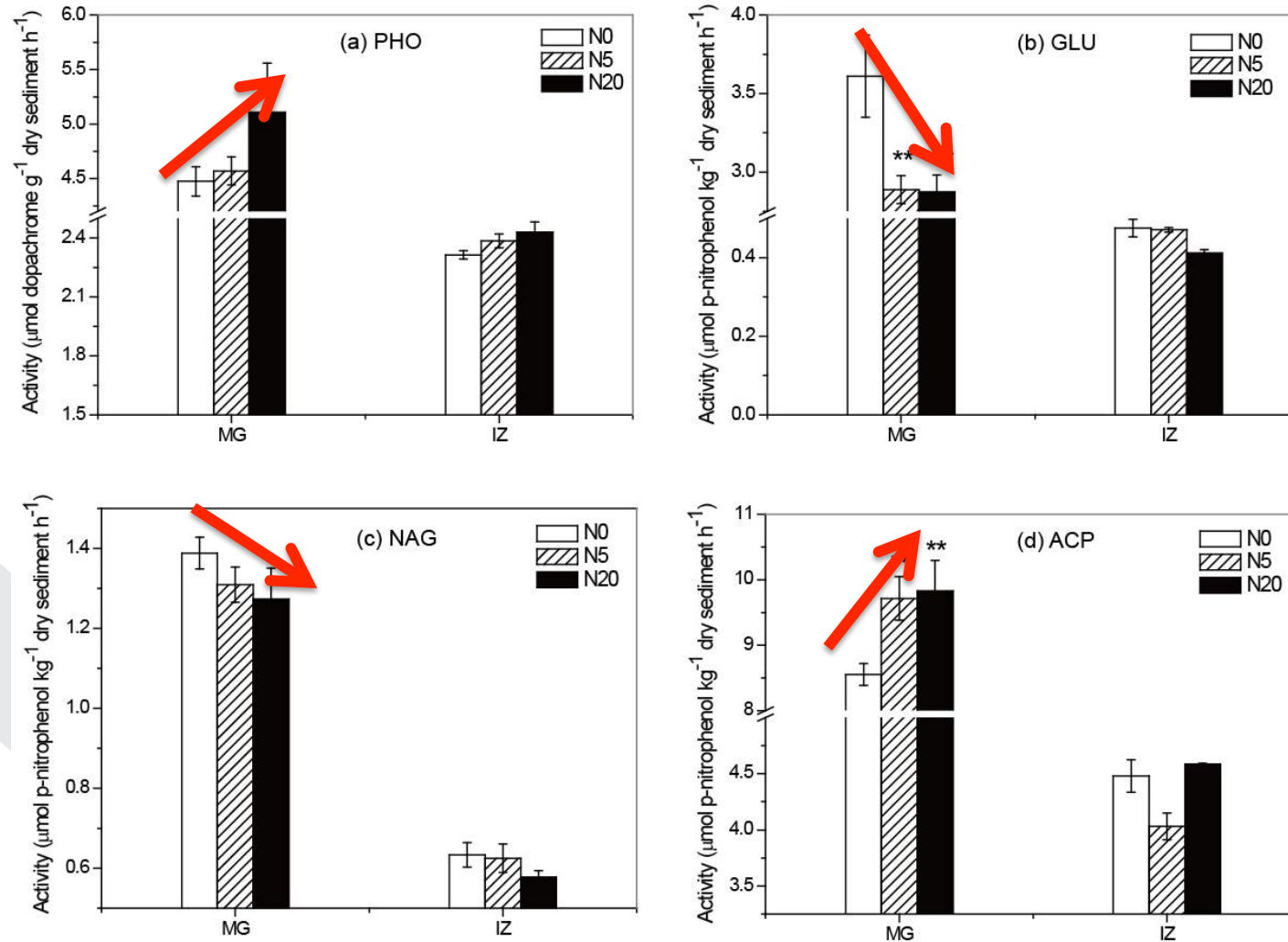
- The activity of **POD** was affected the **most** significantly.
- The other four enzymes were slightly variable relative to the control.



