Assessing impacts of copper on soil enzyme activities in regard to their natural spatiotemporal variation under long-term different land uses

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Abstract

Enzymes are known to be sensitive indicators of soil quality. Nevertheless, for a relevant interpretation of activity levels, especially in contaminated soils, the natural spatiotemporal variability of enzyme activities needs to be considered. We first monitored the variability of enzymatic activities (acid and alkaline phosphatases, β-glucosidase, N-acetyl-β-glucosaminidase, urease and dehydrogenase) in two agricultural sites in north-western France during a seasonal April – October cycle. For both sites, two types of long-term different agricultural managements, grassland and intensive cropping were considered. Our results revealed great variability of enzymatic activities in space and over time, yet more pronounced for grassland than for cropped soils. Then, we assessed the impact of copper on enzymes activity on Terrestrial Model Ecosystems (TMEs) filled with undisturbed soil, and incubated for 70 days in open-field conditions. Copper was added at two concentration levels corresponding to a regulated annual agronomic input (2 mg kg^{-1}) or to a high soil contamination (200 mg kg^{-1}). In comparison to effects of natural spatial variability

of soil conditions, copper addition did not show significant impacts on enzymatic activities. Finally, our results confirmed that for assessing effective impacts of contaminants in soils under real field conditions, natural spatiotemporal soil variability must be considered.

Keywords: Grassland; Intensive crop; Terrestrial Model Ecosystems; Soil quality; Enzyme; Bioindicators

1. Introduction

Crop soils are anthropogenic ecosystems, often intensively exploited to meet human needs. Hence, threatened by the agricultural practices. The use of various organic amendments, the spreading of sewage sludge, as well as application of fertilizers and pesticides contribute to soil contamination by organic or inorganic chemicals. Among contaminants introduced into soil, heavy metals, because of their non-degradability, may have serious consequences with respect to soil biological activity (Giller et al., 1998; Mikanova, 2006). Copper has been widely used in agricultural practices against fungal or bacterial pests. Furthermore, land application of organic waste is also a potential source of metal contamination (Kunito et al., 2001; Madejon et al., 2001). Although the regulated amount of Cu corresponds to 2 mg kg^{-1} of dry soil for the superficial soil layer (0-10 cm) per year, Cu concentrations may sometimes reach levels exceeding European guidelines of 100 mg $kg^{-1}$for agricultural soils (Flores-Vélez et al., 1996). Metal contamination may affect the structure of microbial communities and their functions in terrestrial ecosystems (Laguerre et al., 2006; Ranjard et al., 2006; Wang et al., 2007). For assessment of environmental risk related to metal contamination, the study of relevant bioindicators of soil quality is essential.

During the last decades, several biological endpoints have been proposed to assess soil quality (Kandeler, 2007). Among these endpoints, enzymes essentially produced by soil microorganisms were mentioned as key-actors of soil behaviour Being involved in reallocation of organic matter, biogeochemical cycles and soil structure (Olander and Vitoussek, 2000; Nannipieri et al., 2003). Since soil enzymatic activities reflect the structure and functions of microbial communities, they are considered as integrative bioindicators. Hence, soil enzymatic activities are often used for monitoring impacts of soil management, agricultural practices or contaminations on soil health (Kandeler et al., 1996; Deng and Tabatabaï, 1997; Gianfreda et al., 2005; Mamy et al., 2011).

It is generally admitted that high activity levels of enzymes indicate a good soil quality whereas low levels may relate to toxic effects of contaminants on biological processes (Kandeler et

al., 1996; Mikanova, 2006). In literature, metal impacts on enzymatic activities are currently assessed on disturbed soil samples (i.e., sieved and homogenised), then incubated under controlled conditions in the laboratory (Ekenler and Tabatabaï, 2002; Gianfreda et al., 2005; Demanou et al., 2006). Therefore, results of such experimental approaches are often difficult to interpret in terms of open-field situations. Furthermore, the activity levels of enzymes also depend on different environmental factors such as climate, soil structure, physicochemical characteristics or nutrient availability for microorganisms (Olander and Vitoussek, 2000; Kandeler, 2007). Also, since soils often are heterogeneous and contrasting ecosystems, only few studies considered until now, the spatial and temporal variability of enzymatic activities in soils at the plot scale. Usually, such *in situ* investigations were performed via a limited number of measurements at one single time point (Gianfreda et al., 2005; Bell and Henry, 2011). Since environmental or anthropogenic factors affect the metabolism of microorganism, it is necessary to integrate such confounding factors when interpreting enzymatic indicators to assess the effective impact of contaminants on soil behaviour in real field conditions.

