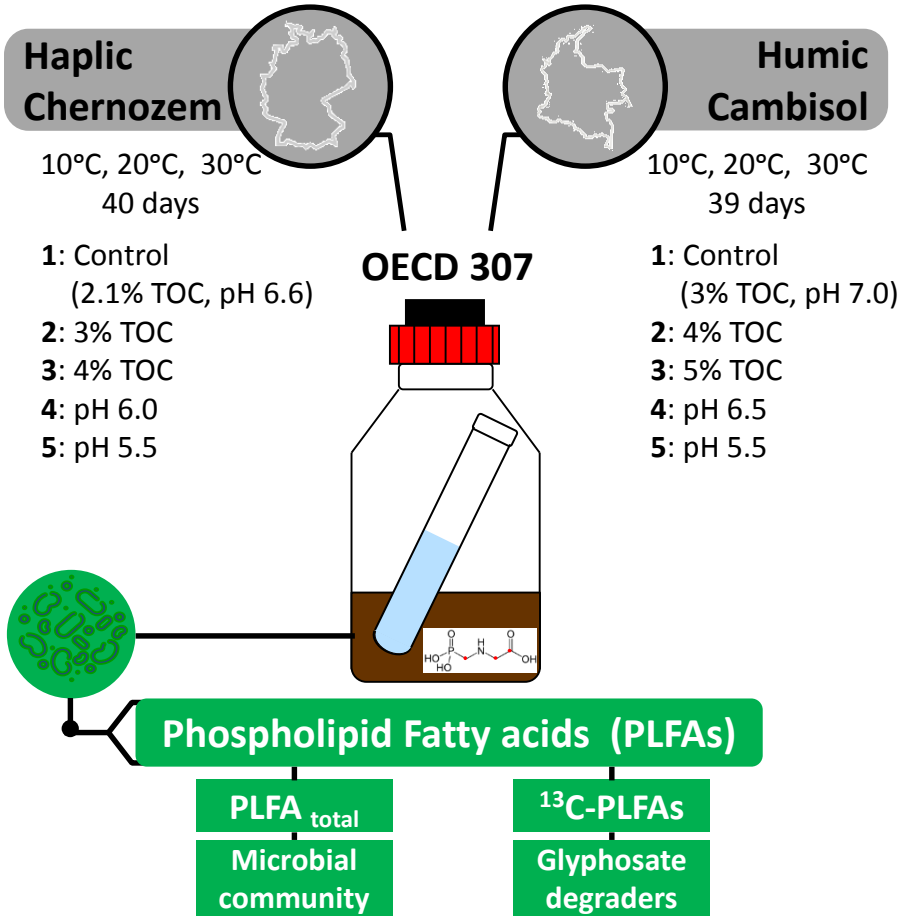
Highlights:

- Microbial activity of the humic Cambisol was higher than that of the haplic Chernozem
- Enhanced TOC of two soils shifted the microbiome towards Gram-positive bacteria
- The proportion of active glyphosate degraders was greater in the Haplic Chernozem
- Enhanced TOC shifted glyphosate degraders of two soils towards Gram-positive bacteria

Graphical Abstract



Microbial community composition and glyphosate degraders of two soils under the influence of temperature, total organic carbon and pH

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Abstract

Glyphosate can be degraded by soil microorganisms rapidly and is impacted by temperature and soil properties. Enhanced temperature and total organic carbon (TOC) as well as reduced pH increased the rate of $^{13}C_3{}^{15}N$-glyphosate conversion to CO_2 and biogenic non-extractable residues (bioNERs) in a Haplic Chernozem (Muskus et al., 2019) and in a Humic Cambisol (Muskus et al., 2020). To date; however, the combined effect of temperature and TOC or pH on microbial community composition and glyphosate degraders in these two soils has not been investigated. Phospholipid fatty acid [PLFA] biomarker analysis combined with ^{13}C labeling was employed to

investigate the effect of two soil properties (pH, TOC) and of three temperatures (10°C, 20°C, 30°C) on soil microorganisms. Before incubation, the properties of a Haplic Chernozem and a Humic Cambisol were adjusted to obtain five treatments: (a) Control (Haplic Chernozem: 2.1% TOC and pH 6.6; Humic Cambisol: 3% TOC and pH 7.0), (b) 3% TOC (Haplic Chernozem) or 4% TOC (Humic Cambisol), (c) 4% TOC (Haplic Chernozem) or 5% TOC (Humic Cambisol), (d) pH 6.0 (Haplic Chernozem) or pH 6.5 (Humic Cambisol), and (e) pH 5.5 for both soils. All treatments were amended with 50 mg kg^{-1} glyphosate and incubated at 10°C, 20°C or 30°C. We observed an increase in respiration, microbial biomass and glyphosate mineralization with incubation temperature. Although respiration and microbial biomass in the Humic Cambisol was higher, the microorganisms in the Haplic Chernozem were more active in glyphosate degradation. Increased TOC shifted the microbiome and the ^{13}C-glyphosate degraders towards Gram-positive bacteria in both soils. However, the abundance of ^{13}C-PLFAs indicative for the starvation of Gram-negative bacteria increased with increasing TOC or decreasing pH at higher temperatures. Gram-negative bacteria thus may have been involved in earlier stages of glyphosate degradation.

Keywords: glyphosate, mineralization, pH, TOC, soil respiration, PLFAs

1. INTRODUCTION

Glyphosate (N-phosphonomethylglycine) is globally the most applied herbicide (Benbrook., 2016; Li et al., 2016) and its use is increasing rapidly (from 67 million kg ha^{-1} in 1995 to 826 million kg ha^{-1} in 2014; Benbrook, 2016). Glyphosate residues are detected in all environmental compartments and their adverse effects on living biota are currently raising public concerns (Hvězdová et al., 2017; Milan et al., 2018). Numerous reports show that microorganisms can quickly convert glyphosate to CO_2 in various soils (Araújo et al., 2003; Benslama and Boulahrouf, 2013; Carlisle and Trevors, 1986; Wardle and Parkinson, 1990). Different Gram-positive and Gram-negative bacteria, and fungi can utilize glyphosate as a source of carbon, nitrogen or phosphorus, e.g. *Pseudomonas* spp., *Rhizobium* sp., *Agrobacterium* sp., *Arthrobacter* sp. GLP S. *meliloti*, *Ochrobactrum anthropi*, *Agrobacterium radiobacter* and *Penicillium* sp. (Adams et al.. 2008; Bjarne Hove-Jensen et al., 2014; Liu et al.. 1991; Pipke et al., 1987; Wackett et al., 1987).

Microbial degradation of glyphosate can proceed along two metabolic pathways: via the sarcosine or the aminomethylphosphonic acid (AMPA) pathway (Araújo et al.. 2003; Balthazor and Hallas, 1986; Hove-Jensen et al., 2014; Giesy et al., 2000; Jacob et al., 1988; McAuliffe et al.; 1990; Rueppel et al., 1977). The initial "sarcosine pathway" yields glycine which is presumably integrated into the proteins as the monomeric building block (Wang et al., 2016). The latter degradation of glyphosate via the "AMPA pathway" produces glyoxylate and AMPA. The AMPA is further metabolized at much slower rates than produced (Wang et al., 2016). The shift of the metabolic pathway of glyphosate towards the AMPA pathway is presumably regulated by the C:N stoichiometry of both the resource (e.g. sarcosine) and the microorganisms (Mooshammer et al. 2014). In order to avoid the excess of N in the cells, microorganisms shift to C metabolism based only on glyoxylate and the N excretion as AMPA (Brock et al., 2019). Nevertheless, microorganisms assimilated the carbon and nitrogen from glyphosate into their

biomass during the two pathways of glyphosate degradation in soil (Muskus et al., 2019; Muskus et al., 2020) and water-sediment (Wang et al., 2016).

