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Fate of glyphosate and its degradation products AMPA, glycine and sarcosine in an agricultural soil: Implications for environmental risk assessment

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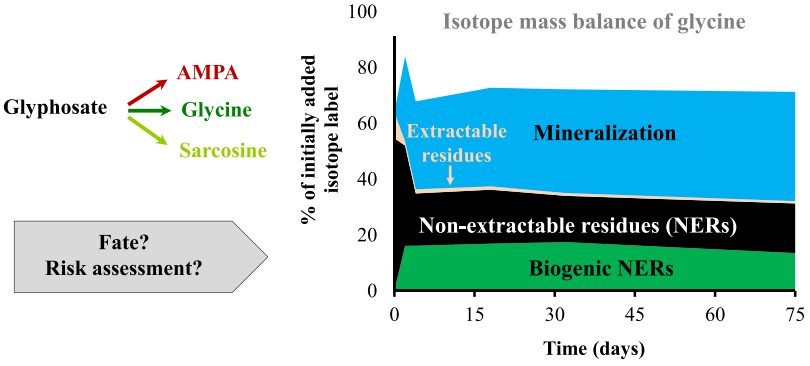
HIGHLIGHTS

- $^{13}C/^{15}N$-mass balances of glyphosate and degradation products fate were determined.
- Only traces of glyphosate and about 30% of AMPA were extracted from soil.
- High amounts of $NERs_{biogenic}$ were determined for glyphosate, glycine and sarcosine.
- The NERs from AMPA were mainly $NERs_{unknown}$ and thus potentially $NERs_{xenobiotic}$.

GRAPHICAL ABSTRACT



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ABSTRACT

Glyphosate can be biodegraded via the aminomethylphosphonic acid (AMPA) and the sarcosine/glycine pathway leading to the formation of three intermediate products AMPA, sarcosine or glycine. The fate of the three intermediate compounds of glyphosate biodegradation including nature of non-extractable residues (NERs; harmless biogenic [$NERs_{biogenic}$] versus hazardous xenobiotic [$NERs_{xenobiotic}$]) in soils has not been investigated yet. This information is crucial for an assessment of environmental risks related to the speciation of glyphosate-derived NERs which may stem from glyphosate intermediates. Therefore, we incubated ^{13}C- and ^{15}N-labeled glyphosate (2-^{13}C,^{15}N-glyphosate) and its degradation product AMPA (^{13}C,^{15}N-AMPA), sarcosine ($^{13}C_3$,^{15}N-sarcosine) or glycine ($^{13}C_2$,^{15}N-glycine) in an agricultural soil separately for a period of 75 days. $^{13}C_2$-glycine and $^{13}C_3$-sarcosine mineralized rapidly compared to 2-^{13}C-glyphosate and ^{13}C-AMPA. The mineralization of ^{13}C-AMPA was lowest among all four compounds due to its persistent nature. Only 0.5% of the initially added 2-^{13}C,^{15}N-glyphosate and still about 30% of the initially added ^{13}C,^{15}N-AMPA was extracted from soil after 75 days. The NERs formed from ^{13}C,^{15}N-AMPA were mostly $NERs_{xenobiotic}$ as compared to other three compounds for which significant amounts of $NERs_{biogenic}$ were determined. We noticed 2-^{13}C,^{15}N-glyphosate was biodegraded

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via two biodegradation pathways simultaneously; however, the sarcosine/glycine pathway with the formation of harmless $NERs_{biogenic}$ presumably dominated.

1. Introduction

Glyphosate is one of the most widely used herbicide worldwide due to its great efficacy against a wide variety of weeds [5]. Glyphosate and its transformation product aminomethylphosphonic acid (AMPA) were most frequently found pesticides in agricultural European Union soils [34]. Such a widespread occurrence of both glyphosate and AMPA in soils triggers public concern about the safety of glyphosate use for the environment and humans [35]. Glyphosate can be biodegraded via two well-documented pathways: the AMPA and the sarcosine pathway [10, 35,40]. The two pathways of glyphosate biodegradation result in formation of different intermediate products that have different environmental fate and implications for environmental risk assessment [24,40]. For instance, the AMPA pathway produces persistent AMPA and glyoxylate which may further form amino acid glycine [40]; see Fig. 1). In contrast, the sarcosine pathway yields sarcosine which is readily oxidized to glycine [31,37]. However, Li et al. [22] suggested that the C-N bond of glyphosate also can be cleaved directly to glycine bypassing sarcosine formation.

Isotope mass balance of the glyphosate fate comprising mineralization, extractable parent compound & its degradation products and non-extractable residues (NERs) was well documented in various soils [28] and in planted filters [19]. In contrast, the isotope mass balance studies of the fate of AMPA, glycine or sarcosine in soils are still lacking. Previous studies reported only half-life dissipation (DT_{50}) of AMPA (151–173 days; [4,15] in soils, as well as of glycine (0.89 day; [36] and sarcosine (0.99 day; [36] in the soil-water system.

The NERs that can be only determined using isotope tracers are often a 'black box' in the mass balance study of the chemical fate in soils due to their unknown identity [33]. The NERs are remaining residues of an isotope labeled parent chemical or its degradation product(s) in soils that cannot be extracted using aquatic or organic solvents [23]. The parent chemical or its degradation product(s) can be strongly sorbed to soils as hazardous xenobiotic NERs ($NERs_{xenobiotic}$) with a remobilization potential and delaying the environmental risk [23,33]. However, a chemical also can undergo microbial degradation accompanied with the formation of CO_2 and microbial biomass [33,21]. After the death of microorganisms, biomass compounds and in particular proteins are stabilized in soil matrix as harmless biogenic NERs ($NERs_{biogenic}$) [29, 33]. The $NERs_{biogenic}$ can be a result of assimilation of inorganic C and N (CO_2 or NH_4^+) or monomeric molecules (e.g. amino acids) from a biodegraded compound into microbial biomass [39]. When the $NERs_{biogenic}$ constitute a major portion of the NERs, the environmental risks associated with the $NERs_{xenobiotic}$ formation will be overestimated [29]. The lack of information about the NER speciation is thus a 'bottleneck' in fate studies of chemicals since it impedes an assessment of environmental risks related to the $NERs_{xenobiotic}$ [33,21].

The intermediates of glyphosate, AMPA, glycine or sarcosine may determine the NER speciation resulting from the glyphosate degradation (Fig. 1). We hypothesize that an enhanced transformation of glyphosate to AMPA in the AMPA pathway will result in an increased formation of hazardous $NERs_{xenobiotic}$. The AMPA (DT_{50}: 151–173 days) is more resistant to biodegradation than glyphosate (DT_{50}: 7–60 days) [4,15, 37]; and it is thus expected to be mainly sorbed to soils as $NERs_{xenobiotic}$ with release potential to waters [39,4,6]. In contrast, enhanced degradation of glyphosate via the sarcosine/glycine pathway accompanied with the glycine formation may yield $NERs_{biogenic}$. Both glycine and sarcosine are biomolecules, which are readily transformed to CO_2 and microbial biomass [12,22]. The glycine may be either assimilated as a monomeric 'building block' into microbial biomass and then into the $NERs_{biogenic}$ or mineralized to CO_2 or NH_4^+ which are then integrated into the biomass (see Fig. 1 and **S1**).

