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

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Abstract: Glyphosate can be biodegraded via the aminomethylphosponic acid (AMPA) and the sarcosine/glycine pathway leading to the formation of three intermediate products AMPA, sarcosine or glycine that may have different environmental implications. The fate of three intermediate compounds of glyphosate biodegradation in soils has not been investigated in detail yet. Therefore, we studied the biodegradation and formation of biogenic non-extractable residues ($NERs_{biogenic}$) of glyphosate and its three degradation products in an agricultural soil. The soil was spiked with ^{13}C- and ^{15}N-labeled glyphosate (2-^{13}C,^{15}N-glyphosate), AMPA (^{13}C,^{15}N-AMPA), sarcosine ($^{13}C_3$,^{15}N-sarcosine) or glycine ($^{13}C_2$,^{15}N-glycine) and was incubated for a period of 75 days. $^{13}C_2$-glycine and $^{13}C_3$-sarcosine mineralized very rapidly within the first four days of

incubation as 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 xenobiotic as compared to other three compounds for which significant amounts of $NERs_{biogenic}$ were determined. We also found 2-^{13}C,^{15}N-glyphosate was biodegraded via two biodegradation pathways simultaneously; however, the sarcosine/glycine pathway with the formation of harmless $NERs_{biogenic}$ presumably dominated.

Keywords

Biodegradation; stable isotope probing; non-extractable residues; biogenic non-extractable residues; amino acids

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.

1. Introduction

Glyphosate is one of the most widely used herbicide worldwide due to its great efficacy against a wide variety of weeds (Benbrook, 2016). Recently, the International Agency for Research on Cancer (IARC) has classified this molecule as probably carcinogenic to humans and aquatic organisms (Wang et al., 2014). Glyphosate can be biodegraded via two pathways: the AMPA and the sarcosine pathway (Fu et al., 2017; Singh et al., 2020). The two pathways of glyphosate biodegradation result in formation of different intermediate products that have different environmental fate and implications for environmental risk assessment (Dick and Quinn, 1995). For instance, the AMPA pathway produces persistent AMPA and glyoxylate which may further form amino acid glycine (Sviridov et al., 2012; see **Figure 1**). In contrast, the sarcosine pathway yields sarcosine which is readily oxidized to glycine (Pérez Rodríguez et al., 2019; Sviridov et al., 2012; Tang et al., 2019). However, Li et al. (2018) suggested that the C-N bond of glyphosate also can be cleaved directly to glycine bypassing sarcosine formation. Each degradation product of glyphosate, i.e. AMPA, glycine or sarcosine also can determine the speciation of so-called non-extractable residues (NERs) resulting from glyphosate degradation (**Figure 1).**

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 (Roberts, 1984). 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 (Barriuso et al., 2008). However, a chemical also can undergo microbial degradation accompanied by formation of CO_2 and microbial biomass (Kästner et al., 2014). After the death of microorganisms, biomass compounds and in particular proteins are stabilized in soil matrix as

harmless biogenic NERs ($NERs_{biogenic}$; Nowak et al., 2011). 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 (Wang et al., 2016). When the $NERs_{biogenic}$ constitute a major portion of the NERs, the environmental risks associated with the $NER_{xenobiotic}$ formation will be overestimated (Nowak et al., 2011). 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 $NERs_{xenobiotic}$ (Kästner et al., 2014).

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 is more resistant to biodegradation than glyphosate (Battaglin et al., 2014; Tang et al., 2019); and it is thus expected to be mainly sorbed to soils as $NERs_{xenobiotic}$ with release potential to waters (Battaglin et al., 2014; Brock et al., 2019; Wang et al., 2016). Therefore, AMPA is found in surface and groundwater samples more frequently than glyphosate (Battaglin et al., 2014). In contrast, enhanced degradation of glyphosate via the sarcosine/glycine pathway accompanied with the glycine formation will yield mainly $NERs_{biogenic}$. Both glycine and sarcosine are biomolecules, which are readily transformed to CO_2 and microbial biomass (González-Valenzuela and Dussán, 2018; Li et al., 2018). The glycine can 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 **Figure 1** and **S1**).

Formation of three degradation products of glyphosate and their proportions: AMPA, glycine and sarcosine can therefore determine environmental risks associated with the $NER_{xenobiotic}$ formation during glyphosate degradation in soil. To date, the environmental fate and in particular the formation of $NERs_{biogenic}$ from the three glyphosate degradation products have not been

investigated. This information may help to predict more accurately the overall fate and NER speciation (hazardous $NERs_{xenobiotic}$ versus harmless $NERs_{biogenic}$) of glyphosate in soil. 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 $NER_{biogenic}$ 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 (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. (2019) 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°C 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 (60g 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}; Silva et. al, 2018). 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 (OECD, 2002). 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. (2011).

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 x 5 mm; Chrompack, Middleburg, Netherlands; Girardi et al., 2013).

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 ($^{13}C/^{15}N$-$ERs_{unknown}$). 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. 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 (Jing et al., 2021). 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-$ERs_{unknown}$ (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 (Girardi et al., 2013). The temperature of the oxidation oven was 1020°C and the one of the reduction oven was 650°C. The amount of NERs 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 50W-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. (2011) and later also reported by Muskus et al. (2019). 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. (2011) and Muskus et al. (2019). 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 (Nowak et al., 2011; Wang et al., 2016). In addition, proteins were proven to be most stable microbial biomass compounds in organic matter pool of soil (Hong et al., 2022) and thereby to be most reliable biomarker for calculation of total $NERs_{biogenic}$. The difference between the $^{13}C/^{15}N$-NERs and $^{13}C/^{15}N$-$NERs_{biogenic}$ was shown as $^{13}C/^{15}N_{NERsunknown}$ 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 **Figure 5**) were calculated as a difference between the ^{15}N-$ERs_{unknown}$ 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 $^{13}C/^{15}N$-$ERs_{unknown}$ 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-$ERs_{unknown}$ are much higher than the ^{13}C-$ERs_{unknown}$, 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% to 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 2M 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 (He et al., 2018), 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 (Jones, 1999; McFarland et al., 2010) **Figure 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 $NER_{biogenic}$ 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 (Mamy et al., 2005; Muskus et al., 2019).

