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Degradation of glyphosate in a Colombian soil is influenced by temperature, total organic carbon content and pH

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Abstract

Glyphosate is one of the most used herbicides in the world. The fate of glyphosate in tropical soils may be different from that in soils from temperate regions. In particular, the amounts and types of non-extractable residues (NER) may differ considerably, resulting in different relative contributions of xenoNER (sorbed and sequestered parent compound) and bioNER (biomass residues of degraders). In addition, environmental conditions and agricultural practices leading to total organic carbon (TOC) or pH variation can alter the degradation of glyphosate. The aim of this study is thus to investigate how the glyphosate degradation and turnover are influenced by varying temperature, pH and TOC of sandy loam soil from Colombia. The pH or TOC of a Colombian soil was modified to yield five treatments: control (pH 7.0, TOC 3%), 4% TOC, 5% TOC, pH 6.5, and pH 5.5. Each treatment received 50 mg kg^{-1} of $^{13}C_3{}^{15}N$-glyphosate and was incubated at 10°C, 20°C and 30°C for 40 days. Rising temperature increased the mineralization of $^{13}C_3{}^{15}N$-glyphosate from 13-20% (10°C) to 32-39% (20°C) and 41-51% (30°C) and decreased the amounts of extractable $^{13}C_3{}^{15}N$-glyphosate after 40 days of incubation from 13-26% (10°C) to 4.6-12% (20°C) and 1.2-3.2% (30°C). Extractable $^{13}C_3{}^{15}N$-glyphosate increased with higher TOC and higher pH. Total ^{13}C-NER were similar in all treatments and at all temperatures (47%-60%), indicating that none of the factors studied affected the amount of total ^{13}C-NER. However, ^{13}C-bioNER dominated within the ^{13}C-NER pool in the control and the 4% TOC treatment (76-88% of total ^{13}C-NER at 20°C and 30°C), whereas in soil with 5% TOC and pH 6.5 or 5.5 ^{13}C-bioNER were lower (47-61% at 20°C and 30°C). In contrast, the ^{15}N-bioNER pool was small (between 14-39% of the ^{15}N-NER). Thus, more than 60% of ^{15}N-NER is potentially hazardous xenobiotic NER which need careful attention in the future.

Keywords: glyphosate, ^{13}C and ^{15}N mass balance, degradation pathway, biogenic NER, tropical soil

Capsule:

Temperature controlled mineralization and extractable glyphosate, whereas total organic carbon and pH influenced formation of xenobiotic and biogenic non-extractable residues.

1. INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] is most widely applied herbicide worldwide in agriculture, horticulture and in urban areas (Benbrook, 2016; Hanke et al., 2010). Widespread occurrence of glyphosate and its transformation product aminomethylphosphonic acid (AMPA) in the environment and the current classification of glyphosate as potential carcinogen are causes for public concern about glyphosate use (Bolognesi et al., 2009; Camacho and Mejía, 2017; Myers et al., 2016; Polanco et al., 2014; Solomon et al., 2007; Tarazona et al., 2017; Van Bruggen et al., 2018; Williams et al., 2016). The environment in tropical countries, e.g. Colombia, is at higher risk of contamination and poisoning with glyphosate than the temperate environments due to repeated pesticide application throughout the year and lack of regulations defining the limits of pesticide use (Sanchez-Bayo and Hyne, 2011). Glyphosate is also used for aerial spraying in the illicit drug crop eradication program "Plan Colombia" (Oficina de las Naciones Unidas contra la Droga y el Delito-UNODC, 2017). From 2001 to 2016, glyphosate was applied by plane to about 285,000 hectares of illicit drug plantations in Colombia (Oficina de las Naciones Unidas contra la Droga y el Delito-UNODC, 2017). To date, more than 50 glyphosate-based products are registered in the Colombian market and an increase in their use is observed (Gobierno de Colombia, 2018). The knowledge about the fate of glyphosate and AMPA in Colombian soils is limited. To date, several reports on fate of glyphosate in Brazilian soil have been published (de Andréa et al., 2003; Okada et al., 2017) and only one with Colombian soil (Cuervo and Fuentes, 2014).

Abiotic degradation of glyphosate is usually slower than microbial degradation (Barrett and McBride, 2005; Chen and Liu, 2007; Jaisi et al., 2016; Lund-Høie and Friestad, 1986; Paudel et al., 2015). The high stability of the C-P bond of glyphosate impedes hydrolysis, photolysis and chemolysis processes (Franz et al., 1997; Hove-Jensen et al., 2014; Rueppel et al., 1977; Safdar et al., 2013; World Health Organization, 2010).

In contrast, soil microorganisms can rapidly degrade glyphosate via the sarcosine or the AMPA pathway (Nandula, 2010; Rueppel et al., 1977). The cleavage of the C-P bond of glyphosate by the enzyme C-P lyase in the sarcosine pathway yields sarcosine and glycine; whereas in the AMPA pathway, an oxidoreductase enzyme breaks the C-N bond, forming AMPA and glyoxylate (Pipke et al., 1987; Rueppel et al., 1977; Safdar et al., 2013; Zhan et al., 2018). The two degradation pathways of glyphosate were reported to occur in water-sediment (Wang et al., 2016) and in soil (Muskus et al., 2019).

The extent of glyphosate degradation in soil was balanced with radiocarbon (^{14}C) in previous 307 OECD tests (Kästner et al., 2014). The ^{14}C mass balance encompasses mineralization ($^{14}CO_2$), ^{14}C in extractable glyphosate and AMPA, and ^{14}C in non-extractable residues (NER) (Barriuso et al., 2008). Glyphosate or AMPA could be adsorbed onto solid surface (Wang et al., 2016) or covalently bound via the amino or phosphonic acid group to the solid matrix forming xenobiotic NER (xenoNER) with high or low remobilization risk, depending on the strength and reversibility of the bonds or interactions (Kästner et al., 2014). However, analysis of ^{14}C-glyphosate-NER is limited to quantification of ^{14}C which cannot be extracted with aquatic solvents, but does not allow elucidation of the chemical identity of the NER (Barriuso et al., 2008; Kästner et al., 2014). Therefore, the high content of ^{14}C-glyphosate-NER of unknown identity raises a concern over the potential environmental risks (Kästner et al., 2014).

Recent studies indicated that at least a part of the NER quantified based on radiolabel may consist of microbial biomass residues as bacteria and fungi able to degrade a given compound would use part of the compound-derived C for building their biomass (Nowak et al., 2013, 2011). Biomass residues after death of degrader organisms may be stabilized in soil and form biogenic NER (bioNER). BioNER are harmless since they are formed by microorganisms incorporating the carbon or nitrogen of a chemical into their biomolecules (e.g. amino acids, fatty acids; Kästner et al., 2014; Nowak et al., 2011). A degradation study with stable isotope of carbon (^{13}C) and nitrogen (^{15}N) revealed that the majority of NER from glyphosate (Wang et al., 2016) in water-sediment could be assigned to bioNER.