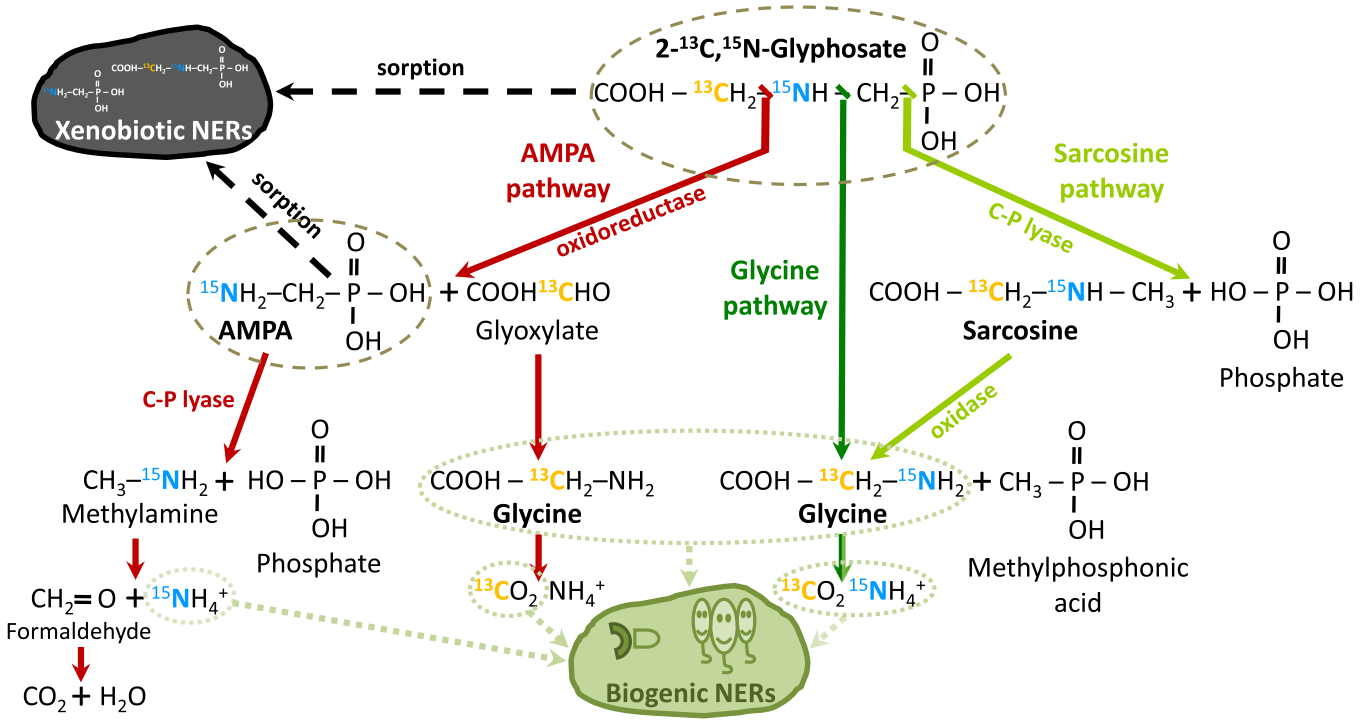

Fig. 1. Formation of degradation products of 2-^{13}C,^{15}N-glyphosate and speciation of non-extractable residues (NERs: xenobiotic or biogenic NERs) as a consequence of three degradation pathways.

Formation of three degradation products of glyphosate and their proportions: AMPA, glycine and sarcosine can therefore determine environmental risks associated with the $NERs_{xenobiotic}$ formation during the glyphosate degradation in soil. To date, mass balance of the fate of the three glyphosate degradation products and in particular the formation of $NERs_{biogenic}$ has not been reported. This information may help to predict more accurately the NER speciation (hazardous $NERs_{xenobiotic}$ versus harmless $NERs_{biogenic}$) of glyphosate in soil and which may stem from glyphosate intermediates. Therefore, the objectives of this study were (i) to elucidate the fate of glyphosate & its three degradation products: AMPA, glycine and sarcosine in soil microcosm experiments, and (ii) to determine the $NERs_{biogenic}$ formation from these compounds using stable isotope double-labeling approach ($^{13}C + {}^{15}N$). The ^{13}C- and ^{15}N-mass balance of the fate of 2-^{13}C,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine was determined and comprised of mineralization (CO_2), extractable residues (ERs) of parent compound & its degradation products and NERs. The $NERs_{biogenic}$ were based on the quantification of ^{13}C- or ^{15}N-amino acids (^{13}C- or ^{15}N-AAs) hydrolyzed from soil proteins.

2. Materials and methods

2.1. Reference soil

The soil used in this study was a haplic Chernozem collected from the topsoil of the Static Fertilization Experiment in Bad Lauchstädt (51° 22′ 0″ N, 11° 50′ 0″ E) located in Saxony-Anhalt, Germany. We used a Haplic Chernozem as a reference soil for this study, since this soil is commonly used for agriculture in Europe. The plot in Bad Lauchstädt received organic fertilizers (30 t ha^{-1} farmyard manure) every second year and had previous history of glyphosate (as Roundup) application. The soil had silty loam texture with following particle size classes: clay, 21%; silt, 68%; and sand, 11%. The other soil characteristics were previously described by Muskus et al. [27] such as total nitrogen, 0.17%; total organic carbon (TOC), 2.1%; pH, 6.6. The maximum water holding capacity of the soil was 47 ± 1.9% (based on our measurements in the laboratory). Soil was sieved at 2 mm and stored in cold room at 4 ℃ until start of incubation experiments. Soil moisture content was adjusted to 60% of maximum water holding capacity.

2.2. Chemicals and reagents

The unlabeled molecules of glyphosate (99% purity), sarcosine (98% purity) and glycine (99.7% purity) were purchased from Sigma-Aldrich, Germany. The unlabeled AMPA (99% purity) was purchased from Alfa Aesar, Thermo Fisher (Kandel) GmbH. Co-labeled 2-^{13}C,^{15}N-glyphosate (98% purity) was purchased from Sigma-Aldrich, Germany. The isotopic enrichment of the labeled glyphosate was 99% for ^{13}C and 98% for ^{15}N. Labeled degradation products of glyphosate including $^{13}C_3$,^{15}N-sarcosine (^{13}C: 99%; ^{15}N: 98%) and $^{13}C_2$,^{15}N-glycine (^{13}C: 99%; ^{15}N: 99%) were purchased from Cambridge Isotope Laboratories, Inc. USA. Labeled ^{13}C,^{15}N-AMPA (^{13}C: 99%; ^{15}N: 98%) was purchased from Toronto Research Chemicals, Canada. All the other chemicals used in this study were purchased from Carl Roth (Karlsruhe, Germany) or VWR/Merck (Darmstadt, Germany).

2.3. Incubation experiment

Sieved soil (60 g dry-equivalent) was spiked with 50 mg kg^{-1} soil (in Milli-Q) of unlabeled or labeled compound separately, i.e. glyphosate, AMPA, glycine or sarcosine and then placed into 500 mL glass bottles. Soil samples spiked with unlabeled compounds were used to correct for natural abundance of ^{13}C and ^{15}N. The applied amounts of tested compounds, especially of glyphosate and AMPA were much higher than these found in soils (2 mg kg^{-1}; [1,2,34]). However, sufficiently high initial amounts of the ^{13}C and ^{15}N compounds were necessary for reliable analysis of ^{13}C and ^{15}N incorporations into AAs (see **Section 2.4**) that is not masked by ^{13}C and ^{15}N isotope natural abundances. Due to a limited availability and high costs, labeled glyphosate used in this study was only labeled at carbon position 2 (C position 2) and at N (2-^{13}C,^{15}N-glyphosate). In contrast, all C and N atoms of three degradation products were labeled (^{13}C,^{15}N-AMPA, $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine). Each incubation vessel contained a small insert with a 2 M NaOH solution in order to trap CO_2. Spiked soil was incubated at 20 °C in dark for a maximum period of 75 days and according to OECD 307 guidelines [30]. The soil humidity was maintained throughout the incubation experiment at 60% of maximum water holding capacity and the NaOH solution was replaced regularly during the incubation period. During the 75-day long incubation, CO_2 evolved by soil respiration (total $^{12}C + {}^{13}C$-CO_2) and from parent compound mineralization ($^{13}CO_2$) was estimated after 2, 4, 10, 18, 24, 32, 41/46, 50, 61/63 and 75 days. In addition, destructive soil samplings were conducted at 0, 2, 4, 18, 32 and 75 days to determine extractable residues of parent compound & its degradation products, total NERs, and to estimate $NERs_{biogenic}$ based on the AA contents hydrolyzed from proteins in soil.

2.4. Mass balance

The mass balance of the fate of 2-^{13}C,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine in soil was determined by estimating mineralization ($^{13}CO_2$), analyzing extractable residues (parent compound & major degradation products) and NERs. ^{13}C- and ^{15}N-AAs hydrolyzed from the proteins in soil (total pool which includes living biomass and non-living organic matter pool) representing $NERs_{biogenic}$ were determined as described previously by Nowak et al. [29].

