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Microbial Communities as Affected by Clarithromycin Addition in Four Acid Soils (NW Iberian Peninsula)

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A laboratory experiment was carried out to investigate the response of the microbial communities in acid agricultural soils located in the NW Iberian Peninsula to the presence of clarithromycin. Four soils, with different organic C content and similar pH, and seven different concentrations of clarithromycin (0.49, 1.95, 7.81, 31.25, 125, 500 and 2,000 mg kg^{-1} of soil) were used, and microbial estimates were made after 8 and 42 incubation days. The phospholipid fatty acids (PLFA) technique was used to estimate the total microbial biomass and biomass of specific microbial groups as well as the microbial community structure (PLFA pattern). The microbial biomass (total and specific groups) was different in the four studied soils, the lowest values being exhibited by soils with the lowest organic C. The antibiotic addition showed a positive effect on microbial biomass (total and specific groups), especially at the highest dose; the effect being similar or even more accentuated with time passed after the addition (42 days ≥8 days). Principal component analysis (PCA) of the PLFA data carried out with the whole data set showed that the main determining factors of the microbial structure followed the order: soil > time incubation ≥ antibiotic dose. When the PCA was performed individually for each incubation time, the results indicated that microbial communities of the four soils were different. Likewise, for each soil, different microbial communities were observed depending on antibiotic concentration. The microbial biomass and PLFA pattern data were coincidentally showing that the clarithromycin addition favored fungi and G^- bacteria more that bacteria and G^+ bacteria; the effect being dose-dependent. Our data (microbial biomass, PLFA pattern) also demonstrated that the effect of clarithromycin addition on microbial communities in these four acid agricultural soils persisted even after 42 incubation days.

Keywords: polluted soil, phospholipid fatty acids, persistent effects, antibiotics, microbial biomass

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INTRODUCTION

Antibiotics, used worldwide for human health, are excreted in feces and urine as the origin compound and/or as secondary metabolites, reaching the environment and causing serious damage to the soil and aquatic ecosystems (Thiele-Bruhn, 2003; Kümmerer, 2009). These emergent contaminants of human origin, which are present in wastewater destined for

wastewater treatment plants (WWTPs), can enter into soil following both agricultural irrigation with liquid effluents and the application of solid effluents (sewage sludge) as organic amendments to the soil (Fijalkowski et al., 2017; Aydin et al., 2022). Thus, residues of both parent veterinary and pharmaceutical compounds and their degradation products have been detected in soils (Biel-Maeso et al., 2018; Conde-Cid et al., 2018; Nuñez-Delgado et al., 2019; Mejías et al., 2021). These findings are being taken into account by the regulatory bodies of the European Union, leading in the last two decades to increased investigations concerning the presence of these emergent contaminants such as antimicrobial compounds on soil-plant ecosystems (Caracciolo et al., 2015; Tasho and Cho, 2016; Pan and Chu, 2017; Madikizela et al., 2018; Gworek et al., 2021; Bolesta et al., 2022; Yin et al., 2023).

Soil microorganisms play a major role in soil quality and long-term sustainability of agricultural terrestrial ecosystems since they control the breakdown of organic matter and hence the net fluxes of carbon and nutrients through the decomposition, mineralization, and immobilization processes (Pankhurst et al., 1996; Nannipieri et al., 2003). However, most studies concerning the impact of emergent contaminants such as antibiotics on terrestrial ecosystems are focused on their presence, behaviour, and risk assessment on human health and aquatic systems (Li, 2014; Bastos et al., 2020; Aydin et al., 2022). It is well known that antimicrobials for veterinary and human medicine can drastically modify non-target organisms living in the agricultural soils and hence alter biodiversity and ecosystem functions. Surprisingly, despite interest in the topic, the investigations addressing the impact of antibiotics on autochthonous microorganisms in the terrestrial ecosystems are scarce. The investigations concerning the impact of different groups of pharmaceutical compounds on microbial communities of agricultural soils located through the world were collected in a recent review by Cycón et al. (2019). The review clearly evidences that the response of microorganisms to the presence of these compounds measured by means of different parameters related to their mass, activity, and diversity (microbial C, total PLFA biomass and specific microbial groups PLFA biomass, soil respiration, bacterial growth, soil enzymes, microbial community structure), were variable; thus, although a negative temporal effect was often observed on microorganisms living in the soil, non effect, or even a positive effect, could also be detected. This is in agreement with recent investigations conducted by our research group performed with acid agricultural soils located in Galicia (NW Iberian Peninsula) added with antibiotics of different groups (tetracyclines, sulfonamides, fluorquinones, penicillins, cephalosphorine, diaminopyridine, β-lactams) (Santás-Miguel et al., 2020a; Santás-Miguel et al., 2020b; Santás-Miguel et al., 2020c; Rodríguez-González et al., 2021; Santás-Miguel et al., 2021; Rodríguez-González et al., 2022; Santás-Miguel et al., 2022; Rodríguez-González et al., 2023).