# A decrease of bacterial and fungal abundances after biochar addition

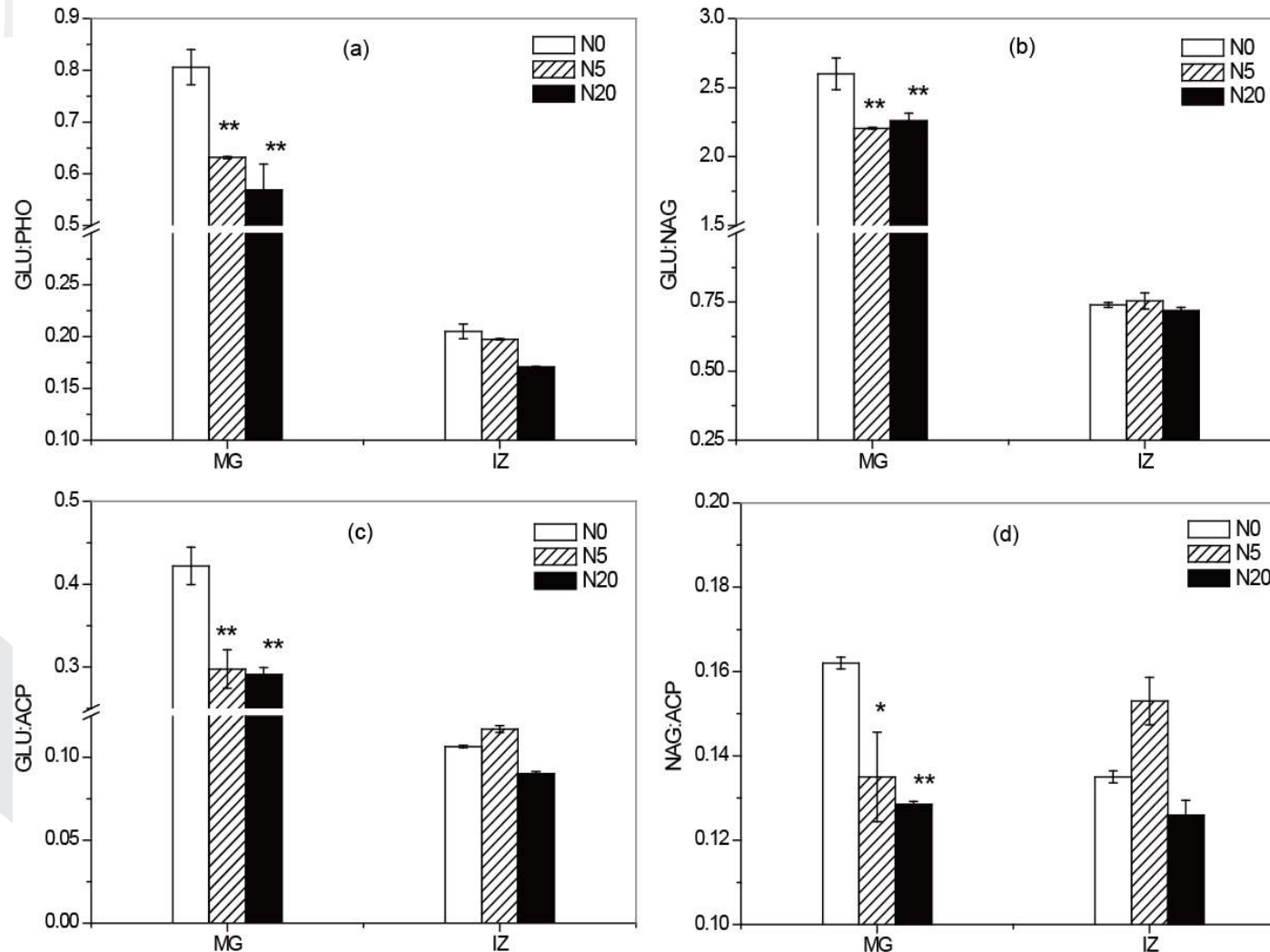


# Dynamics of enzyme activity after N addition



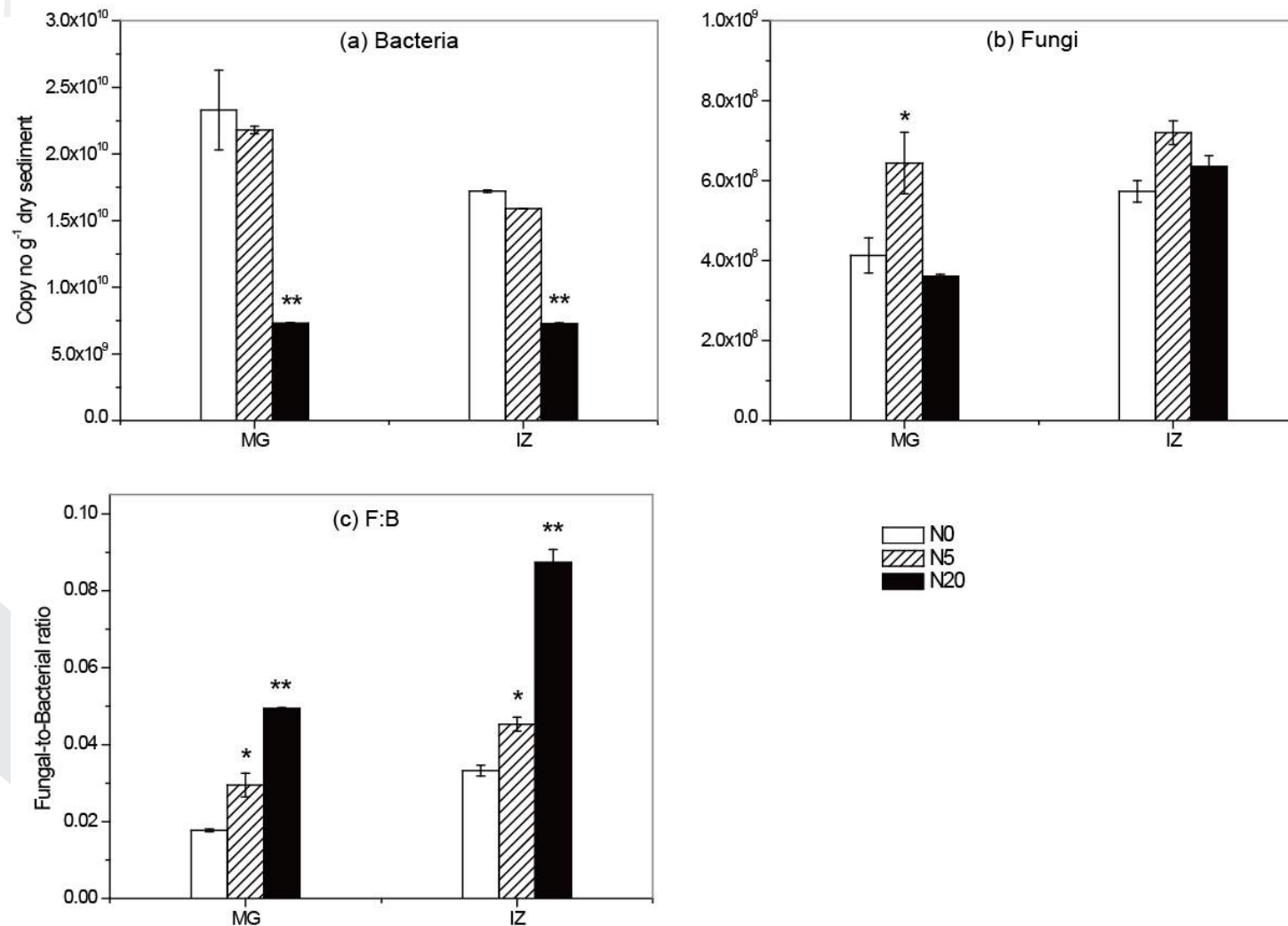
In general, **PHO** and **ACP** activity **increased**, but **NAG** and **GLU** activity **decreased** by N addition.

# Dynamics of enzymatic stoichiometry after N addition



MG sediment was more responsive to N addition, since GLU:PHO, GLU:NAG, GLU:ACP, and NAG:ACP were significantly decreased by N addition.

# Dynamics of microbial abundance after N addition



Bacterial abundance decreased by N addition, but fungal enhanced, especially at low N-addition. Moreover, F:B significantly increased.

# Assessment Methods

## Calculations

● **Equation 1:**  $\Delta C (\%) = [(C_{\text{treatment}} - C_{\text{control}}) / C_{\text{control}}] \times 100$

$\Delta C$ , sediment organic C (SOC);  $C_{\text{treatment}}$ , C content in treated sediment;  $C_{\text{control}}$ , C of biochar added in sediment.

● **Equation 2:**  $\Delta EA (\%) = [(EA_{\text{treatment}} - EA_{\text{control}}) / EA_{\text{control}}] \times 100$

