



Earthworms accelerate cellulose decomposition through the enhancement of fungal biomass during vermicomposting

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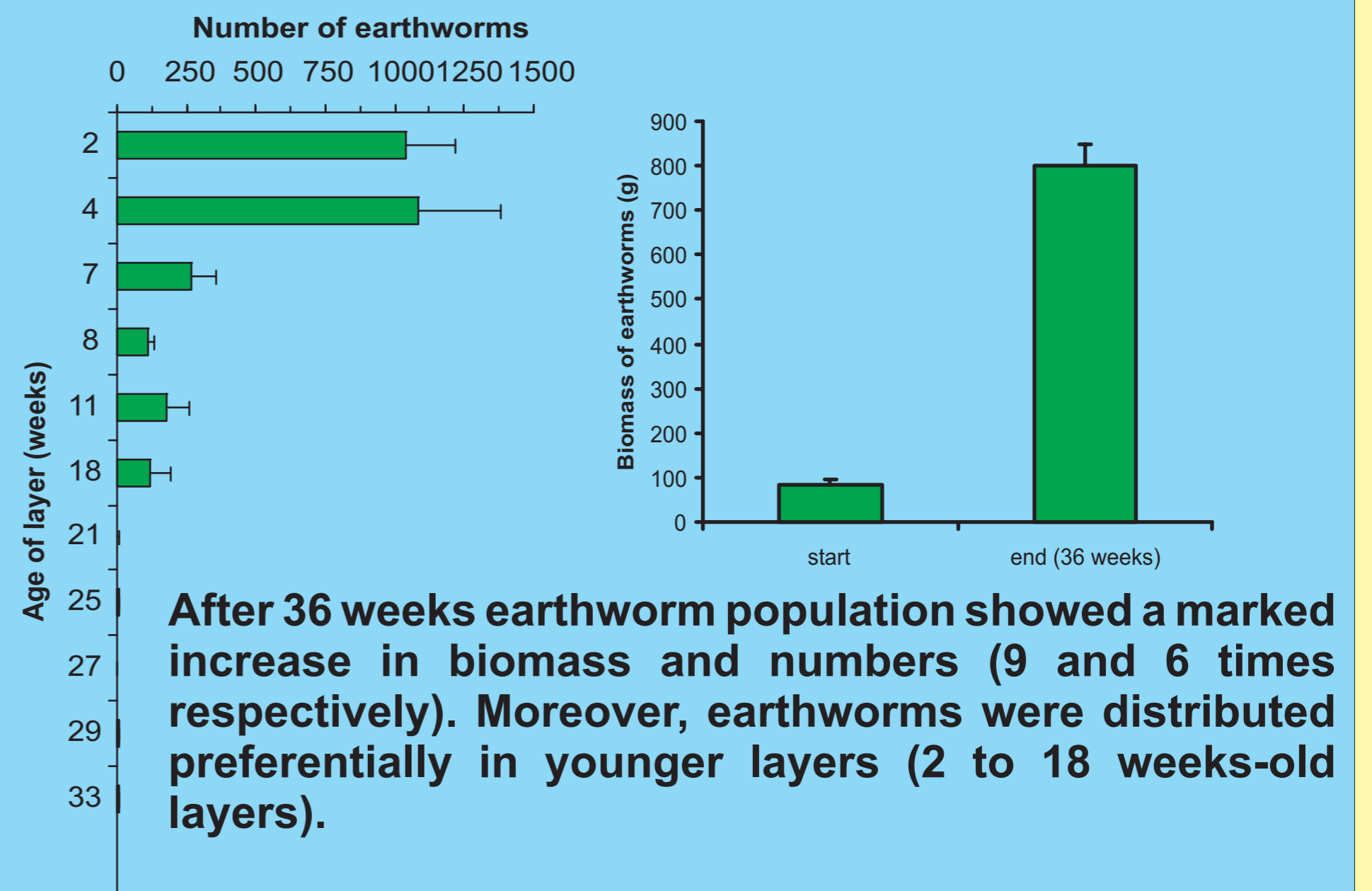
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RATIONALE

Cellulose is the most abundant polymer in nature and constitutes a large pool of carbon for microorganisms, the main agents responsible for soil organic matter decomposition. Cellulolysis occurs as the result of the combined action of fungi and bacteria with different nutrient requirements. Earthworms influence decomposition indirectly by affecting microbial population structure and dynamics, and also directly because the guts of some species possess cellulolytic activity. Here we assess whether the earthworm *Eisenia fetida* (Savigny, 1826) digest cellulose directly (i.e. with its associated gut microflora), and also whether the effects of *E. fetida* on microbial biomass and activity lead to a change in the equilibrium between fungi and bacteria. By enhancing fungal communities *E. fetida* would presumably trigger more efficient cellulose decomposition. To evaluate the role of *E. fetida* in cellulose decomposition we carried out an experiment in which pig slurry, a microbial-rich substrate, was treated in small-scale vermireactors with and without earthworms.

