Science of the Total Environment 907 (2024) 168206

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microbial activity, community composition and degraders in the glyphosate-spiked soil are driven by glycine formation

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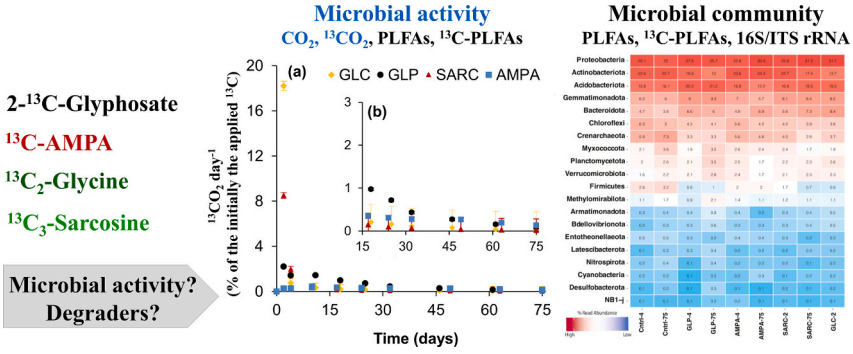
HIGHLIGHTS

- Soil microbial activity and degraders of glyphosate and its three degradation products was studied
- Soil microbial activity and mineralization rates of glycine were highest
- Gram negative bacteria were major degraders of all compounds
- Due to similar shifts in abundance of degraders, we deduce that glyphosate was degraded mainly to glycine
- Glycine indirectly changed microbial activity and community composition of soil

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jay Gan

Keywords:
Sarcosine
Glycine
AMPA
(^{13}C)-PLFAs
DNA Metabarcoding

ABSTRACT

Widely-used glyphosate may produce aminomethylphosphonic acid (AMPA), glycine and sarcosine. To date, little is known about effects of these degradation products on soil microorganisms and their potential degraders. Here, we incubated a soil spiked either with 2-^{13}C-glyphosate, ^{13}C-AMPA, $^{13}C_3$-sarcosine or $^{13}C_2$-glycine for 75 days. Respiration ($CO_{2\ tot}$) and mineralization rates of the compound ($^{13}CO_2$) were estimated in addition to phospholipid fatty acids ($PLFAs_{tot}$ and ^{13}C-PLFAs) as biomarkers to identify four groups of microorganisms (Gram-negative & Gram-positive bacteria, actinobacteria, fungi). 16S/ITS rRNA amplicon sequencing was also conducted to identify the microbial community at the phylum and genus level. The $CO_{2\ tot}$ and $^{13}CO_2$ rates were highest on day 2 in all treatments, as follows: glycine ($CO_{2\ tot}$: 1.09 μmol g^{-1}; $^{13}CO_2$: 18 %) > sarcosine ($CO_{2\ tot}$: 0.89 μmol g^{-1}; $^{13}CO_2$: 8.5 %) > glyphosate ($CO_{2\ tot}$: 0.67 μmol g^{-1}; $^{13}CO_2$: 2.2 %) > AMPA ($CO_{2\ tot}$: 0.53 μmol g^{-1}; $^{13}CO_2$: 0.3 %). Both the $PLFAs_{tot}$ and ^{13}C-PLFAs were highest in glycine ($PLFAs_{tot}$: 0.054–0.047 μmol g^{-1}; ^{13}C-PLFAs: 0.2–0.4 %) and glyphosate ($PLFAs_{tot}$: 0.049–0.047 μmol g^{-1}; ^{13}C-PLFAs: 0.1–0.3 %) treatments compared to sarcosine and AMPA treatments. Gram negative bacteria were major microbial group of soil microbiome as well as primary degraders of all compounds. In contrast, Gram-positive bacteria, actinobacteria and fungi could have been consumers of primary degraders. Certain genera e.g. *Gemmatimonas*, *Arenimonas* and *Massilia* showed increased abundance in certain treatments indicating their potential involvement in

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https://doi.org/10.1016/j.scitotenv.2023.168206
Received 22 August 2023; Received in revised form 22 October 2023; Accepted 27 October 2023
Available online 30 October 2023

biodegradation. Based on similar time-dependent microbial activity and shifts in abundances of (^{13}C-)PLFAs and 16S rRNA genera, we deduced that glyphosate was mainly degraded to glycine, and presumably at elevated amounts. We reported for the first time that the glycine presumably altered microbial activity and community composition rather than glyphosate directly. Future studies should thus also consider the potential impacts of degradation products of the parent compound on soil microbiomes.

1. Introduction

Glyphosate [N-(phosphonomethyl) glycine] has emerged as one of the most widely used herbicides (by volume and land area treated) worldwide because of its great efficacy against large number of weeds (Battaglin et al., 2014; Benbrook, 2016; Wang et al., 2014). As a consequence of glyphosate application, an activity and community composition of soil microbiome can be altered and through that also soil key ecological functions, e.g. nutrient cycling. An enhanced respiration of soil amended with glyphosate especially with its increasing concentration (Aslam et al., 2014; Haney et al., 2000; Lane et al., 2012; Nguyen et al., 2016; Van Bruggen et al., 2021; Weatherley et al., 2022) suggested that glyphosate stimulated the activity of microorganisms. In contrast, microbial community composition as shown by phospholipid fatty acid (PLFA) analysis, namely the proportions of Gram-positive & Gram-negative bacteria, actinobacteria and fungi were unchanged (Lane et al., 2012; Nguyen et al., 2018). However, shifts in bacterial community composition at the phylum level towards Acidobacteria, Actinobacteria, Bacteroidetes and Proteobacteria in response to glyphosate application to soils were noticed by Guijarro et al. (2018) who applied 16S rRNA gene amplicon sequencing method. Mathew et al. (2023) also revealed that glyphosate altered the abundance or frequency of some individual taxa of root-associated fungi and bacteria which might be due to their sensitivity to glyphosate.

Soil microorganisms degraded $^{13}C_3$-glyphosate to CO_2 and proteinaceous biomass (Aslam et al., 2023; Muskus et al., 2019), and four groups of degraders were identified using ^{13}C-PLFA approach (Muskus et al., 2022). The Gram-negative bacteria predominated the initial phases, whereas the Gram-positive bacteria, actinobacteria and fungi prevailed the later phases of the $^{13}C_3$-glyphosate degradation (Muskus et al., 2022). To date, mostly Gram-negative bacteria isolated from soil or raw sludge were reported to utilize glyphosate as a P (phosphorus) source. The following genera were identified: *Achromobacter* (Ermakova et al., 2017; Sviridov et al., 2012), *Agrobacterium* (Wackett et al., 1987), *Comamonas* (Firdous et al., 2020), *Flavobacterium* (Balthazor and Hallas, 1986), *Ochrobactrum* (Hadi et al., 2013; Sviridov et al., 2012) and *Pseudomonas* (Dick and Quinn, 1995). In addition, the Gram-positive bacterium *Bacillus* (Fan et al., 2012) and actinobacterium *Streptomycetes* (Obojska et al., 1999) were also capable to degrade glyphosate. Beyond bacteria, several genera of fungal glyphosate degraders were isolated from soils, e.g. *Aspergillus, Mucor, Scopulariopsis, Trichoderma* (Krzyśko-Łupicka and Orlik, 1997) and *Penicillium* (Klimek et al., 2001; Krzyśko-Łupicka and Orlik, 1997).

Glyphosate can be degraded to persistent aminomethylphosphonic acid (AMPA) and to either easily biodegradable sarcosine or glycine (Aslam et al., 2023; Carretta et al., 2021; Pérez Rodríguez et al., 2019; Sun et al., 2019a, 2019b). Therefore, not directly glyphosate, but its degradation product(s) may indirectly change soil microbial activity and community composition as well as define glyphosate degraders. Furthermore, previous studies investigated either the impact of glyphosate addition to soils on microbial activity or on community composition (Lane et al., 2012; Nguyen et al., 2016). No previous study has compared the impacts of glyphosate and its three degradation products (AMPA, glycine and sarcosine) on soil microbial activity and community composition as well as their potential degraders. Such study could clarify whether the impacts of glyphosate addition to soils on microorganisms and degraders are caused directly by glyphosate or indirectly by its degradation product. To address this current gap of research, we performed a comparative assessment of microbial activity and community composition using PLFA and 16S/ITS rRNA analyses in response to glyphosate and its three degradation products addition to soil. Furthermore, we also identified four groups of microbial degraders (Gram-positive & Gram-negative bacteria, actinobacteria and fungi) of both glyphosate and each degradation product using ^{13}C-PLFA approach.