The originality of this present study was to combine experimental approaches performed *in situ* and in Terrestrial Model Ecosystems (TMEs) in order to assess the real impact of Cu on soil quality and behaviour at the plot scale. For that, we first determined the spatiotemporal variability of enzymatic activities in soils under different agricultural managements (grassland and intensive crop) in the spring, summer and autumn, thus miming a seasonal cycle. Then, the metal impact on soil enzymatic activities was monitored in TMEs containing undisturbed surface soil samples which were incubated in open-field conditions. Two Cu concentrations were selected: 2 mg kg^{-1} as a regulated agronomic contribution, and 200 mg kg^{-1}corresponding to consistent soil contamination. A global indicator of soil microbial activity, the dehydrogenase, as well as five hydrolase activities involved in the biogeochemical P, C and N cycles: acid and alkaline phosphatases, β-glucosidase, N-acetyl-β-glucosaminidase, and urease were measured during experiments.

2. Material and methods

2.1. Sites and soil characteristics

Field experiments were conducted at two locations: Saint-Georges-sur-Fontaine and Yvetot. The soils in these two sites are developed on loess deposits on limestone plateaus in Normandy, and representative for the major agricultural sector in north-western France. The climate was temperate oceanic with a mean annual temperature and rainfall of 10°C and 800 mm, respectively. The soils are Luvisols (IUSS Working Group WRB, 2006), with 18.0% clay, 67.2% silt and 14.8% sand for Saint-Georges-sur-Fontaine, and 18.2% clay, 62.1% silt and 19.7% sand for Yvetot. Selected physicochemical characteristics are presented in Table 1. For each site, two types of agricultural managements were considered: grassland and conventional arable land use. The grasslands were under permanent cover and untilled for more than 25 years. By contrast, arable soils were used for long-term intensive cropping with a rotation of wheat, maize, flax or beat, annually ploughed and fertilized.

2.2. Soil sampling

Multiple sampling was carried out in the 0-10 cm surface layer of soils in April, June and October 2005, representing main seasonal variations of enzyme activity. Twenty replicates spaced 25 m x 25 m were taken in each plot in order to account for spatial variability of the plots. For each replicate, 10 soil cores were collected from a circle of 1-m diameter, and mixed to constitute a composite sample. Each composite sample (3-4 kg) was sieved (< 2 mm) and stored at 4°C prior to measurement of enzyme activities, performed less than one week after sampling.

2.3. Assays of enzymatic activities

Analysis of dehydrogenase activity (DH; E.C. 1.1.1.1) was adapted from Schaefer (1963). In 30-mL centrifuge tubes, 5 g of sieved soil were mixed with 24 ml of distilled water and 1 ml of substrate at 5% TTC (1,3,5-triphenyltetrazolium chloride). Controls were prepared without TTC.

After incubation at 25°C for 16 h, 20 ml of acetone were added and the mixture was kept in the dark for 2 h and shaked every hour. Finally, TTC was added to controls and all mixtures were centrifuged at 6,000 g for 5 min. The absorbance of the supernatant (red colour in case of formazan formed, TPF) was measured at 485 nm. Results were expressed in nmoles TPF g^{-1} of dry soil h^{-1}.