The fate of glyphosate including biodegradation and (bio)NER formation can be affected by soil physico-chemical (texture, organic material content, pH) and biological properties (microbial community) or climatic conditions (Bergström et al., 2011; Borggaard and Gimsing, 2008). A recent study by Muskus et al., 2019 showed that temperature, pH and total organic carbon (TOC) variations influenced the mineralization kinetics of glyphosate as well as the amount of extractable glyphosate and the extent of bioNER formation over time in a German soil. Temperature was the main factor controlling the mineralization kinetics of $^{13}C_3$-glyphosate, whereas increased TOC content boosted formation of ^{13}C- and ^{15}N-bioNER (Muskus et al., 2019). Most glyphosate degradation studies are limited to soils originating from temperate regions (Arbeli and Fuentes, 2007). Tropical soils have different physico-chemical and biological properties which may result in the divergent degradation of glyphosate (Arbeli and Fuentes, 2007; Sanchez-Bayo and Hyne, 2011). Due to the high annual temperatures and precipitation, soils in tropics are more deficient in TOC and richer in free iron and aluminum oxides than the soils from temperate regions (Sanchez-Bayo and Hyne, 2011). To date, it is little known about the degradation potential of glyphosate in tropical soil and how soil properties or temperature affect the fate of glyphosate. In particular, the importance of bioNER formation has not yet been studied.

The aim of the present study is thus to investigate the effect of soil properties (TOC and pH) and temperature on the fate of glyphosate, with a special focus on biodegradation and bioNER formation in a Colombian soil. The original TOC level and pH value of a tropical sandy loam soil (control) were modified to obtain five treatments: 1) control (3% TOC, pH 7.0), 2) 4% TOC, 3) 5% TOC, 4) pH 6.5 and 5) pH 5.5. The five treatments were incubated with ^{13}C and ^{15}N-labelled glyphosate at 10°C, 20°C and 30°C. After 40 days of incubation, the mass balance of ^{13}C and ^{15}N label was determined. In addition, the extent of ^{13}C- and ^{15}N incorporation from $^{13}C_3{}^{15}N$-glyphosate into bioNER was quantified.

2. MATERIALS AND METHODS

2.1. Reference tropical soil

The reference soil used for incubation experiment was taken from an Andean Raspberry (*Rubus glaucus*) cultivation area with slopes of up to 75%. This site was located in a rural area east of Piedecuesta city in Santander, Colombia (N7°00'54.6"W72°59'). The Ap horizon (0-5 cm) of the sandy loam soil (humic Cambisols according to FAO classification) was sampled after removal of the litter layer. Weather on the sampling day was dry and the air temperature was 18°C. The soil samples were sieved through a 2 mm sieve and texture was determined using a 152H hydrometer according to ASTM D422-63 (2007). The soil contained 73% sand, 7% silt and 20% clay. The pH (H_2O) of the soil was 7.0; TOC was 3% (w/w); the maximum water holding capacity (WHC_{max}) was 40% and electrical conductivity was 0.85 dS m^{-1}. No information about the history of pesticide application in that area was available.

2.2. Farmyard manure

For modification of the TOC content of the soil, the original soil was amended with appropriate amounts of cow farmyard manure (FYM). The FYM was collected from the long-term experimental area “Static Fertilization Experiment” in Bad Lauchstädt, Germany. The characteristics of the FYM were as follows (w/w): 34% TOC, 2.5% total N, 0.75% P, 2.91% K, 4.18% Ca and 0.75% Mg. The pH (H_2O) of the FYM was 8.7. Due to amendment with the alkaline FYM, the pH of soil increased to 7.1 (4% TOC treatment), and to 7.2 (5% TOC treatment).

2.3. Chemicals

Labeled $^{13}C_3{}^{15}N$-glyphosate was provided by Iso-Sciences, Trevose, PA, USA at 99 at% ^{13}C isotopic purity, 98 at% ^{15}N isotopic purity and 98% chemical purity. All other chemicals used in this study were obtained from VWR/Merck, Darmstadt, Germany or Carl Roth, Karlsruhe, Germany.

2.4. TOC and pH variations, spiking procedure and incubation conditions

In order to investigate the influence of variations in soil properties (pH or TOC content) on the environmental fate of glyphosate, five different treatments: 1) control (pH 7.0, TOC 3%), 2) 4% TOC, 3) 5% TOC, 4) pH 6.5 and 5) pH 5.5 were arranged (see **Scheme 1**). Each treatment was prepared in triplicate and incubated at 10°C, 20°C and 30°C for 40 days according to OECD guideline 307 (OECD, 2002).

TOC variation. The TOC of control soil (3% TOC; w/w) was modified using FYM to obtain two additional levels of TOC: 4% TOC (w/w) and 5% TOC (w/w). The control soil was mixed with dried, pulverized and sieved (through a 2 mm screen) FYM. 2.5% (w/w) of FYM was added to the control soil to yield 4% TOC, whereas 5% (w/w) of FYM was added to obtain 5% TOC. After an equilibration time of 7 days at 20°C, the TOC contents of the modified soils were checked using an elemental analyzer-combustion-isotope ratio mass spectrometer (EA-C-IRMS; Finnigan MAT 253, Thermo Electron, Bremen, Germany; (Girardi et al., 2013).

pH variation. The pH of the soil was lowered from the initial value (7.0) to either pH 6.5 or pH 5.5 using sulfuric acid (H_2SO_4). Sulfuric acid was used to modify pH since it is able to decrease pH quickly without major harm to the soil bacterial population (Muskus et al., 2019). To reduce pH of the control soil (pH 7.0) to pH 6.0, 1 mL of 0.1 M H_2SO_4 was added to 15 g of soil, while 1 mL of 1 M H_2SO_4 was added to adjust the pH control soil to pH 5.5. After the addition of 1 mL of 0.1 M H_2SO_4 or 1 M H_2SO_4, the modified soil was stored for 7 days at 20°C to equilibrate. The actual values of soil pH obtained by the pH adjustments were confirmed by measurement.

Spiking procedure and incubation conditions. Each treatment received either $^{13}C_3{}^{15}N$-labeled glyphosate or unlabeled glyphosate (control) or no glyphosate (blank); each in triplicate (see also **Scheme 1)**.

The natural abundances of ^{13}C and ^{15}N were corrected using the control and the blank. Unlabeled or $^{13}C_3{}^{15}N$-labeled glyphosate was dissolved in methanol and soil was spiked to yield a final concentration of 50 mg glyphosate kg^{-1}. This corresponded to ten-fold higher concentration of glyphosate than the agronomic application rate, which, however, was needed for obtaining a reliable isotopic enrichment against the ^{13}C and ^{15}N natural abundance, even after partial mineralization of the compound. After spiking, the soil water content was adjusted with distilled water to 60% of the WHC_{max} and 20 g of soil was placed into 500 ml Duran glass bottles. Then, all microcosms were incubated at either 10°C, 20°C or 30°C in the dark for 40 days. The CO_2 originating from soil microbial respiration and from glyphosate mineralization (total CO_2 and

$^{13}CO_2$) was adsorbed into 2M NaOH and analyzed after 3, 5, 10, 24, 31 and 40 days. The total amount of soil was sampled after 40 days, and the carbon and nitrogen mass balances were determined. In addition, proteinaceous bioNER were analyzed (see below for details).

2.5. Carbon and nitrogen mass balance

The carbon (^{13}C) and nitrogen (^{15}N) mass balance encompassed mineralization ($^{13}CO_2$), extractable $^{13}C_3{}^{15}N$-glyphosate and $^{13}C^{15}N$-AMPA as well as carbon and nitrogen in non-extractable residue (total ^{13}C- or ^{15}N-NER).