3. Results and discussion

3.1. Mineralization

We observed distinct patterns of compound mineralization in our experiment (**Figure 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 soil (Muskus et al., 2019; Wang et al., 2016). At the end (75 days) of incubation, about 39±0.3% of initially added ^{13}C were mineralized and this result is comparable to that found in soils with a similar texture (26-35% of initially applied ^{13}C; Aslam et al., 2014; Gimsing et al., 2004; Nguyen et al., 2018). 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 (Carretta et al., 2021; Grandcoin et al., 2017) 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.

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). Both compounds are quickly utilized by microorganisms as a carbon source in anabolic and catabolic reactions (Greenwood and Lees, 1960; Jones, 1999; McFarland et al., 2010; Zhang et al., 2019). Sarcosine is also commonly known precursor to glycine during glyphosate biodegradation through sarcosine pathway which could further follow the degradation pattern of glycine (see **Figure S1;** Ermakova et al., 2017; Sun et al., 2019). This thus could 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$_{unknown}$ on day 0 with EA-irMS in the 2-^{13}C-glyphosate study (see **Table S2**). Thereafter, the amounts of ^{13}C-ERs$_{unknown}$ decreased rapidly to only 0.7±0.3% at end of the incubation. In contrast, the contents of ^{15}N-ERs$_{unknown}$ 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$_{unknown}$ 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$_{unknown}$ (except for day 75) and ^{15}N-ERs (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 to 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. (2019) 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. (2019) 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. (2019) 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. (2019) 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$_{unknown}$ 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$_{unknown}$ 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 to 30±1% on day 75 (**Table S2**). The dissipation half-life (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 (Battaglin et al., 2014; Dick and Quinn, 1995; Gros et al., 2020). This also explains why AMPA is more frequently than glyphosate detected in various land and water resources (Alonso et al., 2018; Aparicio et al., 2013; Battaglin et al., 2014; Silva et al., 2018).

Due to soil matrix effects, the measurements of $^{13}C/^{15}N\text{-}ERs_{glycine}$ and $^{13}C/^{15}N\text{-}ERs_{sarcosine}$ by LC-MS/MS were highly uncertain; therefore, we relied only on the ^{13}C- and $^{15}N\text{-}ERs_{unknown}$ 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 $^{13}C\text{-}ERs_{unknown}$ for on day 0 (**Table S2).** Sarcosine and glycine are both easily biodegradable molecules (Sun et al., 2019); therefore, only small amounts of $^{13}C\text{-}ERs_{unknown}$ 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. (2019) who had found that methyl-d_3-sarcosine (DT_{50} of 0.99 day^{-1}) dissipated a bit slower than d_5-glycine (0.89 day^{-1}) in soil-water system. In contrast to $^{13}C\text{-}ERs_{unknown}$, the $^{15}N\text{-}ERs_{unknown}$ 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 **Table S2**). The higher estimates of $^{15}N\text{-}ERs_{unknown}$ than the $^{13}C\text{-}ERs_{unknown}$ 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 (**Figure 3**). The amounts of

355 ^{13}C-AAs in the 2-^{13}C-glyphosate study increased after 18 days (5.3-5.6% at 2-18 day samplings,
356 8.8% on day 32 and 10.9% on day 75 of initially applied ^{13}C) indicating consistent breakdown of
357 2-^{13}C-glyphosate and utilization by soil microorganisms. The ^{13}C-AAs results are comparable
358 with those of Muskus et al. (2019) who reported that around 10% of initial $^{13}C_3$,^{15}N-glyphosate
359 equivalents were measured in ^{13}C-AAs after 40 days of incubation. The ^{13}C-glycine, ^{13}C-
360 glutamate and ^{13}C-alanine were also the dominant ^{13}C-AAs in agreement with Muskus et al.
361 (2019). The ^{13}C incorporation from both $^{13}C_2$-glycine and $^{13}C_3$-sarcosine into AAs was different
362 from that of 2-^{13}C-glyphosate. We determined about 8% of initially applied ^{13}C in $^{13}C_{AAs}$ which
363 remained constant till the penultimate sampling date and decreased to about 6% on day 75 in the
364 $^{13}C_2$-glycine study. The ^{13}C-AAs in the $^{13}C_3$-sarcosine study were initially lower (6% of initially
365 applied ^{13}C) than the one from $^{13}C_2$-glycine, but it increased to about 9% which remained stable
366 till the day 75. Similarly, to what was observed for 2-^{13}C-glyphosate, ^{13}C-glycine, ^{13}C-glutamate
367 and ^{13}C-alanine were also the dominant ^{13}C-AAs for $^{13}C_3$-sarcosine and $^{13}C_2$-glycine.

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

373 The labeling pattern of AAs with ^{15}N for ^{15}N-glyphosate, ^{15}N-sarcosine and ^{15}N-glycine was
374 comparable to that of ^{13}C. However, higher amounts of ^{15}N-AAs were found for ^{15}N-glyphosate
375 at 18-75 day samplings (9-13% of initially applied ^{15}N; see in **Figure S2**) than the $^{13}C_{AAs}$. In
376 contrast to ^{13}C-AAs, we found that ^{15}N-AAs in the ^{15}N-AMPA study were labeled with ^{15}N at all
377 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. 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 (Wang et al., 2016). 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 (Madigan et al., 2011). 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 (Muskus et al. 2019).

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 (**Figure 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 (**Figure 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 (**Figure 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 study 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. The amounts of ^{13}C/^{15}N-NERs (^{13}C/^{15}N-NERs$_{biogenic}$ + ^{13}C/^{15}N-NERs$_{unknown}$) as well as their speciation also differentiated among four tested compounds. The highest ^{13}C/^{15}N-NERs 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 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. A big portion of ^{13}C- and ^{15}N-NERs of 2-^{13}C,^{15}N-glyphosate, $^{13}C_2$,^{15}N-glycine and $^{13}C_3$,^{15}N-sarcosine were harmless ^{13}C/^{15}N-NERs$_{biogenic}$. However, 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 sorbed AMPA to soil matrix as hazardous NERs$_{xenobiotic}$ with a remobilization potential.