Mineralization. The mineralization ($^{13}CO_2$) of 2-^{13}C,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine was calculated from the total amount of CO_2 ($^{12}C + {}^{13}C$-CO_2 contents) and its isotopic composition (at% $^{13}C/^{12}C$). The total amount of CO_2 in NaOH traps was measured by means of a total organic carbon analyzer (Multi N/C 21005, Jena, Germany). The isotopic composition of CO_2 was determined by 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 a separation from other permanent gases on a Porabond Q-HT Plot FS column (50 m x 0.32 mm × 5 mm; Chrompack, Middleburg, Netherlands; [11].

Extractable residues (ERs). The remaining 2-^{13}C,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, $^{13}C_2$,^{15}N-glycine or $^{13}C_3$,^{15}N-sarcosine was extracted from 1 g of soil into 20 mL of a 40 mM sodium borate buffer solution (pH 8). The soil-sodium borate buffer mixture in a 50 mL centrifuge tube was allowed to shake on overhead shaker for 1 h. After shaking, centrifuge tubes carrying samples were centrifuged at 2362 g for 10 min. The soil supernatants were then transferred to 20 mL falcon tubes and accordingly 1 mL and 2 mL of each sample were taken for elemental analyzer-isotope ratio mass spectrometry (EA-irMS) and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analyses. For EA-irMS, 1 mL of soil extract was air-dried in tin capsules and the sample was then combusted to $^{13}C/^{12}C$-CO_2 or $^{15}N_2/^{14}N_2$ in order to estimate the total ^{13}C and ^{15}N contents in the soil extracts ($^{13}C/^{15}N$-ERs_{total}).

Purification of ERs with SPE. Prior to LC-MS/MS analysis, 2 mL of the extract was purified over OASIS HLB 6 mL (200 mg) SPE cartridges. Each SPE cartridge was first conditioned with 2 mL of methanol, dried under vacuum for 10 min and then 2 mL of water was added prior to the addition of the sample. After the sample had passed through the column, the internal standard glufosinate was added to each sample. For derivatization of the glyphosate, AMPA and glufosinate, 1 mL aliquot of purified extract was first mixed with 50 μL 0.1 M EDTA-Na_4 and vortexed to release glyphosate from the potential glyphosate-metal complexes. Thereafter the derivatization was initiated by adding 100 μL of a 0.5 M borate buffer and 500 μL of a 1 mg mL^{-1} fluorenylmethyloxycarbonyl

chloride (FMOC-Cl) solution in acetonitrile. The mixture was agitated on an orbital shaker (300 rpm, 25 °C, 60 min). Afterwards, the reaction was terminated by adding 20 μL of formic acid. All the derivatized samples were passed through 0.2 μm nylon filters before LC-MS/MS analysis.

LC-MS analysis. The LC-MS/MS consisted of a 1260 Infinity II LC system (Agilent, Santa Clara, USA) coupled to a QTRAP 6500 MS (AB Sciex, Darmstadt, Germany) with an electrospray ionization (ESI) source. A ZORBAX Extend-C18 column (2.1 × 100 mm, 3.5 μm particle size; Narrow Bore RR, Agilent, US) was used to separate the analytes. Glyphosate and AMPA were separated with a gradient of 5 mM ammonium acetate (pH 9) and methanol as mobile phases and detected in negative ion mode. The limits of quantification (LOQ) were 0.04 μg L^{-1} for glyphosate and 0.12 μg L^{-1} for AMPA. The LC method for glyphosate and AMPA analysis has been described previously [18]. The quantification of ^{15}N-AMPA was based on the calibration curve of unlabeled AMPA, because ^{15}N-AMPA standard was not commercially available. The retention time of FMOC-glyphosate was 9.3 min and the retention time of FMOC-AMPA was 12.6 min. The calibration curve for both glyphosate and AMPA had a linear range over 0.05 − 50 μg L^{-1} with $R^2 > 0.99$ (1/x weighted). The precision (RSD) measured at 0.05, 2 and 50 μg L^{-1} were < 2% for glyphosate and < 7% for AMPA. The total run time was 28 min for each sample. Blank samples injections were applied to avoid any cross contamination whereas the soil extracts were used to ensure a correct detection and recovery of the compounds. The sample batch quantification was calculated through a calibration curve measured at the beginning and at the end of each batch. The results of recovery tests from this experiment showed that the soil matrix did not interfere with the ionization process.

We also tried to estimate concentrations of $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine in soil samples using LC-MS/MS. However, the quantification was not reliable due to interference of soil matrices; therefore, we analyzed total amounts of ^{13}C and ^{15}N in soil extracts using EA-irMS. Equal amounts of ^{13}C- and ^{15}N-ERs_{total} (in % of initial ^{13}C or ^{15}N equivalents added with the labeled compound) indicated that the labels are assigned to either untransformed $^{13}C_2$,^{15}N-glycine or $^{13}C_3$,^{15}N-sarcosine. The amounts of glycine and sarcosine in the soil extracts were low since the total ^{13}C in the soil extracts measured by EA-irMS were < 4% of the initially added ^{13}C already after 2 days of incubation (see Section 3.2).

Non-extractable residues (NERs). The remaining soil pellets after extraction and centrifugation were air-dried and grounded using mortar and pestle. About 3–5 mg of sample was combusted using EA-irMS (Finnigan MAT 253, Thermo Electron, Bremen, Germany) coupled to Euro EA 3000 (Eurovector, Milano, Italy) as described by [11]. The temperature of the oxidation oven was 1020 °C and the one of the reduction oven was 650 °C. The amount of NERs (showed here as $NERs_{total}$) was calculated based on comparison of the ^{13}C and ^{15}N excess in labeled samples over the corresponding unlabeled samples.

Amino acids (AAs). The AAs in the soil were hydrolyzed from proteins using concentrated HCl (6 M) at 110 °C for 22 hr. The hydrolyzate was purified over cation exchange resin (DOWEX 50 W-X8) and derivatized before analysis by gas chromatography-mass spectrometry (GC-MS). The adapted methods of extraction, purification and derivatization have been described previously by Nowak et al. [29] and later also reported by Muskus et al. [27]. The identity and quantity of AAs were analyzed with the use of GC-MS (HP 6890, Agilent) using a BPX-5 column (30 m × 0.32 mm × 0.25 μm) for separation. The isotopic composition of ^{13}C and ^{15}N of each AA was measured by GC-irMS (Finnigan MAT 253 coupled to a Trace GC, Thermo Electron, Bremen, Germany) using a BPX-5 column (50 m × 0.32 m × 0.5 μm, SGE International, Darmstadt, Germany). The details on the analytical conditions for AA separation by GC-MS and GC-irMS were reported by Nowak et al. [29] and Muskus et al. [27]. An external standard containing all detectable AAs in the sample was used for quantification and identification of the AAs in each measurement. L-norleucine was used as an internal standard to estimate any losses during the extraction, clean-up and derivatization.

2.5. Data analysis

All incubation experiments were carried out with three repetitions and all results are presented as averages and with standard deviations. Mineralization ($^{13}CO_2$) of each compound molecule was estimated at 2, 4, 10, 18, 24, 32, 41/46, 50, 61/63 and 75 days of incubation. The ^{13}C/^{15}N-ERs, ^{13}C/^{15}N-NERs and ^{13}C/^{15}N-AAs were determined at day 0, 4, 18, 32 and 75 days of incubation. The measured ^{13}C/^{15}N-AA contents were used for calculation of total ^{13}C/^{15}N-$NERs_{biogenic}$ ($AAs*2 = NERs_{biogenic}$) as proteins are the major components of microbial biomass and account for about 50% of the total biomass [29,39]. In addition, proteins were proven to be most stable microbial biomass compounds in organic matter pool of soil [17] and thereby to be most reliable biomarker for calculation of total $NERs_{biogenic}$. The difference between the ^{13}C/^{15}N-$NERs_{total}$ and ^{13}C/^{15}N-$NERs_{biogenic}$ was shown as ^{13}C/^{15}N-$NER_{sunknown}$ which could be ^{13}C/^{15}N-$NERs_{xenobiotic}$ and possibly other ^{13}C/^{15}N-$NERs_{biogenic}$. The ^{15}N-$ERs_{unknown}$ for ^{15}N-glyphosate and ^{15}N-AMPA (shown in Fig. 5) were calculated as a difference between the ^{15}N-ERs_{total} measured by EA-irMS and the extractable parent chemical (^{15}N-$ERs_{glyphosate}$ or ^{15}N-ERs_{AMPA}) determined with LC-MS/MS. The ^{15}N-$ERs_{unknown}$ thus represents the ^{15}N-ERs that are neither parent chemical glyphosate nor its transformation product AMPA and could be an inorganic ^{15}N (e.g. NH_4^+ or NO_x). Due to the high uncertainty of $^{13}C_2^{15}$N-glycine and $^{13}C_3^{15}$N-sarcosine measurements by LC-MS/MS, we relied only on ^{13}C/^{15}N-ERs_{total} measured with EA-irMS. Therefore, the ^{13}C/^{15}N-$ERs_{unknown}$ for $^{13}C_2^{15}$N-glycine and $^{13}C_3^{15}$N-sarcosine could represent the parent compound $^{13}C_2$,^{15}N-glycine or $^{13}C_3$,^{15}N-sarcosine. Similar percentages of the ^{13}C-$ERs_{unknown}$ and ^{15}N-$ERs_{unknown}$ indicate that ^{13}C/^{15}N-$ERs_{unknown}$ contain exclusively the parent compound $^{13}C_2^{15}$N-glycine or $^{13}C_3^{15}$N-sarcosine. However, if the ^{15}N-ERs_{total} are much higher than the ^{13}C-ERs_{total}, most of the ^{15}N in the ^{15}N-$ERs_{unknown}$ will not be the parent compound, but presumably an inorganic ^{15}N (e.g. NH_4^+ or NO_x).