EA: Enzyme activity

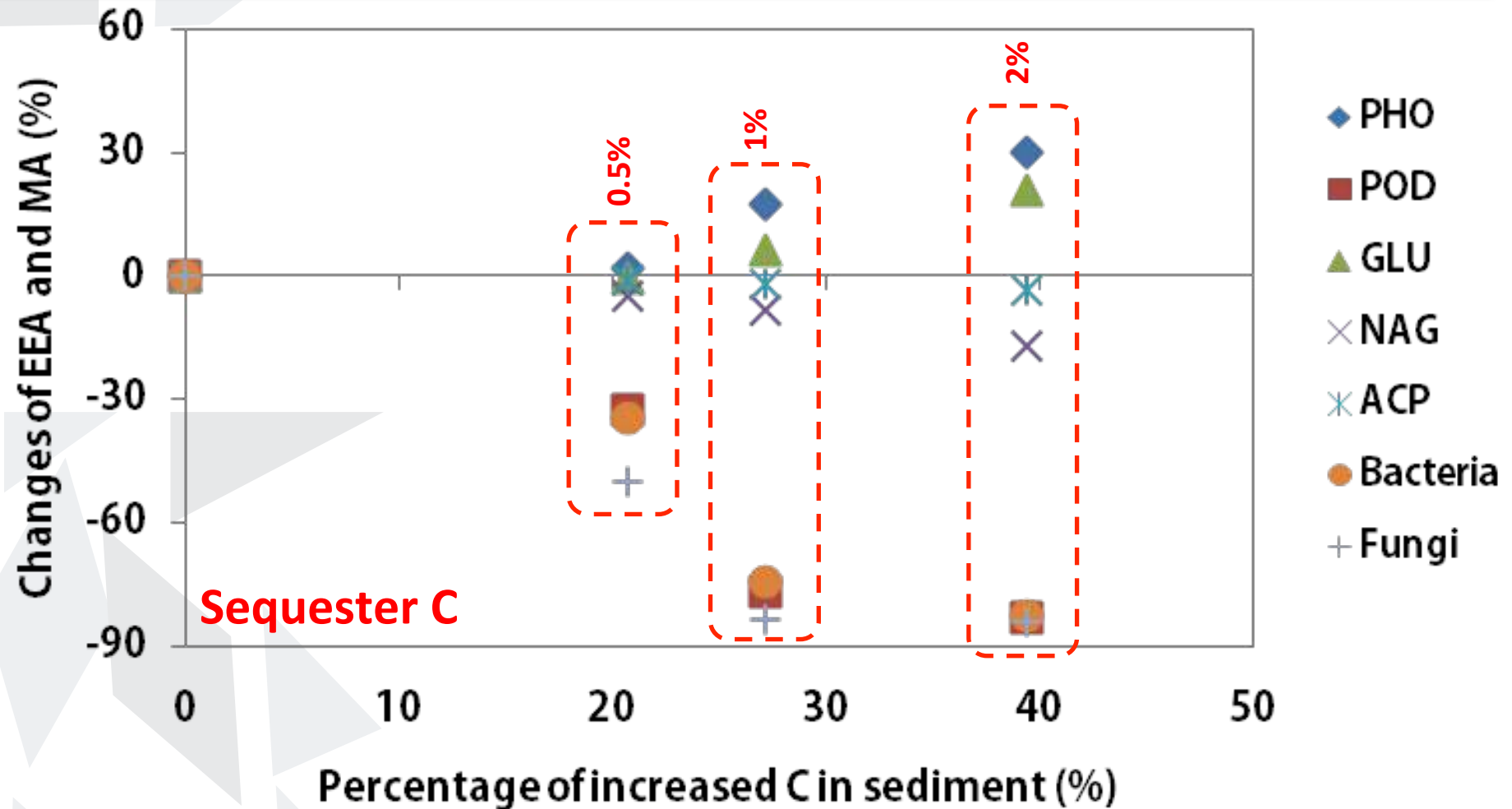
● **Equation 3:**  $\Delta MA (\%) = [(MA_{\text{treatment}} - MA_{\text{control}}) / MA_{\text{control}}] \times 100$

