



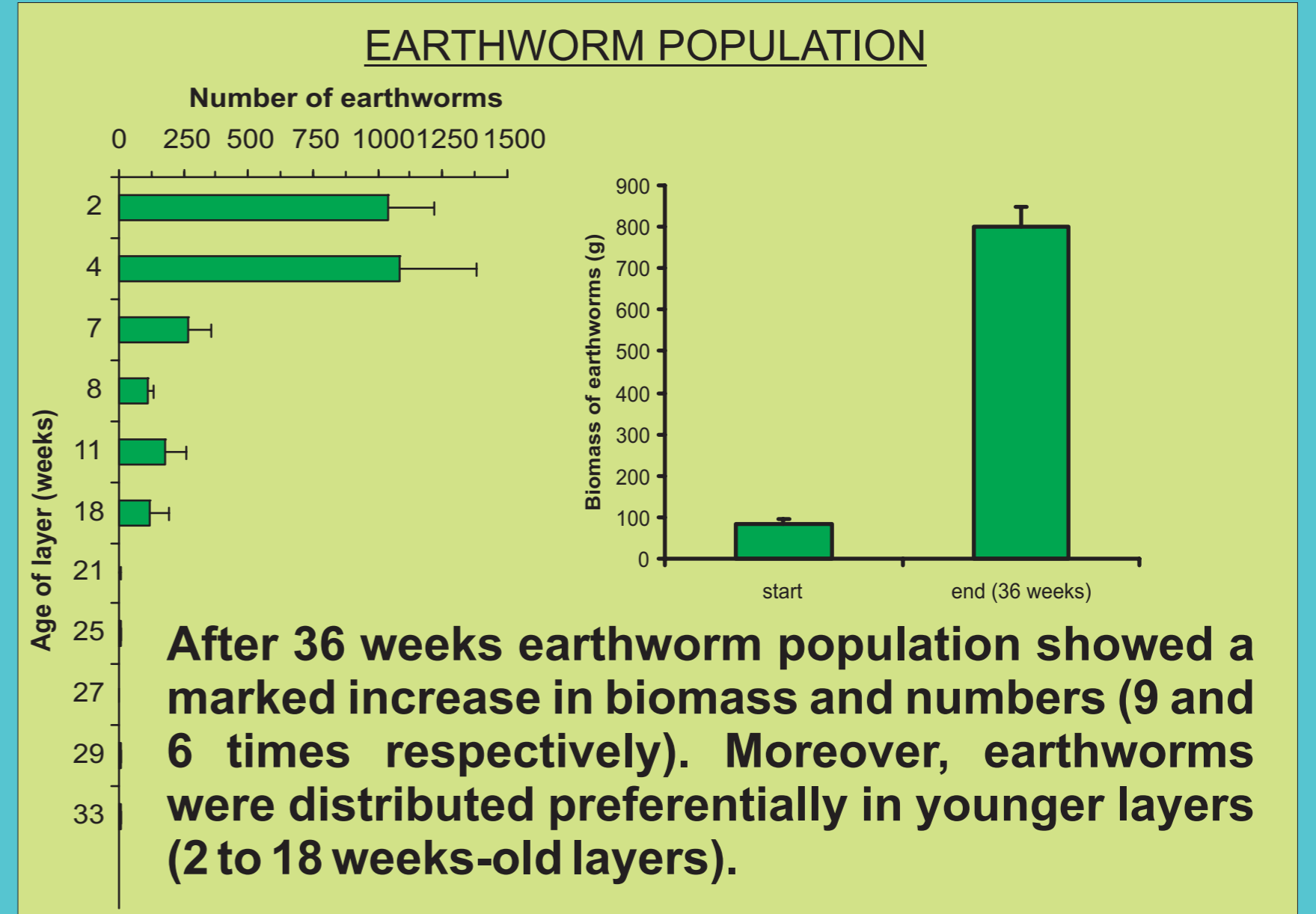
# Earthworms trigger enzymatic activities through the increase of microbial biomass and activity during vermicomposting of pig slurry

Manuel Aira, Cristina Lazcano and Jorge Domínguez

Departamento de Ecoloxía e Bioloxía Animal. Universidade de Vigo. Vigo. E-36310. Spain.  
E-mail: aira@uvigo.es

## RATIONALE

Enzyme activities have been used widely as an index of soil fertility or ecosystem status because they are involved in the biological transformations of native and foreign compounds in soils. Several enzymatic activities have been measured to describe organic matter decomposition in two microbial-driven processes, composting and vermicomposting. Thus, it is necessary to determine the relationships between microbial populations and enzymatic activity during organic matter decomposition; further, it is also important to quantify the amount of extracellular enzyme activity. Such knowledge would lead to better understanding of how earthworms and microorganisms interact during the decomposition of the organic matter.



