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Soil Enzymatic Activities and Microbial Community Structure in Soils Polluted with Tetracycline Antibiotics

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Abstract: A laboratory experiment was performed to examine the medium-term influence of three tetracycline antibiotics (chlortetracycline, CTC; tetracycline, TC and oxytetracycline, OTC) at different concentrations in four agricultural soils with similar pH and different soil organic content. After a 42-days incubation period, three different soil enzymes (β-glucosidase, urease, and phosphomonoesterase) were estimated, as well as the phospholipid fatty acids (PLFAs). A residual effect was observed on all microbial parameters measured in the four soils affecting to the soil enzymes activity and soil microbial communities structure (PLFA pattern). A different microbial sensitivity to antibiotics was detected depending on both, soil type and the microbial property considered. Specifically, in general, no antibiotic effect or even a slight positive effect was observed for phosphomonoesterase and β-glucosidase enzyme activities, respectively, while a negative effect was detected for urease activity values, particularly at higher doses of the antibiotics in a soil with a low organic matter content. The principal component analysis performed with the PLFAs data obtained for all soil samples showed different microbial communities depending mainly on soil type, followed by the antibiotic added to the soil (CTC, TC or OTC) and, in a lesser extent, by its concentration. In general, the PLFA patterns showed similar microbial communities structure due to OTC and TC addition in comparison to the microbial communities structure of soil treated with CTC. These results could be environmentally relevant, especially as regards potential effects of antibiotics on the soil microbiome and hence on health risk assessment of these antibiotics in soils.

Keywords: agricultural soils; veterinary antibiotics contamination; oxytetracycline; chlortetracycline; soil enzymes; PLFAs

1. Introduction

Antibiotics, for both veterinary and human medicine, have been widely used during the past decades and, upon reaching environmental compartments, are recognized as persistent contaminants. These emerging contaminants and their metabolites can enter the soil environment via animal manure, slurry, or sludge, used as organic fertilizer in agricultural or forest soils [1,2] or as an amendment for recovering degraded soils [3]. Ob-viously, investigations into this subject, focusing on potential risk assessment and searching for eventual environmental damage due to the presence of antibiotics in terrestrial ecosystems, are of high interest in the case of cultivated agricultural soils, due to the possible

negative consequences for the food chain and human health, whereas they could have lower relevance for degraded forest soils. However, limited information is still avai-lable related to the dynamics and behavior of antibiotics in soil agricultural ecosystems.

Veterinary antibiotics have been and still are extensively used in animal farms for animal disease control, and a high proportion of those administered are excreted in animal feces and urines, with potential great impact on the environment. Among antibiotics, te-tracyclines are a family characterized by having broad-spectrum favorable antimicrobial properties and the absence of major adverse side-effects, leading to their extensive use in therapy for human and animal infections [4]. Due to their extensive usage, most of the actual evidence suggests that tetracycline antibiotics are omnipresent compounds found in the terrestrial and aquatic environment [2,5,6]. They reach the environment mainly through the spreading of manure or slurries, as well as through various activities related to food production and direct animal contact. Once they reach the soil, the parent chemical compounds and/or their metabolites (which may be even more toxic) can enter surface water bodies and the food chain [7]. This residual effect of veterinary antibiotics is of great importance in soils with long history in the use of farm manure where the doses, method, and time of application are not generally controlled. It was reported that, depending on soil properties and application, tetracycline and oxytetracycline can persist in surface water and soil for over 1 year, thus indicating a moderate degradation of antibiotics of the tetracycline group [8,9]. However, despite interest, there is limited information available on the impacts of antibiotics on soil ecosystems, especially with respect to soil microbial communities, although microorganisms play an important role in many soil processes, such as organic matter turnover and nutrient cycling, thus affecting soil functioning [10]. Santás-Miguel et al. [11], in a recent laboratory study, added with different concentrations of tetracycline (TC) to 22 soils incubated under controlled conditions of moisture and temperature for 42 days, and they reported that, initially, tetracycline negatively affected bacterial activity measured by means of the cellular incorporation of labeled leucine, whereas this effect was drastically reduced at the end of the incubation time. Subsequently, Santás-Miguel et al. [12] studied the toxicity of oxytetracycline (OTC) and chlortetracycline (CTC) toward bacterial communities for 42 days and observed a negative effect of both antibio-tics on the growth of bacterial communities. The effect of CTC decreased with incubation time; however, the effect of OTC on bacterial communities persisted over time. Furthermore, regarding the additional needs of research, it must be noted that information avai-lable about the effect of tetracycline antibiotics on other aspects of soil microorganisms is very scarce, such as microbial biomass or specific activity of specific groups related to the cycles of the most important sources of energy (C) and nutrients (N, P) and microbial community structure.