MA: Microbial abundance

## Measured parameters

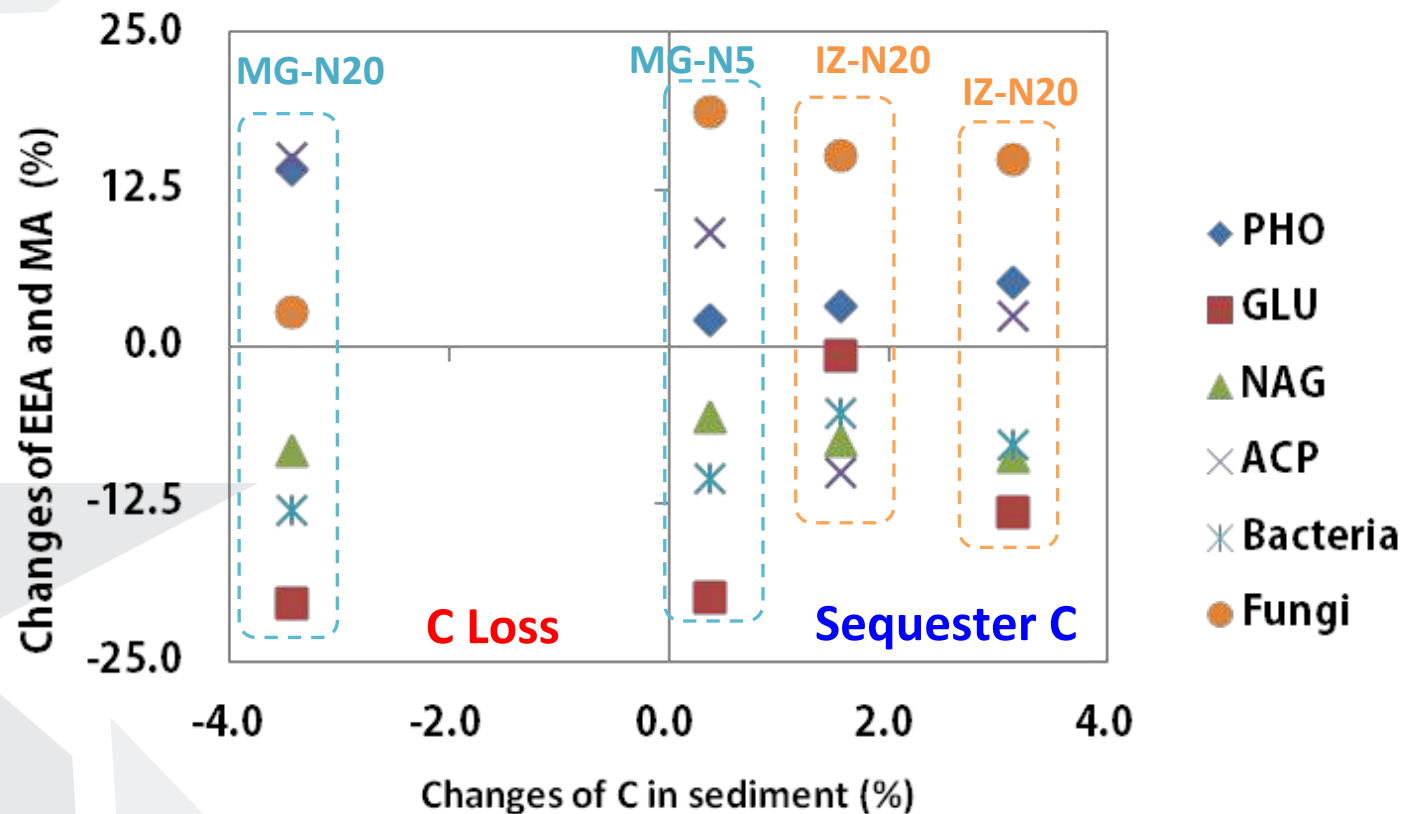
- ✚ Sediment organic C (SOC), and total N and P, and soluble phenolics
- ✚ PHO, peroxidase (POD), GLU, NAG and ACP activity assayed spectrophotometrically
- ✚ Microbial (bacterial and fungal) abundance estimated by qPCR
- ✚ Microbial community by NGS