The World Health Organization (2017) considers the macrolides to be critical antibimicrobials of the highest priority, besides being the second most commonly used group of antibiotics in Europe (European Centre for Disease Prevention and Control, 2018). Clarithromycin is one of the most frequently prescribed macrolides in human medicine, therefore environmental risk investigations about the presence of this compound in soils are necessary (McLaughlin and Belknap, 2008; Baumann et al., 2015; Senta et al., 2019; Aydin et al., 2022). Recently, we examined the bacterial growth of 12 agricultural soils, both with no additions and with the addition of increasing concentrations of clarithromycin over time (1, 8, and 42 incubation days) (Rodríguez-González et al., 2021). In general, the data showed an initial inhibitory (toxic) effect which disappeared over time; however, in some cases, surprisingly, bacterial growth rates reached values higher than those in the corresponding unpolluted soil after 42 days of incubation. The aim of this laboratory experiment is to determine whether these bacterial growth changes were accompanied by changes in biomass and microbial community structure.

MATERIAL AND METHODS

Soils

The study was conducted with four agricultural soils located in the temperate humid zone (Galicia, NW Iberian Peninsula) which have not been previously treated with antibiotics. For each soil, 15–20 subsamples, collected randomly from the top 20 cm of the A horizon, were mixed, sieved (<2 mm), thoroughly homogenized, and air-dried. The main soil properties, which have been previously determined by Rodríguez-González et al. (2022), are shown in **Table 1**. They showed different texture, similar pH (ranges of pH in water 5.1–5.6), and different organic matter content (1.61%–6.8% C).

Experimental Set Up

Of each of the four air-dried soil samples, 100 g was moistened to up to 70% of the water holding capacity and incubated in the dark at 22°C for 15 days to recover and achieve the stabilization of bacterial growth (Meisner et al., 2013). After that, they were

TABLE 1 | Main properties of the four acid soils studied (adapted from Rodríguez-González et al., 2022).

Soil	3	5	10	12
pH_W	5.6	5.7	5.2	5.1
pH_{KCl}	4.8	4.9	4.7	4.5
C (%)	1.1	4.8	3.2	6.8
N (%)	0.18	0.40	0.41	0.59
C/N	6.1	12.0	7.8	11.5
eCEC ($cmol_c\ kg^{-1}$)	6.0	17.4	4.1	5.5
OC ($mg\ kg^{-1}$)	243.1	332.9	327.1	318.4
Sand (%)	44	58	55	34
Silt (%)	33	20	28	38
Clay (%)	23	22	17	28
Texture	loam	Sandy clay loam	Sandy loam	Clay loam
Fe_o ($g\ kg^{-1}$)	2.2	2.8	8.9	8.9
Al_o ($g\ kg^{-1}$)	1.0	5.3	3.1	8.7

pH_W is pH measured in water; pH_{KCl} is pH measured in 0.1 M KCl; C is total carbon; eCEC is Cationic Exchange Capacity; OC is organic carbon; Al_o, Fe_o: Al and Fe extracted with ammonium oxalate.

separated into 8 centrifuge tubes (12 g in each tube) to be spiked with 7 different concentrations of clarithromycin (0.49, 1.95, 7.81, 31.25, 125, 500, and 2,000 mg kg^{-1} of soil), using talc as carrier to facilitate the mixture of the antibiotic with the soil and a blank to which the same amount of talc (48 mg) was added, but without antibiotic. These concentrations allowed the study of the effects of the antibiotic in a range that encompasses the toxicity of the antibiotic on soil bacteria from blank (no toxicity) to levels that suppress bacterial growth at high levels (Rodriguez-Gonzalez et al., 2021). The different mixtures of soil and antibiotic were incubated under controlled conditions (22°C, 70% of water holding capacity, darkness). After 8 days of incubation, 2 g samples were extracted in triplicate from each mixture and PLFA analysis was made. The same process was repeated after 42 days. Sampling was destructive for each treatment-time combination, resulting in a total of 192 microcosms (4 soils × 8 concentrations × 3 replicates × 2 times). The PLFA estimates were made using a representative composite soil sample of each antibiotic treatment obtained by mixing the three incubation replicates.

Phospholipid Fatty Acids (PLFA) Analysis

The total biomass (TotalPLFA) and the biomass of the specific microbial groups as well as the microbial community structure were estimated using the procedure and the nomenclature described by Frostegård et al. (1993). A detailed description of the method is given by Rodríguez-González et al. (2022). TotalPLFA was estimated as the sum of all the extracted PLFAs. The following PLFAs fatty acids were used as indicators of biomass of specific microbial groups: bacterial biomass (BactPLFA), the sum of i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:1ω8, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, and cy19:0 PLFAs; fungal biomass (FungPLFA), 18:2ω6 PLFA; actinobacteria biomass (ActPLFA), the sum of 10Me16:0, 10Me17:0, and 10Me18:0 PLFAs; Gram-positive bacteria biomass (G^+PLFA), the sum of i14:0, i15:0, i16:0, a15:0, i17:0, and a17:0 PLFAs; and Gram-negative bacteria biomas (G^-PLFA), the sum of cy17:0, cy19:0, 16:1ω7c, and 18:1ω7 PLFAs (Santás-Miguel et al., 2020b). In order to detect environmental effects (soil, incubation, antibiotic addition) on the soil microbial community structure, the concentrations of all the individual PLFAs, expressed in mole percent and logarithmically transformed, were subjected to principal component analysis (PCA). All statistical analyses were made using the SPSS 15.0 statistical package.