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 hazardous NERs$_{xenobiotic}$ formation in soil which

may remobilize later therefore delaying the environmental risk. Moreover, AMPA which is formed as major intermediate compound will stay longer in the soil compared to glyphosate since it is biodegraded slowly and sorbed to soil matrix as $NERs_{xenobiotic}$ as showed in the soil incubated with $^{13}C,^{15}N$-AMPA. However, when glyphosate is biodegraded via sarcosine/glycine pathway, the NERs 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 was attributed to harmless $^{13}C/^{15}N$-$NERs_{biogenic}$ in the soil incubated with 2-$^{13}C,^{15}N$-glyphosate (**Figure 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 that of for ^{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. The $^{13}C_2,^{15}N$-glycine produced from the sarcosine/glycine pathway was one of the predominant AAs for 2-$^{13}C,^{15}N$-glyphosate as well as for $^{13}C_2,^{15}N$-glycine and $^{13}C_3,^{15}N$-sarcosine as shown in **Figure 3** and **S2**. The co-labeled $^{13}C_2,^{15}N$-glycine was presumably assimilated firstly into microbial biomass as a monomer. 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 **Figure 1**). In this case, minimal amounts of ^{15}N-$ERs_{unknown}$ would be measured in the ^{15}N-glyphosate study. Therefore, the sarcosine/glycine degradation pathway most probably

prevailed over the AMPA degradation pathway during the biodegradation of 2-^{13}C,^{15}N-glyphosate. However, 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 occur 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 **Figure 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 (**Figure S1**) and other biomolecules like AAs (**Figure 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 contributed significantly to harmless ^{13}C/^{15}N-$NER_{biogenic}$ formation. Therefore, the determination of mass balance of major degradation product(s) including $NERs_{biogenic}$ from other environmentally relevant chemicals could improve future persistency testing of chemicals.

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Figures

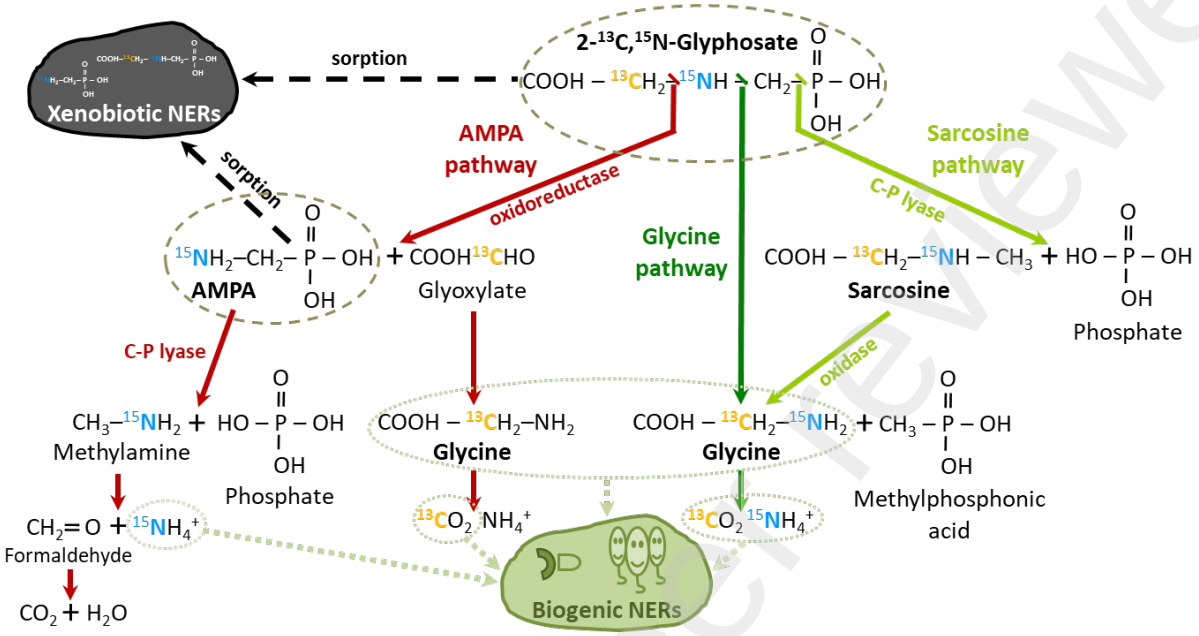
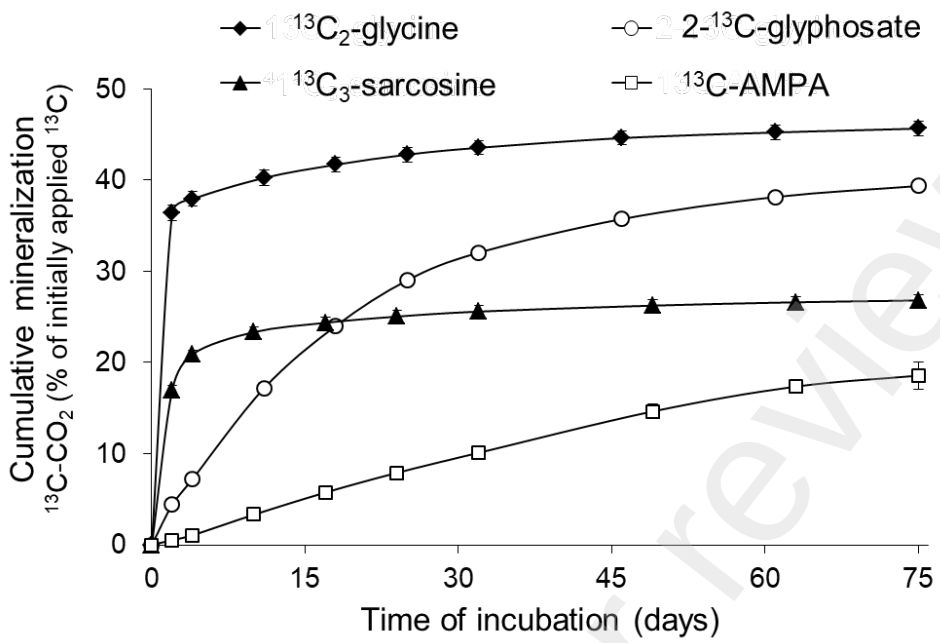


Figure 1: Formation of degradation products of 2-^{13}C,^{15}N-glyphosate and speciation of non-extractable residues (NERs: xenobiotic or biogenic NERs) as a consequence of three degradation pathways.