Total recovery in the mass balances for ^{13}C ranged from 81% to 89% for 2-^{13}C-glyphosate (see Table S1), 82–90% for ^{13}C-AMPA, and 64–90% for $^{13}C_2$-glycine. The recovery of ^{13}C for $^{13}C_3$-sarcosine was much lower (49–62%). We did not lose the ^{13}C and ^{15}N labels in ERs and NERs as well as minimal losses should be in CO_2 because we had a 2 M NaOH solution for trapping the CO_2 inside the air-tight incubation vessel. We might have lost some ^{13}C label from $^{13}C_3$-sarcosine as ^{13}C-formaldehyde which is volatile in ambient temperatures [16], since we did not place inside the incubation vessel any trap for organic volatiles. The ^{13}C-formaldehyde might have been formed from ^{13}C-methanol during the $^{13}C_3$-sarcosine oxidation to $^{13}C_2$-glycine and ^{13}C-methanol [26]; see also in Fig. S1. The total recovery of ^{15}N varied between 78% and 97% for ^{15}N-glyphosate, between 79% and 89% for ^{15}N-AMPA, between 53% and 73% ^{15}N for ^{15}N-glycine, and 56% and 66% for ^{15}N-sarcosine. We might have lost gaseous $^{15}N_2$ or $^{15}N_2O$, especially for both readily biodegradable ^{15}N-glycine and ^{15}N-sarcosine, for which the total recovery of ^{15}N was low. However, transformations of compounds to gaseous ^{13}C-formaldehyde and $^{15}N_2$/$^{15}N_2O$ were not the main focus of this study, which was centered on the biodegradation processes and in particular on the $NERs_{biogenic}$ assessment.

The results are shown as percentages of the ^{13}C and ^{15}N in the respective fraction in relation to initially applied ^{13}C- or ^{15}N-labeled compounds. The detailed calculation of ^{13}C and ^{15}N labels in CO_2, ERs (EA-irMS), NERs and AAs is explained in **text 1** in **SI**. The analytical uncertainty of ^{13}C and ^{15}N isotope signatures based on Gaussian error propagation in each fraction was < 1% and < 5% (of atom percent [at %] ^{13}C or at% ^{15}N) for unlabeled and labeled samples, respectively. The dissipation half-life (DT_{50}) of glyphosate, AMPA, sarcosine and glycine was estimated using single first order kinetics as described previously for glyphosate and other compounds [27].

3. Results and discussion

3.1. Mineralization

We observed distinct patterns of compound mineralization in our experiment (Fig. 2). Mineralization of 2-^{13}C-glyphosate occurred without a lag phase, and it increased by day 46. Soil used in this experiment was sampled from a field which had previous history of glyphosate application as Roundup; therefore, glyphosate degrading microorganisms were most likely already present in the haplic Chernozem soil [27,39]. At the end (75 days) of incubation, about 39 ± 0.3% of initially added ^{13}C was mineralized. This result is comparable to that found in soils with a similar texture (26–35% of initially applied ^{14}C; [3, 28] and in the haplic Chernozem (35% of initially added $^{13}C_3$-glyphosate on day 39; [27]. In contrast, mineralization of ^{13}C-AMPA was slowest and lowest among all tested compounds, especially during the first four days of incubation (1.1 ± 0.03% of initially applied ^{13}C). The slowest mineralization of AMPA in early days exhibited its persistent nature [13,7] and absence of AMPA degrading enzyme in soil microorganisms. After the four-day lag phase, ^{13}C-AMPA mineralization increased progressively, and it amounted to 19 ± 1.5% of initially applied ^{13}C at the end. To date, no reports on mineralization of ^{13}C or ^{14}C-labeled AMPA in soils are available. Nevertheless, AMPA was showed to be utilized as a P source by bacterial isolates within 30–120 h of incubation of pure cultures [32].

Mineralization patterns of $^{13}C_2$-glycine and $^{13}C_3$-sarcosine were quite distinct from that of 2-^{13}C-glyphosate and ^{13}C-AMPA. In both cases, most of the mineralization (80% and 63% of total cumulative mineralization for $^{13}C_2$-glycine and $^{13}C_3$-sarcosine, respectively) occurred during early days of the incubation (i.e. 2 days). Cumulative mineralization of $^{13}C_3$-sarcosine was lower (27 ± 0.7% of initially applied ^{13}C) than that of $^{13}C_2$-glycine (46 ± 0.8% of initially applied ^{13}C) Faster degradation of glycine as compared to sarcosine was also shown previously by Sun et al. [36]. Both compounds are quickly utilized by microorganisms as a C source in anabolic and catabolic reactions [14,20,26,41]. Sarcosine is also commonly known precursor to glycine during glyphosate biodegradation through sarcosine pathway which could further follow the degradation pattern of glycine, see Fig. S1 [9,36]. This thus could also explain slower mineralization of $^{13}C_3$-sarcosine as compared to that of $^{13}C_2$-glycine in this study.

3.2. Extractable residues (ERs)

About 41 ± 5.9% of initially applied ^{13}C and 59 ± 10% of initially applied ^{15}N were measured as ^{13}C- and ^{15}N-ERs$_{total}$ on day 0 with EA-irMS in the 2-^{13}C-glyphosate study (see Table S2). Thereafter, the amounts of ^{13}C-ERs$_{total}$ decreased rapidly to only 0.7 ± 0.3% at end of the incubation. In contrast, the contents of ^{15}N-ERs$_{total}$ reduced much slower and we could determine around 28 ± 2% of initially applied ^{15}N on day 75. The ^{13}C/^{15}N-ERs$_{glyphosate}$ comprised a major portion of the ^{13}C- (except for day 75) and ^{15}N-ERs$_{total}$ but only in the first four days of incubation (see LC-MS/MS results in Table S2). The higher estimates of ^{13}C/^{15}N-ERs$_{glyphosate}$ measured by LC-MS/MS than the ^{13}C-ERs$_{total}$ (except for day 75) and ^{15}N-ERs$_{total}$ (only for day 2 and 4) by EA-irMS suggest higher accuracy of the LC-MS/MS measurement than the EA-irMS. The amounts of ^{13}C/^{15}N-ERs$_{glyphosate}$ decreased quickly from 58 ± 1% of initially applied ^{13}C and ^{15}N on day 0–0.5 ± 0.02% on day 75. We also measured small amounts of ^{15}N-ERs$_{AMPA}$ derived from ^{15}N-glyphosate degradation and whose amounts were between 1.2 ± 0.02% and 2.5 ± 0.05% of initially applied ^{15}N. The ^{13}C/^{15}N-ERs$_{glyphosate}$ (0.5 ± 0.02%) in this study were comparable with those of Muskus et al. [27] who also reported 0.8% of ^{13}C/^{15}N-ERs$_{glyphosate}$ at the end of incubation period (40 days). However, formation of ^{13}C/^{15}N-ERs$_{AMPA}$ in the study by Muskus et al. [27] during $^{13}C_3$,^{15}N-glyphosate degradation was greater (4.6% after 40 days) compared to our results (1.2–2.5%). This difference may be related to a different microbial activity or different sorption capacity of soils. Muskus et al. [27] conducted their study in similar conditions (20 °C) using soil from same agricultural field but from another plot which in addition to farmyard manure also received NPK fertilizers. The presence of P from the fertilizer in the experiment by Muskus et al. [27] may have inhibited the sorption of ^{13}C,^{15}N-AMPA to soil affecting the higher ^{13}C/^{15}N-ERs$_{AMPA}$.