RESULTS AND DISCUSSION

The values of total (TotalPLFA) and specific microbial biomass (FungPLFA, BactPLFA, G^-PLFA, G^+PLFA, and ActPLFA) obtained for soil groups with nothing added and with different doses of the antibiotic clarithromycin added after 8 and 42 days of its application are shown in **Figures 1**, **2**, respectively. In general, the values observed for the two sampling times were quite similar. Therefore, for each soil type, the data obtained for un-treated and treated samples after 8 and 42 days of incubation were grouped together. Furthermore, in order to facilitate the interpretation of the results, the mean values of soil samples added with the 7 doses of clarithromycin were compared with the corresponding soil without antibiotic addition. In control S3 soil, total biomass showed values (mean values ±SE) of 35 ± 6 nmol g^{-1} and FungPLFA, BactPLFA, G^-PLFA, G^+PLFA, and ActPLFA values of 0.47 ± 0.16, 17.8 ± 3.1, 6.6 ± 0.8, 9.9 ± 2.1, and 2.7 ± 0.5 nmol g^{-1}, respectively. Similar values were found in the samples treated with clarithromycin, in which TotalPLFA, FungPLFA, BactPLFA, G^-PLFA, G^+PLFA, and ActPLFA values were 37 ± 3, 0.52 ± 0.07, 18.4 ± 1.1, 7 ± 0.5, 10.2 ± 0.5, and 2.9 ± 0.6 nmol g^{-1}, respectively. In S5, S10, and S12 soils, the magnitude levels of the total and specific biomass values were quite similar and much higher than those observed for S3 soil (**Figures 1**, **2**). These results can be explained by the organic C content of the soils, which is found to be positively correlated with the biomass estimates (Díaz-Raviña et al., 1988; Díaz-Raviña et al., 1993; Leirós et al., 2000). In general, as a consequence of antibiotic addition, the values of total and specific biomass of the S5, S10, and S12 soils increased. Thus, TotalPLFA biomass in these soils ranged from 117 ± 20 and 168 ± 33 nmol g^{-1} in control samples and from 162 ± 30 and 224 ± 56 nmol g^{-1} in antibiotic treated samples. FungPLFA biomass showed the lowest values of the specific microbial groups, ranging from 1.31 ± 0.32 and 1.88 ± 0.56 nmol g^{-1} in control soils and from 1.49 ± 0.47 and 4.16 ± 3.5 nmol g^{-1} in the soils added with clarithromycin. In contrast, BactPLFA biomass showed the highest values of the specific groups ranging from 49.3 ± 8.6 and 80.8 ± 18.5 nmol g^{-1} in un-treated control soils, and from 70.3 ± 26.4 and 104.2 ± 24.8 nmol g^{-1} in antibiotic polluted soils. G^-PLFA biomass values varied from 16 ± 2.3 and 31.2 ± 10.2 nmol g^{-1} in control soils and from 26.7 ± 14 and 35 ± 7.9 nmol g^{-1} in treated soils, while G^+PLFA biomass ranged from 29.3 ± 5.8 and 40.3 ± 9.7 nmol g^{-1} in control soils and from 41.2 ± 11.5 and 64.15 ± 15.33 nmol g^{-1} in antibiotic polluted soils. Finally, ActPLFA biomass ranged from 10.9 ± 1.9 and 14.9 ± 3.2 nmol g^{-1} in un-treated control soils and from 14 ± 4.6 and 20 ± 5.1 nmol g^{-1} in antibiotic polluted soils.

The total and specific biomass PLFA values in both the control studied soils and the corresponding soils added with clarithromycin, were within the reported range given for other soils of the same area added with other organic compounds such as atrazine (Mahía et al., 2011), tetracyclines, cyproflaxin, trimethoprim, and amoxicillin (Santás-Miguel et al., 2021; Rodríguez-González et al., 2022). Likewise, the relative importance of different specific groups (FungPLFA, BactPLFA, G^-PLFA, G^+PLFA, and ActPLFA) with respect to the TotalPLFA, as well as the ratios of Fung/Bact PLFA and Gr^-/G^+ PLFA, were similar to those reported in the mentioned studies. In agreement with a previous study, the lowest total and specific biomass values were exhibited by the soil with the lowest organic matter content (Rodríguez-González et al., 2022).