The degradation rate of glyphosate depends on climatic conditions, soil properties and soil microbiome (Alexa et al. 2008; 2009; Getenga & Kengar, 2004; Gimsing et al., 2004; 2007; Lauber et al., 2009a; Rousk et al., 2010; Zhalnina et al., 2014). Enhanced temperature and TOC or reduced pH increased the rate of $^{13}C_3{}^{15}N$-glyphosate transformation to CO_2 and biogenic non-extractable residues (bioNERs) in a Haplic Chernozem (Muskus et al., 2019) and in a Humic Cambisol (Muskus et al., 2020). The combined effect of temperature and TOC or pH also can affect the activity and composition of microbial community, as well as glyphosate degraders of both soils. This effect; however, has not been shown yet.

Phospholipid fatty acids (PLFAs) are membrane constituents of living cells; the specific PLFA patterns of different organisms enable to distinguish between the main groups of the soil microbiome, i.e. Gram-positive bacteria, Gram-negative bacteria, actinobacteria and fungi (Boschker and Middelburg, 2002; Frostegård and Bååth, 1996; Kaur et al., 2005). The PLFA analysis is therefore used for the identification of the microbial groups actively involved in the microbial turnover of a substrate and for tracing of the active microbes over time (Amelung, 2003; Banks et al., 2014; Glaser and Gross, 2005; Haney et al., 2018; Kaur et al., 2005; Schwab et al., 2017; Yao et al., 2015; Zelles, 1999). When an isotope-labeled substrate is degraded by microorganisms, a certain amount of the isotope label will be incorporated into the PLFAs of the degrader organisms; this allows identifying degraders based on the isotope enrichment of PLFAs indicative for specific groups of bacteria or fungi (Boschker et al., 1998; Coban et al, 2015).

We hypothesize that the variation of temperature and soil parameter (pH or TOC), in particular the TOC addition, will increase the activity of the soil microbiome and the glyphosate degraders. In addition, this effect may differ between two soils: Haplic Chernozem and Humic Cambisol. In the present study, we thus investigated the effect of decreasing soil pH or increasing TOC on the activity of the soil microbiome and $^{13}C_3{}^{15}N$-glyphosate degraders and their interaction with

incubation temperature (10°C, 20°C, 30°C) in the Haplic Chernozem and the Humic Cambisol. We identified shifts in the composition of the soil microbiome and between the specific groups of glyphosate degraders based on $PLFA_{total}$ and ^{13}C-PLFA analyses, respectively. The ^{13}C- and ^{15}N-mass balances of $^{13}C_3{}^{15}N$-glyphosate turnover have been already determined for both soils in the previous two studies by Muskus et al. (2019; 2020). Mineralization of $^{13}C_3$-glyphosate over the course of 39/40 days has been already published in Muskus et al. (2019, 2020). Here, only the final mineralization data of $^{13}C_3$-glyphosate ($^{13}CO_2$) has been shown for the two soils in order to aid the interpretation and discussion of respiration (CO_{2total}), $PLFA_{total}$ and ^{13}C-PLFA results.

2. MATERIALS AND METHODS

2.1 Chemicals

Labeled $^{13}C_3$-glyphosate was acquired from Iso-Sciences Company (Trevose. PA. USA). The isotopic purity was 99 atom% ^{13}C. The chemical purity of glyphosate was 98%. Other chemicals used in this study were obtained from Carl Roth, Karlsruhe, Germany or VWR/Merck, Darmstadt, Germany.

2.2 Reference soils and farmyard manure

Haplic Chernozem. Multiple samples from the Ap soil horizon of the long-term "Static Fertilization Experiment" located in Bad Lauchstädt, Germany N51°23'23.6"E11°52'48.3" were collected into a 60 L container (for details see also Muskus et al., 2019). The sampled plot was amended with 30 t ha^{-1} of farmyard manure every second year for more than 100 years. It also received annual amendments of 12 kg P ha^{-1} and 50 kg K ha^{-1}. Many different pesticides including glyphosate were sprayed for more than 30 years according to normal agricultural practice in the region. The soil was a silt loam (21% clay, 68% silt, 11% sand) with 2.1% (w/w)

total organic carbon (TOC) and 0.17% (w/w) total nitrogen (Körschens, 2002). In the year of sampling, the pH (H_2O) was 6.6.

Humic Cambisol. Multiple samples from the Ap soil horizon from an area under traditional raspberry cultivation, located in the Andean rural eastside of the municipality of Piedecuesta in Santander, Colombia were collected into a 40 L container and (N7°00'54.6"W72°59'; for details see also Muskus et al., 2020). The soil was a sandy loam soil (7% clay, 20% silt, 73% sand, measured with a 152H hydrometer). The pH (H_2O) was 7.0; TOC was 3% (w/w) and nitrogen was 0.27% (w/w). No information about site history, soil amendments and pesticide applications is available for this site.

Farmyard manure (FYM). To increase the TOC concentration of the two soils, the same cow FYM was used as for the annual amendments in the long-term experimental area "Static Fertilization Experiment" in Bad Lauchstädt, Germany (Muskus et al., 2019; Muskus et al., 2020). The pH (H_2O) of FYM was 8.7. It had 2.5% total N (w/w) and 34 % TOC (w/w). Prior to the incubation experiment, soil and FYM samples were homogenized and sieved through a 2 mm diameter mesh.

2.3 Experimental setup and incubation

The experimental setup for the reference Haplic Chernozem and Humic Cambisol is illustrated in **Figure 1**. The details on the experimental set-up and a soil parameter (TOC and pH) modification for two soils can be found in the two studies by Muskus et al. (2019, 2020). Here, only a brief description on the soil modifications is given. The soils were manipulated to increase TOC and decrease pH, resulting in three levels of each factor (control without any modification and two manipulated levels). Three replicates of each treatment were incubated at three different temperatures (10°C, 20°C and 30°C).

TOC modification. The original TOC of the control Haplic Chernozem (2.1%) was increased to 3% and 4% by addition of 3.5% and 7% of air-dried FYM (w/w). About 2.5% (w/w) of FYM was

added to the control Humic Cambisol (3% TOC) to obtain 4% TOC, whereas 5% (w/w) of FYM was added to obtain 5% TOC. The TOC contents of each treatment after FYM addition was controlled using an elemental analyzer-combustion-isotope ratio mass spectrometry (EA-C-IRMS; Finnigan MAT 253. Thermo Electron, Bremen, Germany; (Girardi et al., 2013) after equilibration for 7 days. Further details on the preparation of TOC modifications are available in Muskus et al. (2019).

pH modification. Sulfuric acid (H_2SO_4) was used to adjust the pH of the reference Haplic Chernozem and Humic Cambisol. H_2SO_4 concentrations and amounts to add to each soil were determined as described in Muskus et al., (2019). To reduce the pH of the control Haplic Chernozem (pH 6.6) to pH 6.0 or the Humic Cambisol (pH 7.0) to pH 6.5, 1 mL of 0.1 M H_2SO_4 was added to 20 g of soil. Approximately 1 mL of 1 M H_2SO_4 was added to adjust the pH of the control Haplic Chernozem or Humic Cambisol to pH 5.5. More details on treatment preparations can be found in Muskus et al. (2019). Although 1 mL of 0.1 M H_2SO_4 was added to each soil, we obtained different pH levels for the Haplic Chernozem (6.0) and for the Humic Cambisol (6.5). This difference can be attributed to the different buffering capacity of each soil. The buffering capacity of Humic Cambisol was obviously higher than that of the Haplic Chernozem and it might be due to the greater TOC content of Humic Cambisol (3%) than the Haplic Chernozem (2.1%).

Each treatment was incubated as a blank (no glyphosate) or amended with 50 mg kg^{-1} either unlabeled glyphosate or $^{13}C_3{}^{15}N$-labeled glyphosate. Ten-fold higher concentration of glyphosate than the recommended agricultural application dose of 5 mg kg^{-1} was needed for reliable detection and quantification of the isotopic enrichment against the ^{13}C natural abundances which were obtained from the blanks and controls. All treatments were adjusted to 60% of the maximum water holding capacity and incubated in triplicates at 10°C, 20°C and 30°C according to the OECD guideline 307 (OECD. 2002); for details see Muskus et al. (2019). Total CO_2 (soil microbial respiration) and $^{13}CO_2$ from mineralization of $^{13}C_3$-glyphosate was trapped in 2 M

NaOH solution which was sampled and measured at regular time intervals (3, 5, 10, 21 and 40 days for the Haplic Chernozem and 3, 5, 10, 21 and 39 days for the Humic Cambisol). The total amount of soil was sampled destructively at the end of the incubation period for PLFA analysis. The accelerated degradation of glyphosate in two soils was noticed in the first two weeks of incubation; thereafter it reached plateau (Muskus et al., 2019, 2020). Therefore, the difference of one day in the final sampling point (39 days versus 40 days) of the two soils did not affect the accuracy of results.