EARTHWORM POPULATION



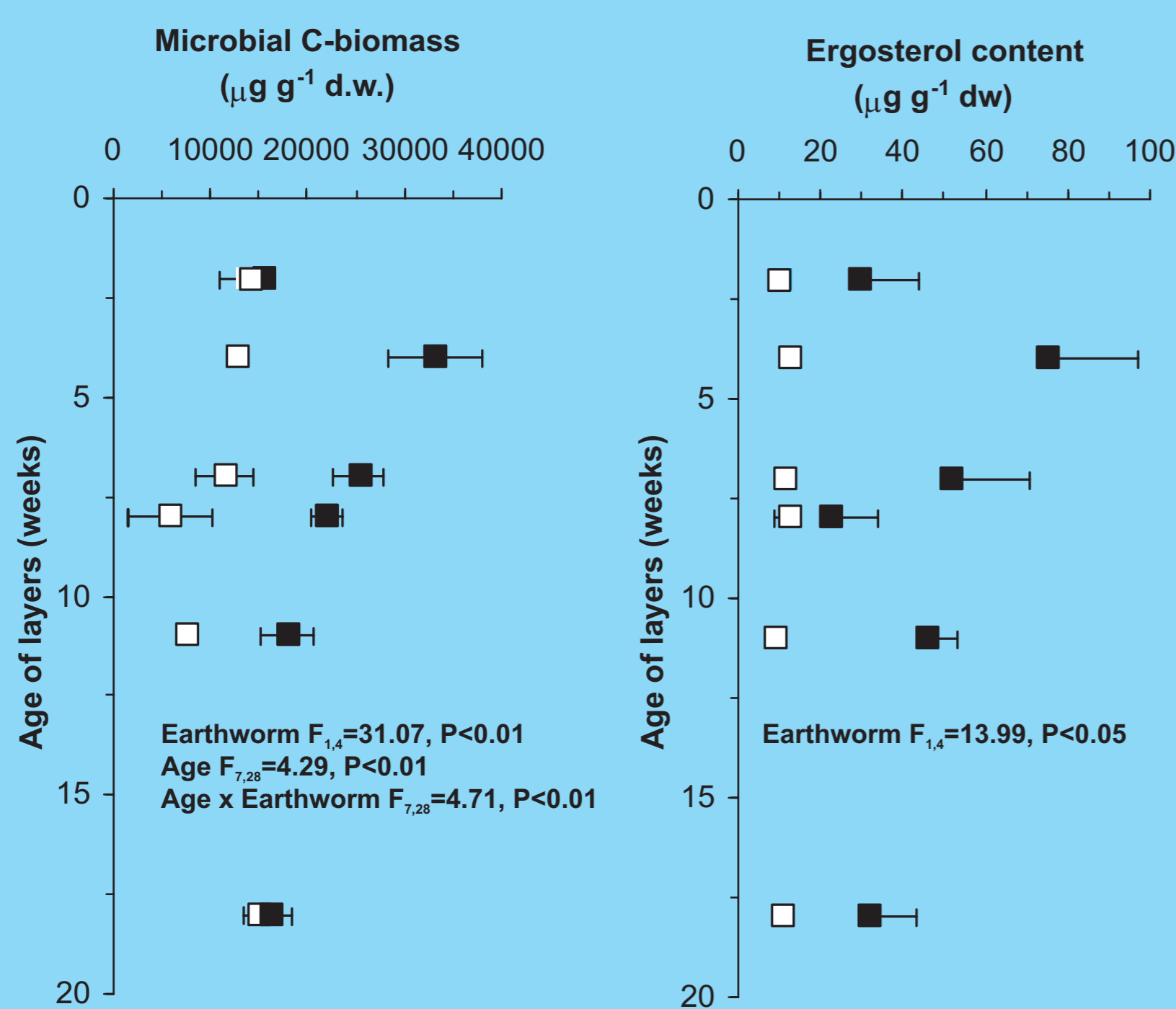
Experimental design

We designed continuous feeding vermireactors with separated layers to date them. We set up three reactors with earthworm (500 mature initial population, ca. 85 g) and three reactors without (control). New layers with fresh pig slurry (3 kg fresh weight) 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. We only sampled layers in which there was earthworm presence (see Earthworm populations).

