

# Functional soil microbial diversity across Europe estimated by EEA, MicroResp and BIOLOG

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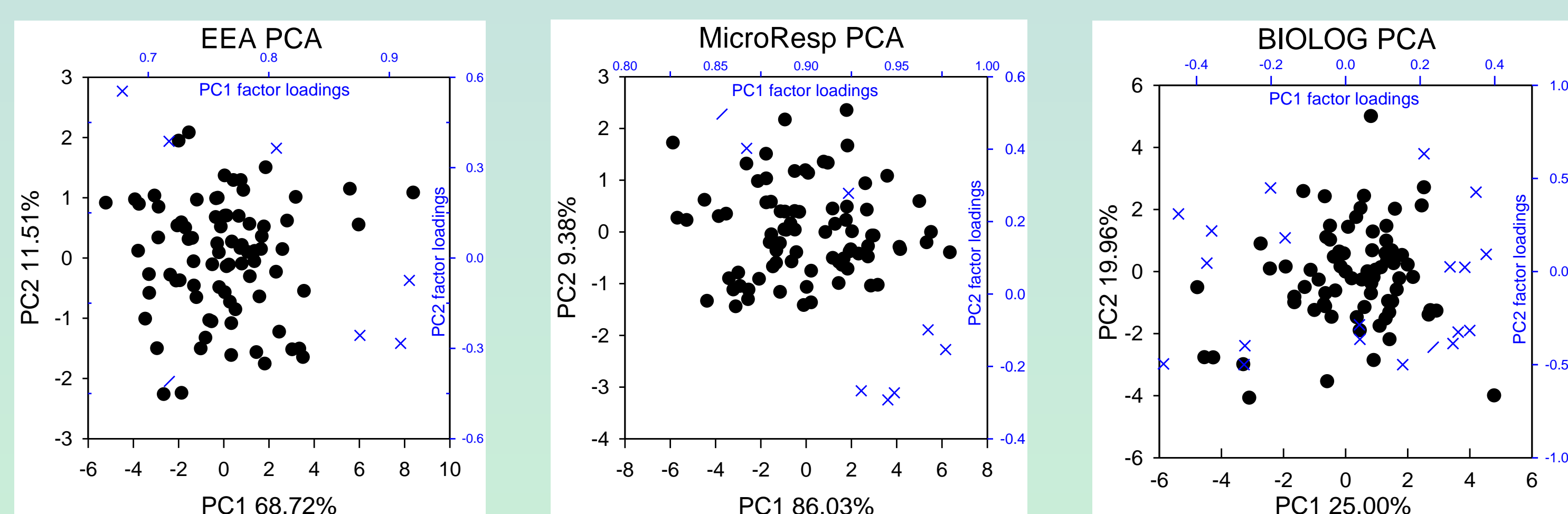
**Objective:** Compare the results of three techniques: Exo-Enzyme Activity (EEA), MicroResp and BIOLOG of functional soil microbial diversity in 81 soils from a European transect.