Considering the above, the aim of the present study was to determine the potential impact of the presence of antibiotics on soil microorganisms. Accordingly, we examined if changes in the enzymatic activities (with specific reference to C, N, and P cycles) and microbial community structure (performed by means of an analysis of phospholipid fatty acids) induced by the residual effect of tetracycline antibiotics occur in the medium term (42 incubation days). The results are relevant from an overall environmental point of view since the potential risk to microbial health of antibiotics in soils is evaluated, which is closely related to the crop quality and, hence, could affect human health.

2. Material and Methods

2.1. Soil Samples and General Characterization

Four soils cultivated with potato–wheat rotation sampled in A Limia (Galicia, Spain) and no detectable concentrations of antibiotics were selected from a set of soils previously analyzed by Conce-Cid et al. [1]. This area has an average altitude of 640 m above sea level. The mean annual temperature is 11 °C, with a total mean annual precipitation of 881 mm, irregularly distributed through the year. The soils were classified as Mollic Umbrisols (Anthric) according to the IUSS Working Group WRB [13]. Soil samples were

co-llected after harvests of the potato crop and before sowing of wheat. Ten soil subsamples were taken from the A horizon (0–20 cm depth) of each agricultural soil and subsequently mixed into a composite representative soil sample (approximately 3 kg). Once in the laboratory, composite soil samples were air-dried, sieved through a 2 mm mesh, thoroughly homogenized, and stored in polyethylene bottles until analysis. Standard methods were used for physical and chemical characterization of a wide range of soil samples [1,11,14]. Briefly, soil pH values in water ranged from 4.7 to 5.0, pH in KCl ranged from 4.3 to 4.4, organic C content was between 10.7 and 33.9 $g \cdot kg^{-1}$, organic C dissolved in water ranged from 0.21 to 0.28 $g \cdot kg^{-1}$, and available P ranged from 0.11 to 0.23 $g \cdot kg^{-1}$ (Table 1). The eff-ective cation exchange capacity ranged from 4.1 to 6.4 $cmol_c \cdot kg^{-1}$, and the soil texture ranged from sandy loam to sandy clay loam (Table 1).

Table 1. Properties of the four soils studied in the laboratory experiment (adapted from Conde-Cid et al. [1]).

Soil	1	2	3	4
pH_{Water}	4.8	5.0	5.0	4.7
pH_{KCl}	4.2	4.4	4.3	4.3
C ($g \cdot kg^{-1}$)	10.7	21.4	25.3	33.9
N ($g \cdot kg^{-1}$)	0.9	2.0	2.3	3.1
eCEC * ($cmolc \cdot kg^{-1}$)	4.1	5.3	6.4	5.9
DOC ** ($g \cdot kg^{-1}$)	0.21	0.28	0.31	0.24
P_{Olsen} *** ($g \cdot kg^{-1}$)	0.23	0.19	0.11	0.12
Texture	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay loam

* Effective cation exchange capacity; ** water-dissolved organic C; *** available phosphorus.

2.2. Experiment Design

Air-dried soil samples were rewetted up to 60–80% water holding capacity and incubated at 22 °C for 1 week in order to reactivate the bacterial activity in the four soils, taking into account that this period of time would allow stabilization of soil microbial activity after moisture adjustment [15]. After this time, each soil was distributed in 72 polypropy-lene tubes (100 mL) (3 antibiotics × 3 replicates × 8 antibiotic concentrations), adding 10 g of soil (dry weight) to each tube. The total number of microcosms was 288 (72 per soil). Three antibiotics of the tetracycline group (chlortetracycline, CTC; tetracycline, TC; and oxytetracycline, OTC) were added at different doses to the soil samples. The antibiotics, all three as hydrochlorides, were supplied by Sigma-Aldrich (USA). The final antibiotic concentrations (for CTC, TC, and OTC) in each soil were 0.00, 0.49, 1.95, 7.81, 31.25, 125, 500, and 2000 $mg \cdot kg^{-1}$ of soil. The concentrations were previously selected [12] in order to obtain almost complete inhibition of soil bacterial growth, thus offering estimates of to-xicity indices in a more reliable way [16]. The antibiotics were added to the soils using inert talc powder as a carrier for equalizing the amount of dry material added to each soil sample, as well as to facilitate mixing of the substances into soil [17]. Talc (CAS 14807-96-6) was supplied by Sigma–Aldrich (Steinheim, Germany).