2.4 Soil respiration and mineralization of $^{13}C_3$-glyphosate

Soil respiration (total concentration of CO_2 in the NaOH traps: CO_{2total}) was determined by TIC analysis (Shimadzu TOC-5050 analyzer, Duisburg, Germany). Mineralization of $^{13}C_3$-glyphosate ($^{13}CO_2$) was quantified based on combined analysis of total concentration of CO_2 ($^{12}C+^{13}C$-CO_2) and its isotopic composition ($^{12}C/^{13}C$ at%). The CO_2 was separated from other permanent gases on a BPX-5 column (50 m×0.32 m×5 μm) and its isotopic composition was measured using gas chromatography-combustion-isotope ratio-mass spectrometry (GC-C-irMS). The separation conditions for GC-C-irMS were described previously (Girardi et al., 2013).

2.5 PLFA biomarker analysis

The PLFAs were extracted from the soils using a mixture of phosphate buffer, methanol and chloroform (Bligh and Dyer, 2010). The chloroform phase containing the PLFA was first separated from the aqueous phase, dried using sodium sulphate and then purified over silica gel as described previously (Nowak et al., 2011). Thereafter, the PLFAs were derivatized to fatty acid methyl esters (FAMEs) with methanol/trimethylchlorosilane (9:1 v/v). FAMEs were identified and quantified after separation on a BPX-5 column (30 m×0.25 mm×0.25 μm) using a gas chromatograph-mass spectrometer (Agilent 6890 GC, 5973N MS, Agilent, Waldbronn, Germany). The isotopic composition of FAMEs in each sample was determined using a GC-C-

irMS Finnigan MAT 252, Thermo Electron, Bremen, Germany, linked to a GC Hewlett Packard 6890 GC (Agilent Technologies, Waldbronn, Germany) equipped with a 50 m × 0.32 m × 5 µm Porabondt Q-HT Plot FS column (Chrompack, Middleburg, Netherlands). More information about the separation conditions of FAMEs and quantification of FAMEs can be found in Nowak et al. (2011) and Girardi et al. (2013).

For data presentation, PLFA were grouped according to their assignments to specific groups of microorganisms. Seven groups were identified (see **Table 1)**: 1) General microbial marker, 2) Gram-positive bacteria, 3) actinobacteria, 4) Gram-negative bacteria, 5) starvation marker, 6) fungi and 7) others (including 20:1; 18:2; a16:0, 15:1br, 16:1w5, 17:1br, 18:0br, 18:1w5, which are not indicative of any special group of microorganisms). Gram-positive bacteria were represented by saturated branched PLFAs (iso and anteiso isomers) and by 10-methyl branched PLFAs (Actinobacteria). Monounsaturated PLFAs are indicative for Gram-negative bacteria, whereas their starvation is indicated by the cyclopropyl PLFAs (Kaur et al., 2005). Cyclopropyl PLFAs are developed in the membranes of Gram-negative bacteria in response to any environmental change e.g. absence of accessible carbon-substrates. Polyunsaturated PLFAs are indicators of fungi (Zelles, 1999). In contrast to other groups of PLFAs, saturated straight chain PLFAs represent general microbial marker which cannot be assigned to any specific microorganism group. Therefore, only PLFAs indicative to any specific group are considered in the interpretation and discussion of the microbial community composition data.

2.6 Data analysis and statistics

All data on respiration (CO_{2total}), mineralization ($^{13}CO_2$) and PLFA biomarkers ($PLFAs_{total}$; ^{13}C-PLFAs) are presented as averages of triplicates with standard deviations. The total amount of PLFAs ($PLFAs_{total}$) is used to indicate changes in microbial biomass, whereas the glyphosate degraders are described by the ^{13}C-PLFAs. The $PLFAs_{total}$ and ^{13}C-PLFAs were analyzed in the control soils with 50 mg kg^{-1} glyphosate, in the two TOC and in the two pH levels at each

temperature. Due to very high number of samples for PLFAs extraction and time-consuming analysis, we did not analyze the PLFAs in the blank control soils (without glyphosate). We analyzed only control blanks for the cumulative soil respiration only, and this information was sufficient to monitor the effects of glyphosate addition on microbial activity of two soils. Cumulative soil respiration is given in µmol CO_2 g^{-1} soil and the $PLFAs_{total}$ in µmol PLFAs g^{-1} soil on day 39 (Haplic Chernozem) and 40 (Humic Cambisol). Cumulative mineralization of glyphosate ($^{13}CO_2$) and ^{13}C-PLFAs are presented as percentages of the initially applied $^{13}C_3$-glyphosate $^{13}CO_2$ on day 39 (Haplic Chernozem) and 40 (Humic Cambisol).

A two-way ANOVA (ANalysis Of VAriance) with post-hoc Tukey HSD (Honestly Significant Difference) test was used to analyze the significance of each factor separately (temperature and TOC or pH variation) on PLFAs ($PLFAs_{total}$ and ^{13}C-PLFAs) in Haplic Chernozem or Humic Cambisol. The difference was considered to be significant when p value was <0.05 and the results are shown in Supplementary Material (SI). The Tukey's honestly significant difference test was used for separation of means using the IBM SPSS and data mining software (Version 25, Copyright IBM Corp 1989, 2017).

The multivariance analysis (MANOVA) was used to reveal significant correlations between temperature and soil parameter (TOC or pH) and soil type on the microbial composition of the soil microbiome ($PLFAs_{total}$ groups) and of the glyphosate degraders (^{13}C-PLFAs groups). MANOVA results are also available in the SI. The effect of multiple parameters (temperature, TOC, pH, mineralization, respiration) on the shifts in the PLFA pattern of soil microbiome ($PLFAs_{total}$ groups) and in the ^{13}C PLFA pattern (^{13}C-PLFAs groups) were analyzed by principal component analysis (PCA). In addition, the abundances of each $PLFAs_{total}$ or ^{13}C-PLFAs group in the Haplic Chernozem or the Humic Cambisol at different levels of temperature, TOC or pH were visualized in a heat-map. The level of significance for all statistical tests was set to $p<0.05$. PCA and heat-map visualization were done in XLSTAT version 2020.3.

240 ## 3. RESULTS

241 ### 3.1 Soil respiration (CO_{2total}) and microbial biomass based on $PLFAs_{total}$

242 The respiration of the blank control Humic Cambisol (without glyphosate) was three to five times
243 higher (23±0.2 – 110±0.3 µmol CO_2 g^{-1}) than of the Haplic Chernozem blank control (8.8±0.2 –
244 24±0.4 µmol CO_2 g^{-1}). Addition of 50 mg glyphosate kg^{-1} to soil increased respiration of both
245 Haplic Chernozem control (by 7-11%) and the Humic Cambisol control (by 4-15%; see the
246 controls with glyphosate in **Table S1**) incubated at 10°C and 20°C as compared to the control
247 blanks (without glyphosate). Respiration increased with raising the incubation temperature in all
248 treatments. An enhanced TOC content of soil (both blank and with glyphosate) promoted
249 microbial activity as indicated by the increased respiration of Haplic Chernozem (three- to five-
250 fold) and Humic Cambisol (< two-fold). The highest respiration was noticed for highest TOC
251 levels of both Haplic Chernozem (4% TOC; 43±0.4 – 120±0.8 µmol CO_2 g^{-1}) and of Humic
252 Cambisol (5% TOC; 37±0.2 – 143±0.3 µmol CO_2 g^{-1}). Reduction of soil pH to 6.0 or 5.5 also
253 increased the respiration of two soils. About 24 – 45% increase in the soil respiration at each
254 temperature as compared to the blank control (8.8±0.2 – 24±0.4 µmol CO_2 g^{-1}) and the control
255 with glyphosate (9.9±0.3 – 23±0.7 µmol CO_2 g^{-1}) was noticed for the Haplic Chernozem. The 7-
256 14% enhanced respiration of Humic Cambisol with pH 5.5 was found at 10°C and at 20°C as
257 compared to the blank control (23±0.2 and 57±0.3 µmol CO_2 g^{-1}, respectively) and to the control
258 with glyphosate (27±0.8 and 59±0.4 µmol CO_2 g^{-1}, respectively).