Around 50 ± 8.5% of initially applied ^{13}C and 45 ± 9% of initially applied ^{15}N were measured as ^{13}C- and ^{15}N-ERs$_{total}$ on day 0 with EA-irMS for ^{13}C,^{15}N-AMPA (Table S2). The measured ^{13}C- and ^{15}N-ERs$_{AMPA}$ by LC-MS/MS comprised a major portion of both ^{13}C- and ^{15}N-ERs$_{total}$ during the 75-day long incubation (Table S2). The amounts of ^{13}C- and ^{15}N-ERs$_{AMPA}$ (LC-MS/MS) decreased slowly from 55 ± 6.6% of initially applied ^{13}C/^{15}N on day 0–30 ± 1% on day 75 (Table S2). The DT$_{50}$ of 2-^{13}C,^{15}N-glyphosate and ^{13}C,^{15}N-AMPA estimated in our study was accordingly 12 days and 76 days (Table S2). This finding is in a good accordance with widely reported much slower dissipation of AMPA (DT$_{50}$ of 151–173 days) than glyphosate (DT$_{50}$ of 7–60 days) in previous studies [15,4]. This also explains why AMPA is more frequently than glyphosate detected in various land and water resources [1,2,34,4]. Slightly higher amounts of ^{15}N-ERs$_{total}$ (37% of initially added ^{15}N) estimated with EA-irMS as compared to ERs$_{AMPA}$ (30%) determined with LC-MS on day 75 suggests a presence of other ^{15}N-compounds than the parent compound AMPA. Since AMPA degrading bacterial strains have been recently reported [32], we presume degradation of AMPA to methylamine and finally to NH_4^+ occurred in this study (see Fig. S1). We thus believe that a small ^{15}N-excess (7%) in the ^{15}N-ERs (difference between the ^{15}N-ERs$_{total}$ and ERs$_{AMPA}$) could be $^{15}NH_4^+$ released from AMPA breakdown. The $^{15}NH_4^+$ may have been used by soil microorganisms for biomass synthesis as supported by amino acid data in Section 3.3.

Due to soil matrix effects, the measurements of ^{13}C/^{15}N-ERs$_{glycine}$ and ^{13}C/^{15}N-ERs$_{sarcosine}$ by LC-MS/MS were highly uncertain; therefore, we relied only on the ^{13}C- and ^{15}N-ERs$_{total}$ measured by EA-irMS. About 49 ± 0.9% of initial $^{13}C_3$-sarcosine equivalents and 9.2 ± 0.3% of initial $^{13}C_2$-glycine equivalents were measured in the ^{13}C-ERs$_{total}$ on day 0 (Table S2). Sarcosine and glycine are both easily biodegradable molecules [36]; therefore, only small amounts of ^{13}C-ERs$_{total}$ were measured after 2 days in the $^{13}C_2$,^{15}N-glycine (0.8–1.6%) and $^{13}C_3$,^{15}N-sarcosine (0.6–3.9%) experiments. The sarcosine dissipated a bit slower (DT$_{50}$: 0.85 day; see Table S2) than glycine (0.79 day). Similar result was obtained by Sun et al. [36] who had found that methyl-d$_3$-sarcosine (DT$_{50}$ of 0.99 day) dissipated a bit slower than d$_5$-glycine (0.89 day) in soil-water system. In contrast to ^{13}C-ERs$_{total}$, the ^{15}N-ERs$_{total}$ were nearly constant until the end of incubation and for both compounds (^{15}N-sarcosine: 38–42%, ^{15}N-glycine: 41–46% and except for day 0; see in

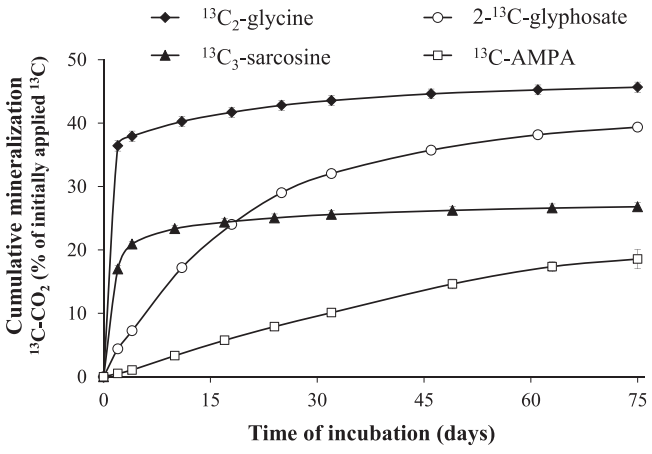

Fig. 2. Cumulative mineralization of 2-^{13}C-glyphosate and its degradation products (^{13}C-AMPA, $^{13}C_2$-glycine and $^{13}C_3$-sarcosine) in soil during 75-day incubation and shown in % of initially applied ^{13}C.

Table S2). The higher estimates of ^{15}N-ERs$_{total}$ than the ^{13}C-ERs$_{total}$ for $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine as well as for 2-^{13}C,^{15}N-glyphosate suggest that presumably an inorganic ^{15}N (e.g. NH_4^+ or NO_x, for details please refer to **Section 2.5**) derived from microbial transformation of these compounds was extracted from soils.

3.3. Amino acids (^{13}C-AAs and ^{15}N-AAs)

The ^{13}C was incorporated into AAs from 2-^{13}C-glyphosate and from its two degradation products $^{13}C_2$-glycine and $^{13}C_3$-sarcosine already on the first sampling day 2 (Fig. 3). The amounts of ^{13}C-AAs in the 2-^{13}C-glyphosate study increased after 18 days (5.3–5.6% at 2–18 day samplings, 8.8% on day 32% and 10.9% on day 75 of initially applied ^{13}C) indicating consistent breakdown of 2-^{13}C-glyphosate and utilization by soil microorganisms. The ^{13}C-AAs results are comparable with those of Muskus et al. [27] who reported that around 10% of initial $^{13}C_3$,^{15}N-glyphosate equivalents were measured in ^{13}C-AAs after 40 days of incubation. The ^{13}C-glycine, ^{13}C-glutamate and ^{13}C-alanine were also the dominant ^{13}C-AAs in agreement with Muskus et al. [27]. The ^{13}C incorporation from both $^{13}C_2$-glycine and $^{13}C_3$-sarcosine into AAs was different from that of 2-^{13}C-glyphosate. We determined about 8% of initially applied ^{13}C in $^{13}C_{AAs}$ which remained constant till the penultimate sampling date and decreased to about 6% on day 75 in the $^{13}C_2$-glycine study. The ^{13}C-AAs in the $^{13}C_3$-sarcosine study were initially lower (6% of initially applied ^{13}C) than the one from $^{13}C_2$-glycine, but it increased to about 9% which remained stable till the day 75. Similarly, to what was observed for 2-^{13}C-glyphosate, ^{13}C-glycine, ^{13}C-glutamate and ^{13}C-alanine were also the dominant ^{13}C-AAs for $^{13}C_3$-sarcosine and $^{13}C_2$-glycine.

No ^{13}C incorporation from ^{13}C-AMPA into AAs was detected on day 4 suggesting the resistance of this compound to microbial degradation. This is also supported by the mineralization data (Section 3.1) where we observed lowest mineralization of ^{13}C-AMPA among the tested compounds. However, small amounts of ^{13}C-AAs were detected at sampling day 32 (1.3% of initially added ^{13}C) and day 75 (1.1% of the initially added ^{13}C) in the ^{13}C-AMPA experiment.