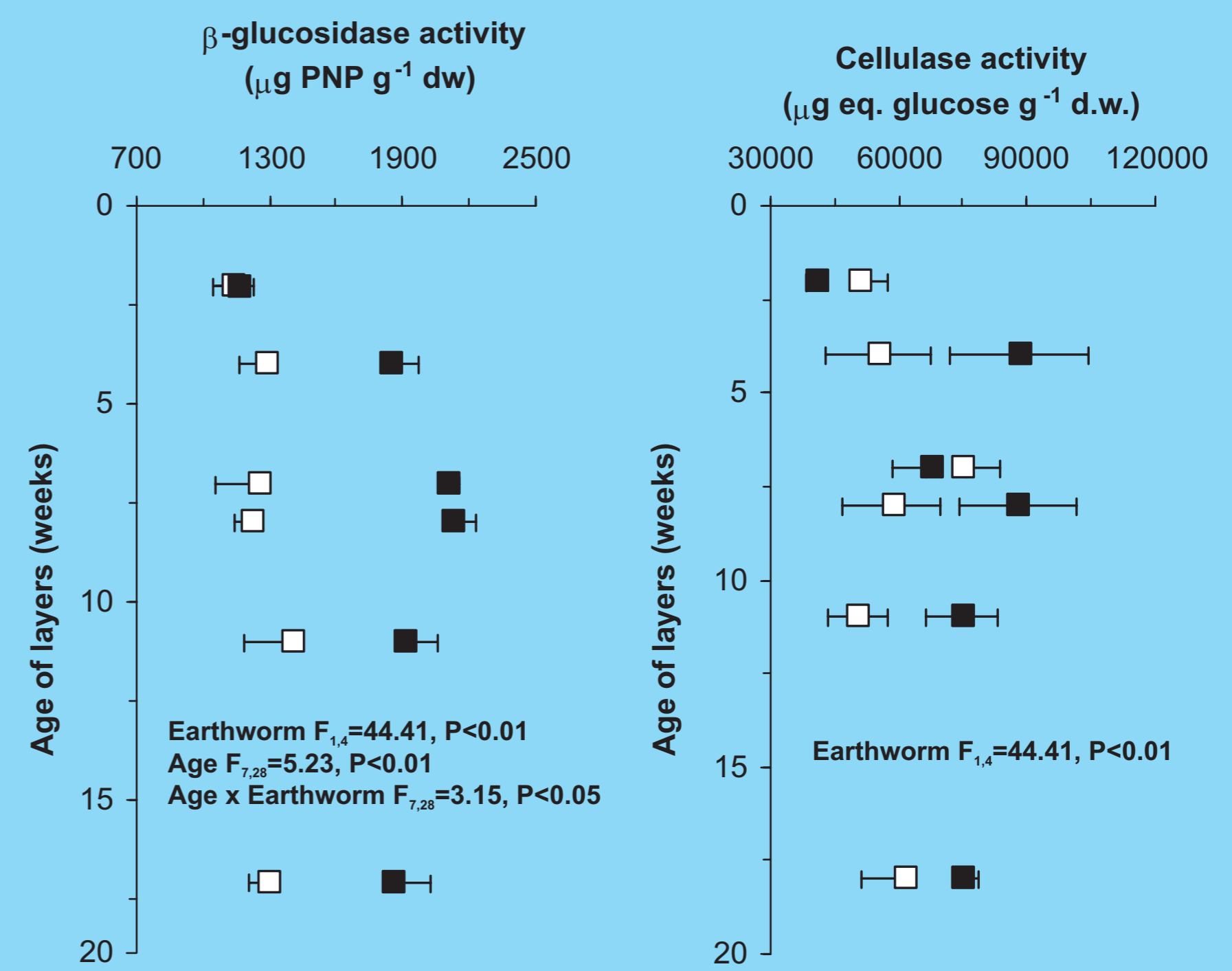
Cellulose and hemicellulose contents in pig slurry were determined by detergent fibre methods. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) values were determined using the FibreBag System®. Microbial biomass C (C_{mic}) was determined by the chloroform fumigation-extraction method in 0.5 M K_2SO_4 extracts. The ergosterol content of pig slurry was extracted by microwave-assisted extraction (MAE) and determined by HPLC analysis. β -glucosidase activity was assessed by determination of the released p-nitrophenol after incubation of samples with p-nitrophenyl glucoside (0.025 M) for 1 hour at 37 °C in a Bio-Rad Microplate Reader at 400 nm. Cellulase activity was estimated by determination of released reducing sugars after incubation of samples with carboxymethylcellulose (CMC) sodium salt (0.7%) for 24 hours at 50 °C in a Bio-Rad Microplate Reader at 690 nm. Data were analyzed under a split plot repeated measures ANOVA design.