## Conclusions:

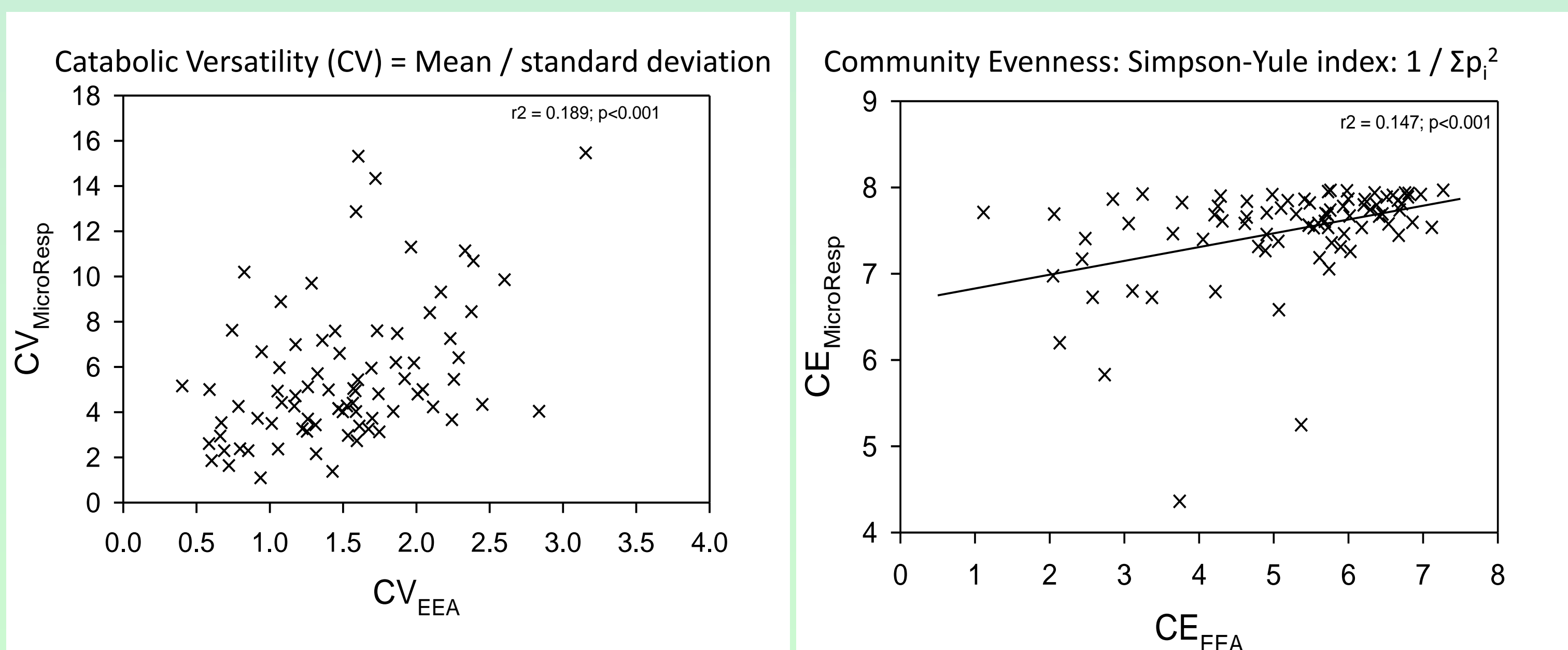
- EEA and MicroResp correlated significantly on total activity measured, Catabolic Versatility, and Community Evenness while BIOLOG did not.
- Principal component 1 and 2 of the three techniques correlated significantly, except BIOLOG vs. EEA.
- BIOLOG data were made inoculum density independent which was not attempted with EEA and MicroResp. Despite this and the high number of variables, PC1 and PC2 of BIOLOG and MicroResp correlated significantly, which is in accordance with [2].
- MicroResp is considered closer to in situ due to sieved soil at 30-60% WHC incubated compared to soil slurries.
- All three techniques deliver fragmented multivariate information on the soil microbial community, but discussion continues about the meaning of the data.
- All three techniques required almost same equipment.
- The protocol of EEA used here, including data treatment, was the fastest.
- The estimated costs were comparable, however, the BIOLOG technique used here required more EcoPlates sample<sup>-1</sup> which increases the costs.

Pearson correlation analysis based on the principal components PC1 and PC2 showed significant correlation (in bold) between all combinations except EEA vs. BIOLOG.

Technique	EEA PC1		MicroResp PC1		EEA PC2		MicroResp PC2	
	p	R <sup>2</sup>	p	R <sup>2</sup>	p	R <sup>2</sup>	p	R <sup>2</sup>
MicroResp	<b>&lt;0.001</b>	<b>0.201</b>	-	-	<b>&lt;0.001</b>	<b>0.179</b>	-	-
BIOLOG	0.108	0.032	<b>0.009</b>	<b>0.084</b>	<b>0.006</b>	<b>0.093</b>	<b>0.002</b>	<b>0.118</b>