Our recent study suggested that glyphosate was mainly degraded via the sarcosine/glycine pathway yielding the glycine or sarcosine degradation product (Aslam et al., 2023). Therefore, we hypothesized that microbial activity and community composition as well as degraders in the soil incubated with glyphosate would be thus similar to that of in soils treated with either glycine or sarcosine but different from the one which received AMPA. If proven true, this would suggest that the impacts of glyphosate addition to soils on microorganisms are possibly caused indirectly by either glycine or sarcosine rather than by glyphosate directly. The objectives of this study were to compare (i) respiration as a proxy for microbial activity, (ii) microbial community composition based on PLFA and 16S/ITS rRNA analyses, (iii) compound mineralization rates and (iv) potential degraders based on ^{13}C-PLFA in soil which was spiked either with 2-^{13}C-glyphosate or one of its three degradation products i.e. ^{13}C-AMPA, $^{13}C_2$-glycine and $^{13}C_3$-sarcosine.

2. Materials and methods

2.1. Reference soil

The soil used in this experiment was a Haplic Chernozem collected from the topsoil (0–10 cm layer) of the Static Fertilization Experiment in Bad Lauchstädt (Saxony-Anhalt, Germany). The soil received farmyard manure (30 t ha^{-1}) every second year and had a previous history of glyphosate application. The soil had 21 % of clay, 68 % of silt, and 11 % of sand, and it is classified as a silty loam based on US Department of Agriculture soil classification system. The total nitrogen was 0.17 %, total organic carbon (TOC) 2.1 %, pH 6.6 (Muskus et al., 2019) and maximum water holding capacity was 47 ± 1.9 % (based on our measurements in the laboratory). Soil was sieved through a 2 mm sieve, and it was stored in a cold room at 4 °C until start of incubation experiments. Soil moisture was adjusted to 60 % of maximum water holding capacity.

2.2. Chemicals and reagents

The unlabeled glyphosate (99 % purity), sarcosine (98 % purity) and glycine (99.7 % purity) were obtained from Sigma-Aldrich, Germany. The unlabeled AMPA (99 % purity) was purchased from Alfa Aesar, Thermo Fisher (Kandel) GmbH. Labeled 2-^{13}C-glyphosate (isotopic purity for ^{13}C: 99 %) was obtained from Sigma-Aldrich, Germany. Labeled degradation products of glyphosate: $^{13}C_3$-sarcosine (^{13}C: 99 %) and $^{13}C_2$-glycine (^{13}C: 99 %) were purchased from Cambridge Isotope Laboratories, Inc. USA, whereas labeled ^{13}C-AMPA (^{13}C: 99 %) from Toronto Research Chemicals, Canada. All labeled chemicals had a chemical purity of 98 %. All the other chemicals were obtained from Carl Roth (Karlsruhe, Germany) or VWR/Merck (Darmstadt, Germany). All solutions of labeled and unlabeled chemicals were prepared in milli-Q water at 50 mg kg^{-1} soil.

2.3. Experimental setup

2.3.1. Incubation

We carried out 75-day long soil incubation experiment to investigate the dynamics of respiration, compound mineralization, microbial community composition and groups of microbial degraders of glyphosate and its three degradation products glycine, sarcosine and AMPA. A reference soil was spiked with either unlabeled (control) or 2-^{13}C-labeled glyphosate, ^{13}C-AMPA, $^{13}C_2$-glycine, $^{13}C_3$-sarcosine at 50 mg kg^{-1} soil and placed into 500-mL Schott bottles. These higher initial spiking concentrations were necessary to overcome the background natural abundance of $^{13}C/^{12}C$ in soils (Muskus et al., 2019; Muskus et al., 2020). Unlabeled controls were used to correct for ^{13}C natural abundance in labeled counterparts. We also incubated a blank soil without compound in parallel to test the effect of the compound addition on soil respiration and microbial community composition. All soils were incubated at 20 °C in dark as per OECD (Organization for Economic Co-operation and Development) 307 guidelines (OECD, 2002). During the incubation, total CO_2 (soil respiration) and $^{13}CO_2$ (compound mineralization) were trapped in 2 M NaOH which was replaced regularly. After 2, 4, 10, 18, 24, 32, 46, 61 and 75 days of incubation, the NaOH traps were taken for analysis of total CO_2 and $^{13}CO_2$. In addition, soil was sampled at 0, 2, 4, 18, 32 and 75 days to analyze PLFAs for groups of microbial community composition (total PLFAs; $PLFAs_{tot}$) and compound's degraders (^{13}C-PLFAs) characterisations. We also took soil samples for DNA metabarcoding analyses (16S/ITS rRNA) in order to complete $PLFAs_{tot}$ analyses with detailed microbial community composition (bacteria and fungi) analysis after 2/4 and 75 days.

2.3.2. Soil respiration and compound mineralization

The total amount of CO_2 in NaOH traps was determined using a total organic carbon analyzer (Multi N/C 21005, Jena, Germany). The isotopic composition of CO_2 ($^{13}C/^{12}C$ at.%) was measured with gas chromatography-isotope ratio mass spectrometry (GC-IRMS; Finnigan MAT 252, Thermo Electron, Bremen, Germany, coupled to Hewlett Packard 6890 GC; Agilent Technologies, Germany) after separation on a BPX-5 column (50 m × 0.32 mm × 5 μm). The temperature parameters of GC-irMS had been previously described by Girardi et al. (2013). The mineralization of each compound ($^{13}CO_2$) was calculated from the total amount of CO_2 and its isotopic composition ($^{13}C/^{12}C$ at.%) (Girardi et al., 2013).

2.3.3. Phospholipid fatty acids (PLFAs)

The extraction, purification and derivatization procedures for PLFAs were described in detail by Nowak et al. (2011). Briefly, the PLFAs were extracted from soil with a mixture of phosphate buffer/methanol/chloroform, purified over silica gel and then derivatized with methanol/trimethylchlorosilane (MeOH/TMCS) to fatty acid methyl esters (FAMEs). The total amounts of PLFAs were determined as FAMEs using gas chromatography–mass spectrometry (GC–MS; HP 6890, Agilent) equipped with a BPX-5 column (30 m × 0.25 m × 0.25 μm). The isotopic composition of FAMEs ($^{13}C/^{12}C$ at.%) was measured with GC-IRMS. The temperature and other parameters for FAMEs separation using GC–MS and GC-IRMS were described by Nowak et al. (2011).

The PLFA biomarker is used to estimate the activity of the following four groups of microorganisms in the environment (Zelles, 1999): I: Gram-positive bacteria (iso- and anteiso-branched PLFAs), II: actinobacteria (sub-group of Gram-positive bacteria; 10-methyl branched PLFAs), III: Gram-negative bacteria (monounsaturated PLFAs) and IV: fungi (polyunsaturated PLFAs). In addition, the starvation of Gram-negative bacteria also can be elucidated based on cyclopropyl PLFAs (Kaur et al., 2005). Saturated straight-chain PLFAs which do not indicate any microbial group were included into the quantitation of $PLFAs_{tot}$ and ^{13}C-PLFAs.

2.3.4. DNA metabarcoding

DNA metabarcoding technique was used to investigate the abundance of microbial populations based on family, genetic and phyla in soils. Briefly, DNA from soil samples was extracted using soil kit and sent to the company for 16S and ITS rRNA sequencing.

2.3.4.1. DNA extraction. DNA was extracted and purified from 0.25 g of soil with the DNeasy PowerSoil Kit (Qiagen, Benelux BV) according to the specifications of the company. The final volume of the DNA was 100 μL, and its concentration was monitored by a Qubit 3.0 fluorometer (Invitrogen, Life technologies) using the Qubit dsDNA HS (high sensitivity) kit (Thermo Fisher Scientific). DNA samples were stored at −80 °C until they were transported to the commercial company Allgenetics (Spain) for PCR amplification and sequencing.