The labeling pattern of AAs with ^{15}N for ^{15}N-glyphosate, ^{15}N-sarcosine and ^{15}N-glycine was comparable to that of ^{13}C. However, higher amounts of ^{15}N-AAs were found for ^{15}N-glyphosate at 18–75 day samplings (9–13% of initially applied ^{15}N; see in Fig. S2) than the $^{13}C_{AAs}$. In contrast to ^{13}C-AAs, we found that ^{15}N-AAs in the ^{15}N-AMPA study were labeled with ^{15}N at all sampling dates. This divergence is associated with the lower ^{15}N natural abundance (0.37%) than that of ^{13}C (1.07%) in soil. Therefore, we cannot exclude a small incorporation of ^{13}C into AAs from ^{13}C-AMPA before the day 32 and which was masked by ^{13}C abundance. However, the ^{15}N-AAs were also low and ranged between 0.4% and 2.4% of initially applied ^{15}N and was lowest among four tested compounds. A slightly higher incorporation of ^{15}N (2.4%) than ^{13}C (1.1%) into AAs from ^{13}C,^{15}N-AMPA suggests that an inorganic $^{15}NH_4^+$ released from AMPA breakdown could have been directly incorporated

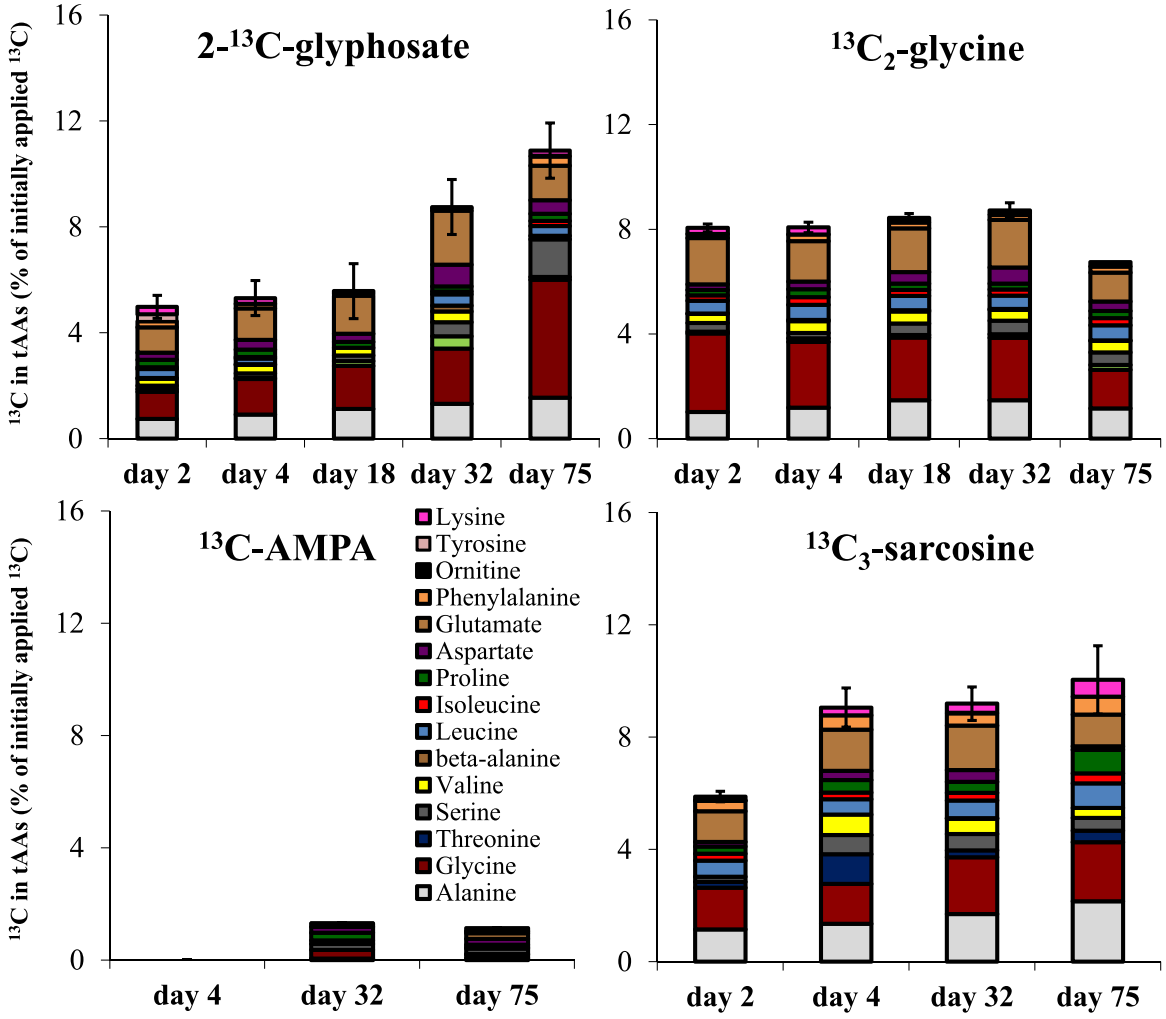

Fig. 3. Contents of ^{13}C-AAs (expressed as % of initially applied ^{13}C) in soil spiked either with 2-^{13}C-glyphosate or its major degradation products (^{13}C-AMPA, $^{13}C_2$-glycine and $^{13}C_3$-sarcosine) during 75-day incubation.

into amino acids as the NH_2-group. The ^{13}C-derived AMPA might have been lost quickly as a gaseous ^{13}C-formaldehyde or $^{13}CO_2$ [16]; see Fig. S1) resulting in a lower assimilation of ^{13}C into AAs than the ^{15}N.

Similar to what observed for ^{13}C-labeling pattern of AAs, the ^{15}N-glycine was the dominant ^{15}N-AA for ^{15}N-glyphosate, ^{15}N-sarcosine and ^{15}N-glycine. The dominant co-labeled amino acid $^{13}C,^{15}N$-glycine was presumably integrated firstly into microbial biomass as a monomeric 'building block' of macromolecular proteins [39]. A direct integration of monomers as building blocks into the macromolecules requires less energy than the biosynthesis of macromolecules derived from single C or N atoms [25]. The direct assimilation of $^{13}C,^{15}N$-glycine suggests that 2-$^{13}C,^{15}N$-glyphosate underwent the sarcosine/glycine pathway. Thereafter, the $^{13}C,^{15}N$-glycine might have been mineralized to $^{13}CO_2$ and $^{15}NH_4^+$. The ^{13}C might have been then used for synthesis of C-backbone of other ^{13}C-AAs, whereas the $^{15}NH_2$-group from ^{15}N-glycine could have been transferred to other ^{15}N-AAs in a process called transamination [27].