Activities of acid phosphatase (PAC; E.C. 3.1.3.2), alkaline phosphatase (PAL; E.C. 3.1.3.1), β-glucosidase (GLU; E.C. 3.2.1.21) and N-acetyl-β-glucosaminidase (NAG; E.C.3.2.1.30) were measured using their respective substrate of conjugated para-nitrophenyl (Sigma-Aldrich) according to a method adapted from Dick et al. (1996). Assays were carried out in 30-ml centrifuge tubes by mixing 1 g of fresh soil, 4 ml of universal buffer (MUB, pH 6; except for PAL, pH 11) and 1 ml of substrate solution (0.05 M), which was omitted in the control samples. After incubation at 25°C for 2 h, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added to stop the reaction. At this step, 1 ml of substrate solution was added in control samples. All samples were centrifuged at 12,000 g for 3 min. The p-nitrophenol (PNP) liberated by enzymatic hydrolysis was determined by UV spectrophotometer at 405 nm. Results were expressed in µmoles PNP g^{-1} of dry soil h^{-1}.

Urease activity (URE; EC 3.5.1.5) was measured according to Kandeler and Gerber (1988) introducing some minor modifications. One gram of fresh soil was dissolved in 50 ml of water and shaken for 10 min. Aliquots of the soil solution (500 µl) were supplemented with 100 µl of 0.4 M urea, which was omitted in the controls. The mixtures were vigorously shaken and incubated at 25°C for 4 h. Then, 100 µl of a salicylate solution (VWR, Hach reagent n°23952-66, 1 bag dissolved in 1 ml of water) were added and the reaction was sustained for 3 min. Finally, 100 µl of a cyanurate solution (VWR, Hach reagent n°23954-66, 1 bag dissolved in 1 ml water) were added. At this step, controls were supplemented with urea solution. After 30 min, the mixtures were centrifuged at 10,000 g for 2 min. The absorbance of the supernatant (blue colour in case of N-NH_4^+ released) was measured at 610 nm. Results were expressed in nmoles N-NH_4^+ g^{-1} of dry soil h^{-1}.

2.4. Impact of copper on soil enzyme activities

Terrestrial Model Ecosystems (TMEs, PVC cylinders of 12.5 cm diameter and 12.5 cm deep) were filled with undisturbed soil columns from 5 representative locations in grassland or cropped soils in the plots of Yvetot. TMEs were incubated in conditions of the open-field for 70 days. Three conditions were examined: control without added Cu, a regulated annual contribution of Cu (2 mg kg^{-1}) and a high Cu contamination level of 200 p mg kg^{-1}. TMEs were spiked with 25 ml of the corresponding concentrated $CuSO_4$ solution (VWR, > 99% purity). Soil pH was found not modified by spiking with $CuCO_4$. Control TMEs received 25 ml of water. For each treatment, enzymatic activities were measured as previously described, from five TMEs after 7, 35 and 70 days of incubation. The representativeness of TMEs was checked by simultaneous measuring of soil enzymatic activities in TMEs and in the plots of Yvetot at the beginning and the end of incubation.

2.5. Statistics

All statistical tests were performed with R software (RDevelopmentCoreTeam, 2004). Analysis of variance (ANOVA) was applied on the datasets to identify (i) the respective importance of agricultural management, localization of sites or sampling date on soil enzymatic activities measured *in situ* and (ii) the respective importance of agricultural management, TME establishment, incubation time or impact of copper on enzymatic activities measured in TMEs. Previously, normality and homogeneity of variances were checked using Wilk-Shapiro test and Bartlett test, respectively. The significant differences between uncontaminated and contaminated TMEs were determined by Mann Whitney test with a significance level of 5%.

3. Results

3.1. Variability of enzymatic activities in grassland and cropped soils

Results of spatiotemporal variability of soil enzymatic activities in the two sites are presented in Fig. 1. Overall, the activity levels of enzymes were 2 to 4 times higher in grassland soils in comparison to cropped soils, for both sites.

For Yvetot (Fig. 1A.), the enzymes with highest spatiotemporal variability were acid and alkaline phosphatases, β-glucosidase, and to a lesser extent, dehydrogenase. Spatiotemporal variability of enzymatic activities was generally greater in grassland soils. For example, 50% values of acid phosphatase and β-glucosidase activities ranged from 1.85 to 1.19 and from 1.15 to 0.61 $\mu mol_{PNP}\ h^{-1}\ g^{-1}$, respectively, in the grassland soils, and from 0.55 to 0.32 and from 0.41 to 0.19 $\mu mol_{PNP}\ h^{-1}\ g^{-1}$ in the soil under intensive cropping. Both the activity level and variability of urease are similar whatever the type of agricultural management of the site.