2.3.4.2. Amplicon sequencing. The DNA concentration in the samples was adjusted to 0.01 ng μL^{-1} and distributed in a 96-well plate for PCR amplification of the 16S rRNA gene. For library preparation, a fragment of the bacterial 16S rRNA region of ~300–350 bp was amplified using the primers 515F (5′ GTG YCA GCM GCC GCG GTA A 3′) (Parada et al., 2016) and 806R (5′ GGA CTA CNV GGG TWT CTA AT 3′) (Apprill et al., 2015). For fungi, the complete fungal ITS2 region of ~300 bp was amplified using the primers ITS86F (5′ GTG AAT CATCGA ATC TTT GAA 3′) (Turenne et al., 1999) and ITS4 (5′ TCC TCC GCT TAT TGA TAT GC 3′) (White et al., 1990). PCRs were carried out in a final volume of 25 μL, containing 2.5 μL of template DNA, 0.5 μM of the primers, 6.5 μL of Supreme NZYTaq 2× Green Master Mix (NZYTech), and ultrapure water up to 25 μL. The reaction mixture was incubated as follows: an initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 50 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. The oligonucleotide indices which are required for multiplexing different libraries in the same sequencing pool were attached in a second PCR round with identical conditions but only 5 cycles and 60 °C as the annealing temperature. A negative control that contained no DNA (BPCR) was included in every PCR round to check for contamination during library preparation. The libraries were run on a 2 % agarose gel stained with GreenSafe (NZYTech) and imaged under UV light to verify the library size. Libraries were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek), following the instructions provided by the manufacturer. Then, they were pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific). The pool was sequenced in a fraction of a MiSeq PE300 run (Illumina).

Quality control and processing of sequencing data. Illumina paired-end raw files consisted of forward and reversed reads sorted by library and their quality scores. The indices and sequencing primers were trimmed during the demultiplexing step. The quality of the FASTQ files was checked using the software FastQC (Andrews, 2010) and summarized using MultiQC (Ewels et al., 2016).

The obtained 16S/ITS amplicon reads were processed using QIIME2 (release 2020.8). DADA2 (Callahan et al., 2016), implemented in QIIME2, was used to: remove the PCR primers, filter the reads according to their quality, denoise, merge the reads, remove the chimeric reads, and cluster the resulting sequences into amplicon sequence variants (ASVs). Due to the fact that the sequencing reads were longer than the 16S/ITS amplicons, non-biological DNA (primers, indices, and sequencing adapters) can appear at the reads ends. Therefore, we used cut-adapt (Martin, 2011) as a primer/adapter removal tool to trim these regions. For visualization, AMPVIS2 package was used in R to plot diversity analyses and heatmaps. All sequences were submitted to the NCBI GenBank Sequence Read Archive under the study accession number PRJNA951409.

2.4. Data analysis

All incubation treatments were carried out with three repetitions and all results are presented as averages and standard deviations, Respiration of soil ($CO_{2\ tot}\ g^{-1}\ day^{-1}$) was based on the quantitation of total CO_2 ($^{12}C + {}^{13}C$) rate per day. Respiration was used as a proxy for microbial activity following the addition of the respective compound to soil. By contrast, the $^{13}CO_2$ daily rates referred to CO_2 released from the mineralization of the compound. The total concentration of PLFAs ($^{12}C + {}^{13}C$; $PLFAs_{tot}$) indicated changes in the microbial biomass quantity and the shifts between microbial groups (four groups mentioned in Section 2.3.3) over 75 days of incubation. The amounts of ^{13}C-PLFAs enabled time-dependent quantification of ^{13}C-label incorporation into microbial biomass (microbial group of degraders) from the ^{13}C-labeled compound and the shifts between the groups of microbial degraders.

The 16S rRNA was only complementary technique to $PLFAs_{tot}$ & ^{13}C-PLFA and $CO_{2\ tot}$ & $^{13}CO_2$ and to demonstrate shifts in microbial community on the phylum & genus level following the addition of a tested substrate. Therefore, 16S/ITS rRNA results are shown as a pooled single measurement of triplicates. The ^{13}C-DNA technique provides much higher taxonomic resolution compared to ^{13}C-PLFAs, but it requires much higher amount of ^{13}C to be incorporated into DNA than into PLFAs (Kästner et al., 2016). The ^{13}C-DNA-SIP method rely on a separation of heavy ^{13}C-DNA from the light counterpart using density-gradient centrifugation (Dumont and Murrell, 2005); therefore, much higher initial amount of the compound than 50 mg kg^{-1} would be necessary.

The calculation of amounts of ^{13}C-label in $^{13}CO_2$ and in ^{13}C-PLFAs were based on the quantitation of their total carbon pools ($^{12}C + {}^{13}C$) and the determination of the ^{13}C excess over the controls as described previously (Wang et al., 2016a, 2016b). The amounts of ^{13}C in the respective fractions were expressed as percentages of the initially applied ^{13}C equivalents. The analytical uncertainty of the total carbon pool in each fraction was <5 %, whereas the uncertainty of at.% ^{13}C isotope signatures was <2 % and <5 % (of at.% ^{13}C) for unlabeled and labeled samples, respectively. A One-Way ANOVA was performed to test for a significant difference in respiration and mineralization rates and microbial biomass/groups using R software; the differences were considered significant when $p < 0.05$. The time-dependent shifts between the four groups of microorganisms ($PLFAs_{tot}$ and ^{13}C-PLFAs) and the microbial populations (16S/ITS rRNA) based on the phylum & genus level were visualized in heat maps using R software.

3. Results and discussion

3.1. Soil respiration rate and microbial biomass

An addition of glycine, sarcosine and glyphosate to soil enhanced respiration accordingly by 91 % (1.09 ± 0.003 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$), 56 % (0.89 ± 0.07 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$) and 18 % (0.67 ± 0.01 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$) as compared to the control without any compound (0.57 ± 0.04 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$) on day 2 (Fig. 1). In contrast, the respiration of soil treated with AMPA (0.53 ± 0.05 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$) was similar to that of the control ($p > 0.05$). For all compounds, the respiration rate was highest on day 2 ($p < 0.05$), then it gradually decreased towards the end (0.15 ± 0.02–0.33 ± 0.08 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$on day 75). Only the soil respiration with sarcosine (0.33 ± 0.08 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$) was slightly higher than for other compounds (0.16 ± 0.018 μmol $CO_{2\ tot}\ g^{-1}\ day^{-1}$) on day 75. Soil respiration played a major role on microbial biomass ($PLFAs_{tot}$) sat an early incubation period (i.e. 2 days), especially for glycine, sarcosine and glyphosate, which are clustered together in Fig. S1 in Supplementary Information (SI).

Evolution of microbial biomass based on $PLFAs_{tot}$ analysis in soils of four treatments (Fig. 2) was similar to the trends of respiration rates. The $PLFAs_{tot}$ were highest in soil spiked with glycine (0.054 ± 0.002 μmol g^{-1}) or glyphosate (0.049 ± 0.0022 μmol g^{-1}) on day 4. Lowest magnitude of $PLFAs_{tot}$ were observed in AMPA and sarcosine treatments, as well as in control ($p < 0.05$) which were at least two times lower than those in glycine and glyphosate treatments. We also observed that the $PLFAs_{tot}$ in both glycine and glyphosate treatments were nearly constant throughout the incubation period. In contrast, the $PLFAs_{tot}$ decreased on day 75 in soils spiked with AMPA or sarcosine as well as in control.