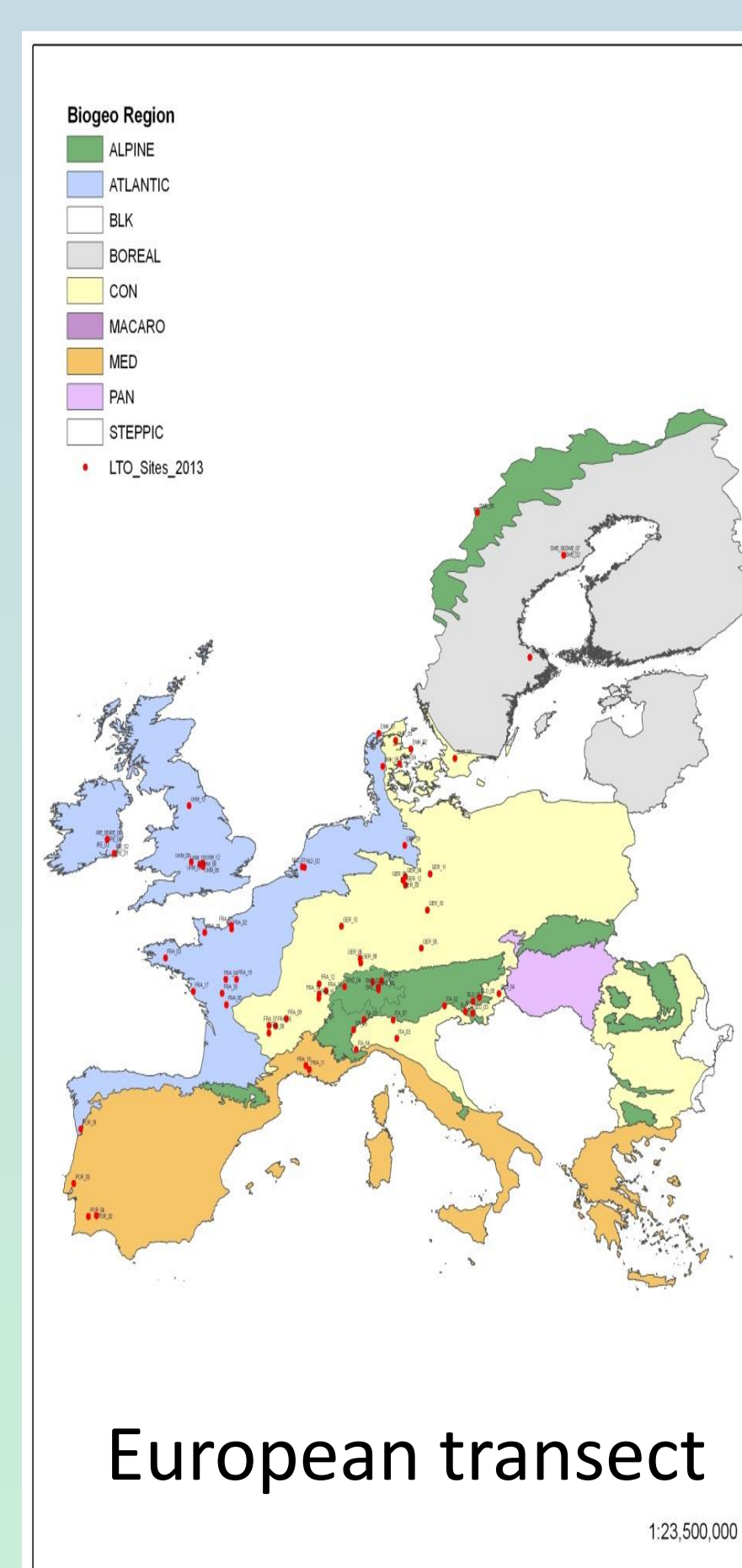


Principal component analysis of each assay separated soils with 45 - 95 % of variance explained by PC1 and PC2.