For Saint-Georges-sur-Fontaine (Fig. 1B.), the highest spatiotemporal variability was observed for acid phosphatase with 50% values ranging from 1.53 to 0.74 $\mu mol_{PNP}\ h^{-1}\ g^{-1}$ in grassland and from 0.69 to 0.16 $\mu mol_{PNP}\ h^{-1}\ g^{-1}$ in cropped soils. Among tested enzymes, dehydrogenase was most little affected by the type of agricultural management of the site. Overall, the variability of soil enzymatic activities was lower than those measured in the Yvetot site.

Results of analysis of variance (Table 2) showed that the agricultural management was the major factor explaining the variability of acid phosphatase and N-acetyl-β-glucosaminidase activities (66 and 42%, respectively). The variability of urease and to a lesser extent of β-glucosidase, was related to the sampling date (93 and 36% respectively). None of the three factors explained the variability of alkaline phosphatase or dehydrogenase.

3.2. Impact of copper on soil enzymatic activities

The response of enzymatic activities to Cu was assessed in TMEs with undisturbed samples from Yvetot, incubated for 70 days in open-field conditions. Results are presented in Fig. 2 for grassland, and in Fig. 3 for the cropped soil. Soil enzymatic activities measured both in uncontaminated or contaminated TMEs varied over time. The variations of enzymatic activities were within the range of spatiotemporal variability measured on disturbed soil samples (Fig. 1).

For the grassland TMEs (Fig. 2), the addition of Cu did not lead to significant effects on the activity levels of enzymes, except for acid phosphatase. A decrease in its activity occurred after 7

days of incubation for exposure at both Cu concentration levels. Only the effect of Cu at 200 mg kg^{-1}persisted during the incubation time. For the TMEs of crop soil (Fig. 3), only acid phosphatase was inhibited by Cu at 2 mg $kg^{-1}$and two enzymatic activities were differentially affected by Cu at 200 mg kg^{-1}after 70 days of incubation. Indeed, dehydrogenase activity was inhibited by Cu whereas that of N-acetyl-β-glucosaminidase was stimulated by the presence of copper.

Acidic and alkaline phosphatases, β-glucosidase and N-acetyl-β-glucosaminidase activities were measured both in TMEs and *in situ* at the site of Yvetot, at the start and the end of TMEs incubation. For grassland, no significant difference of enzymatic variations was observed between TMEs and *in situ,* thus validating our approach of using TMEs for assessing the impact of Cu on soil enzymatic activities. For the cropped soil, only acid phosphatase activity was 1.5 times higher in the plot than in the TMEs (data not shown but integrated in the analysis of variance, Table 3)

Results of analysis of variance (Table 3) confirmed that the absence of effects of establishing TMEs on enzymatic activities. Agricultural management was the major factor explaining the variability of N-acetyl-β-glucosaminidase activity (49%), and the incubation time for urease (89%) and for acid phosphatase (47%). The variability of enzymatic activity was only little related to addition of Cu in TMEs (< 20%).

4. Discussion

4.1. Spatiotemporal variability of enzymatic activities in soils

The first objective of our work was to assess the spatial (20 replicates spaced 25 m x 25 m) and temporal (3 sampling dates during different seasons) variations of enzymatic activities in soils under different agricultural management. The activity levels of various enzymes, involved in the main nutrient cycles, were generally 2-4 times higher in grassland than in cropped soils, in both study sites. Such finding was ascribed to the total contents of carbon and nitrogen of the plots, 2-3 times higher in grasslands than in cropped soils, illustrating the effect of an intensive arable land use leading to decreasing nutrient stocks (Table 1). Such correlations between enzymatic activities

and physicochemical soil characteristics of nutrient stocks were previously reported for phosphatases or β-glucosidase in literature (Deng and Tabatabaï, 1997; Gianfreda et al., 2005; Kandeler, 2007). In our work, the agricultural management was shown as a major factor explaining the variability of acid phosphatase and N-acetyl-β-glucosaminidase contrarily to other enzymatic activities studied. .