Highest amounts of microbial biomass as well as respiration of soil spiked with glycine, sarcosine or glyphosate suggest that these compounds promoted microbial activity, and especially glycine. These findings are in a good agreement with previous studies with glycine (Apostel et al., 2013; McFarland et al., 2010; Xue et al., 2022; Zhang et al., 2019) and glyphosate (Lane et al., 2012; Muskus et al., 2022; Nguyen et al., 2016). Glycine and sarcosine that are a by-product of the glycine (*N*-methylglycine), are amino acids. Amino acids are a source of nutrients, building blocks for protein synthesis and energy to microorganisms (Dai et al., 2022). Two amino acids (alanine and glutamic acid) added to soil were also instantly consumed by soil microorganisms for

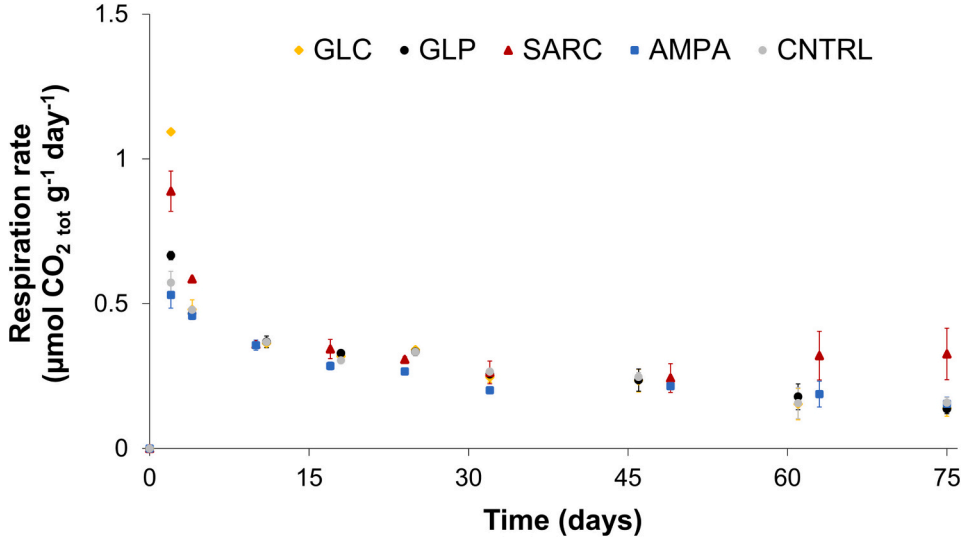

Fig. 1. Respiration rate (μmol $CO_2\ g^{-1}\ day^{-1}$) of control soil (CNTRL) and of soil spiked with glycine (GLC), glyphosate (GLP), sarcosine (SARC) or AMPA during 75-day incubation experiment. Error bars represent standard deviations of means (n = 3).

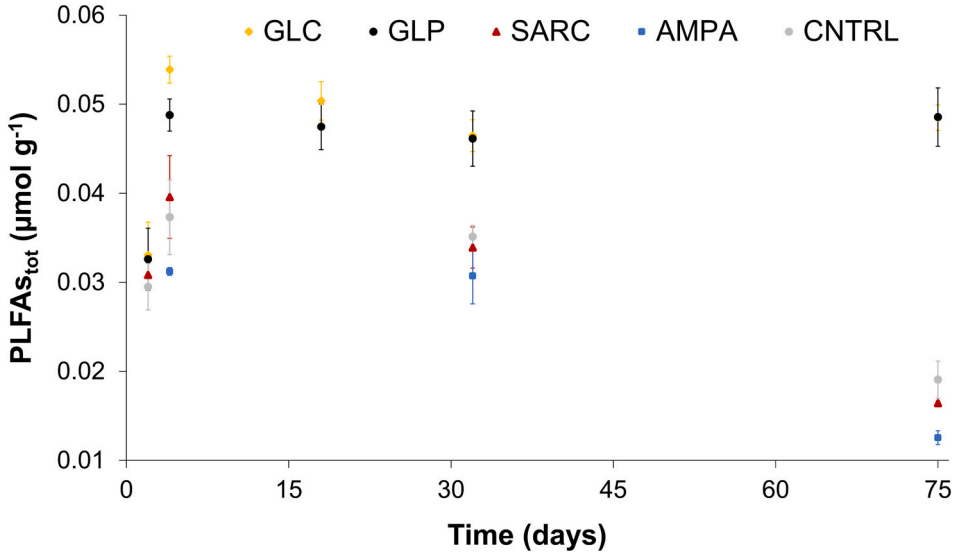

Fig. 2. $PLFA_{stotal}$ (µmol g^{-1}) in control soil (CNTRL) and spiked with glycine (GLC), glyphosate (GLP), sarcosine (SARC) and AMPA during 75-day incubation experiment. Error bars represent standard deviations of means (n = 3).

the synthesis of PLFAs (Apostel et al., 2013). Therefore, an enhanced soil respiration in both glycine and sarcosine treatments in an early phase of incubation (day 2) as compared to other treatments was expected. In line with soil respiration exhibited in this study, most of $^{13}C_2$-glycine (80 % of total $^{13}CO_2$) and $^{13}C_3$-sarcosine (63 % of total $^{13}CO_2$) was mineralized after 2 days of the soil incubation in our previous study by Aslam et al. (2023).

Comparable microbial biomass and respiration of soil spiked with AMPA with that of the control indicate no significant effect of the AMPA addition to soil on microbial activity, despite the fact that AMPA is persistent (Aslam et al., 2023; Battaglin et al., 2014; Domínguez et al., 2016). Based on this finding, it is difficult to estimate whether AMPA had any effect on soil microorganisms. Although the respiration of soil spiked with sarcosine was higher than in control, $PLFAs_{tot}$ data however suggest lower magnitude of microbial biomass in soil. This result is difficult to explain; and it could be due to low sarcosine use efficiency by microorganisms as a C-substrate (Oliver et al., 2021).

3.2. *Microbial community composition based on $PLFAs_{tot}$-group and 16S/ITS rRNA phyla*

Gram-negative bacteria were the most abundant group of microorganisms within the soil microbial community in all treatments (Fig. 3), which were followed by Gram-positive bacteria and then by actinobacteria and fungi. Higher bacterial species richness was noticed for glyphosate (day 4: 1465, day 75: 1775, see SI Fig. S2), glycine (day 2: 1550), sarcosine (day 2: 1625, day 75: 1475) and AMPA (day 4: 1420, day 75: 1460) treatments as compared to control (day 4: 1305, day 75: 1430). Bacterial species abundances were also higher for soils with the compound, i.e., glyphosate (day 4: 6.68, day 75: 6.79), glycine (day 2: 6.69), sarcosine (day 2: 6.77, day 75: 6.65) and AMPA (day 4: 6.68, day 75: 6.61) than in the control (day 4: 6.56, day 75: 6.65, SI Fig. S3). These results suggest that addition of tested compounds to soil increased the diversity of bacterial community as well as their abundances. However, this effect was more apparent for glyphosate, glycine and sarcosine treatments and least in AMPA treatment. The time-dependent changes in richness (SI Fig. S2) and abundance (SI Fig. S3) of bacterial species in each treatment might be related to a different biodegradability of the compound. For instance, an observed increase in richness & abundance of bacterial species in the sarcosine treatment on day 2 versus day 75 is probably related to its ready biodegradability (Carretta et al., 2021). Glyphosate, which is biodegraded a bit slower than sarcosine (Aslam et al., 2023) might have not enhanced the abundance & richness of bacterial species at early phase of degradation (day 4), but later on day 75.

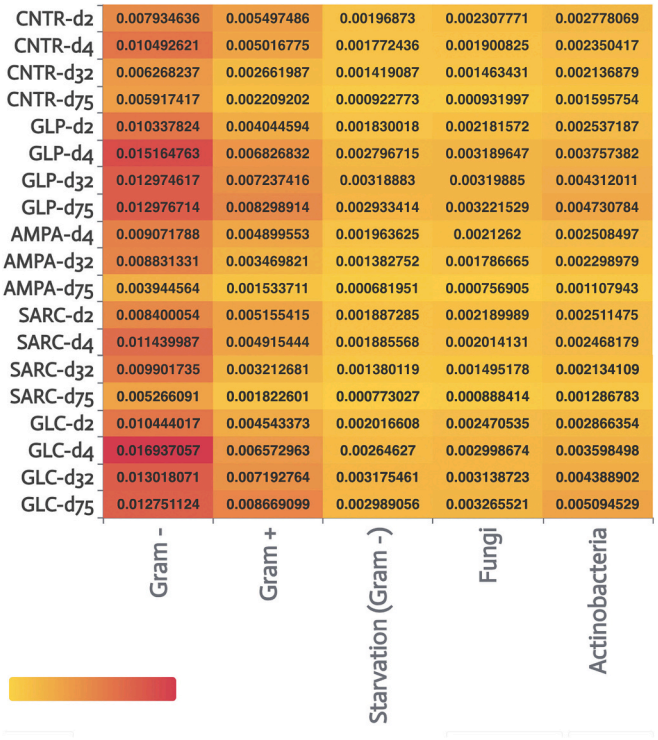

Fig. 3. Heat map representing abundance of four major groups (Gram-positive & Gram-negative bacteria, actinobacteria and fungi) of soil microbial community and starvation biomarker for Gram-negative bacteria based on $PLFA_{stotal}$ (µmol g^{-1}) in control soil (CNTRL) and spiked with glycine (GLC), glyphosate (GLP), sarcosine (SARC) and AMPA during 75-day incubation experiment. Scale in the heat maps shows minimum (light brown color) to maximum (reddish color) contribution of microbial groups.