Mineralization. Total CO_2 from soil microbial respiration and glyphosate mineralization was analyzed with a Total Organic Carbon Analyser Multi N/C 21005, Jena, Germany. The isotopic composition of the CO_2 was measured using a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-irMS; Finnigan MAT 252, Thermo Electron, Bremen, Germany, combined with a Hewlett Packard 6890 GC; Agilent Technologies, Germany) equipped with a Porabondt Q-HT Plot FS column (50 m × 0.32 m × 5 μm; Chrompack, Middleburg, Netherlands, Girardi et al., 2013), and the amount of $^{13}CO_2$ was calculated to assess $^{13}C_3$-glyphosate mineralization. Mineralization of ^{15}N ($^{15}NO_3^-$ and $^{15}NH_4^+$) was not measured in this study. However, we expect this process to be of little importance since the recovery of ^{15}N was relatively high (85-98%; even without considering ^{15}N mineralization.

Extractable $^{13}C_3{}^{15}N$-glyphosate and $^{13}C^{15}N$-AMPA. $^{13}C_3{}^{15}N$ glyphosate and $^{13}C^{15}N$-AMPA were extracted from soil using borate buffer (pH 8.0) and the extract was purified over a HBL OASIS 6 mL (200 mg) column and derivatized with FMOC-Cl as described in Muskus et al., 2019. The derivatized $^{13}C_3{}^{15}N$ glyphosate and $^{13}C^{15}N$-AMPA were separated on a ZORBAX Extend-C18 analytical column (100 × 2.1 mm, 3.5 μm particle size, Agilent) by liquid chromatography - tandem mass spectrometry/mass spectrometry (LC-MS/MS). The detailed conditions for separation and quantitation were described previously (Muskus et al., 2019). The internal standard glufosinate was added to each sample. The limit of detection (LOD) was 1 µg L^{-1} for glyphosate and $^{13}C_3{}^{15}N$-glyphosate and 2 µg L^{-1} for AMPA. The limit of quantification (LOQ) was 10 µg L^{-1} for glyphosate and 20 µg L^{-1} for AMPA. The relative standard deviation (RSD) for the measured soil extracts was 10% for unlabeled glyphosate, 5-10% for AMPA, and 4-5% for $^{13}C_3{}^{15}N$-glyphosate. It was not possible to determine RSD for $^{13}C^{15}N$-AMPA as this compound was not available as authentic standard). The overall recovery of glyphosate and AMPA reached 93% ± 3%.

Carbon and nitrogen in non-extractable residue (NER). After extraction, the soil was air-dried and analyzed for the total amount and the isotopic composition of ^{13}C and ^{15}N-NER by means of an elemental analyzer-combustion-isotope ratio mass spectrometer (EA-C-irMS; Finnigan MAT 253, Thermo Electron, Bremen, Germany; (Girardi et al., 2013) coupled to Flash EA 2000 (Thermo Finnigan). Temperature in the oxidation reactor was 1020°C, whereas the one in the reduction reactor was 650°C.

2.6. Analysis of AAs (proteinaceous bioNER)

The determination of proteinaceous bioNER was based on the hydrolysis of amino acids (AAs) from proteins using 6 M HCl (Nowak et al., 2011). AAs were purified over a cation exchange resin (DOWEX 50W-X8) before a two-step derivatization as described previously (Nowak et al., 2011). The derivatized AAs were quantified by gas chromatography-mass spectrometry (GC-MS, HP 6890, Agilent) using a BPX-5 column (30 m × 0.32 mm × 0.25 µm, Nowak, 2011). The compound-specific ^{13}C- and ^{15}N isotopic compositions of the AAs were measured using a gas chromatography-combustion-isotope ratio-mass spectrometry (GC-C-irMS, Finnigan MAT 253 coupled to a Trace GC, Thermo Electron, Bremen, Germany) and a BPX-5 column (50 m x 0.32 m x 0.5 µm, SGE International, Darmstadt, Germany, Nowak, 2011; Nowak et al., 2013; Wang et al., 2016). L-norleucine was added to each sample before hydrolysis as an internal standard, allowing the calculation of the losses during hydrolysis, extraction, clean-up and derivatization (<10%). The AAs were quantified and identified by comparison of the retention times and mass spectra with the ones obtained from an external standard containing all detectable AAs (alanine, glycine, threonine, valine, leucine, isoleucine, proline aspartate, glutamate, phenylalanine and lysine). ^{13}C and ^{15}N isotopic compositions of the AAs were corrected for shifts during derivatization according to Silfer et al. (1991).

2.8. Data presentation, half-life (DT_{50}) and statistics

The incubation experiments, mass balance and bioNER analyses were performed in triplicates. The results are presented as averages with standard deviation. The mass balance results are shown as percentages of the ^{13}C or ^{15}N in the initially applied $^{13}C_3{}^{15}N$-glyphosate. Total recovery of ^{13}C ranged from 83% to 100%, and of ^{15}N between 71% and 98%. Mineralization of $^{13}C_3$-glyphosate ($^{13}CO_2$) is shown as a cumulative mineralization over the 40-day period. The quantification of $^{13}CO_2$, ^{13}C- or ^{15}N-NER and ^{13}C- or ^{15}N-AAs was based on the measurement of total carbon ($^{12}C+^{13}C$) or total nitrogen ($^{14}N+^{15}N$) in soil and the $^{13}C/^{15}N$ excess over control and blank (Lerch et al., 2009; Wang et al., 2017a, 2017b, 2016).

Total bioNER and xenoNER. About 50% of the bacterial biomass (dw) consists of proteins which are stable in soils (Miltner et al., 2009; Nowak et al., 2011; Wang et al., 2017a). Based on the known 50% protein content in microbial biomass (Nowak et al., 2011), we calculated the approximate amount of the total content of ^{13}C- or ^{15}N-bioNER based on the AAs concentrations after acid hydrolysis of proteins (factor of 2). Therefore, the data are shown as proteins (measured here as AAs) and other bioNER (calculated based on known 50% of protein content in microbial biomass).

The xenobiotic ^{3}C- or ^{15}N-NER were estimated as the difference between measured total ^{3}C- or ^{15}N-NER and calculated ^{13}C- or ^{15}N-bioNER.

Half-life (DT_{50}). The cumulative mineralization data of $^{13}C_3$-glyphosate suggested that a Single First-Order (SFO) model (SFO; **Eq. 1**) is most suitable to describe the degradation kinetics, dissipation rate constants (k; **Eq. 2**) and half-lives (DT_{50}; **Eq. 3**) of $^{13}C_3$-glyphosate (Bento et al., 2016; Bergström et al., 2011; Ghafoor et al., 2011; Mamy and Barriuso, 2005):

$$C_{(t)} = C_o\, e^{-kt} \qquad (\textbf{Eq. 1})$$

$$k = \frac{-\left(\ln C_{(t)} - \ln C_0\right)}{t} \qquad (\textbf{Eq. 2})$$

where C_t is the concentration of glyphosate at time (t) of the incubation experiment, C_o is the initial glyphosate concentration and k is the first order rate constant calculated from the extractable glyphosate at the endpoint.