259 In analogy to respiration results, also highest microbial biomass based on $PLFAs_{total}$ was found in
260 Haplic Chernozem with two highest TOC levels at each incubation temperature (**Table 2,** $p<0.05$;
261 see also **Table S2A**). $PLFAs_{total}$ in Haplic Chernozem with 3% or 4% TOC (0.05 – 0.07±0.002
262 µmol PLFAs g^{-1}) was three- to six-fold higher than in the control (0.01 – 0.02±0.000 µmol
263 PLFAs g^{-1}, $p<0.05$). It is noteworthy that $PLFAs_{total}$ at 30°C in the two highest TOC levels were
264 lower than at 20°C; this could be a result of faster microbial metabolism with an accelerated

resources consumption which results in loss of biomass and decline of the PLFAs at higher temperature. Similarly, lowering pH also increased microbial biomass in Haplic Chernozem which was approximately two and four-fold higher (0.03 – 0.04±0.001 µmol PLFAs g^{-1}, $p<0.05$; see also **Table S2B**) than in the control. This suggests that either increasing TOC level or lowering pH, had a positive effect on the quantity of microbial biomass of Haplic Chernozem.

The amount of $PLFAs_{total}$ in Humic Cambisol (0.09±0.003 – 0.31±0.01 µmol PLFAs g^{-1}) was at least twice higher than in Haplic Chernozem (0.01±0.000 – 0.07±0.002 µmol PLFAs g^{-1}; see **Table 2**) suggesting higher microbial activity of the Humic Cambisol. Microbial biomass was biggest in Humic Cambisol with two highest TOC levels (0.12±0.005 – 0.31±0.01 µmol PLFAs g^{-1}, $p<0.05$; see also **Table S3A**) as compared to the controls (0.09±0.003 – 0.20±0.001 µmol PLFAs g^{-1}, $p<0.05$). Reduction of pH of Humic Cambisol to either pH 5.5 or pH 6.5 also enhanced microbial biomass (0.12±0.006 – 0.19±0.008 µmol PLFAs g^{-1}, $p<0.05$; see also **Table S3B**).

3.2 Groups of microorganisms in soil microbiome based on $PLFAs_{total}$

Increasing temperature (20°C and 30°C) induced a shift of the Haplic Chernozem microbiome towards Gram-negative bacteria in controls (see **Figure 2A**). Same trend was noticed in the Haplic Chernozem with pH 5.5 and 6.0 at 10°C as compared to the control at 10°C (**Figure 2C** and **Figure S1)**. This finding suggests that the shift towards Gram-negative bacteria could be the result of either lowering soil pH or temperature increase or even of both factors. In contrast to the control and to soil with reduced pH at 10°C, increasing TOC of Haplic Chernozem favored the shift of microbiome towards Gram-positive bacteria at each temperature and in particular for the highest TOC level (**Figure 2A**). The shift towards Gram-positive bacteria was also noticed in the Haplic Chernozem with 5.5 and 6.0 at 30°C (**Figure 2C**). The markers indicative for starvation of Gram-negative bacteria increased in the two enhanced TOC as well as in the two reduced pH (except from pH 6.0 at 10°C and 20°C) levels of Haplic Chernozem as compared to their

controls. In contrast, actinobacteria marker was less abundant in the two reduced pH and in the two enhanced TOC at each temperature as compared to controls. Fungal marker was the least abundant marker in all five treatments. However, the fungal marker at increased TOC at all incubation temperatures was more abundant than that in the controls (**Figure S1**).

In contrast to Haplic Chernozem controls, we observed a shift of the Humic Cambisol microbiome towards Gram-positive bacteria in controls at 20°C and 30°C as compared to the control at 10°C (**Figure 2B** and **Figure S2)**. Similarly, increasing TOC (**Figure 2B**) or lowering pH (**Figure 2D**) of the Humic Cambisol to 5.5 at 10°C also shifted the microbiome towards Gram-positive bacteria as compared to the control at 10°C. In contrast, reduction of soil pH to 6.5 at 10°C did not induce changes in the microbial community composition as compared to the control at 10°C. The actinobacteria marker in the soil with 5% TOC at 30°C also increased as compared to the control at 30°C (**Figure 2B**).

The multivariance analysis (MANOVA) for the two soils indicated only a significant difference between the actinobacteria and Gram-positive bacteria of soil microbiome for the isolated variable TOC (Wilk´s λ and $p<0.05$; see also **Table S4**).

3.3 $^{13}C_3$-glyphosate mineralization ($^{13}CO_2$) and incorporation into ^{13}C-PLFAs

The higher microbial activity of Humic Cambisol control ($CO_{2total,}$ **Table S1**) than the Haplic Chernozem control did not enhance the final cumulative mineralization of $^{13}C_3$-glyphosate (see **Table S5**) in Humic Cambisol. Mineralization of $^{13}C_3$-glyphosate is shown as $^{13}CO_2$ in percentage of the initial $^{13}C_3$-glyphosate equivalents. Mineralization of $^{13}C_3$-glyphosate in the controls of Haplic Chernozem (12%±0.9% $^{13}CO_2$) and of Humic Cambisol (13%±0.7% $^{13}CO_2$) soil at 10°C was similar. It was only slightly higher (39%±0.1% $^{13}CO_2$) in the Humic Cambisol control at 20°C than in the Haplic Chernozem control (37%±0.2% $^{13}CO_2$). The temperature was the main factor controlling the mineralization of the applied $^{13}C_3$-glyphosate in both soils (Muskus et al., 2019; Muskus et al., 2020). The mineralization decreased in the order 30°C >

315 20°C > 10°C. Lower pH and higher TOC concentration also increased the mineralization of $^{13}C_3$-
316 glyphosate in both soils. Mineralization of glyphosate was lowest in the Haplic Chernozem
317 control (12%±0.9% – 43%±0.3% $^{13}CO_2$) and highest in the two enhanced TOC levels of the
318 Haplic Chernozem (20%±1.5% – 54%±6.6% $^{13}CO_2$). Similarly, mineralization of $^{13}C_3$-glyphosate
319 in Humic Cambisol with reduced pH (18%±1.1% – 48%±1.4% $^{13}CO_2$) and enhanced TOC
320 (19%±0.5% – 51%±2.0% $^{13}CO_2$) at 10°C and at 30°C was also higher than in the respective
321 controls at 10°C (13%±0.7% $^{13}CO_2$) and 30°C (41%±1.9% $^{13}CO_2$).

322 Higher percentage of ^{13}C from the initial $^{13}C_3$-glyphosate equivalents was incorporated into the
323 PLFAs in the Haplic Chernozem (0.06%±0.01% – 0.25%±0.03% ^{13}C-PLFAs) than in the Humic
324 Cambisol (0.01%±0.00% – 0.13%±0.01% ^{13}C-PLFAs) in each treatment and at each temperature
325 (except for pH 5.5 at 10°C; **Table 3**). This finding suggests a higher incorporation of ^{13}C from
326 $^{13}C_3$-glyphosate into microbial biomass of the Haplic Chernozem although $^{13}C_3$-glyphosate
327 mineralization was comparable in two soils. Temperature did not affect the ^{13}C-PLFAs amounts
328 in the controls of both soils. In analogy to mineralization, increased ^{13}C-PLFAs contents were
329 also noticed in the two enhanced TOC levels of Haplic Chernozem (0.13%±0.02% –
330 0.25%±0.03% ^{13}C-PLFAs, $p<0.05$) and Humic Cambisol (0.09% – 0.13%±0.01% ^{13}C-PLFAs) in
331 which the ^{13}C-PLFAs were always highest (**Table 3**, $p<0.05$). In contrast to two enhanced TOC
332 levels, lowering soil pH reduced the ^{13}C-PLFAs amounts in the Haplic Chernozem with pH 6.0
333 and 5.5 (0.02 – 0.07%±0.01% ^{13}C-PLFAs, except for pH 6.0 at 10°C, $p<0.05$) as compared to the
334 controls (0.08 – 0.10%±0.01% ^{13}C-PLFAs). Similarly, the ^{13}C-PLFAs in two reduced pH levels
335 of the Humic Cambisol at 20°C (0.01 – 0.03%±0.00% ^{13}C-PLFAs, $p<0.05$) were also lower than
336 in the control at 20°C (0.04%±0.00% ^{13}C-PLFAs).