Material and methods

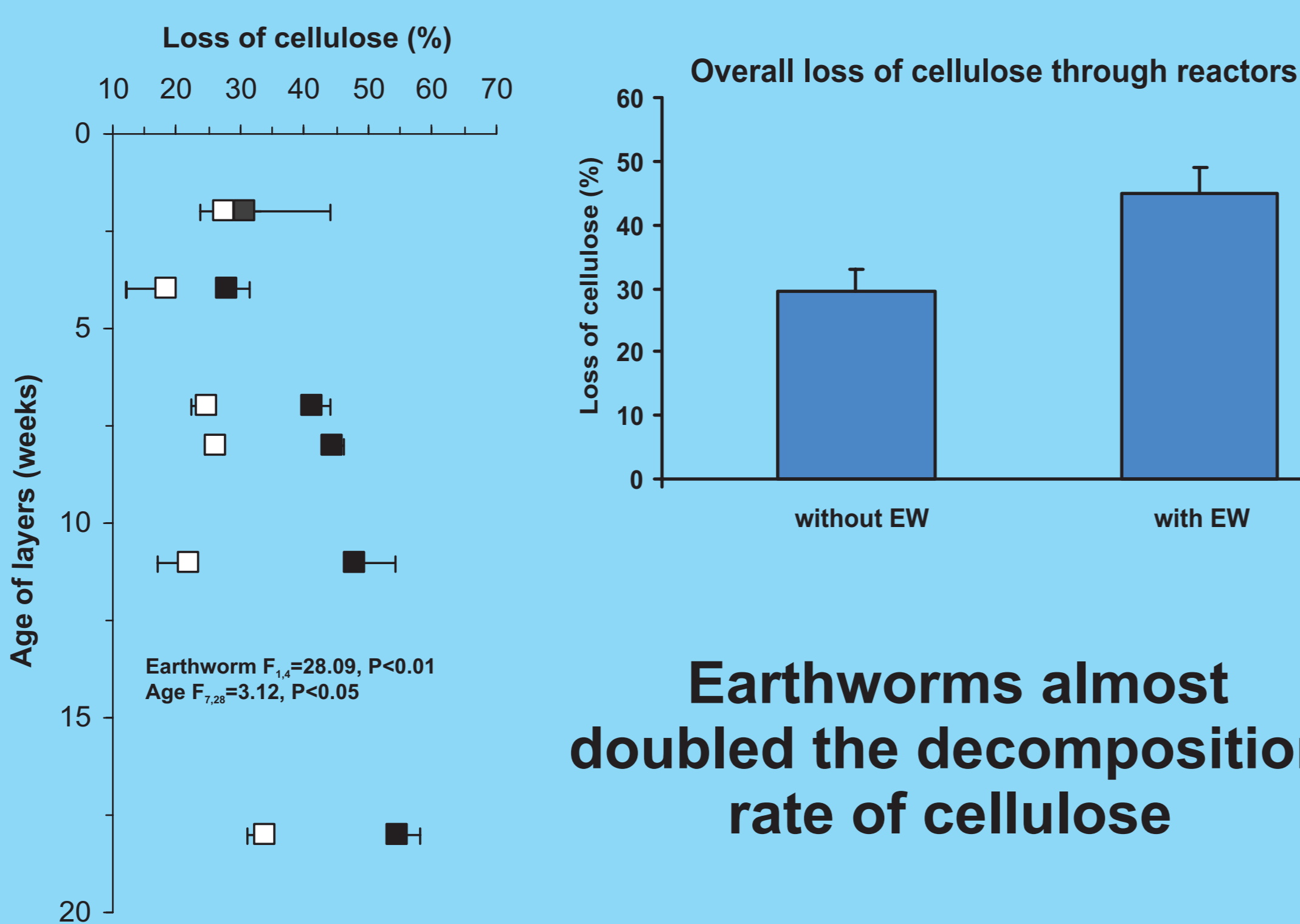
MICROBIAL POPULATIONS



MICROBIAL ACTIVITY



CELLULOLYSIS



CONCLUSIONS

The presence of earthworms significantly increased the rate of cellulose decomposition (0.43% and 0.26% cellulose loss day⁻¹, with and without earthworms respectively). However the direct contribution of *E. fetida* to degradation of cellulose was not significant, although its presence increased microbial biomass and enzyme activity (β -glucosidase and cellulase). Surprisingly, as fungi may be part of the diet of earthworms, the activity of *E. fetida* triggered fungal growth during vermicomposting. We suggest that this activation is a key step leading to more intense and efficient cellulolysis during vermicomposting of organic wastes.