In four studied soils, after the incubation of the soils added with clarithromycin, the values of the total and specific biomass were generally similar (S3 soil) or higher (soils S5, S10, and S12 soils) than those in the equivalent control soils. Consequently, a positive effect of antibiotic addition, with

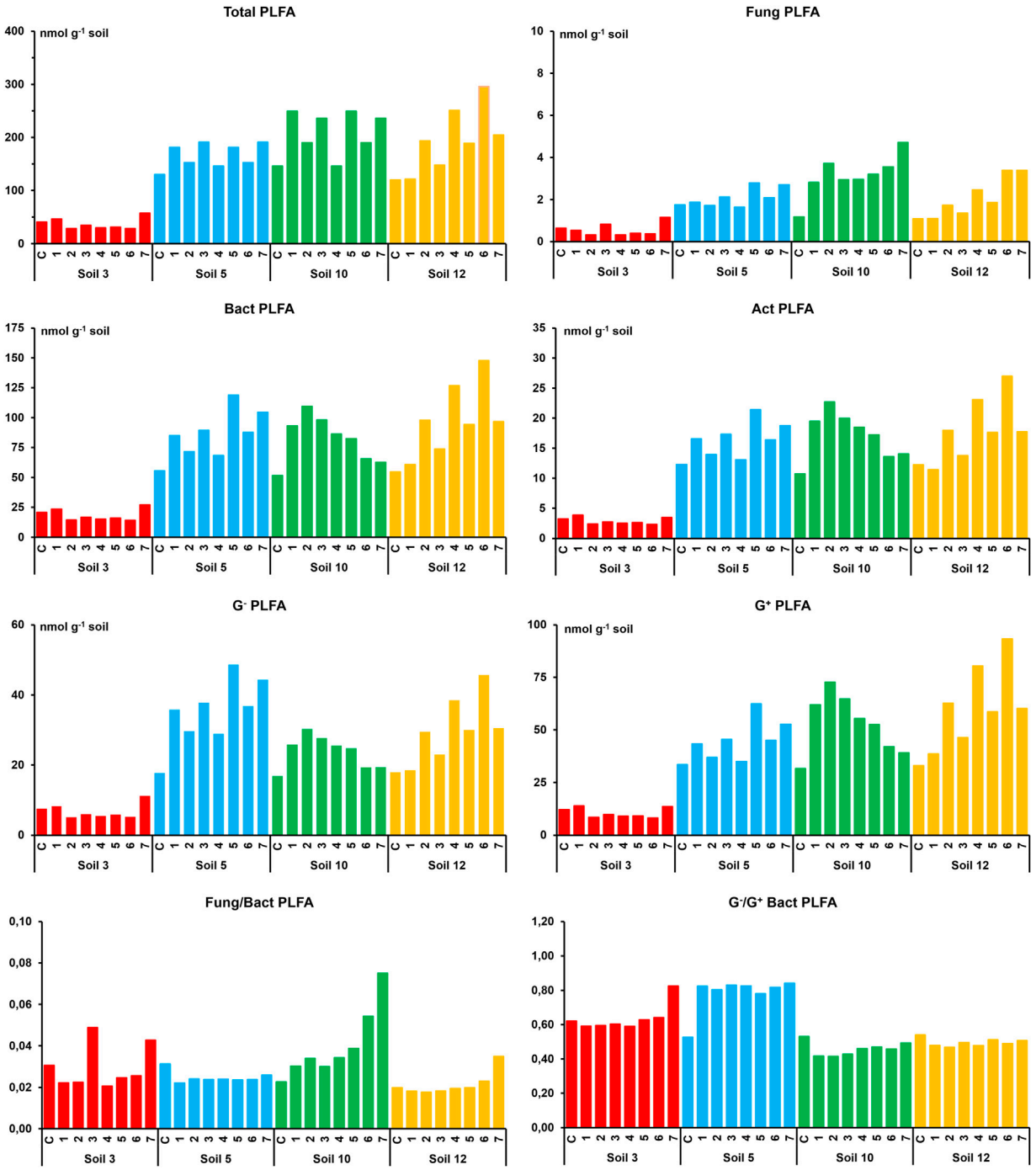

FIGURE 1 | Total and specific microbial biomass values (fungal, bacterial, actinobacteria, Gram-negative bacteria and Gram-positive bacterial) in four studied soils with nothing added (C) and with the different doses of clarithromycin after 8 days of incubation. Doses: 1 (0.49), 2 (1.95), 3 (7.81), 4 (31.25), (5) 125, 6 (500), and 7 (2000) mg kg^{-1} of soil.

increases up to 2 times the control value, was detected at medium- (8 incubation days) and long-term (42 days). Comparison of four soil trends at two sampling times showed slight differences among them, with increases slightly higher at 42 days for S3 and S5 soils while the opposite was observed for S10 and S12 soils. The higher biomass values, which were dose dependent, showed that fungal and $Gram^-$ bacterial groups are more favoured by the antibiotic addition than the bacterial and $Gram^+$ bacterial groups. This trend was evidenced by the values of the Fung/Bact PLFA and $Gram^-/G^+$ PLFA, which is