337 **3.4 Groups of glyphosate degraders based on ^{13}C-PLFAs**

338 Increasing TOC of Haplic Chernozem shifted ^{13}C-glyphosate degraders towards Gram-positive
339 bacteria in all TOC levels and at all incubation temperatures (**Figure 3A** and **Figure S3**). This

result agrees well with the concomitant shift of microbiome towards Gram-positive bacteria in two enhanced TOC levels of the Haplic Chernozem (**Figure 2A**). Similarly to soil with increased TOC, a shift of the glyphosate degraders towards Gram-positive bacteria was also observed in Haplic Chernozem with pH 5.5 at 10°C and 20°C, and with the pH 6.0 at 10°C (**Figure 3C**). This finding contradicts the observed shift of Haplic Chernozem microbiome towards Gram-negative bacteria in the two reduced pH levels (**Figure 2C**). However, an increased abundance of the ^{13}C-PLFAs marker indicative for the starvation of Gram-negative bacteria in the two enhanced TOC levels at 30°C (**Figure 3A**) and in the soil with pH 6.0 at 20°C and 30°C (**Figure 3C**) was also detected. Highest abundance of the ^{13}C-actinobacteria marker was observed in the Haplic Chernozem with pH 5.5 at 30°C.

Similar to what was observed for Haplic Chernozem, an enhanced TOC of Humic Cambisol also promoted the shift of glyphosate degraders towards Gram-positive bacteria (**Figure 3B** and **Figure S4**). This is also in a good accordance with the concurrent shift of microbiome towards Gram-positive bacteria in two increased TOC levels of Humic Cambisol (**Figure 2B**). The shift of glyphosate degraders towards Gram-positive bacteria was also noted in Humic Cambisol with pH 6.5 at each temperature (**Figure 3D**). The starvation marker indicative for starvation of Gram-negative bacteria was most abundant in the soil with pH 6.5 at 20°C. Similar to what was noticed in Haplic Chernozem with pH 5.5 at 30°C (**Figure 3C**), actinobacteria marker in the soil with pH 5.5 at 30°C (**Figure 3D**) was also most abundant among the five treatments of the Humic Cambisol.

Results of the multivariance analysis (MANOVA) of the two soils revealed a significant shift of glyphosate degraders towards fungi and starvation of Gram-negative bacteria for the single effect of temperature, TOC or pH as well as for the combined effect of temperature + TOC or temperature + pH (Wilk´s λ and $p<0.05$; see also **Table S6**).

4. DISCUSSION

4.1 Increased temperature and TOC or pH variation boosted microbial activity

Addition of glyphosate increased only slightly soil respiration in the two soils (7-11% and 4-15% for the Haplic Chernozem and the Humic Cambisol, respectively) as compared to controls without glyphosate. This finding is in good accordance with the observed 10-15% increase of soil respirations following glyphosate addition (Araújo et al., 2003, Wardle and Parkinson, 1990). However, it disagrees with the negligible effect of glyphosate application on soil microbial activity in the study by Liphadzi et al., (2005) and by Zabaloy et al. (2008). Three- to five-fold higher respiration and microbial biomass of Humic Cambisol control than the Haplic Chernozem control indicated for the higher microbial activity of Humic Cambisol than Haplic Chernozem. This difference might be related to the different contents of TOC, total nitrogen and pH in two soils (Xue et al., 2018) all of which were higher in Humic Cambisol. Soil properties like enhanced TOC and pH near 7.0 are well known to stimulate microbial activity and microbial biomass quantity (Sollins et al., 1988). The respiration of forest soils decreased in soils with lower pH (Anderson and Domsch, 1993). Soil pH regulates nutrient availability and ion uptake by the microorganisms, whereas organic carbon and nitrogen are base nutrients supporting microbial growth (Sollins et al., 1988). Furthermore, the C:N stoichiometry of both the accessible resources and the current microbial food demands also affects the activity of soil microorganisms (Mooshammer et al. 2014) . Another explanation for the differences in respiration of the two soils can be a longer storage of Haplic Chernozem at +4ºC than the Humic Cambisol (1 year vs 6 months) prior to incubation. This also can explain a lower microbial activity of Haplic Chernozem.

Increased temperature and TOC boosted microbial activity (respiration and biomass) in two soils. It is obvious that mesophilic temperatures (20ºC-40ºC) are most optimal for microbial activity (Gavrilescu, 2005; Shymko et al., 2011; Singleton, 1994). Similarly, soil amendment with

organic material is well-known to trigger microbial activity through the supply of available organic carbon and nitrogen needed for growth (Chen et al., 2017; Liu et al., 2020; Ma et al., 2018; Orr et al., 2012; Renella et al., 2008; Shymko et al., 2011; Singleton, 1994). This explains the highest soil respiration and microbial biomass in Haplic Chernozem and Humic Cambisol with increased TOC levels.

During the soil manipulation (H_2SO_4 addition and thorough mixing with water) prior to incubation, the availability of C-substrates and other macronutrients to microorganisms could have been enhanced (Aciego Pietri and Brookes, 2008; Binkley and Vitousek, 1989; Kemmitt et al., 2006; Zhao et al., 2011). This could explain an increased microbial activity in the Haplic Chernozem with reduced pH as compared with the control without H_2SO_4 addition and only thoroughly mixed with water. However, we did not measure the nutrients content in the soils prior to incubation; and in addition to the pH, the temperature also might have affected the microbial activity in the soil with reduced pH. We cannot separate the pH factor from the temperature factor in this study; therefore, it is difficult to derive the factor affecting the microbial activity in soil.

4.2 Temperature, TOC and pH treatment changed the composition of soil microbiome

Temperature changed the microbiome composition of both Haplic Chernozem and Humic Cambisol control soils, but different trends were noticed for these two soils. Higher temperature shifted the microbiome of the Haplic Chernozem control soil towards Gram-negative bacteria, but towards Gram-positive bacteria in the Humic Cambisol control soil. These divergences in the two controls are difficult to explain and it might be the result of a different content of nutrients, and pH (Sollins et al., 1988). In addition, the PLFAs were analyzed in both soils only on day 39/40, the initial effect on the compositional structure of microbiome under influence of higher temperatures could have been easily overlooked. The microbial activity of Humic Cambisol control was higher than the Haplic Chernozem control; the observed shift towards Gram-positive

bacteria at higher temperatures thus could have been much faster than in the Haplic Chernozem control. Temperature controls microbial turnover processes in general; for example, the turnover rates of microbial biomass and soil organic matter are higher at high temperature (He et al., 2014; Xiong et al., 2014; Zhou et al., 2012).

FYM addition to Haplic Chernozem and Humic Cambisol (enhanced two TOC levels) favored the shift of the microbiome towards Gram-positive bacteria and actinobacteria. Through the FYM addition, we may have introduced new microorganisms to soil, in particular Gram-positive bacteria and actinobacteria (Shahbaz et al., 2020). Similarly, an increased abundance of actinobacteria in a sandy loam soil amended with a carbon-rich biochar was also found by Zhang et al. (2021). FYM contains high molecular weight polymers that have to be cleaved extracellularly prior to uptake by microorganisms (Ma et al., 2018; Ma et al., 2020). Mostly Gram-positive bacteria, actinobacteria and fungi degrade complex polymeric organic substrates extracellularly, rather than Gram-negative bacteria, which usually take up low molecular weight compounds (Lønne Enggrob et al., 2021; Madigan et al., 2018). This may also explain the shift of microbiome towards Gram-positive bacteria in two soils and a higher abundance of fungal and actinobacteria marker at two increased TOC levels of Haplic Chernozem and Humic Cambisol.