The addition of glyphosate, glycine or sarcosine to soil increased the relative abundances of Gram-negative bacteria on day 4 as compared to control and especially in soils spiked with glycine and glyphosate

(Fig. 3). Similar observations were reported in other degradation studies with readily available substrates like glyphosate (Muskus et al., 2022) and amino acids alanine and glutamic acid (Apostel et al., 2013) in soils. It is noteworthy here that the abundance of Gram-negative bacteria was highest on day 4 in all treatments including the control set-up without a compound. Mixing of soil with water during the preparation of incubation vessels seems to have increased an availability of C and other nutrients in the soil to Gram-negative bacteria explaining their initially highest abundance in all soils (Moore-Kucera and Dick, 2008). After day 4 sampling, contribution of Gram-negative bacteria decreased in all treatments except for AMPA. The observed decrease in the abundance of Gram-negative bacteria is in line with an increasing abundance of the Gram-negative bacteria starvation marker (Fig. 3).

The Gram-negative bacteria were dominated by two phyla Proteobacteria and Acidobacteriota in all treatments (16S rRNA analysis, Fig. 4). These two phyla are widely-known to dominate in the soil bacterial community (Deng et al., 2018; Montecchia et al., 2015). Both phyla play a key role in biogeochemical cycling of elements and have a capability to degrade monomeric and polymeric substrates (Kalam et al., 2020; Mhete et al., 2020). The Gram-negative bacteria were reported to grow rapidly in soils following the addition of easily available substrates (Moore-Kucera and Dick, 2008). After spiking of the reference soil with a test substrate (glyphosate or its degradation product), soil was thoroughly mixed with water prior to incubation. In addition to the test substrate, the availability of other C-substrates and macronutrients to microorganisms and in particular to Gram-negative bacteria might have enhanced as a result of the soil microcosm preparation. This might also explain an enhanced abundance of the Gram-negative bacteria (including Proteobacteria and Acidobacteriota) in all treatments.

The abundance of four phyla Proteobacteria, Acidobacteriota, Gemmatimonadota and Bacteroidota was highest in glyphosate and glycine treatments on day 2/4. These findings are also consistent with results of previous studies. An increased abundance of Proteobacteria, Acidobacteria (Newman et al., 2016; Lupwayi et al., 2020), Gemmatimonadota (Arango et al., 2014), Firmicutes (Lupwayi et al., 2020), and Bacteroidota (Guijarro et al., 2018; Lupwayi et al., 2020) was also found in soils exposed to glyphosate as compared to controls. Moreover, many other pesticides such as imidacloprid, benomyl, and metribuzin (Astaykina et al., 2022); dimethachlor and linuron (Medo et al., 2021) showed positive effect on abundance of Proteobacteria and Acidobacteria. in soil. The Gemmatimonadota phylum has been poorly investigated and its abundance was positively correlated with high C and nutrient contents in soils (Liu et al., 2021; Mujakić et al., 2022). Both glyphosate and glycine as an additional C-substrate thus might have enhanced the abundance of Gemmatimonadota in this study. We also noticed an enhanced abundance of Gemmatimonadota in sarcosine treatment; this suggests that glycine might have been formed from sarcosine in the sarcosine pathway of glyphosate (Zhan et al., 2018). Furthermore, another phylum - Bacteroidota were reported to be copiotrophs growing on nutrient- and C-rich substrates as well as to degrade complex organic C-substrates (Fierer et al., 2012). We have already remarked the highest microbial activity (based on $PLFAs_{tot}$) of soil spiked with glyphosate or glycine in the previous Section 3.1. Therefore, the noticeable abundance of Bacteroidota in these two treatments could have been induced by a C-substrate added, especially the readily biodegradable glycine. Contrastingly, we also noticed an increase in the abundance of Bacteroidota on last day 75 in soils spiked with sarcosine and AMPA as compared to their counterparts on day 2/4 sampling. The increased abundance of Bacteroidota in the sarcosine treatment indicated that bacteria of this phylum could use decaying biomass of primary degraders of sarcosine as a potential C-substrate, whereas that in the AMPA treatment the slowly biodegradable AMPA. Interestingly, we also observed that abundance of Acidobacteriota at the end of incubation (day 75) was lowest in soil spiked with AMPA compared to all treatments including control (Fig. 4). This indicates AMPA might have had toxic impact on microorganisms belonging to this phylum.

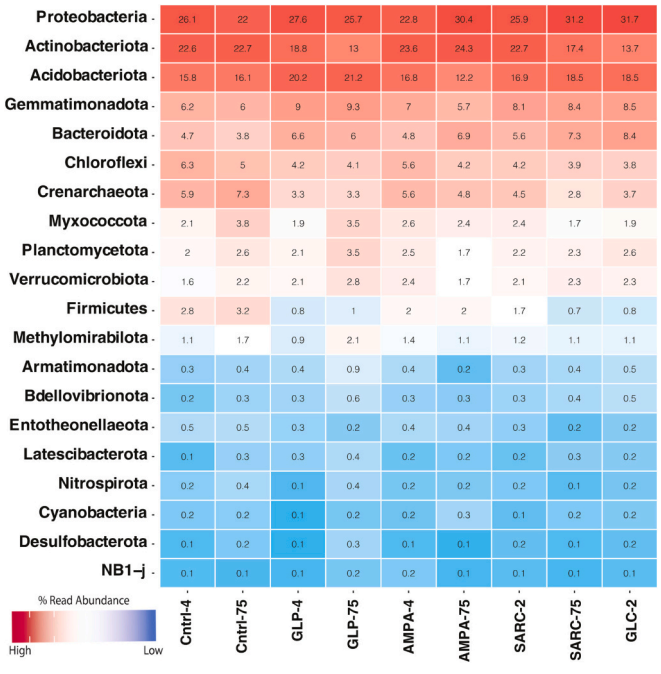

Fig. 4. Heat map of relative abundance of bacterial phyla based on 16S rRNA analysis in control soil (CNTRL) and spiked with glycine (GLC), glyphosate (GLP), sarcosine (SARC) and AMPA on day 2/4 versus day 75. **Gram-negative bacteria:** Proteobacteria, Acidobacteriota, Gemmatimonadota, *Bacteroidota,* Chloroflexi, Crenarchaeota, Myxococcota, Verrucomicrobia, Methylomirabilota, Armatimonadota, Bdellovibrionota, Latescibacterota, Nitospirota, Cyanobacteria, Desulfobacterota. **Gram-positive bacteria:** Firmicutes. **Actinobacteriota** (Gram-positive bacteria phylum). **Unclassified:** Planctomycetota, Entotheonellaeota. Scale in the heat maps shows minimum (blue color) to maximum (reddish color) contribution of microbial groups.

Second most abundant group as indicated by $PLFAs_{tot}$ was Gram-positive bacteria, whereas both actinobacteria and fungi were least abundant in all treatments (Fig. 3). The abundance of Gram-positive bacteria, actinobacteria and fungi were also greatest in soil spiked with glyphosate or glycine and especially after day 4 sampling. The Gram-positive bacteria including actinobacteria and fungi are known to act as decomposers of decaying microbial biomass (necromass) and when easily available C-substrates are depleted (Kramer and Gleixner, 2006; Moore-Kucera and Dick, 2008; Rinnan and Baath, 2009). This thus explains a higher abundance of these three groups (Gram-positive bacteria, actinobacteria and fungi) at the later phase of soil incubation (after 4 days) in this study. Contrastingly, Actinobacteriota phylum (16S rRNA analysis, Fig. 4) was least abundant in soil treatments spiked with glyphosate and glycine as compared to control; whereas that of AMPA was highest. This result is difficult to explain. Nevertheless, the trend in time-dependent abundances of actinobacteria in the $PLFAs_{tot}$ analysis seems to be more consistent than the one when looking at the 16S rRNA data.