3.4. Mass balance

The distribution of ^{13}C and ^{15}N in the ^{13}C- and ^{15}N-mass balance was different among four compounds (Figs. 4 and 5). The $^{13}C/^{15}N$-$ERs_{glyphosate}$, as well as the ^{13}C-$ERs_{unknown}$ in the $^{13}C_2$-glycine and $^{13}C_3$-sarcosine study dissipated rapidly during the incubation period (Figs. 4 and 5). It is noteworthy that ^{15}N-$ERs_{unknown}$ were nearly constant in ^{15}N-glyphosate, ^{15}N-glycine and ^{15}N-sarcosine study (Fig. 5). Only small contents of $^{13}C/^{15}N$-$ERs_{glyphosate}$ were measured by LC-MS/MS after 18 days (< 5% of initially applied ^{13}C, Table S2) at the later period of incubation. Also traces of ^{13}C-$ERs_{unknown}$ (< 4% of initially applied ^{13}C, Table S2) as compared to ^{15}N-$ERs_{unknown}$ (> 38% of initially applied ^{15}N) were measured for $^{13}C_2$-glycine and $^{13}C_3$-sarcosine studies from day 2 onwards. These findings thus suggest that the ^{15}N excess in the ^{15}N-$ERs_{unknown}$ for ^{15}N-glyphosate and most of the ^{15}N in the ^{15}N-$ERs_{unknown}$ for both ^{15}N-glycine and ^{15}N-sarcosine cannot be assigned to the parent compound ^{15}N-glyphosate, ^{15}N-glycine, or ^{15}N-sarcosine, but presumably to inorganic ^{15}N (e.g. NH_4^+ or NO_x). In contrast, the amounts of ^{13}C-ERs_{AMPA} and ^{15}N-ERs_{AMPA} in the $^{13}C,^{15}N$-AMPA study were comparable and ranged between 30% and 55% of initially applied ^{13}C or ^{15}N, except for day 75. The amounts of $^{13}C/^{15}N$-$NERs_{total}$ ($^{13}C/^{15}N$-$NERs_{biogenic}$ + $^{13}C/^{15}N$-$NERs_{unknown}$) as well as their speciation also differentiated among four tested compounds (see also Table S1). The highest $^{13}C/^{15}N$-$NERs_{total}$ were noticed for 2-$^{13}C,^{15}N$-glyphosate (29–56% of initially applied ^{13}C or ^{15}N) throughout the incubation time. The amounts of $^{13}C/^{15}N$-$NERs_{total}$ for $^{13}C,^{15}N$-AMPA (28–40% of initially applied ^{13}C or ^{15}N), $^{13}C_2,^{15}N$-glycine (25–55% of the initially added ^{13}C or ^{15}N) and $^{13}C_3^{15}N$-sarcosine (13–33% of initially applied ^{13}C or ^{15}N) were lower. At the end of incubation, a big portion of ^{13}C- and ^{15}N-$NERs_{total}$ of 2-$^{13}C,^{15}N$-glyphosate (50%), $^{13}C_2,^{15}N$-glycine (40%) and $^{13}C_3,^{15}N$-sarcosine (98%) were harmless $^{13}C/^{15}N$-$NERs_{biogenic}$. Both $^{13}C_2,^{15}N$-glycine and $^{13}C_3,^{15}N$-sarcosine are biomolecules; therefore, the ^{13}C- and the ^{15}N-$NERs_{unknown}$ are expected to contain other harmless $^{13}C/^{15}N$-biomolecules or inorganic ^{15}N (e.g. NH_4^+ or NO_x) sorbed to soil matrix. It is thus likely that the amounts of $^{13}C/^{15}N$-$NERs_{biogenic}$ (AAs*2) derived from both $^{13}C_2,^{15}N$-glycine and $^{13}C_3,^{15}N$-sarcosine are underestimated. In contrast, the $^{13}C/^{15}N$-NERs from $^{13}C,^{15}N$-AMPA were mainly $^{13}C/^{15}N$-$NERs_{unknown}$ which might be AMPA either strongly sorbed/sequestered to soil matrix (type I) or covalently bound to soil matrix (type II) as hazardous $NERs_{xenobiotic}$ [33] with a remobilization potential. The $NERs_{xenobiotic}$ (type I) may pose greater environmental risk due to remobilization as compared to the covalently bound $NERs_{xenobiotic}$ (type II) which are known to have a low potential for remobilization [23,33]. However, we did not differentiate between the two types of NERs from AMPA; therefore, we cannot predict risks related to the NER formation from AMPA. A future study differentiating between the NER type I and II from AMPA would be thus necessary for a more accurate assessment of the risks related to $NERs_{xenobiotic}$ from AMPA.

3.5. Implications for environmental fate and risk assessment

Our results indicate greater environmental risk when glyphosate follows AMPA degradation pathway. This is evident from greater amount of $NERs_{unknown}$ that can comprise hazardous $NERs_{xenobiotic}$ in

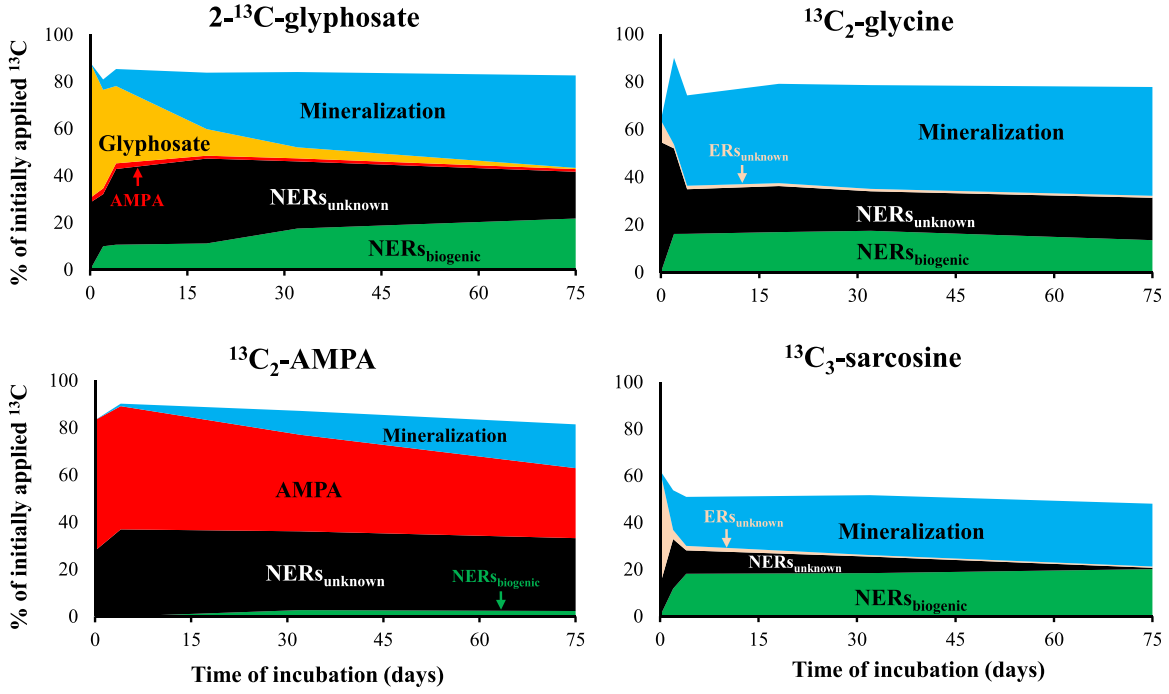

Fig. 4. ^{13}C mass balance of the fate of 2-^{13}C-glyphosate, ^{13}C-AMPA, $^{13}C_3$-sarcosine and $^{13}C_2$-glycine in soil during 75-day incubation and shown as % of initially applied ^{13}C. NERs: non-extractable residues, $NERs_{biogenic}$: biogenic NERs (AAs*2), ERs: extractable residues. $ERs_{unknown}$ for $^{13}C_2$-glycine and $^{13}C_3$-sarcosine may include the parent compound $^{13}C_2$-glycine or $^{13}C_3$-sarcosine or other ^{13}C-organic or inorganic compounds. $NERs_{unknown}$: $NERs_{total}$ – $NERs_{biogenic}$.