## The altered C ( $\Delta C$ ) related to changes of EEA and MA



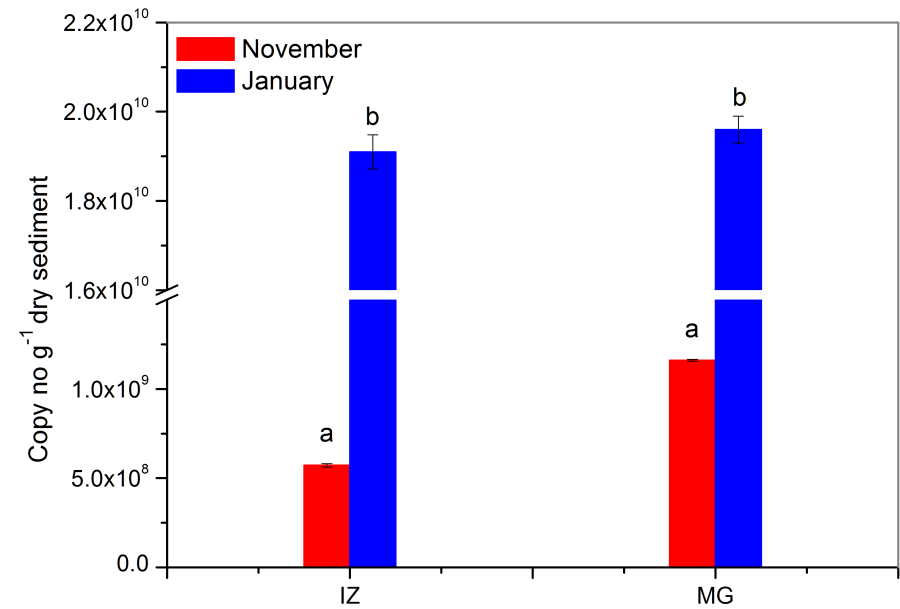
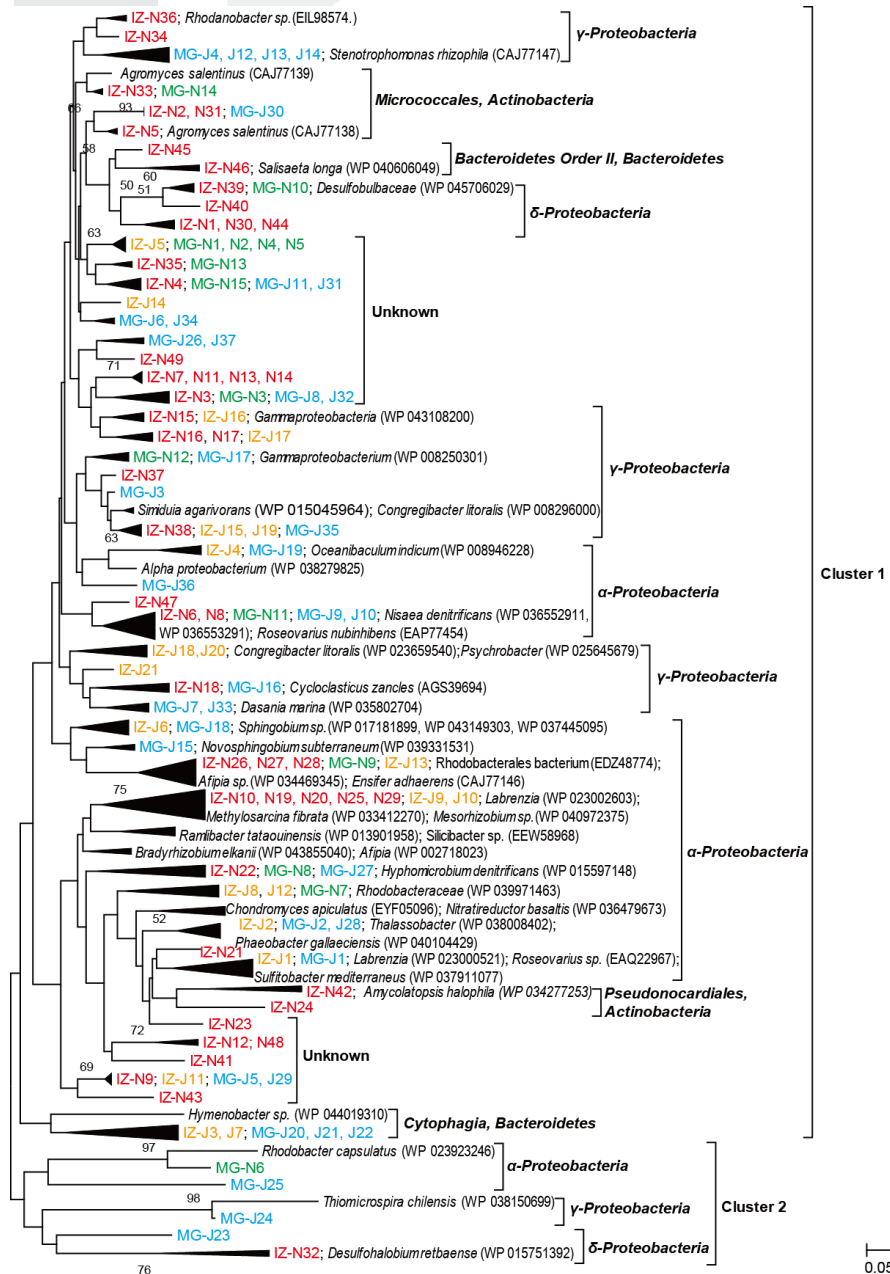
The C sequestration by biochar addition is most likely caused by the remarkable decrease of POD activity together with a decline of bacterial and fungal abundance.

## The altered C ( $\Delta C$ ) related to changes of EEA and MA



- The decreased GLU activities and bacterial abundance after N addition promoted C sequestration in sediment.
- MG-N20 indicated the potential of C loss. The possible reasons are the higher activity of PHO and ACP, and also the improved fungal abundance.