The resulting soil microcosms were incubated in the laboratory under constant conditions of temperature (22 °C in the dark) and moisture content (60–80% of water holding capacity) for 42 days. Distilled water was added occasionally to maintain the soil moisture at a constant level. At the end of the incubation experiment, PLFA analysis and soil enzyme measurements were done. The soil samples were separated into two fractions: one was stored at 4 °C for a time not longer than 1 month and used for the soil enzyme activity analysis, and the other was lyophilized and used for the determination of the PLFAs. The high number of samples to be processed and the high time needed for these analyses made it impossible to perform the various microbial estimations in all 228 microcosms. Soil enzymatic activities (with specific reference to cycles of C (β-glucosidase), N (urease), and P (acid phosphomonoesterase)) were measured in two contrasting soils with different organic matter and texture (soils 1 and 2) using three

incubation replicates (2 soils × 3 antibiotics × 8 antibiotic concentrations × 3 soil incubation replicates = 148 samples). In contrast, the four soils and, thus, all 228 microcosm samples were used for the biomolecular bioindicators (PLFAs); however, for each soil, the three incubation replicates of the same treatment (antibiotic concentration) were mixed and carefully homogenized to get a re-presentative composite soil sample. A total of 96 composite samples (4 soils × 3 antibiotics × 8 concentrations = 96) were analyzed. All chemical reagents were of high purity (analy-tical grade), provided by Sigma-Aldrich (Steinheim, Germany).

2.3. Soil Enzymatic Activities

The β-glucosidase activity was measured following the procedure of Eivazi and Tabatabai [18], which determines the released *p*-nitrophenol after incubation of the soil with a *p*-nitrophenyl glucosidase solution for 3 h at 37 °C. The urease activity was estimated by incubating the soil samples with an aqueous urea solution for 3 h at 37 °C and extracting the NH_4^+ with 1 M KCl and 0.01 M HCl followed by colorimetric NH_4^+ determination using a modified indophenol reaction [19]. The acid phosphomonoesterase activity was analyzed following the method described by Trasar-Cepeda et al. [20], which determines the amount of *p*-nitrophenol release after the incubation of the soils with *p*-nitrophenyl phosphate for 3 h at 37 °C.

2.4. Microbial Community Structure (PLFA Pattern)

The microbial community structure was estimated by means of phospholipid fatty acid (PLFA) analysis [21]. All glass material used in the procedure was heated at 400 °C overnight to remove lipid contaminants. Briefly, lipids were extracted from the 2 g (wet weight) of soil with a chloroform/methanol/citrate buffer mixture (1:2:0.8 *v/v/v*) and separated into neutral lipids, glycolipids, and phospholipids using a prepacked silica column. The phospholipid-containing polar phase was collected and dried under a stream of N_2; thereafter, it was stored at 20 °C and saved for preparations of fatty acid methyl esters. Methyl nondecanoate (19:0) was added to the phospholipid fractions as an internal standard. The samples were subjected to a mild alkaline methanolysis, and the resulting fatty acid methyl esters were identified by gas chromatography (Hewlett Packard gas 5890 chromatograph, Palo Alto, USA, equipped with a flame ionization detector) on the basis of the relative retention times of the fatty acids. Hydrogen was used as a carrier gas, and injections were made in a split mode.

A total of 35 different PLFAs were identified and quantified. The PLFAs were designated in terms of total number of carbon atoms and double bonds, followed by the position of the double bond from the methyl end of the molecule. Furthermore, *cis* and *trans* configurations are indicated by "c" and "t", respectively. The prefixes "a" and "i" indicate anteiso- and iso-branching positions, br indicates the unknown methyl group branching position, "10Me" indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule, and "cy" refers to cyclopropane fatty acids.

2.5. Statistics

All results were expressed on the basis of the oven-dried (105 °C) weight of soil. The values of the different soil enzymatic activities were expressed per g of soil and per h (mean values ± SE of the three incubation replicates). Differences between soil enzyme activities for the soils polluted with each antibiotic concentration and the corresponding control soil without antibiotic addition were determined by ANOVA and Dunnett's post hoc test (considering significance at $p < 0.05$). Concentrations of all the individual PLFAs, expressed in mole percentages and logarithmically transformed ($\log_{10}$ mol.%), were subjected to a principal component analysis (PCA) to elucidate the main differences in the PLFA patterns of the microbial communities of the studied soils. All statistical analyses were carried out using the SPSS 15.0 statistical package.