Whatever the agricultural management of soils, soil enzymes showed a high spatial variability at the plot scale, consistent with an often great heterogeneous distribution of soil structure and composition over short distances. Indeed, soil represent a congregation of mineral and organic aggregates forming various microhabitats for microorganisms, in which the fluxes of nutrient elements, air and water influence the structure of communities and their functions (Nannipieri et al., 2003; Calbrix et al., 2005; Kandeler, 2007). Despite the high intra-plot variability, the statistical analyses did not reveal site effects on *in situ* measured enzymatic activities. This can be explained by the similarity of pedo-climatic characteristics between the sites of Yvetot and Saint-Georges-sur-Fontaine, an encouraging finding for the robustness of such enzymatic tools.

Although soil moisture was highly correlated with the sampling date ($R > 0.95$), only urease activity varied greatly over time at the plot scale, also observed during the incubation of TMEs (> 93%). It is well-known that urease is regulated by various factors including the climate (Krajewska, 2009). Seasonal variations control the biological processes involved in the fluxes of ammonium and agricultural practises such as applications of chemical or organic fertilizers. Little sensitivity of all other enzymes to seasonal variations is consistent with findings of Bell and Henry (2011) who showed that various hydrolase activities including phosphatase, N-acetyl-β-glucosaminidase and β-glucosidase, were not affected by rain events and temperature during one year.

4.2. Effect of Cu on soil enzymatic activities

One original aspect of our work was the establishment of TMEs incubated in open-field

conditions and affected by real climatic variations. In literature, effects of contaminants on enzyme activity was often studied on disturbed soil samples after sieving, and then incubated under controlled conditions (Ekenler and Tabatabaï, 2002; Gianfreda et al., 2005; Demanou et al., 2006). Sieving of soil samples enhances reallocation of organic carbon, and favours bioavailability of organic C to soil microorganisms, stimulating their development and growth. However, it is known that the sensitivity of the enzymatic responses to metals depends on the physiological status in fungi, which constitute one of the largest biomasses in soils (Lebrun et al., 2010). In this study, we chose to compose TMEs from undisturbed soil to preserve the soil structure, and interactions between different soil components and the metabolic status of soil microorganisms. Thus, our experimental approach enables to mime environmental conditions. The relevance of this approach for predicting what happens in the field was confirmed by comparing enzymatic activities measured in TMEs and *in situ.*

Although Cu was added in TMEs to a concentration 100 times higher than regulated contents per year, enzymatic activities were only little affected by the contaminant, independent of the type of considered soil management. Only acid phosphatase was inhibited by Cu at 200 mg kg^{-1} in TMEs of grassland soils of Yvetot. In TMEs of cropped soil, N-acetyl-β-glucosaminidase was stimulated and dehydrogenase was inhibited by Cu at 200 mg kg^{-1}but only after 70 days of incubation in open-field conditions. Therefore, no statistical links were established between the activity levels of enzymes and the presence of copper in grassland or cropped soils when comparing to effects related to natural spatiotemporal variations. Our results corroborate findings of studies that also reported the absence of metal impacts on biological endpoints (Avidano et al., 2005; Gianfreda et al., 2005; Demanou et al., 2006). By contrast, several other studies have mentioned a sensitivity of enzymatic activities measured in soil samples collected from *in situ* multiple metal contaminations including Cu: β-glucosidase (Hinojosa et al., 2004), alkaline phosphatase (Wang et al., 2007), urease and dehydrogenase activities (Mikanova, 2006). Yet, in the latter studies, it was difficult to discriminate the contribution of each metal to the response of enzymatic activities.