610

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

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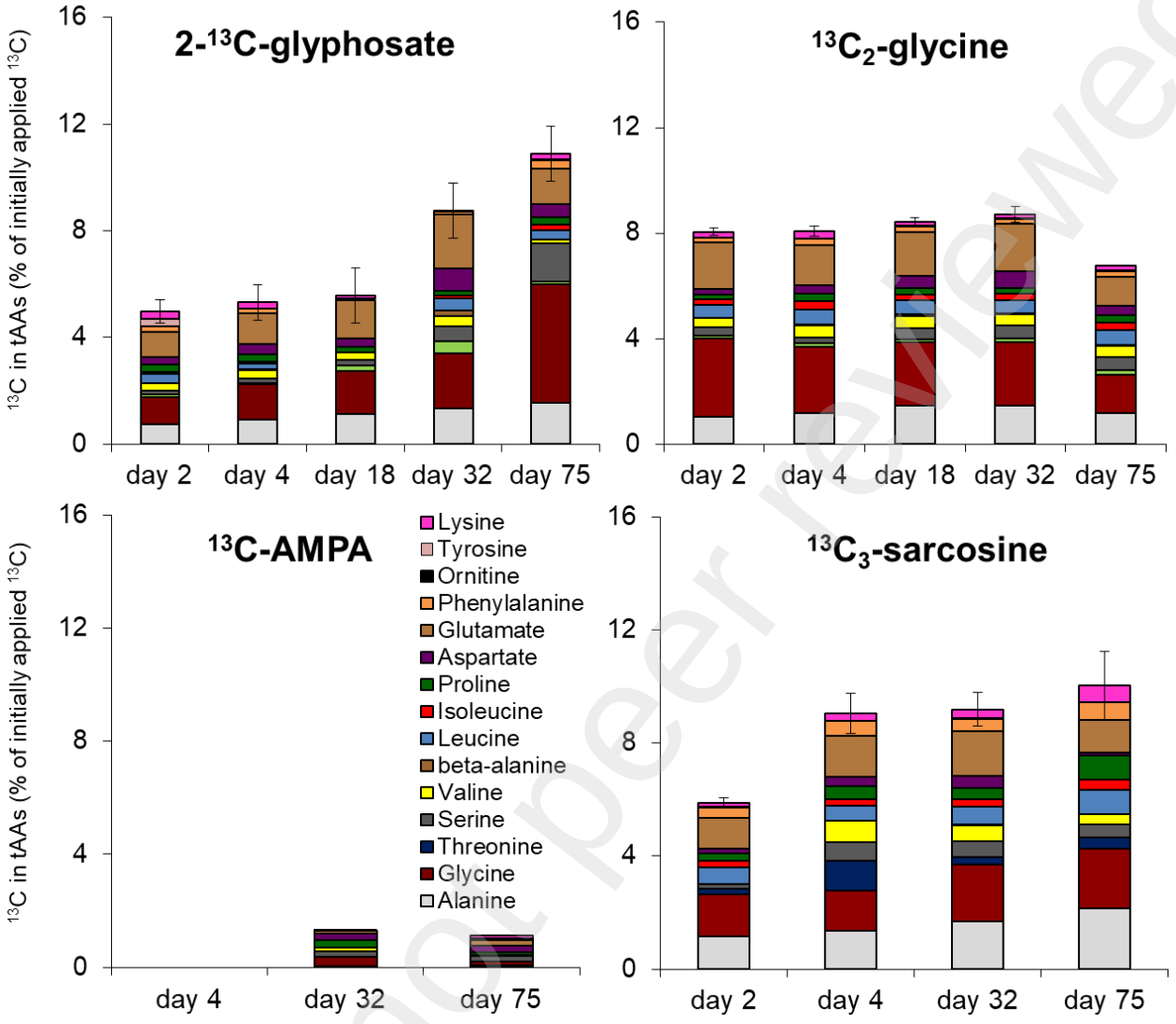

615

616 **Figure 3:** Contents of ^{13}C-AAs (expressed as % of initially applied ^{13}C) in soil spiked either with

617 2-^{13}C-glyphosate or its major degradation products (^{13}C-AMPA, $^{13}C_2$-glycine and $^{13}C_3$-sarcosine)

618 during 75-day incubation.

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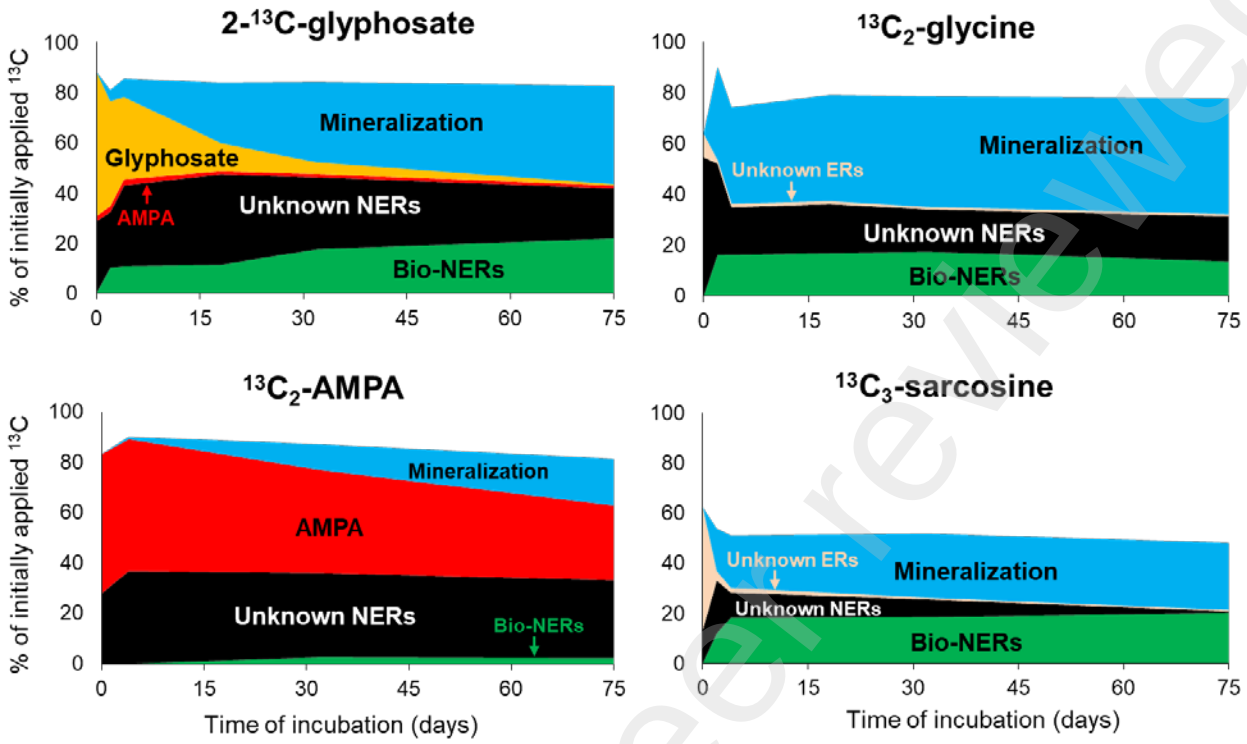