Reduction of pH with H_2SO_2 changed the compositional structure of microbiome towards Gram-negative bacteria in Haplic Chernozem. Contrary to Haplic Chernozem with reduced pH, a shift of Humic Cambisol microbiome towards Gram-positive bacteria was observed at pH 5.5, but only at 10°C. The microbial activity of Humic Cambisol was higher than that of the Haplic Chernozem, and we analyzed $PLFAs_{total}$ at endpoints in both experiments. Therefore, we might have overlooked the shift to Gram-negative bacteria in Humic Cambisol which peaked earlier than in Haplic Chernozem. However, a shift of microbiome towards Gram-negative bacteria was also observed in the controls of Haplic Chernozem at higher temperatures. It is thus unclear whether only temperature or pH variation changed the structural composition of microbiome.

4.3 Temperature, TOC or pH variation affected microbial degradation of glyphosate

Temperature was the main factor controlling mineralization of glyphosate in both soils. Microbial activity is usually highest at mesophilic temperatures (Gavrilescu, 2005; Shymko et al., 2011; Singleton, 1994); therefore, glyphosate mineralization increased at higher incubation temperatures in both soils. Although the microbial activity of the Humic Cambisol control was higher than the control Haplic Chernozem, this did not affect the ultimate mineralization of glyphosate. Mineralization of glyphosate in the Haplic Chernozem control soil after 40 days was similar to that in the Humic Cambisol at 10°C after 39 days, whereas at higher temperatures it was even slightly higher. In analogy to mineralization, the incorporation of ^{13}C from $^{13}C_3$-glyphosate into the PLFAs in Haplic Chernozem was higher than in Humic Cambisol. This indicates that the proportion of active microbes that degraded glyphosate was greater in the Haplic Chernozem. Extractable glyphosate in the Haplic Chernozem (Muskus et al., 2019) was lower than in the Humic Cambisol (Muskus et al., 2020) in each treatment and at each temperature. The possible explanations for different mineralization of glyphosate and incorporation of ^{13}C into the PLFAs in the two soils could be different: (I) number or species of glyphosate degraders, (II) availability of carbon (including glyphosate) and nutrients or (III) soil texture and mineralogy (Alexa et al., 2009; Bergström et al., 2011; Borggaard and Gimsing, 2008; De Andréa et al., 2003; Getenga and Kengara, 2004; Nguyen et al., 2018; Okada et al., 2016; Vinther et al., 2008). Noteworthy is that the two soils originated from different climatic regions; Haplic Chernozem was a silt loam soil from Germany, whereas Humic Cambisol was a sandy loam soil from Colombia. The composition of clays, amounts of iron and aluminum as well as cationic exchange capacity (CEC) are different in soils in temperate regions and in tropics (Sanchez-Bayo and Hyne, 2011). The soils in the tropics often have a large amount of kaolinite and gibbsite, whereas montmorillonite and illite make up most of the clay portion of the soils in the temperate zone (Schroeder et al., 2020; Sollins et al., 1988). Furthermore, soils in tropics are richer in iron and aluminum, and have a lower CEC than the soils from moderate climate (Sollins

et al., 1988). All these differences in the soil property may have affected the different availability of glyphosate (and sorption) and other nutrients to microorganisms in the two soils.

Along with the enhanced mineralization of $^{13}C_3$-glyphosate (Muskus et al., 2019, 2020), the ^{13}C-PLFAs at the two increased TOC levels of both soils were higher than in their controls. This is in a good accordance with the higher amounts of ^{13}C-amino acids in the two soils with the elevated TOC levels as compared to controls (Muskus et al., 2019, 2020). A supply of available organic substrates and nutrients from FYM could have stimulated the activity of microorganisms (Aciego Pietri and Brookes, 2008; Binkley and Vitousek, 1989; Kemmitt et al., 2005; Zhao et al., 2011). Previous studies also suggested that glyphosate is rarely utilized as a sole carbon source (Forlani et al., 1999; Sviridov et al., 2012; Zabaloy et al., 2012). Thus the supply of available additional substrates to glyphosate degraders through the addition of FYM could additionally support glyphosate degradation (Andersson et al., 2000; Kemmitt et al., 2006; Rousk et al., 2009; Vereecken, 2005).

It is difficult to explain the lower amounts of ^{13}C-PLFAs in some pH treatments of the Haplic Chernozem and Humic Cambisol. Possible explanations could be different soil mineralogy or availability of carbon and nutrients as well as the temperature (Borggaard and Gimsing, 2008; Nguyen et al., 2018; Vinther et al., 2008).

4.4 Temperature and TOC or pH variation shifted the composition of glyphosate degraders

Addition of FYM to Haplic Chernozem or Humic Cambisol and pH reduction of Haplic Chernozem shifted the ^{13}C-glyphosate degraders towards Gram-positive bacteria. The different levels of TOC and soil pH influenced microbial growth, biomass size and diversity (Fierer and Jackson, 2006a; Lauber et al.. 2009a; Rousk et al.. 2010; Zelles, 1999). Gram-positive bacteria have a unique capability to survive acidic environments (Cotter and Hill, 2003) and to degrade polymeric organic substrates from FYM (Ma et al., 2018; Ma et al., 2020). Furthermore, a

positive correlation between Gram-negative bacteria PLFAs and high soil pH was also found by Grayston et al. (2004).

An increased amounts of the ^{13}C-PLFA marker indicative for starvation of Gram-negative bacteria (Kaur et al., 2005) were found at the two enhanced TOC-levels or reduced pH of both soils. This could suggest that this group of bacteria could have been more abundant at earlier stages of glyphosate degradation in all treatments. The ^{13}C-PLFAs were only measured at the end of experiment; therefore, we might have overlooked the high abundance of this microbial group at the earlier phase of glyphosate degradation. Gram-negative bacteria have been described to use available substrates like glyphosate or other substrates released to soil from FYM or after pH reduction (Bai et al., 2016; Dungait et al., 2013; Lu et al., 2004; Moore-Kucera and Dick, 2008). In later degradation stages, when the nutrients and glyphosate were reduced, the ^{13}C abundance in Gram-positive bacteria marker could have increased. Gram-positive bacteria and actinobacteria are known for turnover of the necromass of primary degraders of various C-substrates (Billings and Ziegler, 2008; Kramer and Gleixner. 2006; Lu et al., 2004; Moore-Kucera and Dick, 2008; Rinnan and Bååth, 2009, Wang et al, 2021;). In addition to actinobacteria and Gram-positive bacteria, fungi also could have contributed to the degradation of necromass of glyphosate primary degraders in both soils. Fungi have a unique ability to decompose polymeric substrates in soil (Fabian et al., 2017; Li et al., 2015). The fungi, Gram-positive bacteria and actinobacteria thus could have enhanced the availability of the decomposing labeled biomass compounds to consumers of primary glyphosate degraders. Therefore, in later stages of glyphosate degradation, ^{13}C label that had been ingested by Gram-negative bacteria could have been recycled through the microbial food web in various catabolic and anabolic pathways by other microbial groups, including Gram-positive bacteria, actinobacteria or fungi (Wang et al, 2021; Zheng et al, 2021).

It is difficult to derive a single driving factor (temperature and TOC or pH variation) leading to the shifts of microbial community and glyphosate degraders from the only endpoint data. The time-dependent study of ^{13}C label distribution between the respective groups of PLFAs under the

influence of temperature and TOC or pH variation could support the identification of the relevant factor affecting these shifts.

4. CONCLUSIONS

Glyphosate addition to soil slightly increased respiration of both the Haplic Chernozem and the Humic Cambisol. FYM amendment and pH reduction enhanced microbial activity and glyphosate degradation. Although the microbial activity of the Haplic Chernozem was lower than that of the Humic Cambisol, the proportion of actively degrading microbes was greater in the Haplic Chernozem. Gram-negative bacteria were presumably the primary degraders of glyphosate as indicated by the high abundance of ^{13}C in the Gram-negative PLFA starvation marker. The Gram-negative bacteria could have been overlooked at the end-point-samplings of the two experiments. More data on the time-dependent contents of ^{13}C-PLFAs and ^{13}C-fatty acids in necromass would be necessary to clarify this.