# Seasonal variations in mangrove sediments

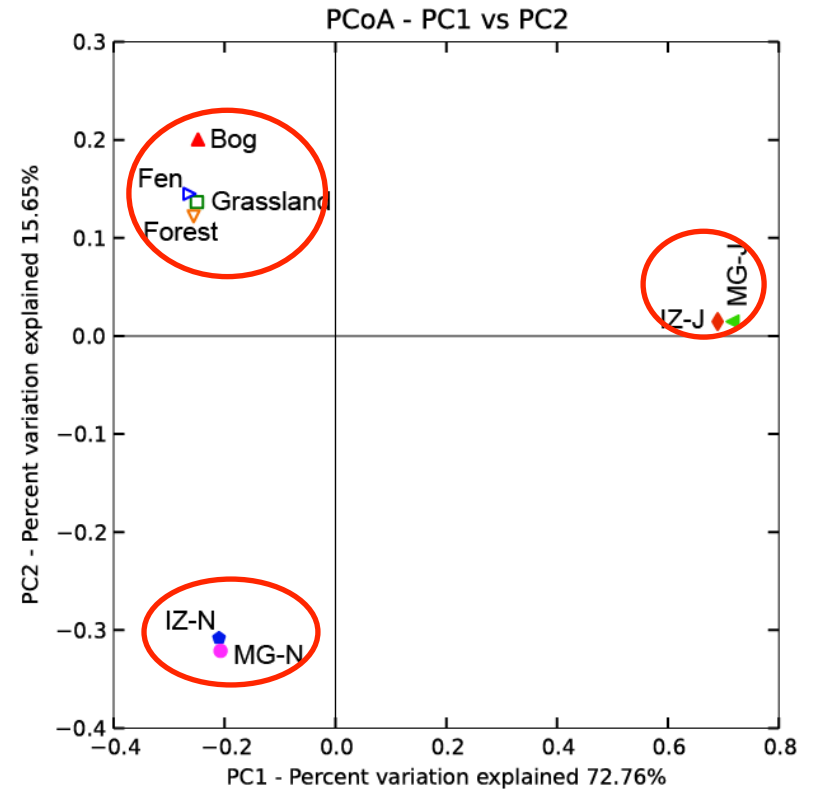
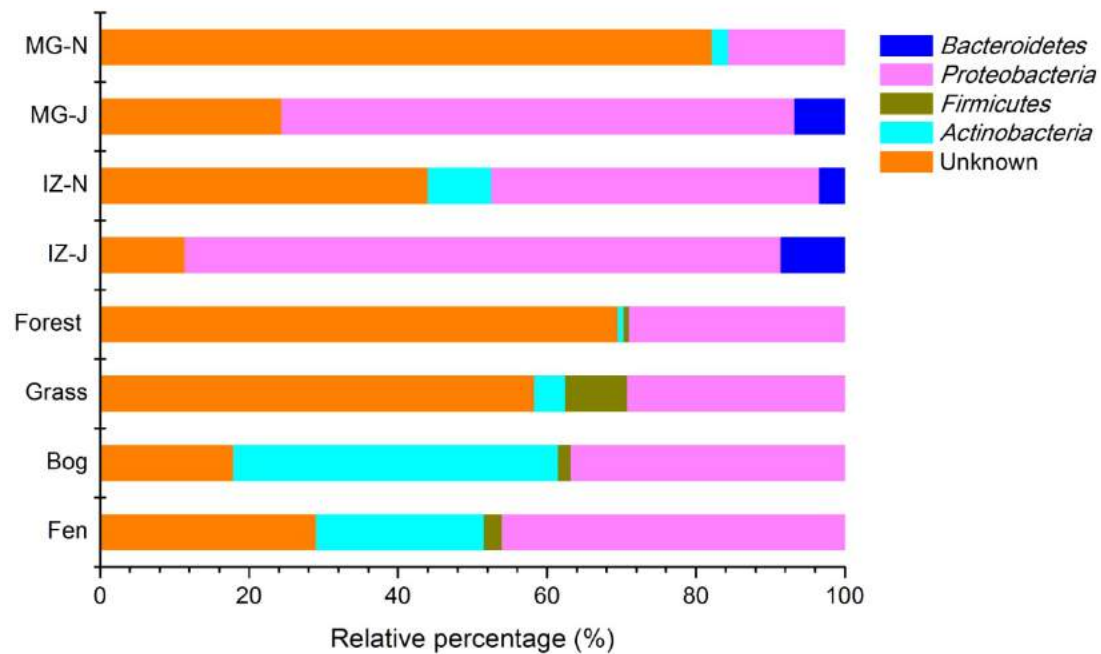


## 4 major groups:

- *Proteobacteria*
  - *Actinobacteria*
  - *Bacteroidetes*
  - *Unknown species*
- α*-, *γ*, and *δ*-*Proteobacteria*,  
*Micrococcales*, and *Pseudonocardiales*,  
*Bacteroidetes* order II, and *Cytophagia*,



# Bacterial laccase-like genes in different ecosystems



- The bacterial laccase-like genes in sediments had strong seasonal variations.
- Among ecosystems, the diversity and distribution of bacterial laccase-like genes varied widely, and this sediment was very different from others reported.