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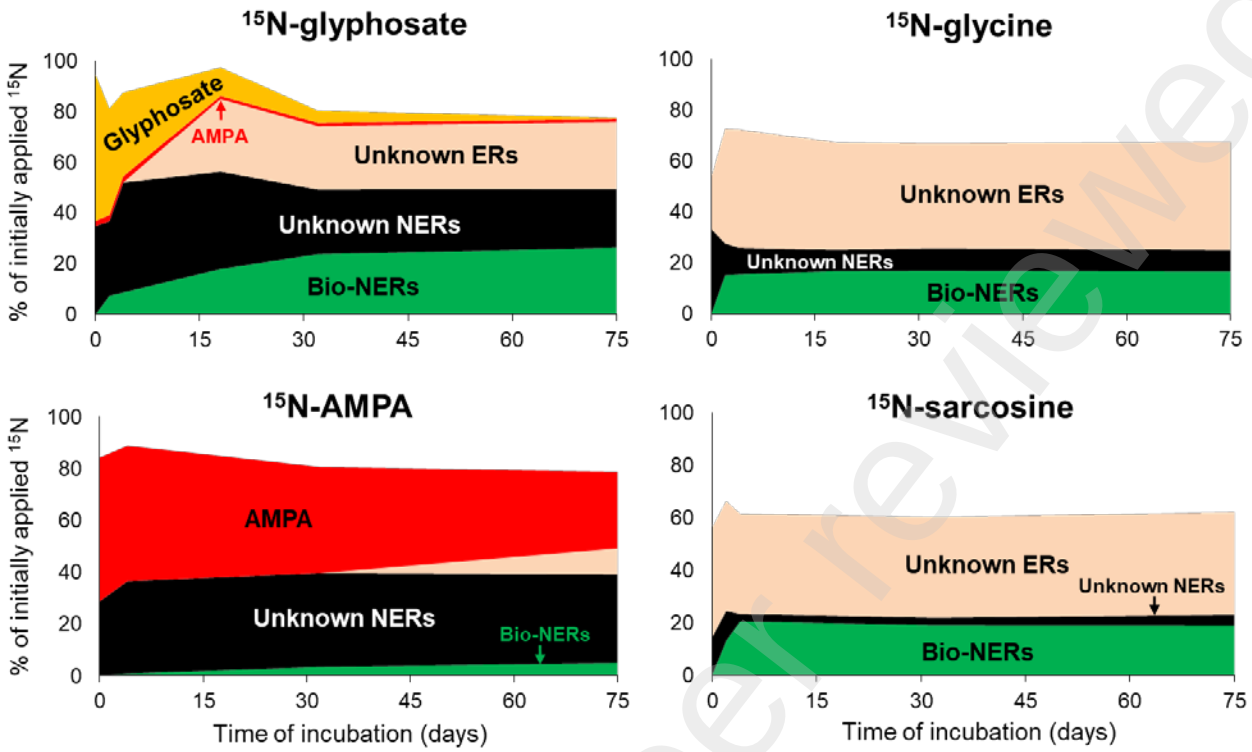
621 **Figure 4:** ^{13}C mass balance of the fate of 2-^{13}C-glyphosate, ^{13}C-AMPA, $^{13}C_3$-sarcosine and $^{13}C_2$-
622 glycine in soil during 75-day incubation and shown as % of initially applied ^{13}C.
623 NERs: non-extractable residues, Bio-NERs: biogenic NERs (Bio-NERs: AAs*2), ERs:
624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

627



628

629 **Figure 5:** ^{15}N mass balance of the fate of ^{15}N-glyphosate, ^{15}N-AMPA, ^{15}N-sarcosine and ^{15}N-
630 glycine in soil during 75-day incubation and shown as % of initially applied ^{15}N.
631 NERs: non-extractable residues, Bio-NERs: biogenic NERs (Bio-NERs: AAs*2), ERs:
632 extractable residues. Unknown ERs for ^{15}N-glyphosate and ^{15}N-AMPA: $ERs_{unknown}$ (EA-irMS) –
633 $ERs_{glyphosate}$ or ERs_{AMPA} (LC-MS/MS). The unknown ERs for ^{15}N-glycine and ^{15}N-sarcosine may
634 include the parent compound ^{15}N-glycine or ^{15}N-sarcosine. Much higher ^{15}N-$ERs_{unknown}$ than the
635 ^{13}C-$ERs_{unknown}$ suggest that most of the ^{15}N in the ^{15}N-$ERs_{unknown}$ for ^{15}N-glycine and ^{15}N-
636 sarcosine will not be the parent compound, but presumably an inorganic ^{15}N (e.g. NH_4 or NO_x).
637 Unknown NERs: total NERs – Bio-NERs.

Supplementary information

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

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Text 1

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Summary of supporting information

Text 1. Calculation of ^{13}C and ^{15}N labels in CO_2, extractable residues (ERs), total NERs and amino acids (AAs).

Table S1: Turnover mass balance of 2-^{13}C,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, $^{13}C_3$,^{15}N-sarcosine and $^{13}C_2$,^{15}N-glycine in soil during 75-day incubation.

Table S2: Extractable ^{13}C- or ^{15}N-residues (^{13}C- or ^{15}N-ERs) of 2-^{13}C,^{15}N-glyphosate or its degradation product ^{13}C,^{15}N-AMPA, $^{13}C_3$,^{15}N-sarcosine and $^{13}C_2$,^{15}N-glycine from soil over 75-day incubation period measured as the **^{13}C-** or **^{15}N-$ERs_{unknown}$** measured by EA-irMS and as the parent chemical or its degradation product (**^{13}C/^{15}N-$ERs_{glyphosate}$**, **^{13}C/^{15}N-ERs_{AMPA}**) by LC-MS/MS. The values are shown as % of initially added compound or ^{13}C/^{15}N label. The dissipation half-life (DT_{50}, days) was estimated using first order kinetics (Mamy et al., 2005; Muskus et al., 2019).