3. Results

3.1. Enzymatic Activity

The enzyme activity concentrations obtained for the two soils studied (Soil 1 and Soil 2) expressed as absolute values (μg *p*-nitrophenol·g^{-1}·h^{-1}, μg NH_4^+·g^{-1}·h^{-1}) are shown in Figure 1. In the case of the control soil sample, Soil 1 showed 8 ± 2 μg *p*-nitrophenol·g^{-1}·h^{-1} activity for glucosidase, 110 ± 11 μg NH_4^+·g^{-1}·h^{-1} activity for urease, and 446 ± 12 μg *p*-nitrophenol·g^{-1}·h^{-1} activity for phosphomonoesterase. For Soil 2, vales in the control soil sample were higher than those observed in Soil 1: 28 ± 2 μg *p*-nitrophenol·g^{-1}·h^{-1}, 156 ± 10 μg NH_4^+·g^{-1}·h^{-1}, and 601 ± 41 μg *p*-nitrophenol·g^{-1}·h^{-1} for glucosidase, urease, and phosphomonoesterase activities, respectively.

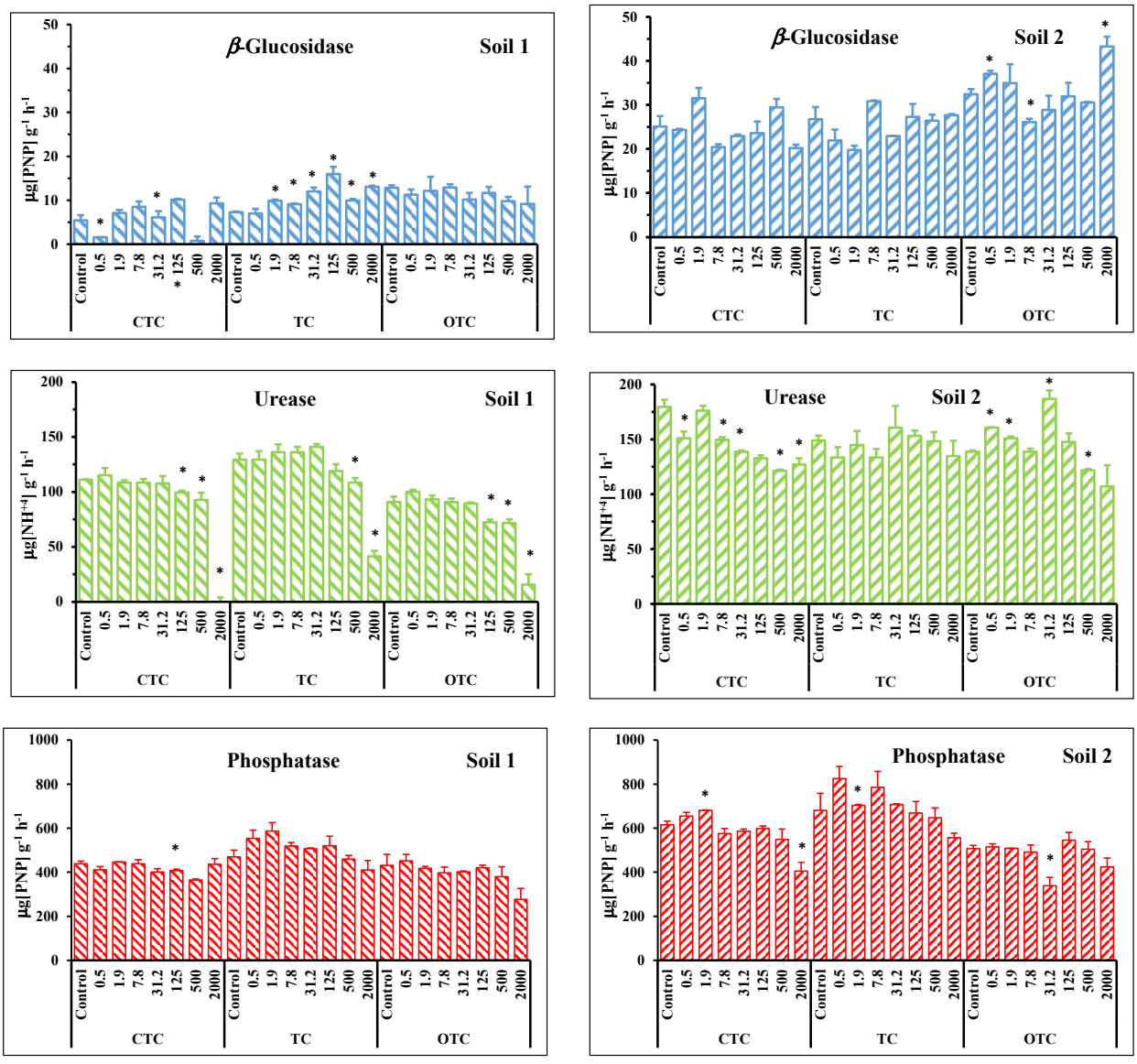

Figure 1. Soil enzymes in the four soils studied treated with different doses of the tetracycline antibiotics (CT chlortetracycline; TC, tetracycline; OTC, oxytetracycline) 42 days after application. Data are expressed per g of soil (absolute va- lues). The error bars represent the standard deviation (n = 3). Asterisks (*) indicate a significant difference compared to the control without addition of antibiotics ($p < 0.05$).

The activity values of the three enzymes were generally not consistently affected by the addition of eight different concentrations of antibiotics, and a dose effect was not detected in β-glucosidase activity, with values slightly increasing with CTC and TC addition in Soil 1,