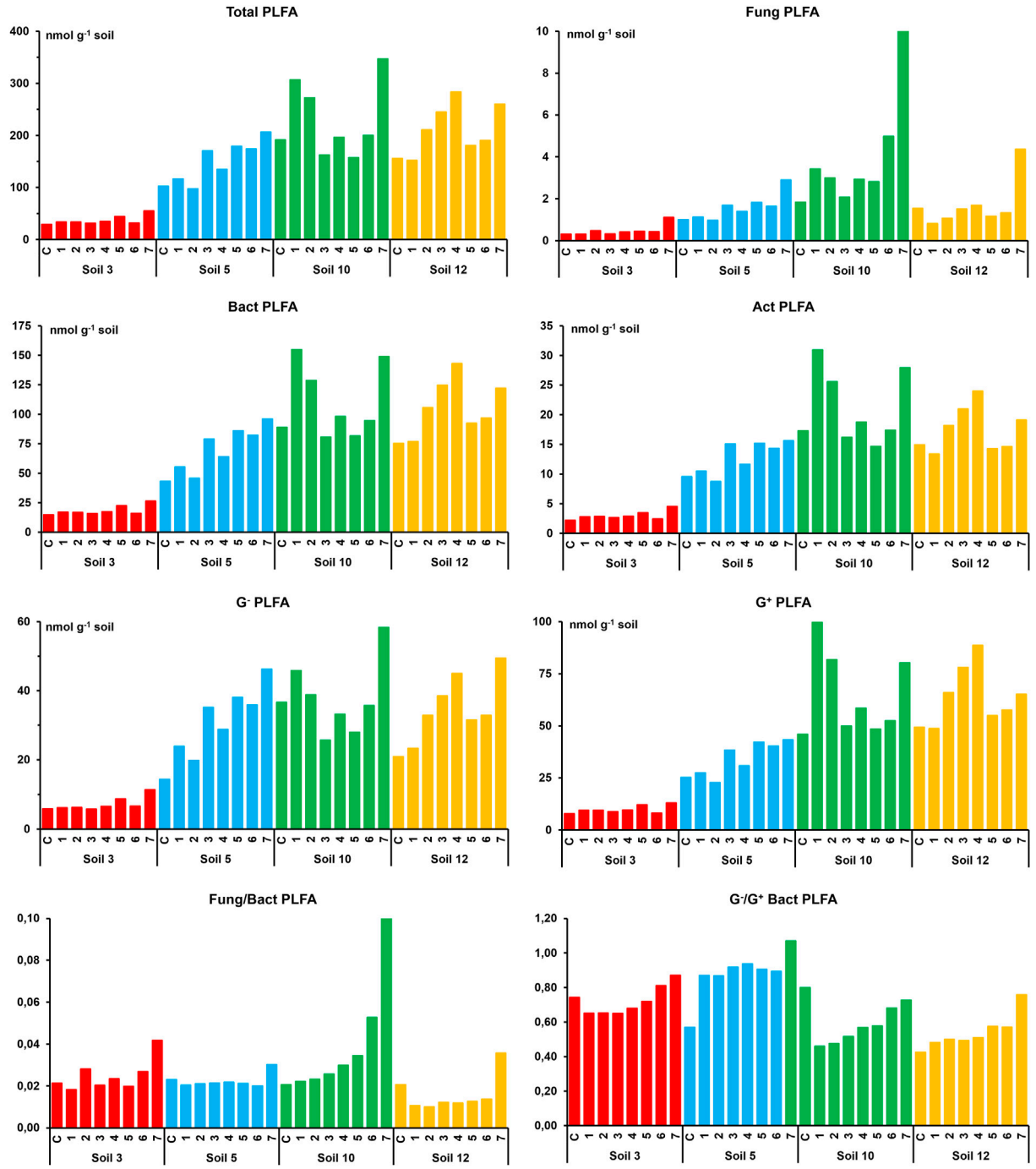

FIGURE 2 | Total and specific microbial biomass values (fungal, bacterial, actinobacteria, Gram-negative bacteria, and Gram-positive bacterial) in four studied soils with nothing added (C) and with the different doses of clarithromycin after 42 days of incubation. Doses: 1 (0.49), 2 (1.95), 3 (7.81), 4 (31.25), (5) 125, 6 (500), and 7 (2000) mg kg^{-1} of soil.

coincident with findings of other studies on this topic (Hammesfahr et al., 2008; Unger et al., 2012; Chen et al., 2013; Rodríguez-González et al., 2022). Inconsistent results were reported in the literature concerning the impact of veterinary and human antibiotics on biomass of soil microbial communities estimated by the PLFA method. According to the results of the present investigation, some authors showed that microbial biomass increased (Cordova-Kreylos and Scow, 2007) while other researchers observed that PLFA biomass values were unaffected or decreased as a consequence of the antibiotic presence (Thiele-Bruhn and Beck, 2005; Cui et al., 2014). The studies also showed that this variable response of microbial