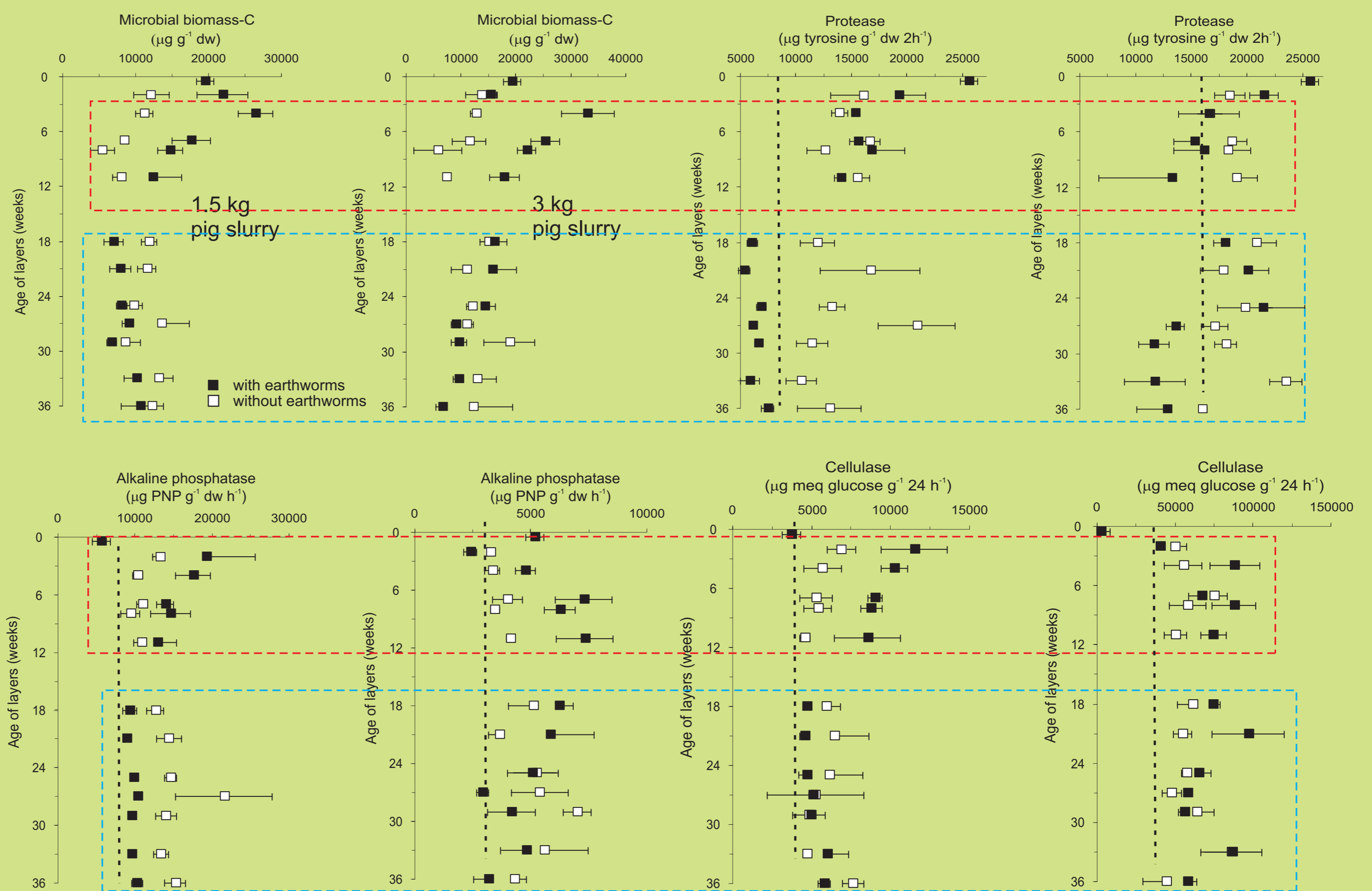
## Material and methods

### Experimental design

We designed continuous feeding vermireactors with separated layers to date them. We set up six reactors with earthworm (500 mature initial population, ca. 85 g) and six reactors without (control). New layers with fresh pig slurry (1.5 and 3 kg fresh weight, six reactors per rate of pig slurry application) were added when the last ones were eaten by the earthworms and the experiment ended after 36 weeks. At the end there were nine layers with an age gradient of 0, 4, 8, 13, 21, 25, 27, 33 and 36 weeks from upper to bottom layers. Five samples of substrate per module were taken at random and gently mixed for biochemical analyses, i.e. microbial biomass C (Cmic),  $\beta$ -glucosidase, cellulase, alkaline phosphatase and protease activities. Data were analyzed under a split plot repeated measures ANOVA design.

	Cmic	Extracellular enzyme activity
$\beta$ -glucosidase	0.45	
1.5 kg	0.66	760 $\mu\text{g PNP g}^{-1} \text{ dw h}^{-1}$
3 kg	0.49	1080 $\mu\text{g PNP g}^{-1} \text{ dw h}^{-1}$
Cellulase	0.35	
1.5 kg	0.60	3250 $\mu\text{g meq glucose g}^{-1} \text{ dw 24 h}^{-1}$
3 kg	0.48	44900 $\mu\text{g meq glucose g}^{-1} \text{ dw 24 h}^{-1}$
Alkaline phosphatase	0.15	
1.5 kg	0.53	8070 $\mu\text{g PNP g}^{-1} \text{ dw h}^{-1}$
3 kg	0.50	2950 $\mu\text{g PNP g}^{-1} \text{ dw h}^{-1}$
Protease	0.33	
1.5 kg	0.45	7710 $\mu\text{g tyrosine g}^{-1} \text{ dw 2 h}^{-1}$
3 kg	0.14	16160 $\mu\text{g tyrosine g}^{-1} \text{ dw 2 h}^{-1}$

Table 1. Correlations between microbial biomass-C (Cmic) and the four enzyme activities analyzed. The first value corresponds to correlation of both rates of pig slurry (1.5 and 3 kg) together, the other two values correspond with the correlation of Cmic and the enzymes in each rate of pig slurry separately. Values marked in red are statistically significant ( $p < 0.0001$ ). We also give the values of extracellular enzyme activity per each rate of pig slurry. To quantify the amount of extracellular enzyme activities we applied the approach of McLaren and Pukite (1973) as in Nannipieri