Further work should concentrate on the detailed investigation of glyphosate degradation in different soil environments and the interaction between the synergistic degraders, e.g. with the help of metagenomics or proteomics. It is still little known whether there are any consequences of the changed proportions of synergistic degraders in the soil microbiome after the addition of glyphosate to soil.

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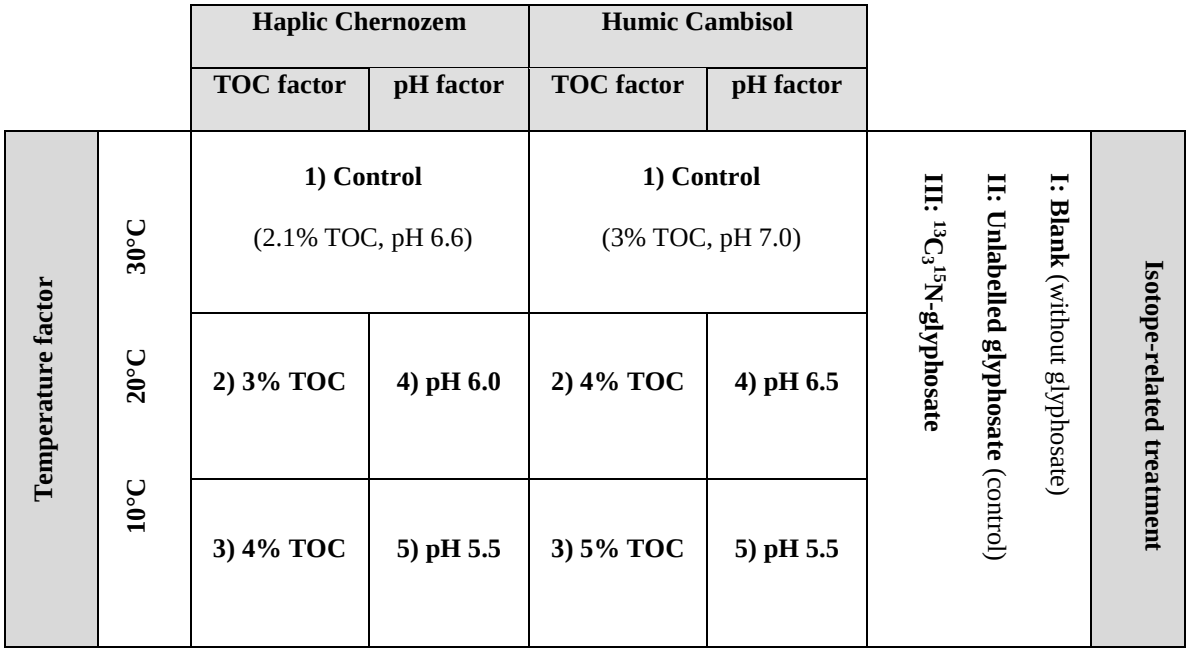

2 **Figure 1**. Experimental setup for Haplic Chernozem and Humic Cambisol with TOC and pH

3 modifications.

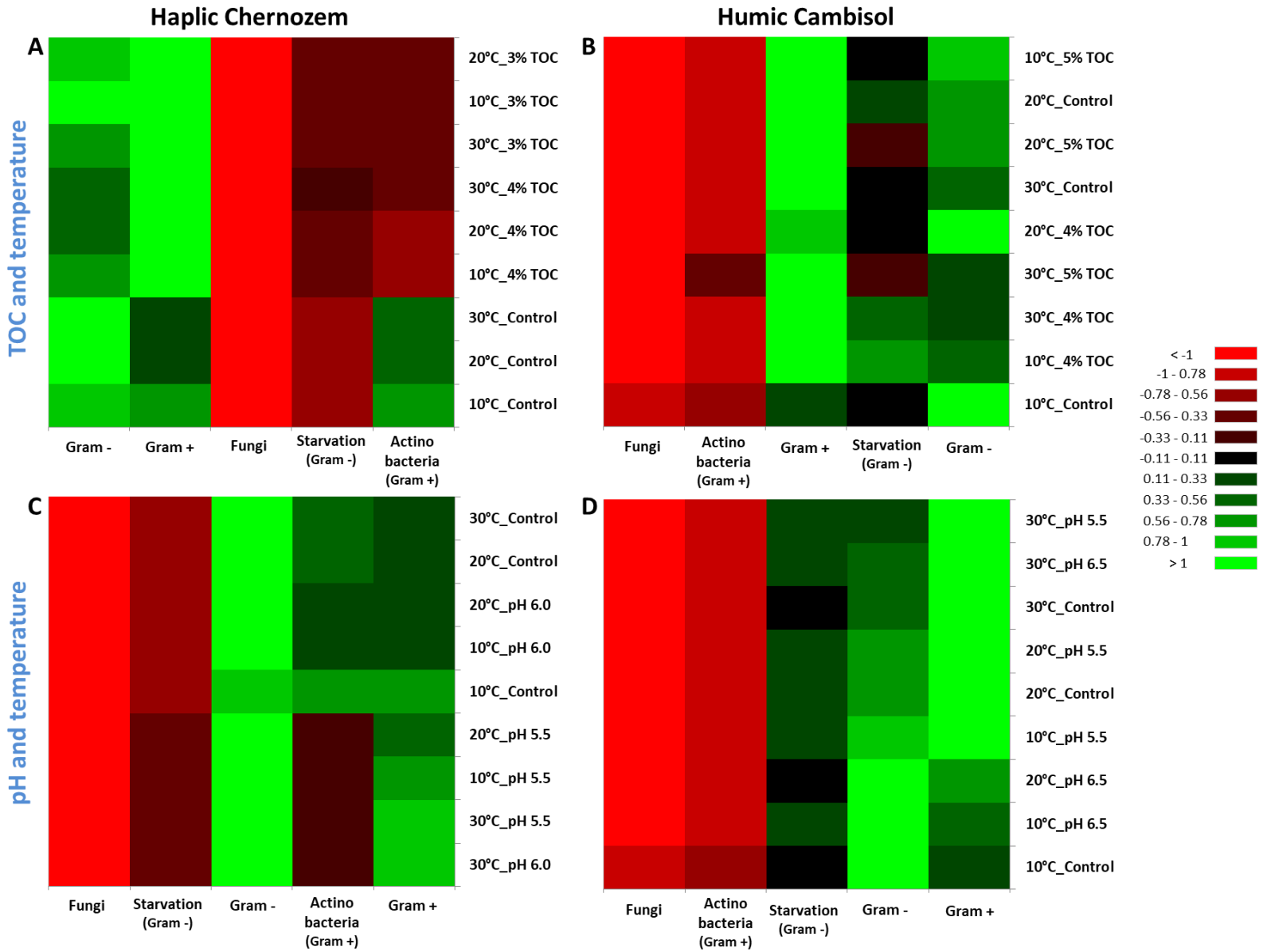

Figure 2. Heat-map of the microbial groups based on $PLFA_{total}$ in a haplic Chernozem and a humic Cambisol soil microbiome with a modified TOC (**A**, **B**) and pH (**C**, **D**). The color scale indicates the relative abundance of five microbial PLFA markers (Gram-positive bacteria, actinobacteria [subgroup of Gram-positive bacteria], Gram-negative bacteria, starvation and fungi) according to PLFAs in the individual treatment. Green represents the most abundant groups, whereas red represents the least abundant group. Control soil: 50 mg kg^{-1} of glyphosate (no modification of pH or TOC).