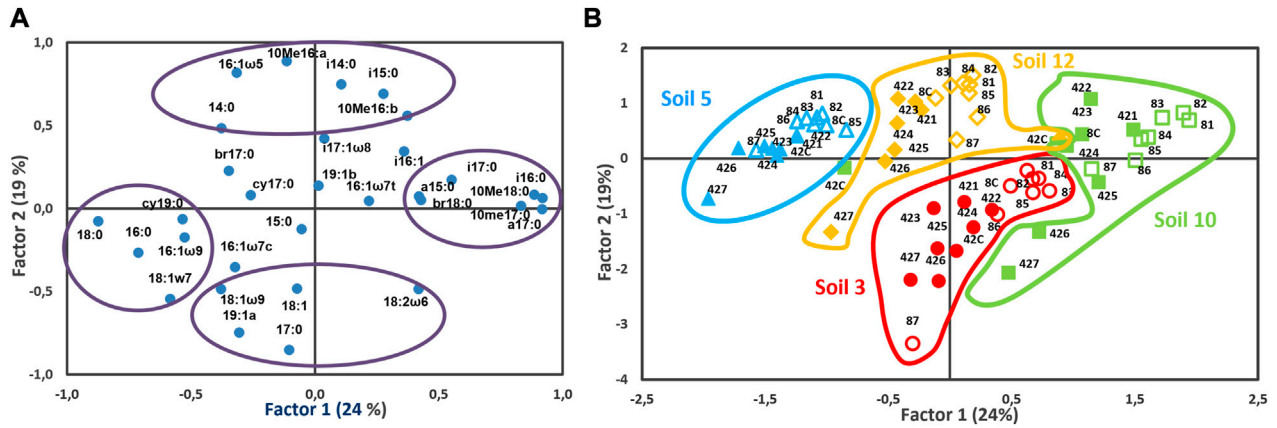

FIGURE 3 | Principal component analysis (**(A)**, variables; **(B)**, samples) performed with the PFLA data set for the four studied soils with nothing added (C) and with the different doses of clarithromycin after 8 (open symbols) and 42 (filled symbols) days of incubation. Doses: 1 (0.49), 2 (1.95), 3 (7.81), 4 (31.25), (5) 125, 6 (500), and 7 (2000) mg kg^{-1} of soil.

communities to the antibiotic addition depended on soil characteristics, type, and dose of antibiotic as well as time passed after its application (Cycón et al., 2019; Santás et al., 2020b; Santás et al., 2021; Rodríguez-González et al., 2022).

Figure 3 shows the results of the principal component analysis performed with the whole PLFA data set obtained for the four studied soils (S3, S5, S10, S12) with nothing added (control soil) and with different concentrations of clarithromycin added (0.49, 1.95, 7.81, 31.25, 125, 500 and 2,000 mg kg^{-1} of soil) after 8 and 42 days of incubation. The plane defined by the first and second component, accounting for the 43% of the variation, separated the soils independently of antibiotic addition and the time passed after its application. Samples of the same soil at two sampling times (8 and 42 days) and the antibiotic addition were grouped together and separated from the rest of the soils. S10 samples, having higher concentrations of i16:0, a17:0, 10Me18:0 and 10Me17:0 PLFAs, were located in the positive part of axis 1, S12 and S3 samples in the middle part and, finally, S5 samples, having higher concentrations of PLFAs 18:0, 16:0, cy19:0, 16:1ω9, and 18:1ω7, in the negative part. The axis 2 clearly separated S3 samples, having higher concentrations of PLFAs, at 17:0, 19:1a, 18:2ω6, 18:1, and 18:1ω9, from the rest of the soils (S5, S10, and S12), with higher values of 10Me16:0, 16:1ω5, i14:0, and i15:0. This axis also differentiated samples showing higher antibiotic concentrations, located in its negative part, which exhibited higher concentrations of PLFAs 18:2ω6 and 18:1ω9, indicative of fungi, and PLFA 19:1a, characteristic of G^- bacteria. Likewise, for each soil, a slight separation of samples incubated for 8 and 42 days was observed along axis 1 as well as a more defined separation of samples according the antibiotic addition, particularly after 42 days of incubation at higher doses.

To properly analyze the influence of the antibiotic addition on microbial community structure without the masking effect of time passed after its application, principal component analysis was made for each time separately (8 and 42 days after incubation) (**Figure 4**). Similar results were observed independently of sampling time (8 and 42 days of incubation). Again, the plane was defined by axis 1 and 2, accounting for 46% of the variation, samples of same soil were grouped together and separated from the other soils. Along axis 1, accounting for 23% of the variation, samples of S3 and S10 were located in the positive part of axis 1, while samples of S5 and S12 were situated in the negative part. The axis 2, accounting for 23% of the variation, clearly distinguished between samples with nothing added and those with an increasing dose of clarithromycin. Thus, in general, for all soils, independently of soil and sampling time, the highest effect of clarithromycin was observed at higher doses. It should be noted that, for all soils, the samples with higher doses of clarithromycin antibiotic had a relatively higher abundance of fungi, as indicated by PLFAs 18:2ω6 and 18:1ω9, and G^- bacteria, as indicated by PLFAs 18:1ω7, 19:1a, 16:1ω7c, and cy19:0.

Therefore, our data clearly demostrated that the main factor determining the source of variation of PLFA profiles among samples are soil characteristics followed by the incubation time and, to a lesser extent, the antibiotic dose. This is consistent with numerous field and laboratory studies concerning phospholipid fatty acid profiles performed with different soils following the impact of numerous stress agents (e.g., wildfire, prescribed fire, soil heating, fire retardants, post-fire rehabilitation treatments, vegetation type, soil management, climatic conditions, herbicide application, heavy metal pollution) which found that microbial communities of the same soil with different disturbances were grouped together and separated from microbial communities of other soils (Bossio et al., 1998; Díaz-Raviña et al., 2006, 2018; Fernández-Calviño et al., 2010; Mahía et al., 2011; Barreiro et al., 2015; Lombao et al., 2015). Likewise, a similar medium-term response of microbial communities was observed following the addition of antibiotics of both veterinary use such as tetracyclines (Santás-Miguel et al., 2021) and human use such as ciprofloxacin, trimethoprim, and amoxicillin (Rodríguez-González et al., 2022) to some soils of the same temperate humid zone.