as well as with the addition of OTC in Soil 2, whereas the effect being more accentuated for Soil 1. In contrast, β-glucosidase activity did not show an appreciable change in soil 1 due to the addition of OTC, nor in soil 2 due to the addition of CTC and TC. Except for TC in soil 2, urease activity values seemed to be negatively affected by the addition of the three antibiotics, with the effects being much more accentuated in soil 1 at the highest antibiotic concentration (2000 mg·kg^{-1}). Phosphomonoesterase activity values hardly changed after the addition of the antibiotics. In both soils, generally, no effect or a slight negative effect was observed after OTC, TC, and OTC addition, with this effect being significant only at some specific concentrations of the antibiotics.

3.2. Microbial Community Structure (PLFA Pattern)

The results of the PCA (samples and variables) performed with the whole PLFA dataset obtained for eight different concentrations of the three antibiotics (CTC, TC, OTC) added to four soils (1, 2, 3, 4) are shown in Figure 2.

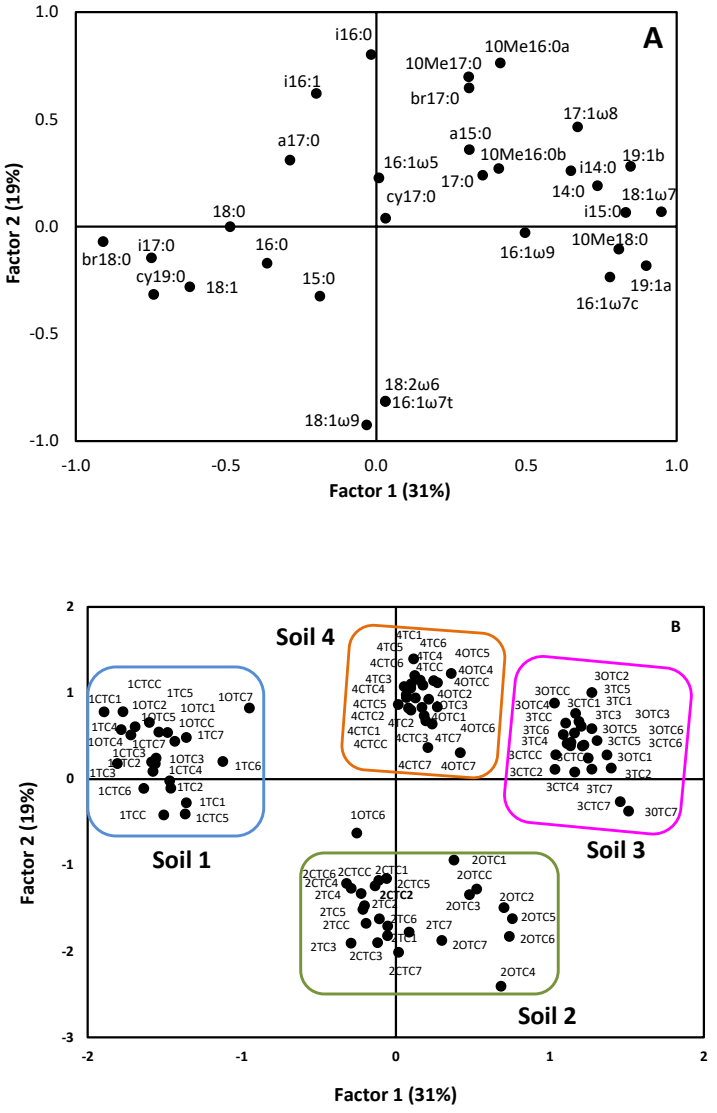

Figure 2. Principal component analysis (**A**, variables; **B**, samples) performed with the whole PLFA dataset for the four soils studied (1, 2, 3 and 4) with eight different concentrations (0.00 (C), 0.49 (1), 1.95 (2), 7.81 (3), 31.25 (4), 125 (5), 500 (6), and 2000 (7) mg·kg^{-1} soil) of the tetracycline antibiotics (CTC, chlortetracycline; TC, tetracycline; OTC, oxytetracycline) 42 days after application.