The time when glyphosate concentration is reduced to the half of the initially applied amount on day 0 (DT_{50}) is calculated by combining **Eq. 1** with **Eq. 2** and obtaining **Eq. 3**:

$$DT_{50} = (\ln 2)/k \qquad (\textbf{Eq. 3})$$

Statistics. A two-way Analysis of Variance (ANOVA) without replication and a Multivariate Analysis of Variance (MANOVA) with the HSD (Honestly Significant Difference) and the Tukey test were performed to test the effect of soil properties (pH or TOC) and temperature on the cumulative mineralization of $^{13}C_3$-glyphosate, the extracted $^{13}C_3{}^{15}N$-glyphosate, $^{13}C^{15}N$-AMPA, ^{13}C- and ^{15}N-AAs. The ANOVA was done with the Microsoft Excel 2010 software, whereas the MANOVA was computed with the IBM SPSS and data mining software (Version 25, Copyright IBM Corp 1989, 2017). The differences between the data were considered as significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Mineralization of glyphosate

Temperature was the main factor controlling mineralization kinetics of $^{13}C_3$-glyphosate as follows 10°C < 20°C < 30°C ($p < 0.05$; see **Fig. 1**), and cumulative mineralization on day 40 ranged from 13-20% at 10°C; 32-39% at 20°C; 41-51% at 30°C. These results are in good accordance with the cumulative mineralization of ^{14}C-glyphosate in similar tropical soils (Al-Rajab and Schiavon, 2010; Gimsing et al., 2004) and in a German loamy sand soil (Muskus et al., 2019); see also **Table S1**.

Mineralization started immediately in all treatments and boosted quickly by day 24; thereafter, it increased slowly till the end. The ultimate cumulative mineralization was lowest in control soil incubated at 10°C (13%), and highest in the 4% TOC and 3% TOC treatments at 30°C (50%). Mineralization of glyphosate without a lag phase could indicate the presence of considerable abundances of glyphosate degraders (Hove-Jensen et al., 2014; Schnurer et al., 2006) or enzymes cleaving glyphosate, e.g. C-P lyase (Eberbach, 1998; Franz et al., 1997; Landry et al., 2005; -; Sørensen et al., 2006; -Strange-Hansen et al., 2004; -).

In addition to temperature, variation of TOC or pH also influenced the mineralization kinetics of $^{13}C_3$-glyphosate. An increased TOC content (4% and 5%) promoted mineralization of $^{13}C_3$-glyphosate at 10°C (19-20% on day 40; $p < 0.05$) and at 30°C (50% on day 40; $p < 0.05$) in comparison with the control (13% at 10°C and 41% at 30°C on day 40; see **Fig. 1a**). An amendment of different soils with organic material also improved the mineralization kinetics of glyphosate being the result of boosted microbial activity (Albers et al., 2009). Lowering pH of the control soil to pH 6.5 or pH 5.5 also accelerated the ultimate mineralization of $^{13}C_3$-glyphosate at 10°C and at 30°C (**Fig. 1b**; $p > 0.05$). Similar results were obtained for a German soil (Muskus et al., 2019). The change of soil pH could have increased the availability of glyphosate or carbon (Andersson et al., 2000; Kemmitt et al., 2006; Rousk et al., 2009; Vereecken, 2005) and other nutrients from soil (Aciego Pietri and Brookes, 2008; Binkley and Vitousek, 1989; Kemmitt et al., 2005; Marschner and Rengel, 2011; Zhao et al., 2011) which stimulated the microbial activity including glyphosate degraders.

3.2. Extractable glyphosate and AMPA

In analogy to mineralization results, the contents of extractable $^{13}C_3{}^{15}N$-glyphosate were also mainly influenced by temperature variation (**Table 1**). The amounts of $^{13}C_3{}^{15}N$-glyphosate were highest at 10°C and lowest at 30°C in all treatments; this agrees with the $^{13}C_3{}^{15}N$-glyphosate degradation in German soil (Muskus et al., 2019).

The amounts of $^{13}C_3{}^{15}N$-glyphosate in 5% TOC at 10°C were similar to the amounts measured in control (25% of initially added $^{13}C_3{}^{15}N$-glyphosate; **Table 1**), while these values were higher than in control at 20°C (12% versus 8.5%; $p < 0.05$) and at 30°C (3.2% versus 1.8%; $p < 0.05$). Similar trend was noticed for $^{13}C_3{}^{15}N$-glyphosate degradation in German soil (Muskus et al., 2019). In their study, higher TOC content (3% and 4%) of soil than in the control (2.1%) also increased the amount of extractable $^{13}C_3{}^{15}N$-glyphosate at 10°C (15-16% in 3% and 4% TOC versus 8.8% in control) and at 20°C and 30°C (1.4-4.6% in 3% and 4% TOC versus 0.1-0.8% in control). However, for all combinations of temperature and TOC, extractable $^{13}C_3{}^{15}N$-glyphosate in the study by Muskus et al. (2019) was lower than in this study. This divergence could be related to the different soil types used in the experiment, which - among others - differed in texture (Colombian soil: sandy loam (this study) versus German soil: loamy sand (Muskus et al., 2019) and probably also mineralogy.

Reduction of soil pH (6.5 and 5.5) diminished the amount of extractable $^{13}C_3{}^{15}N$-glyphosate in soil at 10°C ($p < 0.05$; 13-16% versus 25% in control). This agrees with the results obtained by Muskus et al. (2019), where the amounts of extractable $^{13}C_3{}^{15}N$-glyphosate in German soil at pH to 5.5 and 6.0 were lower than in the unaltered control (pH of 6.6). Soil surfaces and glyphosate both are more negatively charged at high pH (De Jonge et al., 2001; Gimsing et al., 2004; McConnell and Hossner, 1985; Paradelo et al., 2015). This enhances the repulsive forces leading to reduced sorption. Thus, an explanation for a lower extractability of $^{13}C_3{}^{15}N$-glyphosate from soil with lower pH could be the reduced negative charge under these conditions. In addition, the sorptive strength of glyphosate to soil may increase at lower pH due to the formation of complexes between glyphosate and surface-exchange multivalent cations like iron or aluminum (Glass, 1987; Sprankle et al., 1975). The tropical soils are often more abundant in iron and aluminum oxides than soils in temperate regions (Sanchez-Bayo and Hyne, 2011). This might foster ligand exchange reactions between the oxides and glyphosate after acidification and thus increase sorption of glyphosate in tropical soils.

The TOC or pH variation did not influence significantly the extracted amounts of ^{13}C-AMPA when compared to the control soil at 10°C and 30°C (**Table 1**; $p > 0.05$). In contrast, at 20°C the extractable ^{13}C-AMPA in soil with pH 5.5 and pH 6.5 (3.5-4.2%) or with 4% and 5% TOC (3.0-3.9%) were lower than in the control (6.3%). It is difficult to explain the results on extractable AMPA as AMPA is an intermediate being both produced and consumed during incubation, and the amount of extractable ^{13}C-AMPA were measured only at the end of incubation. Low amounts of AMPA were found in most studies after 40 days (Bergström et al., 2011; Muskus et al., 2018; Tang et al., 2019). However, the recent study by Tang et al. (2019) showed that remarkable amounts of AMPA have been extracted after 80-90 days of incubation of soil slurries. However, out incubation experiment has been stopped much earlier, i.e. on day 40. We therefore cannot conclude on longer-term development of AMPA concentrations.