Table S3: Contents of $^{13}C_2$,^{15}N-glycine (expressed as % of initially applied ^{13}C or ^{15}N) and ^{13}C:^{15}N glycine ratio incorporated by microbes after biodegrading of four compounds during 75-day incubation.

Figure S1: Degradation pathways of $^{13}C_3$,^{15}N-sarcosine, $^{13}C_2$,^{15}N-glycine and ^{13}C,^{15}N-AMPA in soil and potential nature of non-extractable residues (NERs; xenobiotic or biogenic NERs).

Figure S2: Contents of ^{15}N-AAs (expressed as % of initially applied ^{15}N) in soil spiked either with ^{15}N-glyphosate or its major degradation products (^{15}N-AMPA, ^{15}N-glycine and ^{15}N-sarcosine) during 75-day incubation.

Text 1. Calculation of ^{13}C and ^{15}N labels in CO_2, extractable residues (ERs), total NERs and amino acids (AAs).

A heavy isotope (^{13}C and ^{15}N) of the introduced substrate is masked by the natural abundance of heavy isotopes in soils. The natural abundance 'overlapping' with the introduced isotopes from the added labeled substrate was corrected with the help of two controls (I: without substrate and II: unlabeled substrate). The estimation of excess of the respective heavy isotope (^{13}C and ^{15}N) over the controls in mineralization ($^{13}CO_2$), total label in $ERs_{unknown}$, NERs and AAs was calculated as shown in **eq. 3**.

$^{13}C/^{15}N$ control I = total C or N × at% (eq. 1)

$^{13}C/^{15}N$ control II = total C or N × at% + C/N-unlabeled substrate × at% unlabeled substrate (eq. 2a)

$^{13}C/^{15}N$ labeled = total C or N × at% + C/N-labeled substrate × at% labeled substrate (eq. 2b)

$^{13}C/^{15}N$ excess = $^{13}C/^{15}N$-labeled **– $^{13}C/^{15}N$ control (average of I + II)** **(eq. 3)**

Where:

at%: at% $^{13}C/^{12}C$ or at% $^{15}N/^{14}N$

Table S1: Turnover mass balance of 2-^{13}C,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, $^{13}C_3$,^{15}N-sarcosine and $^{13}C_2$,^{15}N-glycine in soil during 75-day incubation and shown as % of initially added ^{13}C/^{15}N label.

		% of ^{13}C-labeled molecule				% of ^{15}N-labeled molecule		
Compound	**Time (days)**	**Mineralization**	**ERs***	**NERs (Biogenic)**	**Recovery**	**ERs**	**NERs (Biogenic)**	**Recovery**
2-^{13}C,^{15}N-glyphosate	0	n.d.	60±1	29±1.2 (n.d.)	89±2.2	60±1	35±1.5 (n.d.)	95±2.5
	2	4.4±0.02	44±1.4	32±0.9 (9.9)	81±2.4	44±1.4	37±0.6 (7.4)	81±2
	4	7.3±0.1	35±0.5	43±0.7 (11)	85±1.2	35±0.5	52±0.7 (8.7)	87±1.2
	18	24±0.1	13±0.2	47±1.6 (11)	84±2	41±1.1	56±0.8 (17.9)	97±1
	32	32±0.2	6±0.1	46±0.1 (18)	84±0.4	31±8	49±0.2 (23.7)	80±0.4
	75	39±0.3	1.6±0.04	42±0.5 (22)	83±0.8	28±2.0	49±0.1 (26.4)	78±0.2
^{13}C,^{15}N-AMPA	0	n.d.	55±6.6	28±0.5 (n.d.)	83±7	55±6.6	29±0.3 (n.d.)	84±6
	4	1.1±0.03	52±3	37±0.6 (0)	90±3.6	52±3	37±1 (0.8)	89±4
	32	10±0.5	41±1.2	36±1.4 (2.7)	87±3	41±1.2	40±1.5 (3.3)	81±2.7
	75	19±1.5	30±0.9	33±0.1 (2.3)	82±2.5	40±3.9	40±0.2 (4.9)	79±1
$^{13}C_2$,^{15}N-glycine	0	n.d.	9.2±0.3	55±9 (n.d.)	64±9	20±3.6	34±2 (n.d.)	53±5
	2	36±0.8	1.6±0.1	52±4.5 (16)	90±5	45±0.02	28±0.8 (15)	73±1
	4	38±0.8	1.5±0.05	35±1.2 (16)	75±2	46±2.8	26±0.6 (16)	73±3
	18	42±3	1.2±0.02	36±2.3 (17)	79±3	42±1.9	25±0.8 (17)	67±2.5
	32	44±0.8	1.0±0.1	34±0.2 (17)	79±1	41±2.9	26±0.2 (17)	67±3
	75	46±0.8	0.8±0.1	31±0.5 (14)	78±1.5	42±2.2	25±0.2 (17)	68±2
$^{13}C_3$,^{15}N-sarcosine	0	n.d.	49±0.9	13±2 (n.d.)	62±3	42±2.5	15±1 (n.d.)	56±3
	2	17±0.5	3.9±0.2	33±3 (12)	54±4	42±3.9	24±0.1 (13)	66±4
	4	21±0.4	1.9±0.1	28±0.2 (18)	51±1	38±1	23±1.9 (2.4)	61±3
	32	26±0.6	0.7±0.04	25±0.4 (18)	52 ±1	38±2.7	22±0.7 (19)	60±3.4
	75	27±0.7	0.6±0.01	21±5.7 (20)	49±6	39±2.6	23±0.4 (19)	62±3

n.d.: not determined; values are shown as averages of triplicates ± standard deviation; NERs: non-extractable residues; NERs values in parenthesis represent percentage of biogenic NERs. *ERs: extractable residues: **2-^{13}C,^{15}N-glyphosate** includes 2-^{13}C,^{15}N-glyphosate and ^{15}N-AMPA (**^{13}C/^{15}N-ERs$_{glyphosate}$**, **^{13}C/^{15}N-ERs$_{AMPA}$**) estimated by LC-MS/MS, **^{13}C,^{15}N-AMPA** is ^{13}C,^{15}N-AMPA estimated by LC-MS/MS (**^{13}C/^{15}N-ERs$_{AMPA}$**), **$^{13}C_2$,^{15}N-glycine** and **$^{13}C_3$,^{15}N-sarcosine**: total ^{13}C- or ^{15}N-ERs measured by EA-irMS (**^{13}C/^{15}N-ERs$_{unknown}$**).