# Bacterial laccase-like genes in particle size fractions

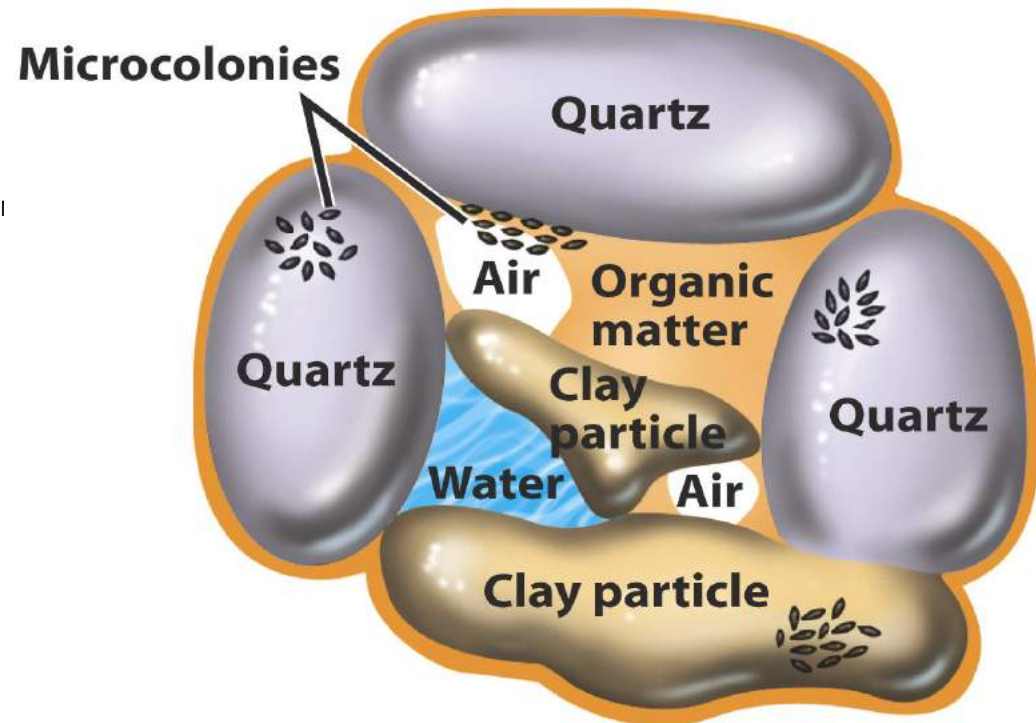
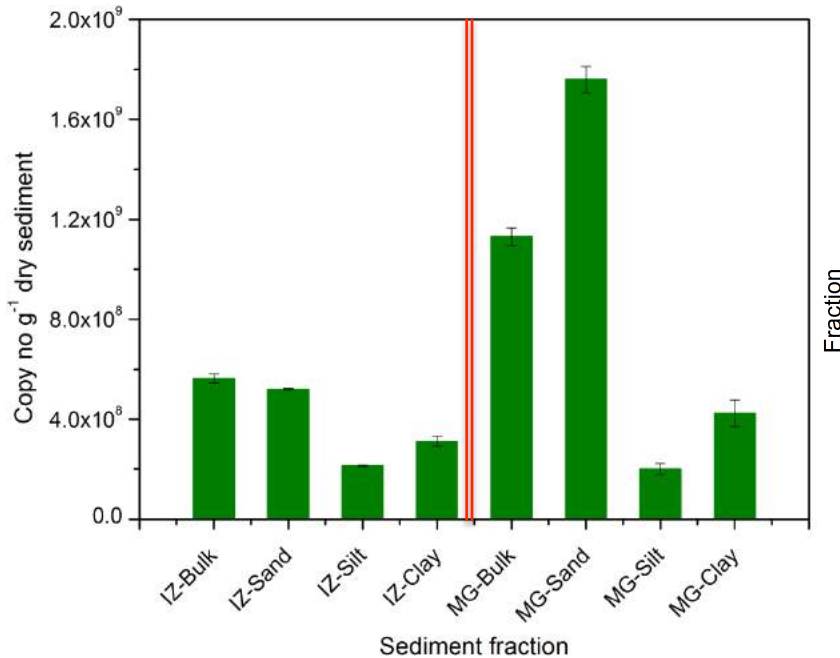


Figure 19-7 Brock Biology of Microorganisms 11/e  
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- The abundance of bacterial laccase-like communities declined: Sand > Silt > Clay, and MG-Sand showed higher abundance than MG-Bulk sediment.
- 9 groups were found in all samples: *Bacteroidetes*, *Caldithrix*, *Cyanobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Firmicutes*, *Proteobacteria*, *Actinobacteria* and Unknown species. And new species were enriched after fractionation.

# Conclusions

- **Biochar decreases peroxidase activity the most, resulting in C sequestration.**
- **Biochar and N addition promote C sequestration due to the decrease of several key enzyme activities and relevant microbial abundance.**
- **Biochar addition reduced both bacterial and fungal abundance, but N addition increased fungal abundance and decrease bacterial abundance**
- **The diversity, distribution and abundance of bacterial laccase-like genes varied between mangrove and mudflat, and, more importantly, high activity is associated with the sand fraction.**



# 米埔自然護理區



## MAI PO NATURE RESERVE



米埔內後海灣拉姆薩爾濕地  
Mai Po Inner Deep Bay Ramsar Site

濕地動植物  
Wetland Plants and Animals