Effects of metals in soils on biological endpoints depend rather on their bioavailability than on total concentrations (van Gestel, 2008). Metal bioavailability is highly related to different intrinsic soil properties such as the nature and contents of organic matter, the soil's moisture content or the ionic composition… In our work, we also measured the EDTA-extractable Cu concentrations in TMEs. For the addition of Cu at 200 mg kg^{-1}in TMEs, we obtained about 105 mg $kg^{-1}$of Cu, i.e. about 50% of bioavailable Cu, whatever the agricultural management (data not shown here). Yet these data confirm that Cu was available for soil microorganisms without modifying the global levels of enzymatic activities. Although Cu is an essential element for all biota, a high Cu input in soil may inducetoxic effects on microorganisms or affect biodiversity by acting as a factor of species selection. For example, Cu may enhance changes in the structure of microbial communities (Laguerre et al., 2006; Wang et al., 2007). The fact that these enzymatic tools do not reflect a change in the structure of microbial communities and metal impact on their functions could be explained by a functional redundancy, i.e. the enzymes tested in this study are commonly present in all microorganisms.

5. Conclusion

Our work provides a first dataset on soil enzymatic activities for an important agricultural region on north-western France. We showed that activity levels of different soil enzymes involved in soil behaviour and major nutriment cycling were subject to great natural spatiotemporal variations at the plot scale. Such natural variability of enzyme activity should be considered interpretating their responses to different human pressures, such as agricultural land management Their activity levels were found generally higher in grassland soils than in soils under intensively arable land use, which was related to the nutrient contents of the soils. However, enzymatic activities were revealed as no good descriptors for contamination of soils by Cu, in regard to natural soil variability. Our work underlined the need of considering spatiotemporal variations of biological endpoints when assessing effective impact of contaminants in real field situations.

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Table 1. Soil physicochemical characteristics of two agricultural sites, Yvetot and Saint-Georges sur-Fontaine, under different agricultural managements. Bold values are means.

	Yvetot		Saint-Georges	
	Grassland	Intensive crop	Grassland	Intensive crop
Total carbon ($g\ kg^{-1}$)	**31.2**	**10.1**	**30.6**	**14.0**
	5.3	*0.2*	*5.8*	*1.3*
Total nitrogen ($g\ kg^{-1}$)	**2.7**	**1.0**	**2.7**	**1.5**
	0.4	*0.1*	*0.4*	*0.1*
C/N ratio	**11.4**	**9.9**	**11.2**	**9.3**
	0.6	*0.6*	*0.6*	*0.1*
CEC ($cmol^{+}\ kg^{-1}$)	**10.2**	**7.1**	**6.8**	**9.5**
	0.0	*0.0*	*0.1*	*0.1*
pH	**5.8**	**6.7**	**5.0**	**6.8**
	0.4	*0.2*	*0.5*	*0.5*
Moisture (%)	**39.1**	**28.8**	**28.9**	**22.4**
	2.5	*0.7*	*2.6*	*1.7*
P_2O_5 ($mg\ kg^{-1}$)	**86.4**	**116.4**	**129.7**	**74.6**
	18.1	*15.9*	*49.7*	*26.4*

Values in italics are standard errors ($n = 20$).

Table 2. Effects of agricultural management, sampling date and localization of sites on enzymatic activities measured in situ by ANOVA: explanatory factors of the variabilityin %. Bold values are the major factors of variability for each enzyme.

Enzymatic activities	Agricultural management	Date	Site	Residual variability
Acid phosphatase	**65.6**	14.7	0.0	19.7
Alkaline phosphatase	0.0	43.4	0.0	**56.6**
β-glucosidase	21.6	**36.7**	31.7	10.0
N-acetyl-β-glucosaminidase	**41.8**	28.3	0.0	29.9
Dehydrogenase	14.7	26.5	3.2	**55.6**
Urease	0.0	**93.7**	0.0	6.3

Table 3. Effects of agricultural management, TME establishment, incubation time and copper impact on enzymatic activities measured from undisturbed soil TMEs by ANOVA: explanatory factors of the variability in %. Bold values are the major factors of variability for each enzyme.