Table S2: Extractable ^{13}C- or ^{15}N-residues (^{13}C- or ^{15}N-ERs) of 2-^{13}C,^{15}N-glyphosate or its degradation product ^{13}C,^{15}N-AMPA, $^{13}C_3$,^{15}N-sarcosine and $^{13}C_2$,^{15}N-glycine from soil over 75-day incubation period measured as the **^{13}C-** or **^{15}N-ERs$_{unknown}$** measured by EA-irMS and as the parent chemical or its degradation product (**^{13}C/^{15}N-ERs$_{glyphosate}$**, **^{13}C/^{15}N-ERs$_{AMPA}$**) by LC-MS/MS. The values are shown as % of initially added compound or ^{13}C/^{15}N label. The dissipation half-life (DT_{50}, days) was estimated using first order kinetics (Mamy et al., 2005; Muskus et al., 2019).

	day 0	**day 2**	**day 4**	**day 18**	**day 32**	**day 75**	**DT_{50} (days)**
2-^{13}C,^{15}N-glyphosate							
ERs$_{unknown}$							
Total ^{13}C	41±5.9	17±3.4	16±4.9	11±1.6	3.1±1.0	0.7±0.3	n.d.
Total ^{15}N	59±10	25±4.6	20±6.4	41±1	31±8	28±2	n.d.
ERs$_{glyphosate}$, ERs$_{AMPA}$							
2-^{13}C,^{15}N-Glyphosate	58±1	42±1.4	33±0.4	11±0.2	4.7±0.1	0.5±0.02	12
^{15}N-AMPA	2.0±2.0	2.5±0.05	2.4±0.05	1.2±0.06	1.3±0.03	1.2±0.02	n.d.
^{13}C,^{15}N-AMPA							
ERs$_{unknown}$							
Total ^{13}C	50±8.5	n.d.	34±4.8	n.d.	28 ±2.8	25±0.8	n.d.
Total ^{15}N	45±9	n.d.	36±4.5	n.d.	36±1.8	37±4	n.d.
ERs$_{AMPA}$							
^{13}C,^{15}N-AMPA	55±6.6	n.d.	52±3	n.d.	41±1.2	30±1	76
$^{13}C_2$,^{15}N-glycine*							
ERs$_{unknown}$							
Total ^{13}C	9.2±0.3	1.6±0.08	1.5±0.05	1.2±0.02	1±0.1	0.8±0.1	0.79
Total ^{15}N	20±3.6	45±0.02	46±2.8	42±1.9	41±2.9	42±2.2	n.d.
$^{13}C_3$,^{15}N-sarcosine*							
ERs$_{unknown}$							
Total ^{13}C	49±0.9	3.9±0.2	1.9±0.1	n.d.	0.7±0.04	0.6±0.01	0.85
Total ^{15}N	42±2.5	42±3.9	38±1	n.d.	38±2.7	39±2.6	n.d.

n.d.: not determined

*measurement of sarcosine and glycine by LC-MS/MS was impeded by soil matrices; therefore, only data of ^{13}C or ^{15}N-ERs$_{unknown}$ measured by EA-irMS are shown.

Table S3: Contents of $^{13}C_2$,^{15}N-glycine (expressed as % of initially applied ^{13}C or ^{15}N) and ^{13}C:^{15}N glycine ratio incorporated by microbes after biodegrading of four compounds during 75-day incubation.

		Glycine		
		^{13}C Contents	**^{15}N Contents**	**^{13}C:^{15}N Ratio***
2-^{13}C,^{15}N-glyphosate	day 2	1.02	2.07	0.492
	day 4	1.35	2.42	0.602
	day 18	1.62	4.65	0.349
	day 32	2.08	5.97	0.349
	day 75	4.45	6.53	0.682
^{13}C,^{15}N-AMPA	day 2	n.d.	n.d.	n.d.
	day 4	N.D.	0.04	
	day 18	n.d.	n.d.	n.d.
	day 32	0.31	0.24	1.294
	day 75	0.12	0.35	0.332
$^{13}C_3$,^{15}N-sarcosine	day 2	1.48	1.80	0.822
	day 4	1.42	2.71	0.524
	day 18	n.d.	n.d.	n.d.
	day 32	2.02	2.54	0.796
	day 75	2.11	2.49	0.844
$^{13}C_2$,^{15}N-glycine	day 2	2.99	3.02	0.99
	day 4	2.51	2.63	0.956
	day 18	2.40	2.38	1.007
	day 32	2.39	2.40	0.991
	day 75	1.47	2.29	0.644

n.d.: not determined, N.D.: not detected

*Ratio of ^{13}C-glycine to ^{15}N-glycine ~ 1 indicates the prevalence of the sarcosine/glycine degradation pathway of 2-^{13}C,^{15}N-glyphosate over the AMPA degradation pathway as well as the oxidation of $^{13}C_3$,^{15}N-sarcosine to $^{13}C_2$,^{15}N-glycine in the $^{13}C_3$,^{15}N-sarcosine degradation pathway.