The results of total and specific microbial biomass and microbial community structure determined by means of PLFA

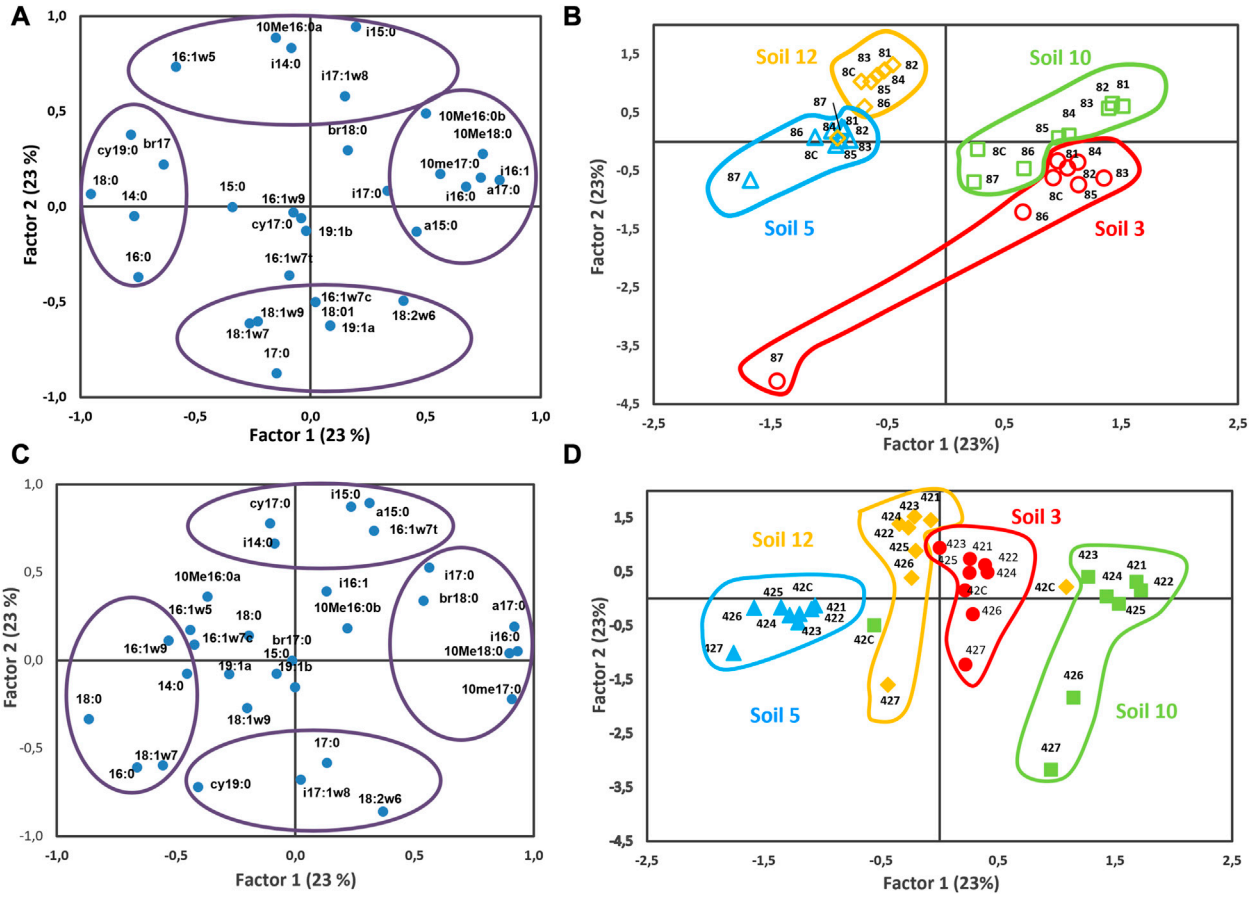

FIGURE 4 | Principal component analysis performed with the PFLA data set for the four studied soils with nothing added (C) and with the different doses of clarithromycin after 8 **(A,B)** and 42 **(C,D)** days of incubation. Doses: 1 (0.49), 2 (1.95), 3 (7.81), 4 (31.25), (5) 125, 6 (500), and 7 (2000) mg kg^{-1} of soil.