The effect of temperature or pH variation on the content of ^{15}N-AMPA is expected to be same as for ^{13}C-AMPA. The only difference is that the amounts of ^{15}N-AMPA are 3-fold higher than the ^{13}C-AMPA since the ^{15}N is referred to the initial amount of only one ^{15}N in glyphosate versus three labeled ^{13}C atoms of $^{13}C_3$-glyphosate.

The degradation kinetics of glyphosate in soils is described to follow SFO kinetic (Eberbach, 1998; Gimsing et al., 2004; Moshier and Penner, 1978), and also the mineralization data from this study supported SFO kinetics. Therefore, we calculated the half-lives (DT_{50}) of glyphosate in all treatments based on the assumption of SFO kinetics. DT_{50} for the control, 4% and 5% TOC was 20 days at 10°C, 11-13 days at 20°C, and 7-8 days at 30°C (see **Table S2a**). For pH 6.5 and 5.5, the DT_{50} was between 13 and 15 days at 10°C, 9-10 days at 20°C, and 6-7 days at 30°C (**Table S2b**). The DT_{50} obtained in our study was lower (except for 10°C) than the DT_{50} of 17-42 days for glyphosate degradation in other studies with similar soils and incubation conditions (Al-Rajab and Schiavon, 2010; Bergström et al., 2011; Cheah et al., 1998). An addition of crop residues on top of a clay loam soil increased the DT_{50} of glyphosate from 28 to 47 days (Cassigneul et al., 2016). This is in contrast to our study, where the DT_{50} of glyphosate in TOC treatments were comparable with the control soil. This divergence is explained by the different organic materials used for amendment (FYM versus crop residues) and the fact that in the TOC treatments of the present study FYM was mixed with the soil and not placed on top.

3.3. Carbon and nitrogen-derived glyphosate in the AAs

The temperature, TOC and pH variation did not influence significantly the total contents of ^{13}C-AAs ($p > 0.05$) which ranged between 11% and 21% of initial $^{13}C_3$-glyphosate equivalents (see **Table 2**). This disagrees with the study by Muskus et al. (2019), where the temperature, TOC and pH variation affected the total amounts of ^{13}C-AAs. However, the amounts of ^{13}C-AAs in our study were comparable to the contents of ^{13}C-AAs in German soil (8-20%; Muskus et al.; 2019).

The total contents of ^{15}N-AAs (3.9-15% of initial ^{15}N-glyphosate equivalents (see **Table 2**) were lower than ^{13}C-AAs. However, the contents of ^{15}N-AAs in the present study were higher than the 2.1-6.2% reported by Muskus et al. (2019). Unlike the ^{13}C-AAs, the temperature and TOC or pH variation affected total amounts of ^{15}N-AAs. The ^{15}N-AAs were highest in the control soil at 10°C (9.6%; **Table 2**) in comparison with that in soil with elevated TOC (4.9-7.6%) or reduced pH (3.9-7.0%). At 20°C, the ^{15}N-AAs in 4%TOC (14%) and pH 5.5 (15%) treatments were higher than in other treatments (6-8.3%). Lower contents of ^{15}N-AAs were detected in the control soil at 30°C (6.9%) than in soils with modified TOC and pH (8.8-12%). Although we observed significant effect of temperature or soil parameter (pH or TOC) variation on the ^{13}C- and ^{15}N-AAs in the present study and in the study by Muskus et al. (2019), it is difficult to explain it. It might be related to the different kinetics and efficiency of microbial metabolism of $^{13}C_3{}^{15}N$-glyphosate in different soils used in both studies. In addition, the amount of ^{13}C- and ^{15}N-AAs were analysed only at the end of incubation. Information about the contents of ^{13}C- and

^{15}N-AAs at different timescales of $^{13}C_3{}^{15}N$-glyphosate degradation is thus necessary to clarify the relevance of temperature or tested soil parameter on the formation^{13}C- or ^{15}N-AA.

3.4. Degradation pathways of glyphosate

Previous studies indicated the relevance of two different degradation pathways of glyphosate: via sarcosine or via AMPA (Pipke et al., 1987; Rueppel et al., 1977; Safdar et al., 2013; Wang et al., 2016; Zhan et al., 2018). The AMPA pathway can be confirmed by the presence of $^{13}C^{15}N$-AMPA and ^{13}C-glycine, which are degradation products of $^{13}C_3{}^{15}N$-glyphosate (Wang et al., 2016). Sarcosine usually cannot be detected in soil since it is converted rapidly to $^{13}C^{15}N$-glycine (Wang et al., 2016). The occurrence of the sarcosine pathway thus can be proven by the presence of both ^{13}C- and ^{15}N-labeled glycine. To estimate the relative importance of the two pathways, the ratio of ^{13}C-glycine to ^{15}N-glycine can be calculated. A ^{13}C-glycine to ^{15}N-glycine ratio close to 1 indicates the presence of $^{13}C^{15}N$-glycine and thus an important contribution of the sarcosine pathway.

Our results show that the AMPA pathway is relevant independent of temperature, pH and TOC since $^{13}C^{15}N$-AMPA was found in all treatments (**Table 1**). The occurrence of $^{13}C^{15}N$-glycine on day 40 in all controls and TOC treatments (**Table S3a)** is indicative of a significant contribution of the sarcosine pathway. Although $^{13}C^{15}N$-glycine was absent in soil at pH 6.0 and 10°C, we cannot exclude some contribution of the sarcosine pathway in that treatment. This amino acid was detected at all pH levels at 20°C and 30°C (**Table S3a**). In addition, ^{15}N-glycine was present in soil with pH 5.5 at 10°C. We thus cannot explain the absence of $^{13}C^{15}N$-glycine in pH 6.0 at 10°C; but it is possible that $^{13}C^{15}N$-glycine was below detection limit. The ratio of ^{13}C-glycine to ^{15}N-glycine in the control was between 1.1 and 1.5, in the treatments with elevated TOC it was 0.3-1.6, whereas at reduced pH it ranged between 0.5 and 1.5 (see **Table S3b**). ^{15}N and ^{13}C can be incorporated into glycine not only from $^{13}C_3{}^{15}N$-glyphosate within the sarcosine pathway, but also during further transformation of $^{13}C^{15}N$-AMPA. Therefore, to identify which pathway is dominant in glyphosate degradation, the changes in contents of $^{13}C^{15}N$-AMPA, ^{13}C-glycine and $^{13}C^{15}N$-glycine over time are needed. We measured the components only at the end of our incubation experiment, i.e. at the later phase of $^{13}C_3{}^{15}N$-glyphosate degradation. Therefore, it is not possible to clearly identify which pathway dominated based on the data available to date.

3.5. BioNER and xenoNER

The total recovery of ^{13}C from $^{13}C_3$-glyphosate at 10°C was between 83% and 99% (see **Table S4**), whereas at 20°C and 30°C, it ranged from 95-100% and 88-99%, respectively. In contrast to the results presented by Muskus et al. (2019), neither temperature nor the soil parameters under study affected the total content of ^{13}C-NER (proteins + other bioNER + xenoNER), which was between 40-60% for all incubation temperatures (see **Fig. 2**). The ^{13}C-NER at 10°C, 20°C and 30°C were similar to ^{13}C-NER in German soil at the same temperatures (Muskus et al., 2019).