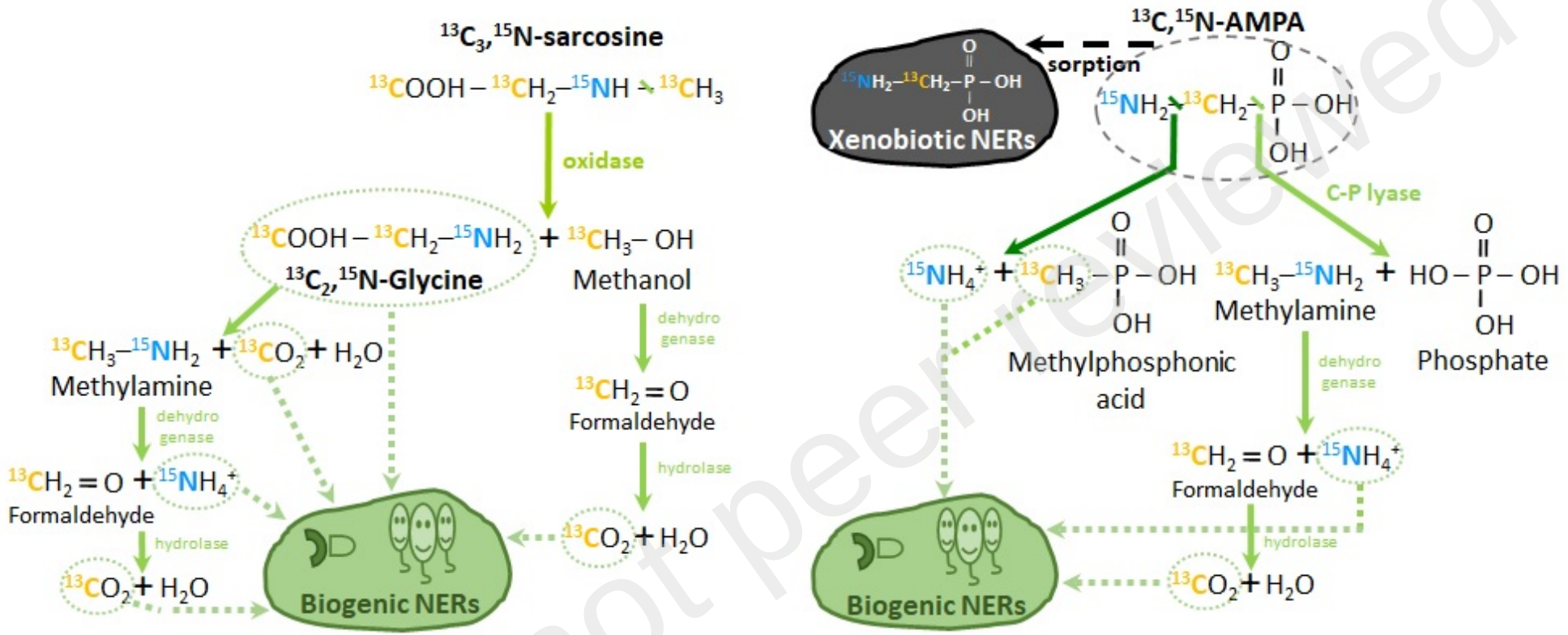

Figure S1: Degradation pathways of $^{13}C_3$,^{15}N-sarcosine, $^{13}C_2$,^{15}N-glycine and ^{13}C,^{15}N-AMPA in soil and potential nature of non-extractable residues (NERs; xenobiotic or biogenic NERs).

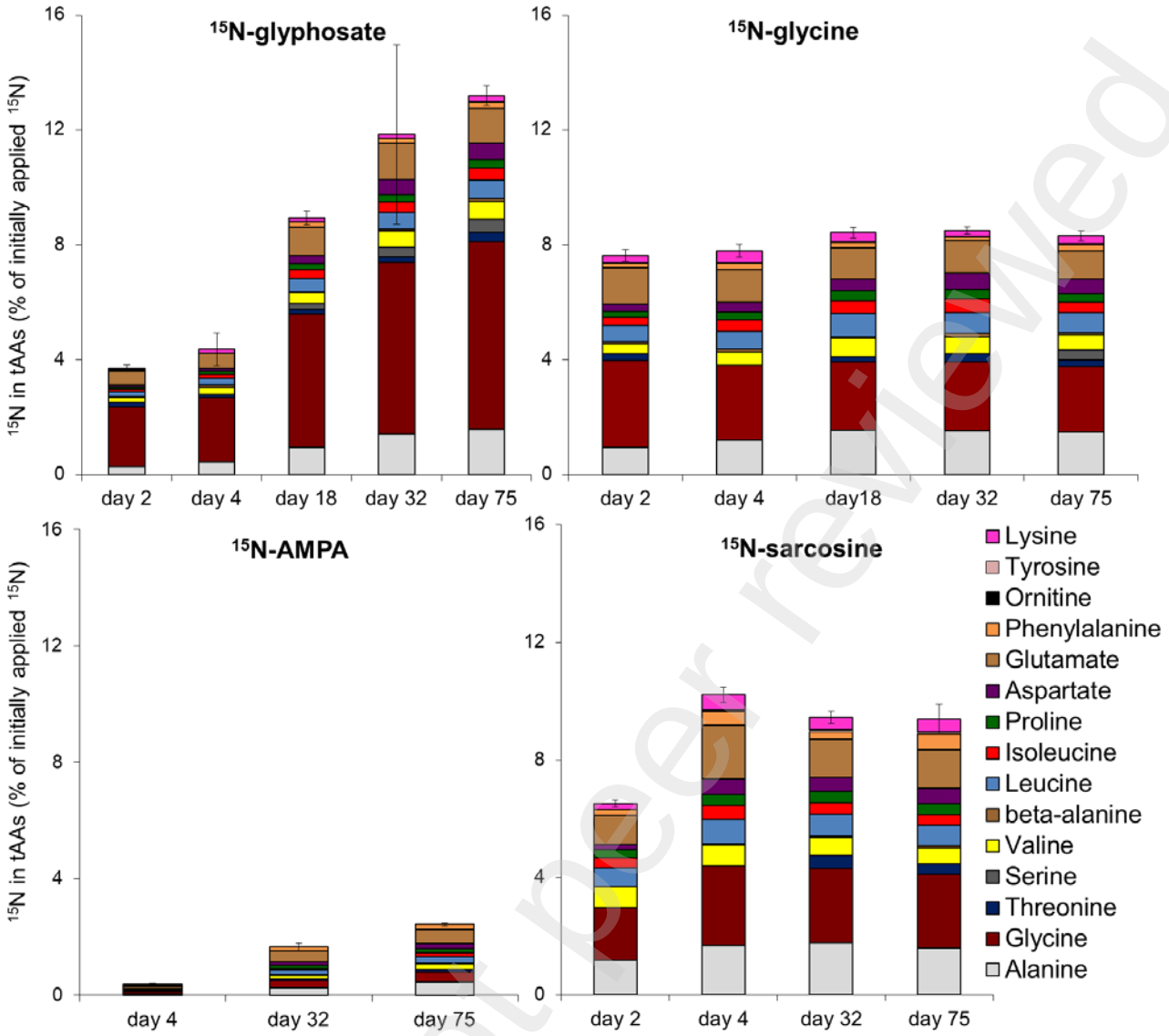

Figure S2: Contents of ^{15}N-AAs (expressed as % of initially applied ^{15}N) in soil spiked either with ^{15}N-glyphosate or its major degradation products (^{15}N-AMPA, ^{15}N-glycine and ^{15}N-sarcosine) during 75-day incubation.

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