The minute plane defined by the first and second factors, which explained the 50% of variation, clearly distinguished the different soils. Soils 3 and 4 were situated in the positive region of Factor 1, while soil 1 was situated in the negative region. Soil 2 was situated in the negative region of Factor 2. It should be noted that all samples of the same soils with different doses of the three antibiotics were grouped together and separated from the rest of the soils. Soil 3 (having positive values along PC1) was characterized by having higher concentrations of PLFAs 18:1ω7, 16:1ω7, 10Me18:0, 10:1a, and 19:1b, whereas soil 4 showed high concentrations of PLFAs i16:0, 10Me17:0, and 10Me16:0a, soil 1 exhibited the predominance of PLFAs br18:0, i17:0, cy19:0, and 18:1, and soil 2 was cha-racterized by having high concentrations of PLFAs 18:1 ω 9, 16:1ω 7, and 18:2ω6.

4. Discussion

Soil microorganisms perform many vital processes and participate in the maintenance of soil health and quality. They play a crucial role in organic matter turnover, nutrient release, stabilization of the soil structure, and biological control by inhibiting the growth of pathogens and, hence, ensuring soil fertility [10]. Therefore, the measurement of several microbial parameters based on mass, activity, and composition (biomass C, soil respiration, N mineralization, dehydrogenase activity, FDA hydrolysis, specific enzymes of C, N and P cycles, PLFA pattern, BIOLOG, etc.) can be used as bioindicators of soil quality. In the current study, we used as indices of microbial activity three enzyme acti-vities related to the C (β-glucosidase), N (urease), and P (acid phosphomonoesterase) cycles. The values of these properties observed for the two soils studied were in the reported range given for soils located in the same temperate humid area [22–24]. Soil 1 (with low organic matter content) exhibited enzyme activity values lower than those observed in Soil 2 (with high organic matter content). This can be explained by the close relationships observed between enzyme activities and organic matter content [22,24,25]. Our data showed that the effect of antibiotic addition on specific enzyme activities depended on both each microbial parameter and each soil type considered. Thus, while no effect due to the presence of the antibiotics or even a slight positive effect was observed for phosphatase and β-glucosidase enzyme activities, a negative effect was detected for urease activity values, particularly at higher doses of the antibiotics in the soil with a low organic matter content. The sensitivity of soil enzymes to the presence of antibiotics was low and followed the order acid phosphomonoesterase < β-glucosidase << urease. This is in accor-dance with the scarce and inconsistent reports on the effects of pharmaceutical antibiotics on soil enzyme activities. For example, it has been reported that tetracycline and oxyte-tracycline inhibit dehydrogenase, arylsulfatase, urease, and acid phosphomonoesterase activities [26–29]. In other studies, dehydrogenase and phosphatase activities were not affected by oxytetracycline addition, even at high doses [30,31]. In contrast, a temporary increase in dehydrogenase activity was found in soils contaminated with chlortetracycline [32]. The inhibition of enzyme activity in antibiotic-treated soils may be related to the inhibition of growth or death of sensitive microorganisms [33]. In turn, the increased activity of enzymes under antibiotic pressure may result from the ability of many bacteria to subsist in the presence of antibiotics using those compounds as a carbon source [34]. In addition, the presence of some antibiotics in soil may cause an overgrowth of fungi, which are generally less sensitive to antibiotics than bacteria. Fungi are major producers of enzymes in soils and, thus, may be responsible for the observed increases in enzyme activity [35].