About 76-88% of total ^{13}C-NER comprised ^{13}C-bioNER (proteins + other bioNER) in the control and the 4% TOC treatment at 20°C and 30°C (see **Fig. 2a;** see also **Table S4**). In the soil with 5% TOC this percentage was lower (64% at 20°C and 55% at 30°C). Addition of organic material can boost not only microbial activity but also sorption of glyphosate or AMPA to the solid matrix. Microbial activity stimulated microbial conversion of glyphosate to CO_2 and formation of bioNER in 4% TOC treatment. In contrast, in 5% TOC treatment, the sorption was more prominent whereas the number of degraders was presumably too low to yield higher mineralization and bioNER formation. An excessive amendment of soil with organic material, e.g. FYM, thus carries the risk to promote the formation of potentially hazardous xenoNER. A lower contribution of ^{13}C-bioNER (50-61%) to ^{13}C-NER in the pH treatments (5.5 and 6.5) was observed at 20°C and 30°C in comparison with the control soil (77-80%; see **Fig. 2b**). This finding suggests that a decrease of pH 7.0 to 6.5 or 5.5 has a negative effect on the extent of bioNER formation. Low soil pH has been reported to increase the sorption potential of glyphosate or AMPA to soil minerals (Glass, 1987; Sprankle et al., 1975). This results in the formation of hazardous xenoNER and lower availability for biotransformation, which is a prerequisite for bioNER formation.

Approximately 81-97% of ^{15}N from initially added ^{15}N-glyphosate could have been recovered at 10°C; whereas the total recovery of ^{15}N at 20°C was 71-98% and 73-89% at 30°C (see **Table S5**). ^{15}N-NER formation increased with rising temperature (**Fig. 3a** and **b;** see also **Table S5**) and amounted to 85-88% of initial ^{15}N-glyphosate equivalents at 30°C, 70-85% at 20°C and 60-67% at 10°C. These values were higher than the ^{13}C-NER and comparable to ^{15}N-NER in German soil (Muskus et al., 2019). A major formation of ^{15}N-xenoNER (> 60% of ^{15}N-NER) was observed for all treatments incubated at 10°C, 20°C and 30°C; the contribution of ^{15}N-bioNER to total ^{15}N-NER was lower (14-30% at 10°C, 24-39% at 20°C, 23-31% at 30°C). This is in contrast to the major contribution of ^{13}C-bioNER to the ^{13}C-NER pool at all temperatures and in all TOC or pH treatments (50-88%). Thus, this finding suggests that $^{13}C_3{}^{15}N$-glyphosate was considerably mineralized by microorganisms as reflected by higher amounts of $^{13}CO_2$ and lower amounts of $^{13}C_3{}^{15}N$-glyphosate at higher temperatures at the end. Therefore, the transformation product ^{15}N-AMPA could have been sorbed to solid matrix forming ^{15}N-xenoNER. As AMPA is mineralized at lower rates than glyphosate (Bergström et al., 2011; Borggaard and Gimsing, 2008), this transformation product presumably contributes to xenoNER rather than bioNER formation. Another explanation for the preferential sorption of AMPA over glyphosate is that higher ^{15}N-bioNER and ^{13}C-bioNER were noticed in 5% TOC treatment than in other treatments. The^{15}N-bioNER contributions to total ^{15}N-NER were substantially higher than the 6-18% reported by Muskus et al., (2019) for the German soil. This discrepancy is difficult to explain since we measured the ^{15}N-bioNER and ^{15}N-NER only on day 40. A potential explanation could be related to the higher extractable ^{15}N-glyphosate and lower ^{15}N-AMPA in the Colombian than in the German soil (Muskus et al., 2019).

4. CONCLUSIONS

Temperature, TOC and pH variation affected the mineralization of glyphosate in soil. An amendment of tropical soil with organic material like FYM can reduce, whereas a decrease of pH can promote the sorption of glyphosate to minerals. An application of FYM in the tropics has to be carefully planned and coordinated with pesticide application since at high rate (> 2.5% FYM) this amendment may enhance the formation of potentially hazardous xenobiotic NER. Any environmental process or agricultural practice causing acidification of tropical soil abundant in iron and aluminium oxides should be controlled in future, since it can promote not only sorption processes but also formation of xenoNER which potentially can be remobilized, thus causing delayed environmental risk. In addition, it is little known about the composition of ^{15}N-NER which is mainly xenobiotic. Thus, further analysis of ^{15}N-NER should be specifically focused on the mechanisms of xenobiotic NER formation and their stability under different climatic conditions.

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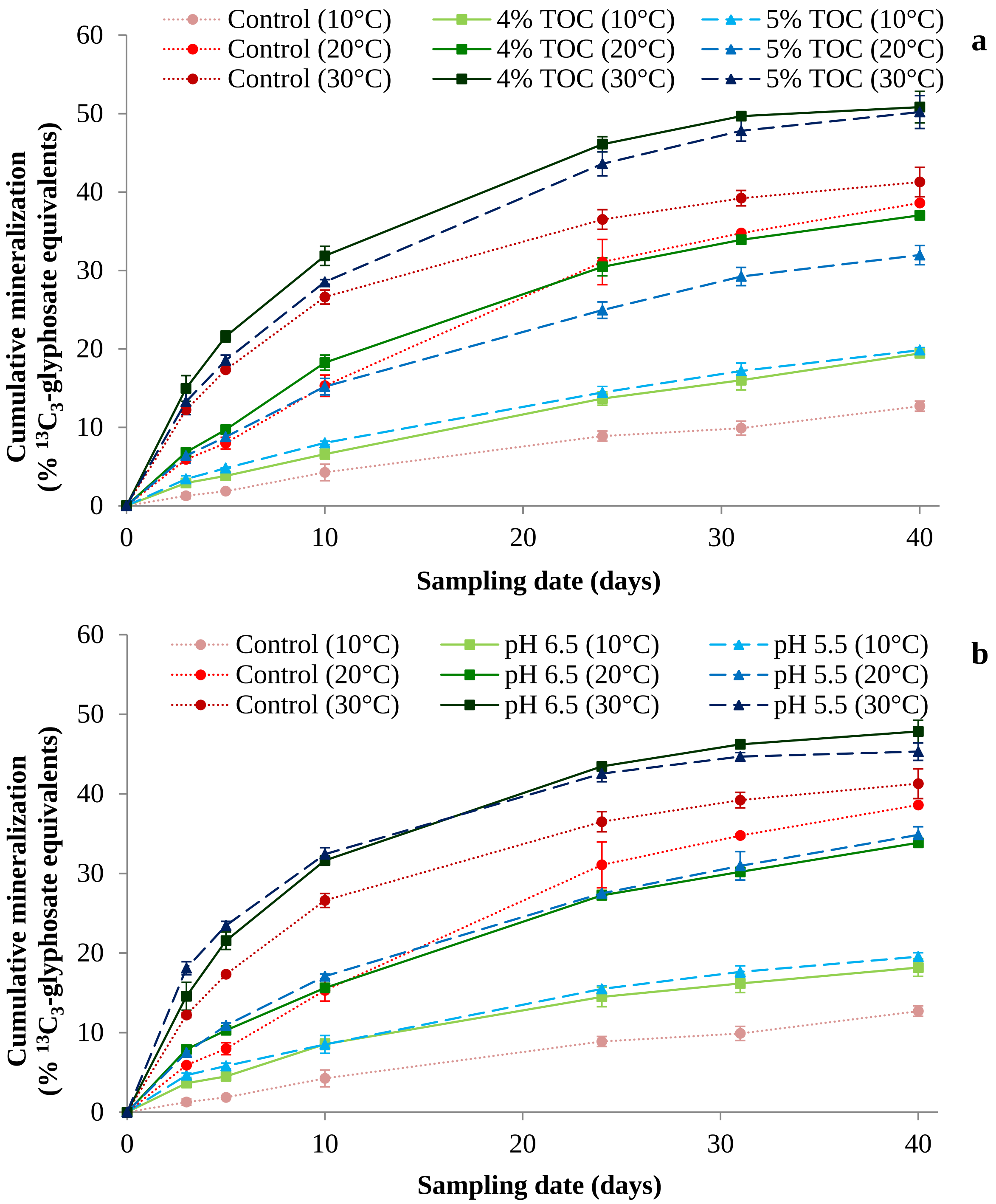