analysis were somehow coincident, showing that: a) the clarithromycin addition favoured fungi and G^- bacteria more than bacteria and G^+ bacterial and b) the effects observed short-term (8 days of incubation) changed very little at medium-term (42 days of indication). The PLFA pattern distinguished different microbial communities following the application of increasing concentrations of clarithromycin; however, this analysis does not allow us to determine if its impact is positive or negative. This information is derived from total and specific microbial biomass data which showed a positive influence on soil microbial communities. The results of a study of Díaz-Raviña et al. (1988) concerning the soil respiration and mineralization of soil organic matter (SOM) in soils with nothing added and with glucose added, located in the temperate humid zone, demonstrated that the C availability is the limiting factor of microbial community activity. Thus, the positive effect of clarithromycin on soil microorganisms can be explained by the use of the antibiotic as a source of C (Liao et al., 2016; Schofield, 2018) and/or the increase of C and nutrient availability derived from the death of non-target bacteria (bactericide effect) (Díaz-Raviña and Bååth, 1996; Rajapaksha et al., 2004). Likewise, an increased mineralization of the native SOM (priming effect) caused by trace concentrations of "tigger solutions" of original chlaritromycin and its secondary metabolites can not be discarded. The magnitude and the duration (8 and 42 days) of the increases of the microbial biomass seem to support the latter hypothesis. Our results are also in agreement with findings of other authors concerning the respiration or bacterial growth rate values following the addition of glucose, amino acids, root exudates (De Nobili et al., 2001), herbicides (Mahía et al., 2008), and antibiotics of veterinary or human origin (Rodríguez-González et al., 2022) to different soils. It should be noted that an increased biomass and bacterial growth rates cannot be necessarily related to an increase in the soil quality status, particularly in these acid soils with low C and nutrient availability where soil quality is closely related to quantity and quality of soil organic matter (SOM) (Carballas et al., 2015). Thus, for example, in soil S3 exhibiting the lowest SOM content and C/N ratio, an increased SOM over a prolonged time (more than 42 days) can provoke a decrease in SOM content and hence also in soil quality.

The values reported in the literature for half-lifes of clarithromycin in soils, estimated in laboratory experiments using labelled compounds, ranged from 8 days to almost no dissipation (Kodešová et al., 2016; Topp et al., 2016). This

behavior can be explained by the characteristics and concentration of the clarithromycin, the soil properties, as well as the time passed after application. In general, the higher half-life values or long-term persistence of clarithromycin were observed in more polluted soils with high sorption capacity (Cycón et al., 2019). This is associated not only with clarithromycin affinity by soil and chemical properties such as pH and properties related to the effective cation exchange properties (Rodríguez-González et al., 2022), but also with the soil microbial properties. The latter hypothesis is confirmed by Kodešová et al. (2016) who found higher dissipation half-lives in soils of better quality and, hence, with better microbial conditions for biodegradation processes than in lower quality soils. The history of field exposure to clarithromycin also showed a great influence on the response of microbial communities to the recent application of this antibiotic to the same soil; thus, repeated annual exposure to the antibiotic provided selective pressure to provoke changes in microbial composition (increase of tolerant microorganisms and decrease of sensitive ones) and, hence, a faster dissipation of clarithromycin (Topp et al., 2016; Lau et al., 2020). Evidence for long-term exposure leading to accelerated biodegradation of organic compounds in soils was previously detected for herbicides (Barriuso and Houot, 1996; Mahía et al., 2011).

In a previous investigation, we examined the bacterial growth rates of soils S3, S5, S10, and S12 not added and added with the same increasing concentration of clarithromycin over time (1, 8, and 42 days) (Rodríguez-González et al., 2022); therefore, data can be properly compared with those obtained in this investigation. For all soils, an inhibitory effect of clarithromycin on bacterial growth was detected; however, this toxicity disappeared gradually with time (8 days) and was no longer found after 42 days. In fact, for soil S3 after 8 days and for all soils after 42 days of incubation, bacterial growth rates increased exponentially between 8 and 42 days at higher doses of the antibiotic. In the present work, a positive effect of antibiotic was shown on total and specific microbial biomass values after both 8 and 42 days, the amended soils reaching values 1.5–2 times higher than those in the corresponding unpolluted soil. Therefore, the data supported our initial hypothesis about the fact that changes in bacterial growth observed as a consequence of clarithromycin addition were accompanied by changes in both microbial community composition and total and specific microbial biomass.

CONCLUSION

In summary, the results of the present study clearly demonstrated residual effects on total and specific microbial biomass (shifts in the positive direction) in these agricultural acid soils as a consequence of the clarithromicyn addition after 42 days of application. Since these microbial PLFAbiomass changes are associated with shifts in the microbial composition (PLFA pattern), it is quite probable that clarithromycin can have also an impact on functional microbial diversity. Further investigations including the measurements of microbial parameters related to mass, activity, microbial community structure, and functional microbial diversity should be conducted in short-, medium- and long-term studies in order to gain a deeper understanding of the persistence period of clarythromycine in terrestrial ecosystems as well as its risk assessment in human health, which is associated with its potential negative impact on soil functioning.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

Conceptualization; MA-E, DF-C, MD-R; Methodology: MA-E, DF-C, MD-R; Software: LR-G, VS-M, ÁM; Data curation: EG-C, ÁM, LR-G, MD-R; Writing-Original draft preparation: LR-G, VS-M, MD-R, ÁM; Visualization: MA-E, DF-C, MD-R, LR-G, VS-M; Investigation: MD-R, LR-G, VS-M, EG-C; Supervision: MA-E, DF-C, MD-R; Validation: MD-R, MA-E, DF-C; Writing-Reviewing and Editing: LR-G, MD-R, VS-M.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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