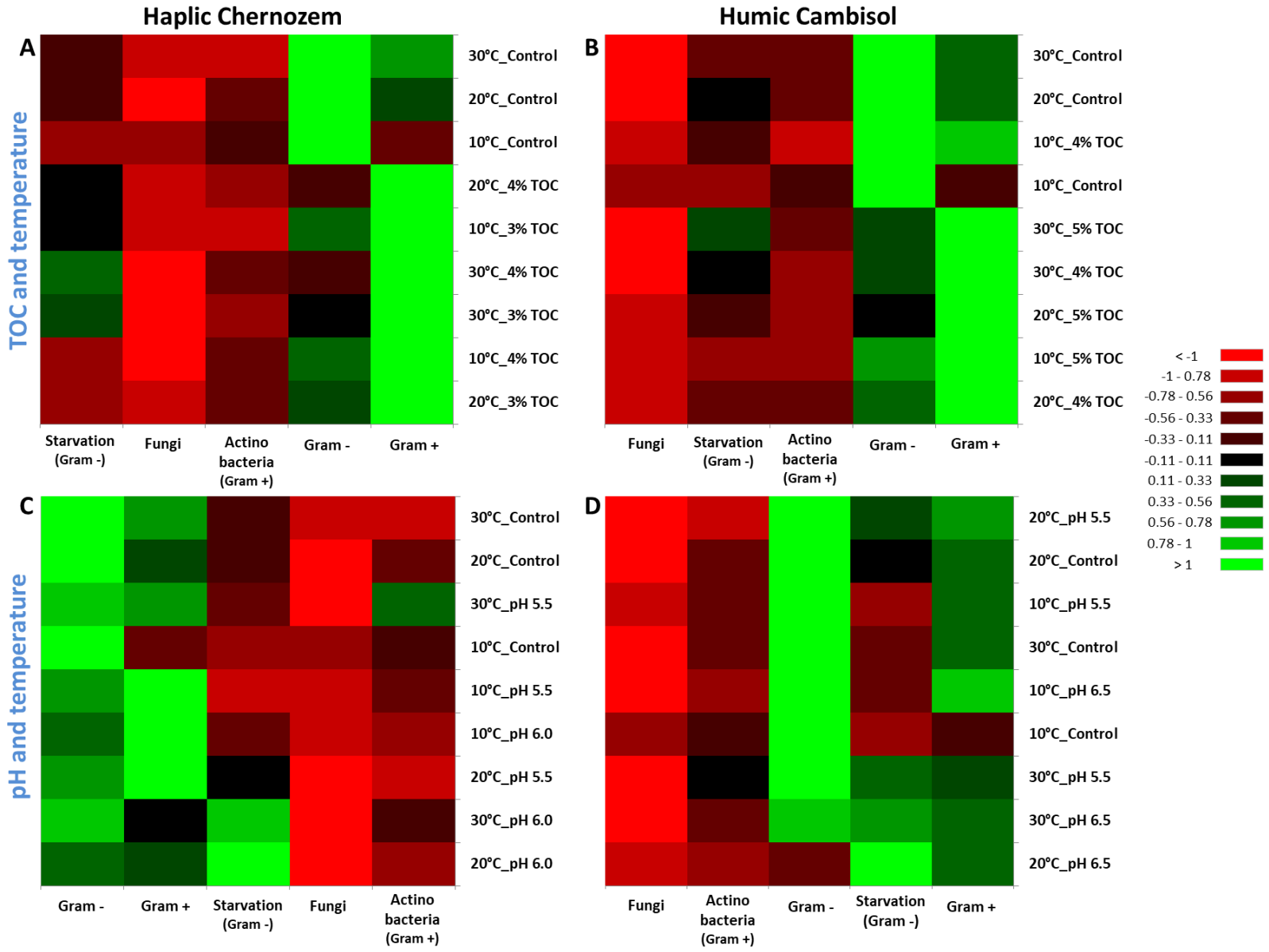

Figure 3. Heat-map of the microbial groups of glyphosate degraders based on ^{13}C-PLFAs in a haplic Chernozem and a humic Cambisol with a modified TOC (**A**, **B**) and pH (**C**, **D**). The color scale indicates the relative abundance of five microbial PLFA markers (Gram-positive bacteria, actinobacteria [subgroup of Gram-positive bacteria], Gram-negative bacteria, starvation and fungi) according to PLFAs in the individual treatment. Green represents the most abundant groups, whereas red represents the least abundant group. Control soil: 50 mg kg^{-1} of glyphosate (no modification of pH or TOC).

Table 1. Groups of microorganisms and representative PLFAs biomarkers (Boschker and Middelburg, 2002; Frostegård and Bååth, 1996; Kaur et al., 2005; Zelles, 1999a, b;).

Group of microorganisms	Representative biomarkers
1) General	14:0, 15:0, 16:0, 18:0, 20:0
2) Gram-positive bacteria	i-14:0, i-15:0, a-15:0, i-16:0, a-16:0, i-17:0, a-17:0
3) Actinobacteria (subgroup of Gram-positive bacteria)	10-Me16:0, 10-Me17:0, 10-Me18:0
4) Gram-negative bacteria	16:1ω7c, 18:1ω9c, 18:1ω7
5) Starvation (Gram-negative bacteria)	cy-17:0, cy-19:0
6) Fungi	18:2ω6,9
7) Others	20:1, 18:2, 15:1 br, 18:0 br, 17:1 br

i: iso, a: anteiso, Me: methyl, cy: cyclopropyl, br: branched, , ω: omega

Table 2. The $PLFAs_{total}$ (µmol CO_2 g^{-1}) in Haplic Chernozem and Humic Cambisol incubated at 10°C, 20°C and 30°C and after 39 or 40 days. Five treatments are shown: control, two TOC and two pH variations. Mean values (n=3) are shown ± standard deviation. Control Haplic Chernozem: 2.1% TOC, pH 6.6. Control Humic Cambisol: 3% TOC, pH 7.0. Both controls: 50 mg kg^{-1} of glyphosate (no modification of pH or TOC).

Haplic Chernozem														
10°C					**20°C**					**30°C**				
Control	**3% TOC**	**4% TOC**	**pH 6.0**	**pH 5.5**	**Control**	**3% TOC**	**4% TOC**	**pH 6.0**	**pH 5.5**	**Control**	**3% TOC**	**4% TOC**	**pH 6.0**	**pH 5.5**
0.02±0.000	0.06±0.002	0.07±0.002	0.03±0.001	0.04±0.001	0.02±0.000	0.07±0.002	0.07±0.002	0.03±0.001	0.03±0.001	0.01±0.000	0.05±0.002	0.06±0.002	0.04±0.001	0.03±0.001
Humic Cambisol														
Control	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**	**Control**	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**	**Control**	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**
0.20±0.001	0.12±0.005	0.19±0.007	0.17±0.008	0.14±0.006	0.16±0.007	0.22±0.009	0.31±0.01	0.18±0.008	0.17±0.007	0.09±0.003	0.21±0.008	0.30±0.01	0.19±0.008	0.12±0.006

Table 3. The ^{13}C-$PLFAs_{total}$ (% of initially added $^{13}C_3$-glyphosate) in Haplic Chernozem and Humic Cambisol incubated at 10°C, 20°C and 30°C and after 40 days. Five treatments are shown: control, two TOC and two pH variations. Mean values (n=3) are shown ± standard deviation. Control Haplic Chernozem: 2.1% TOC, pH 6.6. Control Humic Cambisol: 3% TOC, pH 7.0. Both controls: 50 mg kg^{-1} of glyphosate (no modification of pH or TOC).

[% of initially added $^{13}C_3$-glyphosate equivalents]														
Haplic Chernozem														
10°C					**20°C**					**30°C**				
Control	**3% TOC**	**4% TOC**	**pH 6.0**	**pH 5.5**	**Control**	**3% TOC**	**4% TOC**	**pH 6.0**	**pH 5.5**	**Control**	**3% TOC**	**4% TOC**	**pH 6.0**	**pH 5.5**
0.08±0.02	0.13±0.02	0.14±0.01	0.08±0.01	0.02±0.00	0.10±0.01	0.15±0.02	0.17±0.03	0.06±0.01	0.07±0.01	0.08±0.01	0.21±0.02	0.25±0.03	0.06±0.01	0.07±0.01
Humic Cambisol														
Control	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**	**Control**	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**	**Control**	**4% TOC**	**5% TOC**	**pH 6.5**	**pH 5.5**
0.03±0.01	0.10±0.01	0.11±0.01	0.05±0.00	0.02±0.00	0.04±0.00	0.13±0.01	0.10±0.01	0.01±0.00	0.03±0.00	0.03±0.00	0.09±0.01	0.10±0.01	0.03±0.00	0.03±0.00