Noteworthy here is that the time-dependent abundances of Gram-negative and positive bacteria, actinobacteria and fungi in soil spiked with glyphosate shared similarity with that of glycine treatment (Fig. 3). The Gram-negative, starvation of Gram-negative bacteria and fungi on day 4 as well as Gram-positive bacteria and actinobacteria on day 32 and 75 are clustered together in both glycine & glyphosate treatment (SI Fig. S1). Furthermore, the following four phyla; Proteobacteria, Acidobacteriota, Gemmatimonadota and Bacteroidota (Fig. 4) were nearly equally abundant in these two treatments. The time-dependent similarity of $PLFA_{tot}$-groups and phyla of the glyphosate treatment with that of the glycine one might be attributed to the fact that high amounts of glycine could have been formed during glyphosate biodegradation (Sun et al., 2019a, 2019b). From these findings we thus deduce that

glyphosate might have been biodegraded mainly to glycine rather than to sarcosine or AMPA.

We observed a higher abundance of fungi after 4 days of incubation in glycine and glyphosate treatments compared to control, sarcosine and AMPA treatments (Fig. 3). An addition of glyphosate or AMPA to soil also affected species richness and abundance of fungi. Fungal species richness (ITS rRNA; SI Fig. S4) and abundance (Fig. SI S5) in both glyphosate (richness: 167, abundance: 3.74) and AMPA (richness: 153, abundance: 3.57) treatments were lower than that of in control (richness: 177, abundance: 3.9). We also noticed shifts in the fungi at the phylum level in glyphosate and AMPA samples as compared to control (SI Fig. S6). Ascomycota were more abundant in glyphosate and AMPA treatment as compared to control soil. The Ascomycota prevailed in heavy metal (Narendrula and Nkongolo, 2017) and microplastic (Temporiti et al., 2022) contaminated soils than in the uncontaminated ones. Therefore, an addition of glyphosate or AMPA to soil might have induced the shift of fungi towards the Ascomycota due to either enhanced tolerance to the compound or capability to degrade the compound. It is noteworthy that the abundance of Basidiomycota in the AMPA treatment was greatest; this fungal phylum might have a capability to degrade AMPA in this study. A white-rot Basidiomycota fungi were reported to degrade a great variety of organic contaminants like pharmaceuticals, pesticides, heavy metals and polymeric compounds (Bosco and Mollea, 2019; Henn et al., 2020; Nguyen et al., 2022; Vaksmaa et al., 2023).

3.3. Mineralization of glyphosate and its three degradation products

Daily mineralization rates of ^{13}C labeled compounds ($^{13}CO_2$ day^{-1}) were significantly different from each other especially during early days of incubation experiment (Fig. 5). At day 2 sampling, highest mineralization rate was observed for glycine treatment (18 ± 0.4 % of the initial $^{13}C_2$-glycine equivalents; $p < 0.05$). Second fastest mineralization occurred for sarcosine treatment (8.5 ± 0.3 % of the initial $^{13}C_3$-sarcosine equivalents) followed by glyphosate (2.2 ± 0.01 % of the initial 2-^{13}C-glyphosate equivalents) and slowest in AMPA (0.3 ± 0.02 % of the initial ^{13}C-AMPA equivalents). Mineralization of sarcosine and glycine was fastest in the first 2 days of incubation and after 32 days was negligible (glycine: 0.03–0.1 %; sarcosine: 0.02–0.07 %). The rates of $^{13}CO_2$ in glycine and sarcosine treatments were of highest relevance on day 2/4, as shown in SI Fig. S7. Glycine and sarcosine are readily biodegradable compounds; therefore, both compounds are quickly mineralized by microorganisms (Aslam et al., 2023; McFarland et al., 2010; Sun et al., 2019a, 2019b).

Glyphosate mineralization also decreased over time, but at slower rates than glycine and sarcosine and after 32 days ranged between 0.1 % and 0.4 % of the initial 2-^{13}C-glyphosate equivalents. Mineralization rate of glyphosate in our study was comparable (0.3 ± 0.004 % of the 2-^{13}C-glyphosate equivalents on day 45) with that (0.2 % of the $^{13}C_3$-glyphosate equivalents on day 40 and at 20 °C) reported by Muskus et al. (2022) who incubated the same soil in their study as of ours.

The lowest mineralization rates of AMPA (0.1–0.4 % of the initial ^{13}C-AMPA equivalents) are associated with the persistent nature of this compound in soil (Aslam et al., 2023; Battaglin et al., 2014). Noteworthy here is that the mineralization rate of AMPA was highest among all treatments on day 75 (0.1 ± 0.02 %) suggesting that microorganisms degraded this compound, albeit slowly. This also suggests that microorganisms could utilize the AMPA when other available C-substrates were depleted at the later phase of soil incubation.

3.4. Potential microbial degraders of a compound based on ^{13}C-PLFAs and 16S/ITS rRNA genera

Highest contents of ^{13}C-PLFAs during the entire incubation period and among all treatments were found for glycine (0.2–0.4 % of the initial $^{13}C_2$-glycine equivalents, see Fig. 6) and glyphosate (0.1–0.3 % of the initial 2-^{13}C-glyphosate equivalents) treatments. We observed the lowest amounts of ^{13}C-PLFAs for AMPA treatment (in range of 0.005–0.007 % of the initial ^{13}C-AMPA equivalents).

The dynamics of ^{13}C incorporation into PLFAs differed among four treatments. The maximum amounts of ^{13}C-PLFAs for glycine (0.4 ± 0.03 % of the initial $^{13}C_2$-glycine equivalents) and sarcosine (0.2 ± 0.02 % of the initial $^{13}C_3$-sarcosine equivalents) treatments were measured on day 4. Thereafter, the ^{13}C-PLFAs decreased in both sarcosine and glycine treatments, but for the $^{13}C_3$-sarcosine (73 % of the maximum) it was greater than for $^{13}C_2$-glycine (22 % of the maximum). The rapid incorporation of ^{13}C-label from 2–^{13}C-glycine into PLFAs in different soils was also noticed by Xue et al. (2022). In contrast, the ^{13}C assimilation into PLFAs from 2–^{13}C-glyphosate was slower and reached its maximum during 16–32 days (0.3 % of the initial 2-^{13}C-glyphosate equivalents)

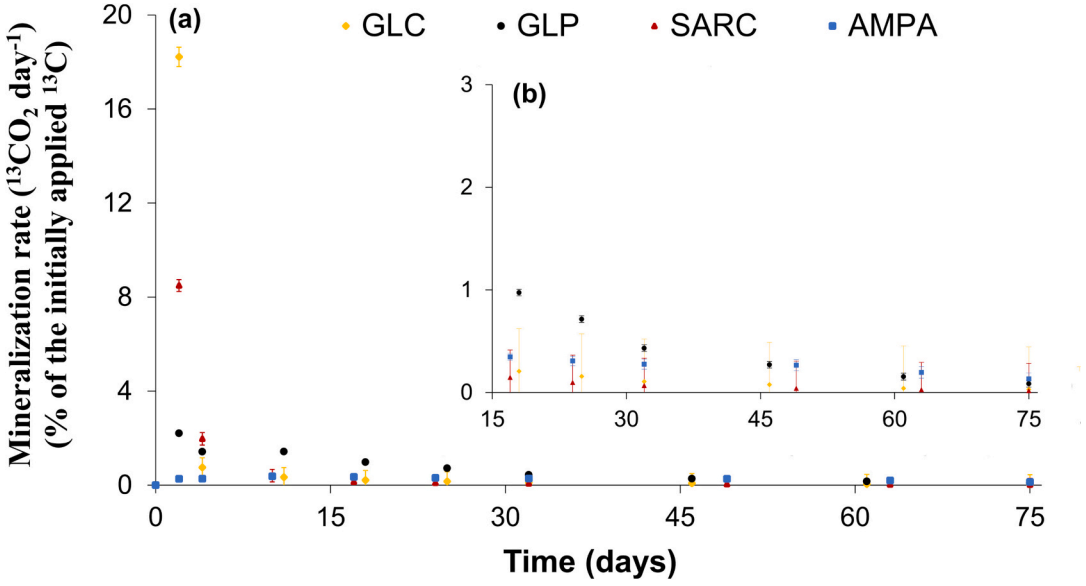

Fig. 5. Net mineralization rates of glyphosate (GLP) and its major transformation products sarcosine (SARC), glycine (GLC) and AMPA in soil during 75-day incubation experiment in % of the initially applied ^{13}C label (a). Data from day15 to day75 are also shown with smaller scale (b). Error bars represent standard deviations of means (n = 3).