Enzymatic activities	Agricultural management	TME	Incubation time	Copper	Residual variability
Acid phosphatase	0.0	0.0	**47.0**	10.8	42.2
Alkaline phosphatase	12.0	14.2	9.6	19.2	**45.0**
β-glucosidase	17.6	0.0	39.3	0.0	**43.1**
N-acetyl-β-glucosaminidase	**49.4**	9.1	13.1	2.0	26.4
Dehydrogenase	1.2	N.D.	27.9	5.6	**65.3**
Urease	0.0	N.D.	**89.2**	2.8	8.0

Fig. 1. Spatiotemporal variability of enzymatic activities in Yvetot (A) and Saint-Georges-sur-Fontaine (B) under two agricultural managements: grasslands (■) and intensive cropping (□). For each enzyme, boxes correspond to 50% of values in which medians are included (*n* = 60; 20 replicates spaced 25 m x 25 m for each of 3 sampling dates). Bares represent the minimal and maximal values. Abbreviations: acid phosphatase (PAC), alkaline phosphatase (PAL), β-glucosidase (GLU), N-acetyl-β-glucosaminidase (NAG), dehydrogenase (DH) and urease (URE).

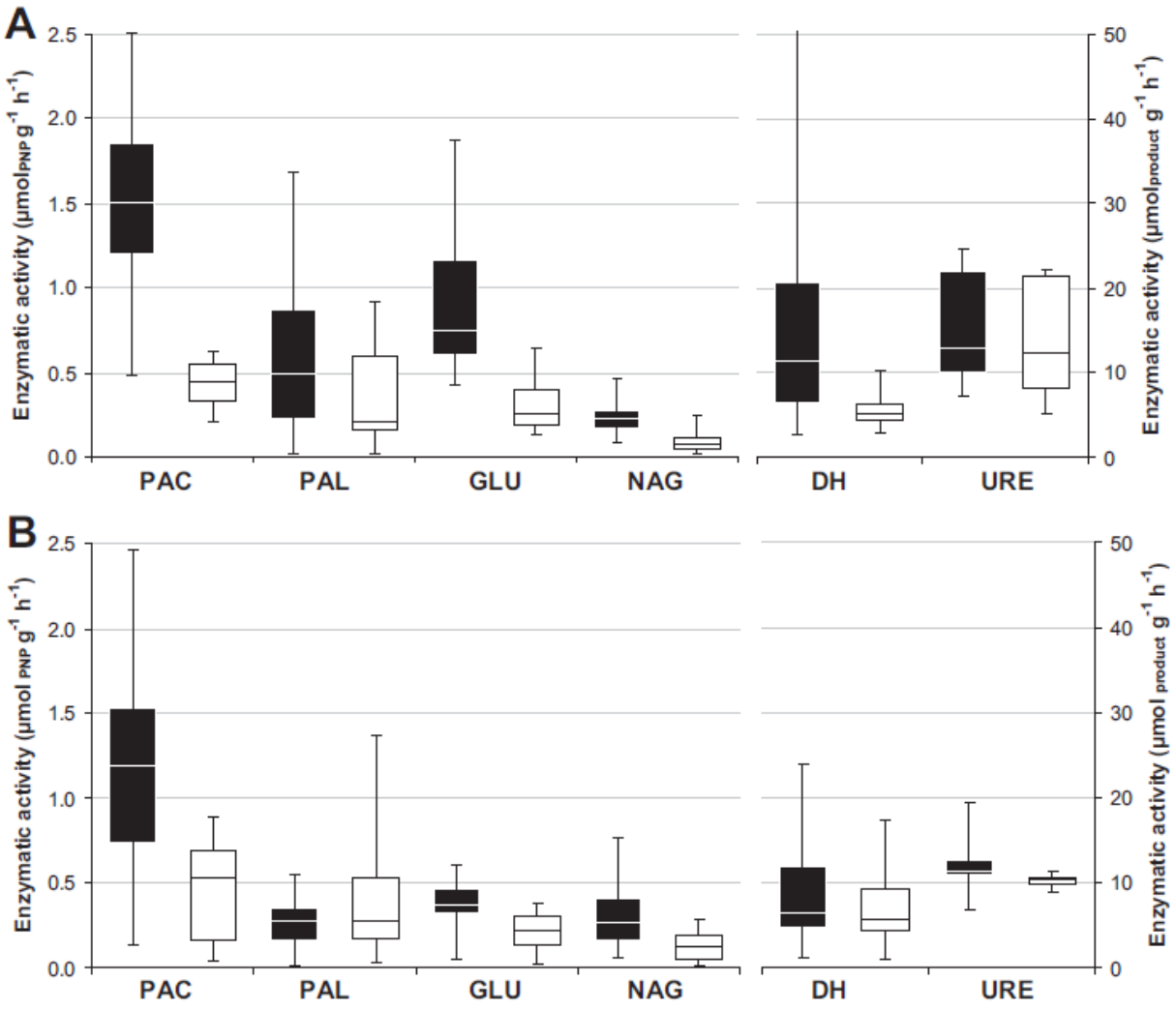

Fig. 2. Enzymatic activities in TMEs filled with undisturbed soil of grassland and incubated in open-field conditions (site of Yvetot). TMEs were either uncontaminated (□) or contaminated by Cu at 2 (▒) or 200 mg kg^{-1} (■). *Significant differences between control and contaminated TMEs ($p < 0.05$).

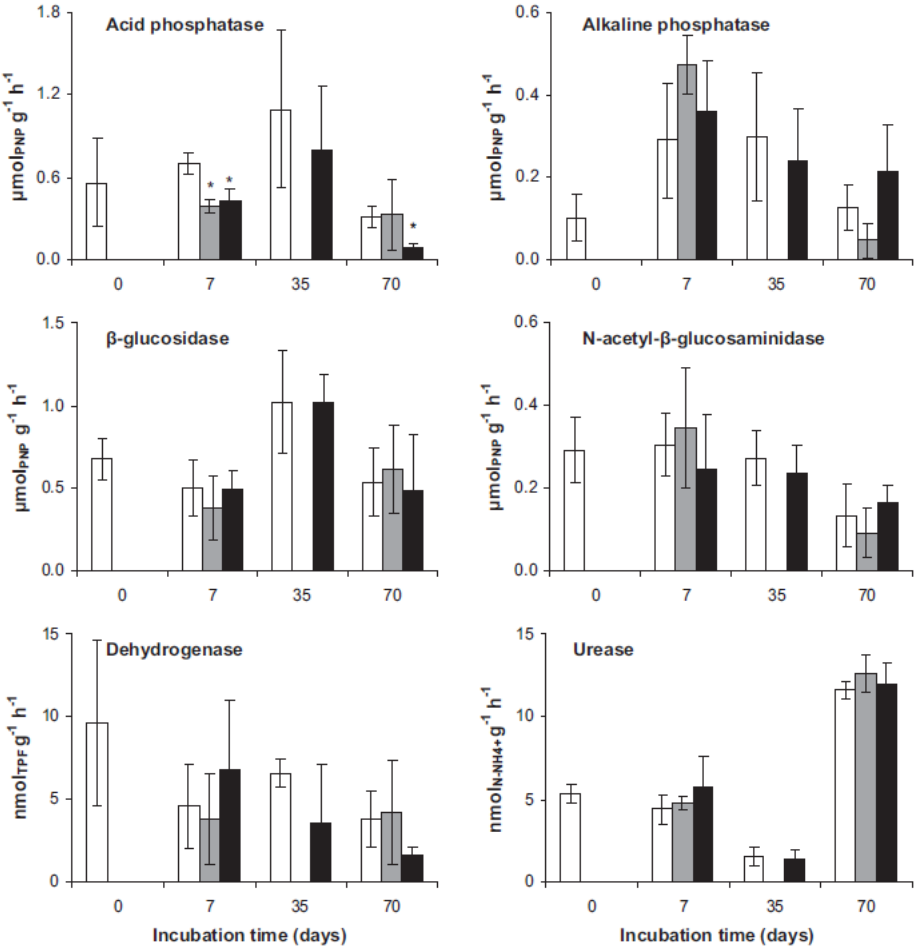

Fig. 3. Enzymatic activities in TMEs filled with undisturbed soil under intensive crops and incubated in open-field conditions (site of Yvetot). TMEs were either uncontaminated (□ or contaminated by Cu at 2 (▒) or 200 mg kg^{-1} (■). *Significant differences between control and contaminated TMEs ($p < 0.05$).