et al. (1996). It consists in plotting the enzyme activity against the microbial biomass and, if the correlation between them is significant, the extrapolation to zero biomass will give the extracellular enzyme activity (Nannipieri et al., 1996; Dilly and Nannipieri, 2001). McLaren AD, Pukite A. Ubiquity of some soil enzymes and isolation of soil organic matter with urease activity. In: Povoledo D, Golterman ML, editors. Humic substances and function in the biosphere. Wageningen, The Netherlands: Pudoc; 1973. pp 187-193. Nannipieri P, Sastre I, Landi L, Lobo MC, Pietramellara G. Determination of extracellular neutral phosphomonoesterase activity in soil. Soil Biol Biochem 1996;28: 107-112. Dilly O, Nannipieri P. Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biol Fertil Soils 2001;34: 64-72.



## CONCLUSIONS

In both rates of pig slurry applied, earthworms stimulated microbial growth which decreased once earthworms left the slurry. This increase was related to the initial activation of the microbial enzymes studied:  $\beta$ -glucosidase, cellulase, alkaline phosphatase and protease as correlations between microbial biomass and enzymes showed (Table 1, and red rectangle in figures). These strong relationships indicated an increase of intracellular enzyme activity. In the aged slurry, the pattern of activity of the four enzymes assayed depended on the rate of pig slurry applied. Thus, in low rate reactors, enzymatic activity through layers appeared to be related to microbial biomass, but in high rate reactors the activity of enzymes was more or less continuous (blue rectangle in figures). Further, these differences in overall enzyme activity agree with the variation found in extracellular enzyme activity (Table 1, and dashed line in figures) suggesting certain dependence on substrate availability. We found that extracellular enzyme activity increased with rate of pig slurry, indicating a baseline of enzyme activity through decomposition. Thus, rate of pig slurry should be chosen carefully if vermicompost is going to be used as organic amendment, since earthworm activity did not decrease it.