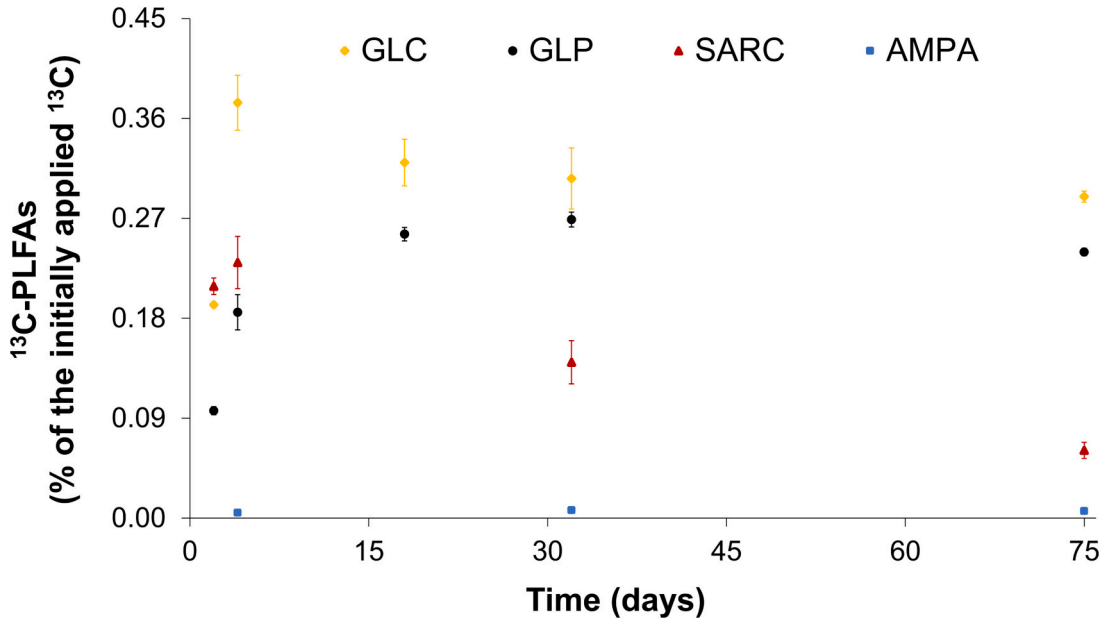

Fig. 6. Incorporation of ^{13}C from ^{13}C-glyphosate (GLP) or its major transformation products ^{13}C-glycine (GLC), ^{13}C-sarcosine (SARC) and ^{13}C-AMPA into PLFAs in soils during 75-day incubation experiment in % of the initially applied ^{13}C label. Error bars represent standard deviations of means (n = 3).

which remained nearly constant till end of experiment. The measured ^{13}C-PLFAs content (0.3 % of the initial 2-^{13}C-glyphosate equivalents) on day 32 in the glyphosate treatment was higher than that in the study by Muskus et al. (2022) who reported only 0.1 % of the initial $^{13}C_3$-glyphosate equivalents in ^{13}C-PLFA in the same soil incubated at 20 °C. Lower assimilation of ^{13}C of $^{13}C_3$-glyphosate into PLFAs in the study by Muskus et al. (2022) compared to this study might be due to the two reasons. First reason might be a lower microbial activity of soil caused by 1-year long storage at 4 °C prior to incubation resulting in a slower biodegradation of $^{13}C_3$-glyphosate and thereby lower ^{13}C-PLFAs in the study by Muskus et al. (2022). The second explanation could be a different labeling of glyphosate with ^{13}C, i.e. all three C were labeled ($^{13}C_3$-glyphosate; Muskus et al., 2022) from which a big portion and especially the ^{13}C-COOH-group could have been directly released as $^{13}CO_2$.

The incorporation of ^{13}C into PLFAs from ^{13}C-AMPA was low and it was nearly constant throughout the entire incubation (0.006 ± 0.001 % of the initial ^{13}C-AMPA equivalents). The AMPA is the most resistant compound to microbial degradation among all tested compounds (Aparicio et al., 2013); hence, the lowest ^{13}C-PLFAs were as expected. Although, ^{13}C incorporation into PLFAs from ^{13}C-AMPA was minimal, this study reports for the first time that ^{13}C label from ^{13}C-AMPA was still utilized as a C source for biomass synthesis (i.e. PLFAs). This finding was also confirmed in our recent study by Aslam et al. (2023) who reported a small incorporation of both ^{13}C and ^{15}N labels from ^{13}C,^{15}N-AMPA into amino acids (1.1 % and 2.4 % of the initially added ^{13}C and ^{15}N, respectively).

Gram-negative bacteria were the most abundant in ^{13}C during biodegradation of all compounds and throughout the entire incubation period (Fig. 7). Gram-negative bacteria were suggested in previous studies to be the major group of degraders of organic pollutants like glyphosate (Muskus et al., 2022), 2,4-dichlorophenoxyacetic acid (2,4-D) (Nowak et al., 2011) or ibuprofen (Nowak et al., 2013) in soils. Increased ^{13}C abundance of the Gram-negative bacteria starvation marker after 4 days in glyphosate and glycine treatments suggests that Gram-negative bacteria were presumably involved in both compounds degradation in early phases of soil incubation. We did not find any of widely reported Gram-negative bacterial degraders of glyphosate such as *Achromobacter* (Ermakova et al., 2017; Sviridov et al., 2012), *Agrobacterium* (Wackett et al., 1987), *Comamonas* (Firdous et al., 2020), *Flavobacterium* (Balthazor and Hallas, 1986), *Ochrobactrum* (Hadi et al., 2013; Sviridov et al., 2012) and *Pseudomonas* (Dick and Quinn, 1995; Peñaloza-Vazquez et al., 1995) in our 16S RNA data. However, we found increased abundances of other genera of Gram-negative bacteria that might have degraded glyphosate as compared to control on day 4 (SI Fig. S8). These are as follows: *RB 41* and *Subgroup_7* (Acidobacteriota phylum), *Gemmatimonas* (Gemmantimonadota phylum) and *Arenimonas* (Proteobacteria phylum). Noteworthy is here that the abundances of these four genera were nearly similar in both glyphosate and glycine treatments at early degradation (day 2 and 4) compared to control. This finding suggests again our assumption that glyphosate was biodegraded mainly to glycine in Section 3.2 (time-dependent shifts between the four classes of $PLFAs_{tot}$ and four phyla). The two genera *RB41* and *Gemmatimonas* were also highly abundant in sarcosine treatment, but the abundance of both *Subgroup_7* and *Arenimonas* was rather similar to these in control soil. Therefore, it is less likely that high amounts of sarcosine were formed from glyphosate. Interestingly, the abundance of genus *Massilia* (Pseudomonadota phylum) on day 75 was highest in AMPA treatment as compared to other treatments indicating a possible contribution of this bacterial group in AMPA biodegradation. There is no direct information available on the role of this genus *Massilia* in AMPA biodegradation; however, some studies suggest that this novel genus may be involved in the degradation of other organic pollutants such as phenanthrene (Du et al., 2021; Gu et al., 2016; Wang et al., 2016a, 2016b).

In addition to the Gram-negative bacteria, both sarcosine and especially glycine treatments exhibited increased ^{13}C abundance in the Gram-positive positive bacterial marker throughout the entire incubation period as compared to AMPA and glyphosate (Fig. 7). This finding is in good agreement with Xue et al. (2022) who showed that Gram-positive bacteria were most important degraders of glycine in a forest soil. However, increased ^{13}C abundance in the starvation marker of Gram-negative bacteria was also found in the glycine treatment after 4 days. Furthermore, Gram-positive & Gram-negative bacterial degraders of glycine on both day 4 and 32 are plotted nearly together in SI Fig. S7 and it correlates well with daily $^{13}CO_2$ rates of glycine. This altogether suggests that the Gram-negative bacteria were rather the primary degraders of glycine which mineralized quickly (see Section 3.3).