Therefore, since various effects on enzymes have been shown for different antibiotics applied to soils, these microbial indices are not good indicators of soil contamination with antibiotics [36]. This is in accordance with a recent study of Nannipieri et al. [37] concerning the limitations to interpreting enzyme data generated by the methods currently avai-lable and using them as bioindicators of soil quality. Previously, Trasar-Cepeda et al. [38], in a study concerning the use of soil enzymes as indicators of soil pollution, concluded that quantification of soil degradation based on these microbial indices requires supplementation with information on other biochemical soil properties. In this regard, activity

measurements based on more reliable techniques, such as the incorporation of labeled substrates (^{3}H leucine) into soil bacteria, are more adequate for this purpose of studying the effect of the presence of antibiotics on microorganisms [36]. This may be due to the fact that they reflect only the activity of living organisms under field conditions ("in situ"), instead of potential activity estimated under laboratory conditions corresponding to both biotic and abiotic enzyme activities [37]. Other disadvantages of enzyme assays are that they often do not match the mode of toxic action of antibiotics (bactericidal and bacteriostatic), making these assays less sensitive than bacterial growth [17,39–41].

In the current study, the results of PLFA pattern characterization performed with the whole dataset of soil samples showed that the relative importance of various environmental variables in governing the composition of microbial communities could be ranked in the following order: soil type >> antibiotic added (CTC, TC or CTC) >> dose of application. Soil type was the most important determinant of the microbial communities tested in this study. In fact, soil samples of the same soil with different antibiotics were grouped together and well separated from the remaining soils along Factor 1, according to the soil organic matter content (positive region: soils 3, 4, and 2; negative region: soil 1) (Figures 2 and 3). Soil 1, with low organic matter content, had a relatively higher abundance of PLFAs br10:0 and i17:0, specific to Gram-positive bacteria, and cyclopropil fatty acid cy19:0, which has been proposed as an indicator of starvation stress conditions (Figure 2). Soil 3 was characterized by having relatively high concentrations of monounsaturated 18:1ω7, 16:1ω7, and 17:1ω8 PLFAs, as well as actinobacteria 10Me18:0, terminally branched saturated i14:0 and i:15:0, and unsaturated 19:1a and 19:1b PLFAs. Soil 4 was characterized by a higher relative abundance of mid-chain branched saturated 10Me16:0 and 10Me17:0 PLFAs and branched i16:0 and br17:0 PLFAs. Soil 2 exhibited a higher re-lative abundance of 18:1ω9, 16:1ω7, and 18:2ω6 PLFAs, characteristic of fungi. It should be noted that the PLFA data also discriminated soil microbial communities corresponding to soil 2 from the remaining soils (1, 3, and 4) along Factor 2. This behavior may be due to soil properties other than those considered in the present study. These results of PLFA pattern are in accordance with the studies of Bossio et al. [42] and Díaz-Raviña et al. [43], who also observed that the PLFA pattern could distinguish between microbial communities of different soils.

When PLFA data were analyzed separately for each soil, PLFA patterns allowed differentiating soil microbial communities receiving the application of the three antibiotics of the tetracycline group used in the current study (CTC, TC, and OTC). In this respect, it should be noted that, for the four soils studied, in the planes defined by Factor 1 and 2 (explaining the 40–60% of the variance), the PLFA pattern could discriminate the soil microbial communities with CTC from those with OTC and TC, which were pooled together along Factor 1. These results are in accordance with recent studies performed with the same soils showing that the toxicity of CTC was higher than that observed for OTC and TC [12]. However, in soil 2, different microbial communities were observed along Factor 2 as a consequence of OTC and TC addition, showing the same behavior as that observed when comparing the microbial composition of the four soils studied (Factor 2 in Figure 2). These results of the PLFA pattern indicated that the exposure to the three tetracycline antibiotics provided sufficient selective pressure to cause shifts in the fatty-acid composition of soil microbial communities, but specific groups of microorganisms were not identified. In addition, an effect of the highest dose of the three different antibiotics was also observed, whereby microbial communities in soils contaminated with 2000 mg of CTC, TC, or CTC per km of soil differed notably from communities in soils contaminated with the remaining concentrations up to 500 mg of antibiotic per kg of soil. Higher reductions in soil enzymes at the highest antibiotic concentrations added were also observed, parti-cularly for urease in soil 1 (Figure 2).