Fig. 1. Cumulative mineralization of initial $^{13}C_3$-glyphosate equivalents at 10°C, 20°C and 30°C (n=3, bars represent standard deviation). **a:** Control soil (3% TOC) and TOC treatments (4% TOC and 5% TOC); **b:** Control soil (pH 7.0.) and pH treatments (pH 6.5 and pH 5.5).

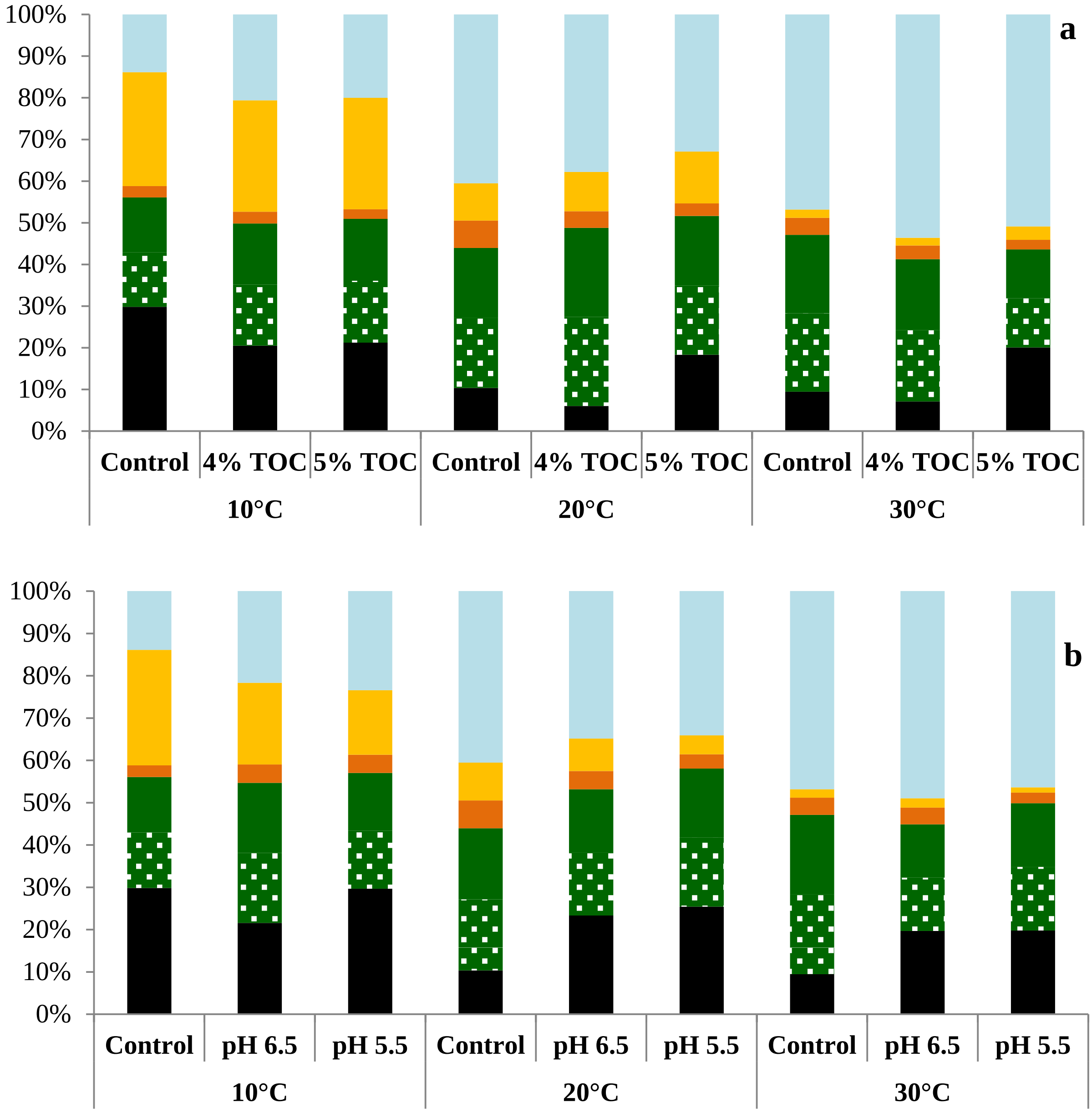

Fig. 2. Mass balance of $^{13}C_3$-glyphosate in % of total ^{13}C (normalized to 100%) in soil treatments incubated at 10°C, 20°C and 30°C for 40 days (n=3). Proteinaceous bioNER are ^{13}C-AAs. Other bioNER were calculated based on known 50% content of proteins (^{13}C-AAs). **a:** Control soil (3% TOC) and TOC treatments (4% TOC and 5% TOC); **b:** Control soil (pH 7.0.) and pH treatments (pH 6.5 and pH 5.5). Mineralization (🟦), glyphosate (🟨), AMPA (🟧), Proteins (🟩), other bioNER (▨), XenoNER (■).