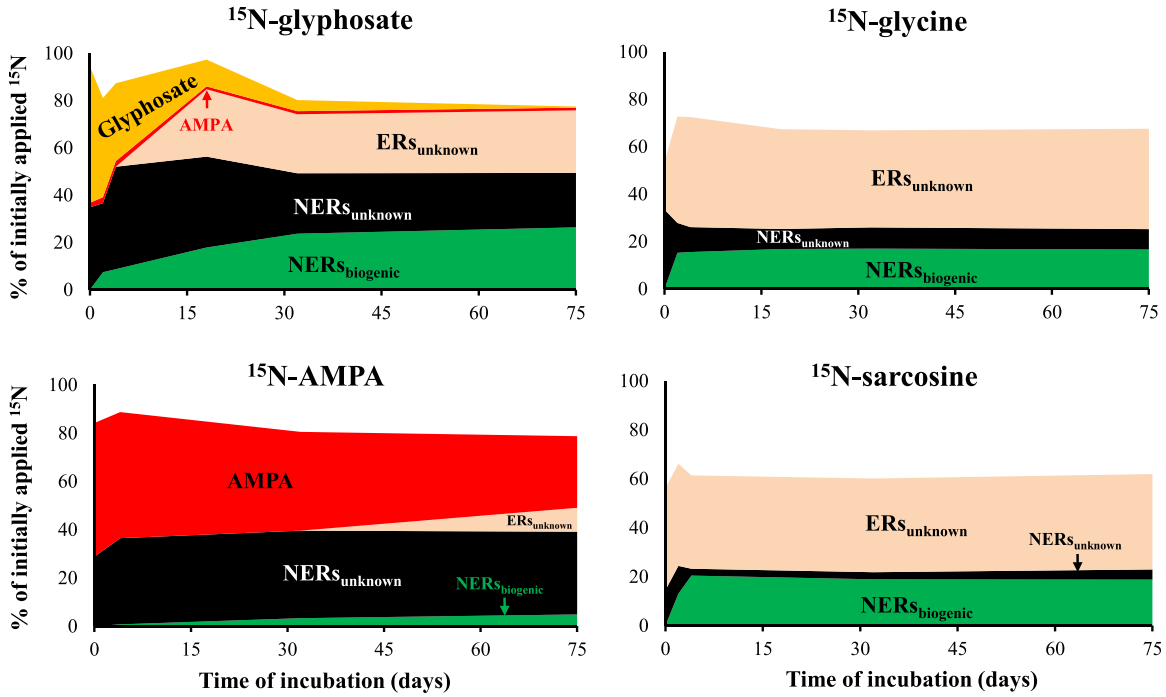

Fig. 5. ^{15}N mass balance of the fate of ^{15}N-glyphosate, ^{15}N-AMPA, ^{15}N-sarcosine and ^{15}N-glycine in soil during 75-day incubation and shown as % of initially applied ^{15}N. NERs: non-extractable residues, $NERs_{biogenic}$: biogenic NERs (AAs*2), ERs: extractable residues. $ERs_{unknown}$ for ^{15}N-glyphosate and ^{15}N-AMPA: ERs_{total} (EA-irMS) – $ERs_{glyphosate}$ or ERs_{AMPA} (LC-MS/MS). The $ERs_{unknown}$ for ^{15}N-glycine and ^{15} N-sarcosine may include the parent compound ^{15}N-glycine or ^{15} N-sarcosine. Much higher ^{15}N-$ERs_{unknown}$ than the ^{13}C-$ERs_{unknown}$ suggest that most of the ^{15}N in the ^{15}N-$ERs_{unknown}$ for ^{15}N-glycine and ^{15} N-sarcosine will not be the parent compound, but presumably an inorganic ^{15}N (e.g. NH_4^+ or NO_x). $NERs_{unknown}$: $NERs_{total}$ – $NERs_{biogenic}$.

soil which may remobilize later therefore delaying the environmental risk. Moreover, AMPA will be mainly sorbed to soil matrix as $NERs_{xenobiotic}$ since it is biodegraded slowly as showed in the soil incubated with ^{13}C,^{15}N-AMPA. High amounts of AMPA residues, both as ERs or $NER_{xenobiotic}$ presents a toxicity risk to soil micro and macro fauna such as earthworms [13,37,7,8] as well as to many species of aquatic ecosystems like Zebrafish [38]. However, when glyphosate is biodegraded via sarcosine/glycine pathway, the $NERs_{total}$ comprise mainly $NERs_{biogenic}$ since the ^{13}C and ^{15}N derived from 2-^{13}C,^{15}N-glyphosate will be used by microorganisms to synthesize biomolecules like AAs.

The 2-^{13}C,^{15}N-glyphosate was biodegraded via two pathways: the sarcosine/glycine and the AMPA pathway simultaneously as we measured both ^{13}C,^{15}N-AMPA (Section 3.2) and ^{13}C,^{15}N-glycine (Section 3.3) in soils. However, a high portion (20–50%) of the ^{13}C/^{15}N-$NERs_{total}$ was attributed to harmless ^{13}C/^{15}N-$NERs_{biogenic}$ in the soil incubated with 2-^{13}C,^{15}N-glyphosate (Figs. 4 and 5). Furthermore, high amounts of ^{15}N-$ERs_{unknown}$ representing presumably inorganic ^{15}N (NH_4^+ or NO_x) in % of initially applied ^{15}N were measured for ^{15}N-glyphosate (25–28%), ^{15}N-glycine (41–46%) and ^{15}N-sarcosine (38–42%) as compared to ^{15}N-AMPA (0–9.9%). This finding thus suggests that ^{15}N-glyphosate underwent similar ^{15}N transformation processes to ^{15}N-glycine or ^{15}N-sarcosine. An evidence for a prevalence of the sarcosine/glycine pathway over the AMPA degradation pathway during the biodegradation of 2-^{13}C,^{15}N-glyphosate is the predominance of $^{13}C_2$,^{15}N-glycine in the total pool of ^{13}C/^{15}N-AAs not only in the 2-^{13}C,^{15}N-glyphosate study, but also in $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine studies as shown in Fig. 3 and **S2**. The co-labeled $^{13}C_2$,^{15}N-glycine was presumably assimilated firstly into microbial biomass as a monomer [39]. Afterwards, the $^{15}NH_2$-group from the $^{13}C_2$,^{15}N-glycine might have been released as $^{15}NH_4^+$ and attributed to ^{15}N-$ERs_{unknown}$. If 2-^{13}C,^{15}N-glyphosate would follow mainly the AMPA pathway, single-labeled ^{13}C-glycine would be only produced, since the ^{15}N would be retained in ^{15}N-AMPA (see Fig. 1). In this case, minimal amounts of ^{15}N-$ERs_{unknown}$ would be measured in the ^{15}N-glyphosate study.

It is difficult to differentiate between the sarcosine and the glycine pathway. In $^{13}C_3$,^{15}N-sarcosine and $^{13}C_2$,^{15}N-glycine experiments, we measured comparable amounts of ^{13}C-glycine and ^{15}N-glycine (ratio of ^{13}C-glycine to ^{15}N-glycine ~ 1 with few exceptions; see Table S3). Therefore, both pathways could have occurred during the biodegradation of 2-^{13}C,^{15}N-glyphosate; and the $^{13}C_3$,^{15}N-sarcosine could have been oxidized to $^{13}C_2$,^{15}N-glycine (see degradation pathways of sarcosine and glycine in Fig. S1). The amounts of ^{15}N-glycine formed from 2-^{13}C,^{15}N-glyphosate were much higher than the ^{13}C-glycine (^{13}C:^{15}N ratio < 0.7). This suggests that the glycine or sarcosine formed from 2-^{13}C,^{15}N-glyphosate was further transformed by microorganisms, presumably to inorganic compounds like CO_2, NH_4^+ or NO_x (Fig. S1) and other biomolecules like AAs (Fig. 3 and **S2**).

To conclude, a precise estimation of the fate of major intermediate compound(s) and its relative proportion(s) could help to elucidate complex fate processes as well as NER speciation of a given chemical in soils. The knowledge about the NER speciation is important for the environmental risk assessment related to the formation of $NERs_{xenobiotic}$ The resulting $NERs_{xenobiotic}$ or $NERs_{biogenic}$ may be formed not directly from the parent chemical but also from its degradation products as it was shown for 2-^{13}C,^{15}N-glyphosate, i.e. $^{13}C_2$,^{15}N-glycine or $^{13}C_3$,^{15}N-sarcosine, which both contributed significantly to harmless ^{13}C/^{15}N-$NER_{biogenic}$ formation. Therefore, the determination of mass balance of the fate of major degradation product(s) including $NERs_{biogenic}$ formation using multiple isotope labeling (^{13}C + ^{15}N) from other environmentally relevant chemicals could improve future persistency testing of chemicals.

Statement of environmental implication

Glyphosate is still a chemical of major environmental concern although its fate in agricultural soils has been extensively investigated. The degradation of glyphosate may result in production of three major degradation products: AMPA, sarcosine and glycine with different

environmental implications. This may include formation of different proportions of hazardous residues with a release potential (sorbed to soil) and harmless biomass residues (result of biological transformation). The fate and speciation of the residue formation of the three degradation products are still elusive. This knowledge could improve the future assessment of environmental risks related to the hazardous residue formation from glyphosate.

CRediT authorship contribution statement

Conceptualization: **Aslam, Jing, Nowak**, Data curation: **Aslam, Jing**, Formal analysis: **Nowak** Funding acquisition: **Aslam, Nowak**, Investigation: **Aslam**, Methodology: **Aslam, Jing**, Project administration: **Nowak**, Resources: **Aslam, Nowak**, Software: **Aslam**, Supervision: **Nowak**, Validation: **Aslam, Jing**, Visualisation: **Aslam**, Writing – original draft: **Aslam, Nowak**, Writing – review & editing: **Nowak**.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Karolina Nowak reports financial support was provided by Helmholtz Centre for Environmental Research - UFZ. Karolina Nowak reports financial support was provided by German Research Foundation. Sohaib Aslam reports was provided by Alexander von Humboldt Foundation.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.130847.

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