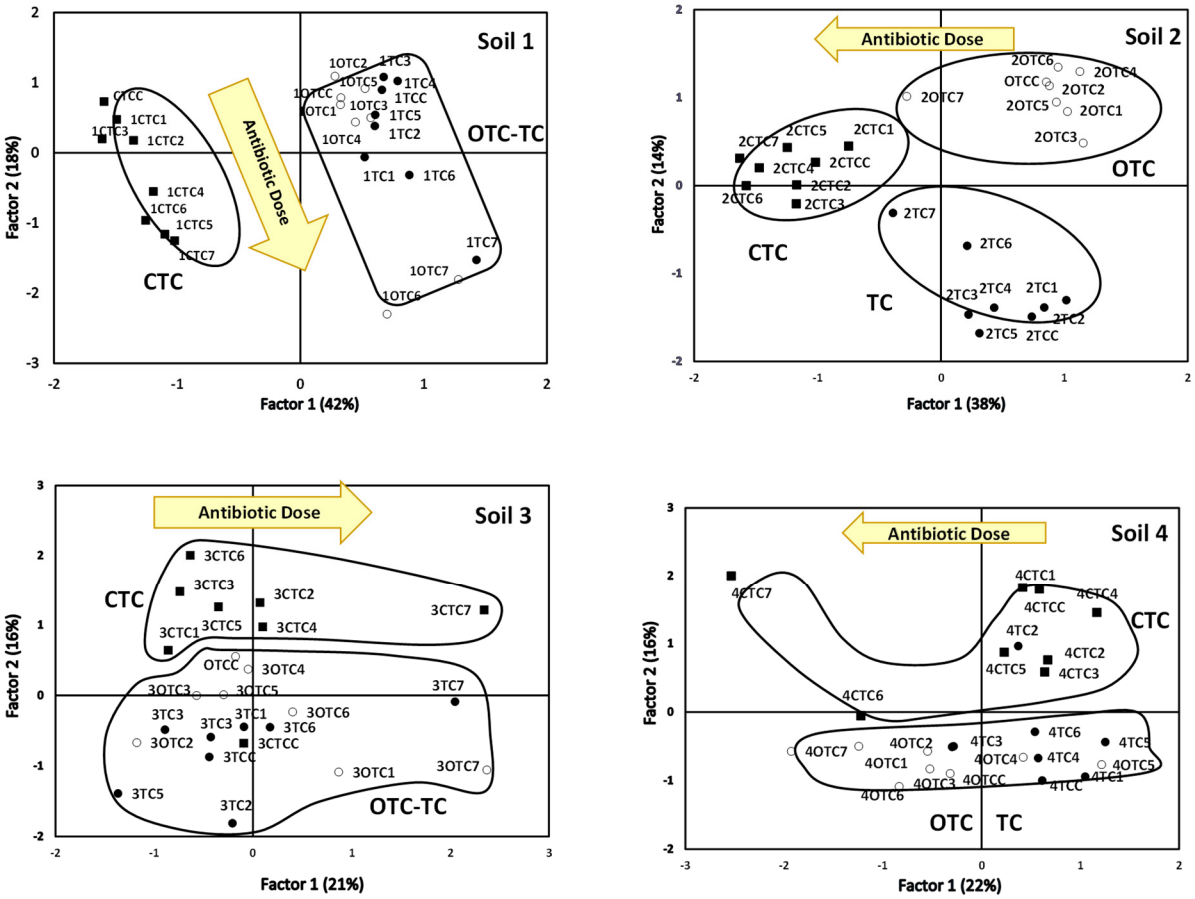

Figure 3. Principal component analysis (samples distribution) performed separately with the PLFA dataset of each studied soil (1, 2, 3, and 4) with eight different concentrations (0.00 (C), 0.49 (1), 1.95 (2), 7.81 (3), 31.25 (4), 125 (5), 500 (6), and 2000 (7) mg·kg^{-1} soil) of the tetracycline antibiotics (CTC, chlortetracycline; TC, tetracycline; OTC, oxytetracycline) 42 days after application.

Investigations concerning the effects of antibiotics for both veterinary and human use on soil microbial microorganisms, showing changes in microbial community composition determined using the PLFA technique, are quite recent. It is relevant that the magnitude of these changes has been found to vary depending on soil properties, dose and type of antibiotic, and time passed after their application [2,36].

5. Conclusions

For the four soils included in the current study, similar results were observed despite different organic matter contents. Specifically, the effect of the addition of eight different concentrations of the tetracycline antibiotics OTC, TC, and CTC to the soils induced changes in soil enzyme activities and microbial composition, particularly in the latter. Slight changes, which must be interpreted with caution, were observed for β-glucosidase, urease, and acid phosphomonoesterase, as a consequence of the presence of these anti-biotics. Moreover, an effect due to the different antibiotics was observed on the microbial composition, detected on the basis of PLFA patterns. The data clearly showed that, independently of the soil considered, microbial communities of soils with TC and OTC were quite similar and differed notably from soils with CTC. Likewise, a slight shift in microbial structure was observed as a consequence of the addition of the highest dose of the three antibiotics. These results can be considered relevant with regard to the environmental impacts of antibiotic pollution, as well as soil health and soil quality, especially considering the integrity and diversity of soil microbiota.

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