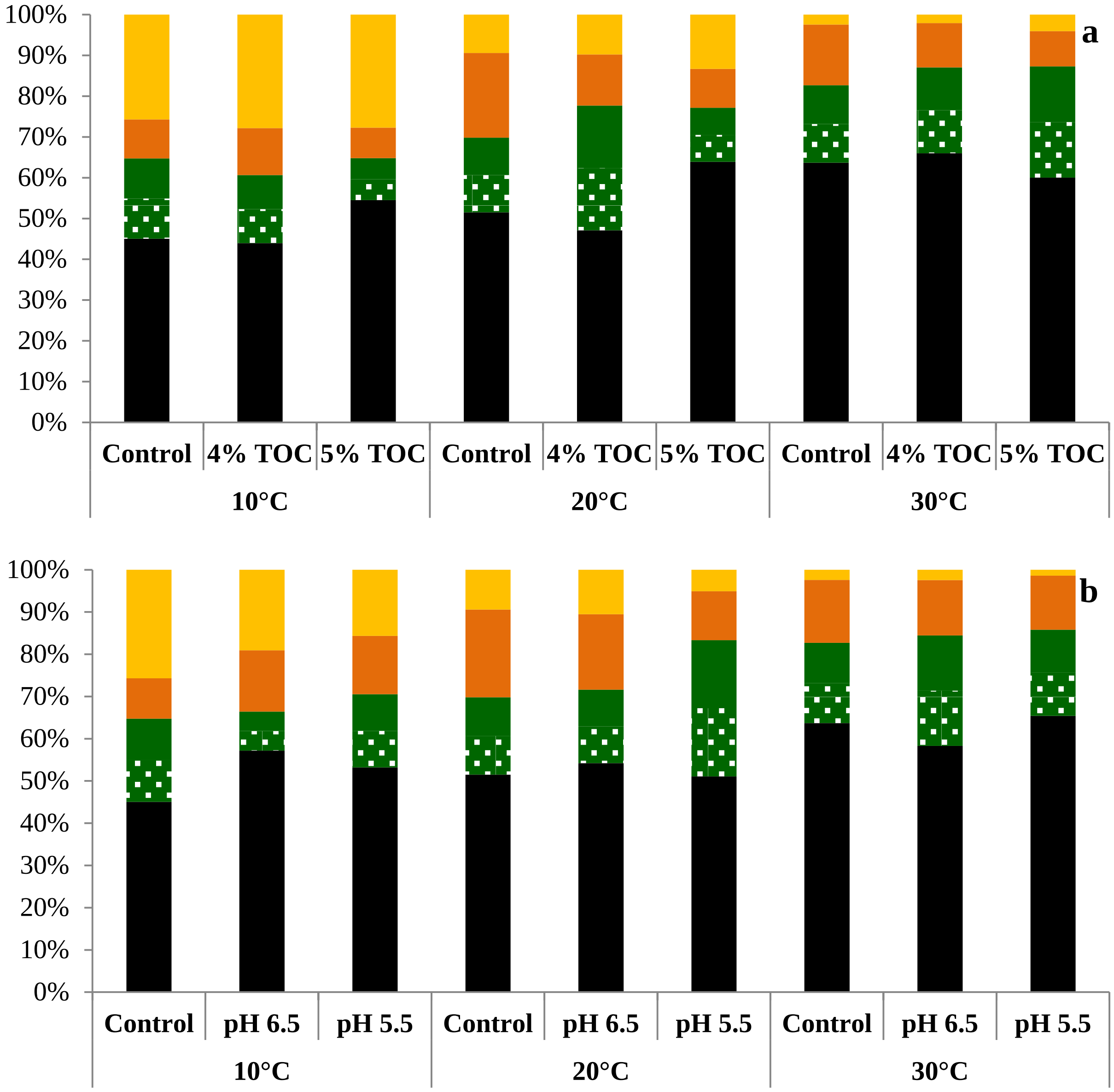

Fig. 3. Mass balance of ^{15}N-glyphosate in % of total ^{15}N (normalized to 100%) in soil treatments incubated at 10°C, 20°C and 30°C for 40 days (n=3). Proteinaceous bioNER are ^{13}C-tAA. Other bioNER were calculated based on known 50% content of proteins (^{13}C-tAAs). **a:** Control soil (3% TOC) and TOC treatments (4% TOC and 5% TOC); **b:** Control soil (pH 7.0.) and pH treatments (pH 6.5 and pH 5.5). Glyphosate (🟨), AMPA (🟧), Proteins (🟩), other bioNER (▨), XenoNER (■).

Scheme 1. Five treatments of a Colombian sandy loam soil: **1)** Control (pH 7.0, TOC 3%), **2)** 4% TOC, **3)** 5% TOC, **4)** pH 6.5, **5)** pH 5.5 and three isotope-related treatments (**I-III**) incubated at **10°C**, **20°C** and **30°C**. The pH of 4% TOC was 7.1, whereas of 5% TOC was 7.2. The pH 5.5 and 6.5 treatments had 3% TOC.

		TOC variation	**pH variation**		
Temperature variation	**30°C**	**1) Control** (3% TOC)	**1) Control** (pH 7.0)	**I: Blank** (without glyphosate) **II: Unlabeled glyphosate** (control) **III: $^{13}C_3{}^{15}N$-glyphosate**	**Isotope-related treatment**
	20°C	**2) 4% TOC**	**4) pH 6.5**		
	10°C	**3) 5% TOC**	**5) pH 5.5**		

Table 1. ^{13}C and ^{15}N-in extractable residues in control soil, TOC and pH treatments at 10°C, 20°C and 30°C after 40 days (n=3, mean values ± standard deviation).

^{13}C- and ^{15}N-in extractable residues [% of $^{13}C_3{}^{15}N$-glyphosate]

Temperature	Compound	Control	4% TOC	5% TOC	pH 6.5	pH 5.5
10°C	**$^{13}C_3{}^{15}N$-Glyphosate**	25 ± 4.7	25 ± 3.7	27 ± 0.2	16 ± 1.8	13 ± 2.1
	^{13}C-AMPA	2.5 ± 0.8	2.7 ± 0.0	2.3 ± 0.4	3.6 ± 0.5	3.6 ± 0.6
20°C	**$^{13}C_3{}^{15}N$-Glyphosate**	8.5 ± 0.8	9.2 ± 0.7	12 ± 1.7	7.5 ± 0.3	4.6 ± 0.3
	^{13}C-AMPA	6.3 ± 0.2	3.9 ± 0.4	3.0 ± 0.2	4.2 ± 0.5	3.5 ± 0.3
30°C	**$^{13}C_3{}^{15}N$-Glyphosate**	1.8 ± 0.2	1.8 ± 0.3	3.2 ± 0.3	2.2 ± 0.3	1.2 ± 0.2
	^{13}C-AMPA	3.6 ± 0.0	3.1 ± 0.7	2.3 ± 0.1	3.9 ± 0.6	2.5 ± 2.2

^{15}N-AMPA: is not shown. The values of ^{15}N in the AMPA are 3-fold higher than the ^{13}C-AMPA since the ^{15}N is referred to the initial amount of only one ^{15}N in glyphosate versus three labeled ^{13}C atoms of $^{13}C_3$-glyphosate.

Table 2. ^{13}C- and ^{15}N-AAs in control soil and TOC and pH treatments at 10°C, 20°C and 30°C after 40 days (n=3, mean values ± standard deviation).

Temperature	**Label**	**Control**	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**
10°C	**^{13}C**	12 ± 1.8	14 ± 2.2	15 ± 7.9	14 ± 8.2	11 ± 3.3
	^{15}N	9.6 ± 0.3	7.6 ± 0.3	4.9 ± 0.3	3.9 ± 1.1	7.0 ± 0.6
20°C	**^{13}C**	16 ± 3.1	21 ± 8.6	16 ± 1.5	15 ± 0.4	17 ± 14
	^{15}N	8.3± 1.0	14 ± 1.0	6.0 ± 0.5	6.2 ± 0.5	15 ± 4.6
30°C	**^{13}C**	17 ± 2.0	16 ± 4.3	12 ± 7.3	12 ± 3.5	15 ± 8.1
	^{15}N	6.9 ± 0.0	9.0 ± 2.0	11 ± 1.3	12 ± 0.2	8.8 ± 2.8