Time-dependent shifts in ^{13}C abundance of fungal degraders were not straightforward in all treatments. However, the ^{13}C-PLFA fungal

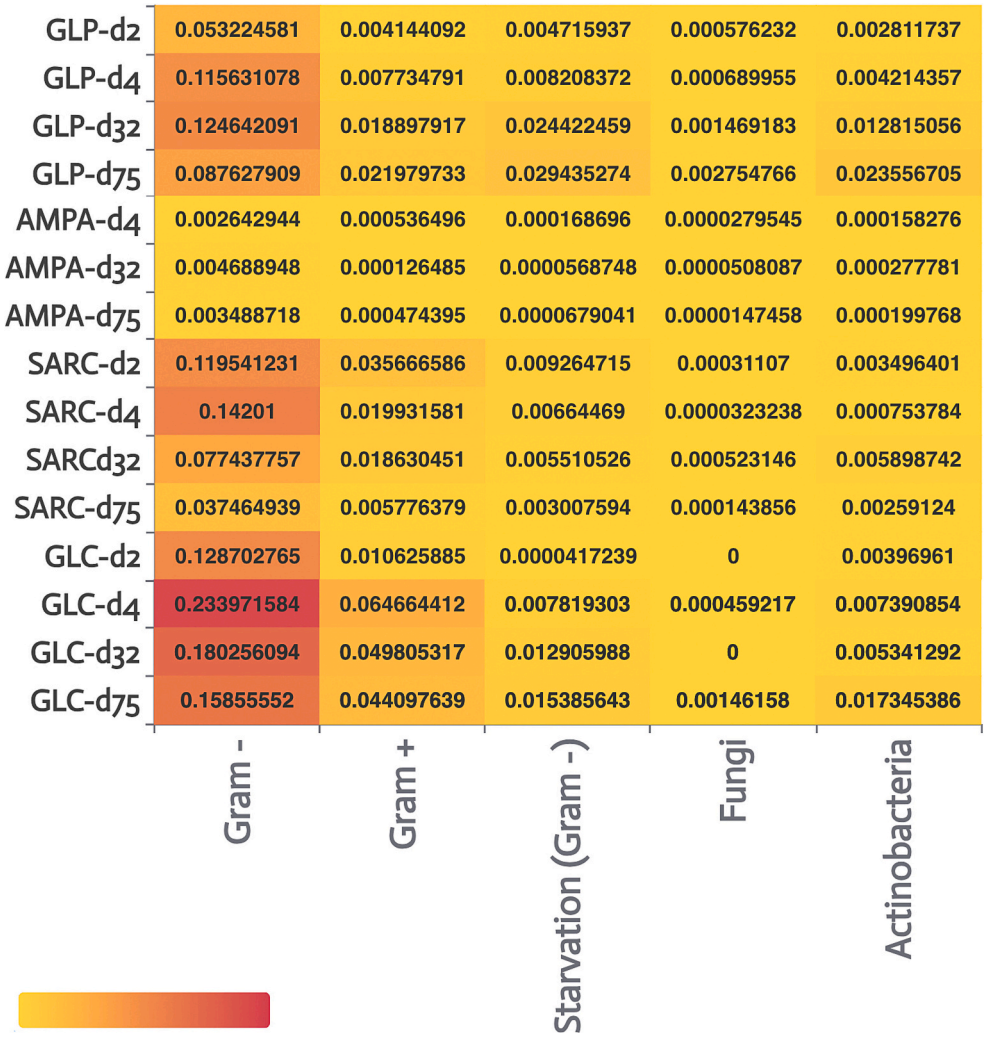

Fig. 7. Heat map representing abundance of major four groups of microbial degraders (Gram-positive & Gram-negative bacteria, actinobacteria and fungi) and starvation biomarker for Gram-negative bacteria based on ^{13}C-PLFA (PLFA-SIP analysis) in soil spiked with glycine (GLC), glyphosate (GLP), sarcosine (SARC) and AMPA during 75-day incubation experiment. Scale in the heat maps shows minimum (light brown color) to maximum (reddish color) contribution of microbial groups.

marker was most abundant in glyphosate treatment at the end of soil incubation, i.e. day 32 and 75. Actinobacteria were most abundant in ^{13}C in the glyphosate treatment on day 75; however, this microbial group was comparably highly abundant in ^{13}C in the glycine treatment on day 75 (Fig. 7). Actinobacteria are also plotted towards glyphosate treament on both day 32 and 75 in SI Fig. S7. Gram-positive bacteria, actinobacteria and fungi were suggested as decomposers of the necromass of degraders that had consumed C-substrates (Kramer and Gleixner, 2006; Moore-Kucera and Dick, 2008; Rinnan and Baath, 2009). Therefore, actinobacteria and fungi prevailed at later phases of glyphosate degradation and Gram-positive bacteria and actinobacteria in glycine treatment.

We found genus of fungi *Sordariomycetes* (OTU 8; Ascomycota phylum) that was highly abundant in glyphosate treatment (SI Fig. S9) on day 75 compared to very low abundance in control and AMPA treatments. In contrast, two *Ascomycota* genera were highly abundant in AMPA treatment as compared to glyphosate treatment and control. Some fungi from Ascomycota phylum (*Penicillium and Aspergillus*) have been found to be efficient degraders of glyphosate herbicide, which produces AMPA as a breakdown product (Correa et al., 2023). Therefore, we suggest that Ascomycota fungi in both glyphosate and AMPA treatments presumably decomposed the necromass of primary glyphosate or AMPA degraders. Interestingly, we found a genus *Sistotrema* (Basidiomycota phylum) only in the AMPA treatment. This finding implies that this genus of fungi could be either AMPA tolerant or AMPA degrader. The study by Op De Beeck et al. (2015) showed that the abundance of *Sistotrema* increased in soils in response to heavy metals pollution suggesting the adaptation of this genus to the pollution.

4. Conclusions

This is the first report comparing the effect of glyphosate and its three degradation products on microbial activity and community composition, as well as identifying potential microbial degraders of each compound. Highest respiration and compound mineralization rates in soil spiked with glycine or glyphosate suggests that microbial activity in both treatments was comparable. Similar time-dependent shifts between the four $PLFAs_{tot}$ classes and abundances of four genera (*RB41, Subgroup_7*, *Gemmatimonas* and *Arenimonas*) in glyphosate and glycine treatments suggest that glyphosate was biodegraded mainly to glycine. This study thus suggests for the first time that an enhanced soil microbial activity (respiration & mineralization rates) and shifts in the microbial community composition might be a result of the increased formation of glycine from glyphosate and not of glyphosate directly.

Further studies investigating the capability of specific bacterial and fungal genera to degrade glyphosate to either glycine or to AMPA

product could support our findings. This could be e.g., stable isotope labeling metagenomics or proteomics with native soil microbial communities as well as single soil-microbial isolates.

CRediT authorship contribution statement

Conceptualization: Aslam, Nowak
Data curation: Aslam, Arslan, Nowak
Formal analysis: Nowak
Funding acquisition: Aslam, Nowak
Investigation: Aslam
Methodology: Aslam, Nowak
Project administration: Nowak
Resources: Aslam, Nowak
Software: Aslam, Arslan
Supervision: Nowak
Validation: Aslam, Arslan
Visualisation: Aslam, Arslan
Writing – original draft: Aslam, Nowak
Writing – review & editing: Nowak
Authors gave final approval of the revised version of this manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Karolina M. Nowak reports financial support was provided by German Research Foundation. Sohaib Aslam reports a relationship with Alexander von Humboldt Foundation that includes:

Data availability

Data will be made available on request.

Acknowledgements

This study was financially supported by the Alexander von Humboldt Foundation, German Research Council (DFG, No 980/3-1) and Helmholtz Centre for Environmental Research-UFZ. The authors thank Anja Miltner (UFZ, Department of Environmental Biotechnology) and Steffen Kümmel (UFZ, Department of Isotope Biogeochemistry) for their assistance in the compound-specific isotope analysis. Dr. Nazish Roy (KAM School of Life Sciences, Forman Christian College, Lahore) is acknowledged for her help in bioinformatic analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168206.

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