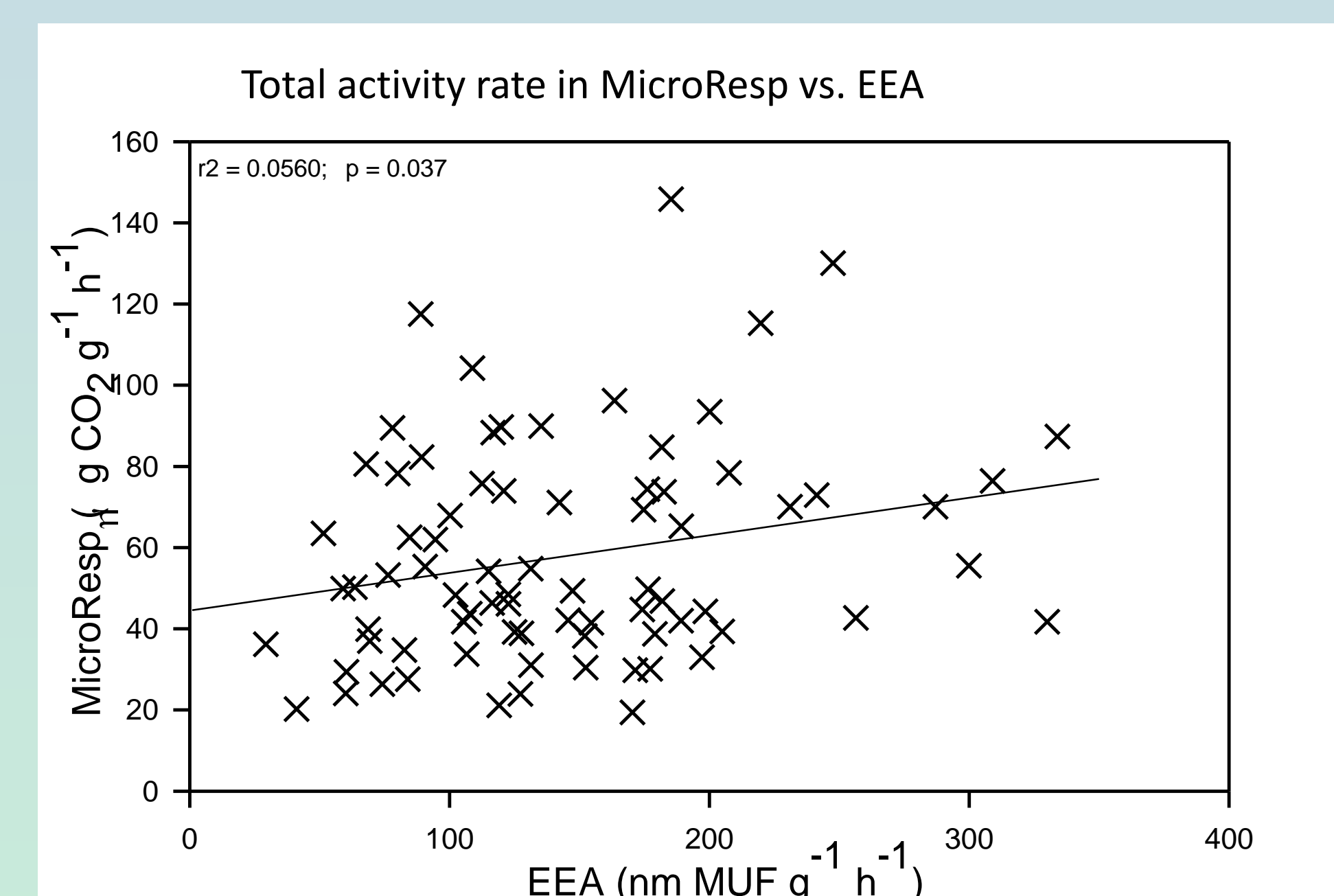


Catabolic diversity and community evenness calculated on log-transformed data, correlated significantly and positively between EEA and MicroResp but neither with BIOLOG. In contrast to [3] who found negative correlation between MicroResp and EEA, however, only 4 out of 8 enzyme-substrates tested in the EEA assays were similar.

MicroResp: C-substrate	EEA: active enzyme
D-(+)-galactose	arylsulfatase
L-malic acid	α-glucosidase
γ amino butyric acid	β-glucosidase
n-acetyl glucosamine	cellobiosidase
D(+) glucose	β-xylosidase
α ketoglutarate	chitinase
citric acid	phosphomonoesterase
	leucin aminopeptidase



81 sites representing a pedo-climatic transect across Europe



The positive correlation might be due to abundance and activity of organisms along with physical and chemical properties of the soils. BIOLOG data were made inoculum density independent according to the Netherlands Soil Monitoring Network (4) and showed no correlation with MicroResp nor EEA.

## Comparison of EEA, MicroResp and BIOLOG

Parameter	EEA	MicroResp	BIOLOG
Variables #	8	7	31
Main Target	Exo-enzymes	Bacteria	Bacteria
Growth dependent	No	No	Yes
Measure	Enzymatic cleavage	CO <sub>2</sub>	Redox change
Soil structure intact	No	Yes	No
pH controlled	Yes	No	Yes
Incubation time	2 h	4-6 h	5 d

## Techniques of microbial functional diversity

**Exo-Enzyme Activity (EEA)** with MUF or AMC labelled substrates measures exoenzymatic hydrolytic activity towards C-substrates. Exo-enzymes can be cell-associated or free and are mainly of bacterial origin. [5]

**MicroResp** measures microbial (mainly bacterial) respiration (CO<sub>2</sub>) in intact soil introduced to C-substrates. Soil is at 30-60% WHC. [1].

**BIOLOG:** EcoPlates with 31 C-substrates. Measures respiration (CO<sub>2</sub>) based on bacterial growth during 1-7 days in microtiterplates with C-substrate. Data calculated to obtain inoculum density independence [4].