



Maize root and shoot litter quality controls short-term CO₂ and N₂O emissions and bacterial community structure of arable soil

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10 **Abstract.** Chemical composition of root and shoot litter controls decomposition and, subsequently, C availability for biological nitrogen transformation processes in soils. While aboveground plant residues have been proven to increase N₂O emissions, studies on root litter effects are scarce. This study aimed 1) to evaluate how fresh maize root litter affects N₂O emissions compared to fresh maize shoot litter, 2) to assess whether N₂O emissions are related to the interaction of C and N mineralization from soil and litter, and 3) to analyze changes in soil microbial community structures related to litter input
15 and N₂O emissions.

To obtain root and shoot litter, Maize plants (*Zea mays* L.) were cultivated with two N fertilizer levels in a greenhouse and harvested. A two-factorial 22-day laboratory incubation experiment was set up with soil from both N levels (N1, N2) and three litter addition treatments (Control, Root, Root+Shoot). We measured hourly CO₂ and N₂O fluxes, analyzed soil nitrate and water extractable organic C (WEOC) concentrations, and determined quality parameters of maize litter. Bacterial
20 community structures were analyzed using 16S rRNA gene sequencing.

Maize litter quality controlled NO₃⁻ and WEOC availability and decomposition related CO₂ emissions. High bioavailability of maize shoot litter strongly increased CO₂ and N₂O emissions, while emissions induced by maize root litter remained low. We identified a strong positive correlation between cumulative CO₂ and N₂O emissions, supporting our hypothesis that litter quality affects denitrification by creating plant litter associated anaerobic microsites. The interdependency of C and N
25 availability was validated by analyses of regression. Moreover, there was a strong positive interaction between soil NO₃⁻ and WEOC concentration resulting in much higher N₂O emissions, when both NO₃⁻ and WEOC were available. A significant correlation was observed between total CO₂ and N₂O emissions, the soil bacterial community composition and the litter level, showing a clear separation of Root+Shoot samples of all remaining samples. Bacterial diversity decreased with higher N level and higher input of easily available C. Altogether, changes in bacterial community structure reflected degradability
30 of maize litter with easily degradable C from maize shoot litter favoring fast growing C cycling and N reducing bacteria of the phyla *Actinobacteria*, *Chloroflexi*, *Firmicutes* and *Proteobacteria*.



1 Introduction

Chemical composition controls decomposition of both roots (Birouste et al., 2012; Redin et al., 2014; Silver and Miya, 2001) and plant litter (Jensen et al., 2005; Kögel-Knabner, 2002; Zhang et al., 2008) and, subsequently, C availability for biological
35 nitrogen transformation processes in soils. When O₂ concentrations are low, denitrifying soil microorganisms may use nitrate (NO₃⁻) as electron acceptor in the respiratory chain to break down organic compounds (Zumft, 1997). This leads to loss of plant available N (Müller and Clough, 2014) and makes soils an important source of the greenhouse gas N₂O (Ciais et al., 2013).

Plant residues have been proven to increase N₂O emissions upon incorporation into soil. When different types of litter were
40 compared, quality parameters of plant residues, such as C:N ratio, lignin:N ratio and chemical composition of structural components explained a large share of variances in N₂O emissions (Baggs et al., 2000; Chen et al., 2013; Millar and Baggs, 2004). Especially in drier soils, denitrification is largely controlled by the supply of readily decomposable organic matter (Azam et al., 2002; Burford and Bremner, 1975; Loecke and Robertson, 2009). Availability of easily degradable C compounds stimulates microbial respiration, limiting O₂ at the microsite level and increasing N₂O emissions (Azam et al.,
45 2002; Chen et al., 2013; Miller et al., 2008). Furthermore, plant litter enhances local anaerobicity by absorbing water from surrounding pores and retaining high moisture concentrations (Kravchenko et al., 2017, 2018).

While effects of aboveground plant residues on N₂O emissions have been studied extensively, studies of root residues on N₂O emissions are scarce. In a temperate forest soil, fine root litter of maize and native tree species did not cause any N₂O emissions, but a very close interrelation between C mineralization of fine root litter and N₂O emission was found in other
50 biomes (Hu et al., 2016). In other studies, lower cumulative N₂O emissions were reported after addition of sugar beet roots compared to leaves (Velthof et al., 2002) and rice roots compared to rice straw (Lou et al., 2007). Furthermore, decomposition dynamics of roots have been studied in great detail, revealing that chemical composition explains most of its variation (Birouste et al., 2012; Johnson et al., 2007; Machinet et al., 2011; Redin et al., 2014; Silver and Miya, 2001; Zhang and Wang, 2015). In general, decomposition rates of hemicelluloses and pectin are higher than that of cellulose, while
55 among cell wall components lignin is most resistant against microbial decomposition (Kögel-Knabner, 2002).

Soil microorganisms are often specialized in specific substrates with fungi being regarded as the main decomposers of plant materials rich in cellulose and lignin, while hemicelluloses and pectin are decomposed by many aerobic and anaerobic bacteria and fungi (Kögel-Knabner, 2002). While the phyla *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* are described as fast growing copiotrophic bacteria that are stimulated by input of easily degradable C compounds (Fierer et al., 2016; Pascault et al., 2013), abundance of *Acidobacteria* decreased following the addition of dissolved organic matter into the soil
60 (Fierer et al., 2016). Similarly, denitrifying microorganisms are found in bacteria, fungi and archaea depending on substrate availability and environmental conditions (Zumft, 1997). Fungi are seen as major contributors to denitrification under aerobic and weakly anaerobic conditions, while bacterial denitrification predominates under strongly anaerobic conditions (Hayatsu et al., 2008). Denitrifying bacteria can be found in most phyla (Zumft, 1997), with dominant populations in



65 *Pseudomonas* and *Alcaligenes* (Gamble et al., 1977; Megonigal et al., 2013). The most abundant denitrifying bacteria in soil are heterotrophic, and, as such, require a source of electrons or reducing equivalents contained in C compounds of organic matter or plant residues. Availability of organic C may thus affect both decomposing and denitrifying soil microorganisms. In most reported studies on decomposition and N₂O emissions, dried and often ground plant material was used. This facilitates a homogenous distribution in soil and minimizes differences between replicates. Nevertheless, drying of fine roots prior to incubation increased their decomposition rate and led to overestimation of decomposition and nutrient cycling rates (Ludovici and Kress, 2006). Additionally, formation of plant litter associated anaerobic hotspots was reduced when ground plant material was homogeneously mixed with the soil, while litter aggregation significantly increased soil N₂O emissions (Loecke and Robertson, 2009). Differences in N₂O emissions between two clover species were observed only with intact (but dried) leaves, but not when ground material was used (Kravchenko et al., 2018).

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75 The aim of this study was 1) to evaluate how fresh maize root litter affects N₂O emissions compared to fresh maize shoot litter, 2) to assess to what extent N₂O emissions are related to the interaction of C and N mineralization from soil and litter, and 3) to analyze the changes in soil microbial community structures related to litter input and N₂O emissions. We hypothesize that easily degradable C compounds stimulate microbial respiration in plant litter associated hotspots leading to high N₂O formation when both C and N availability is high. We further expect that differences in litter chemical quality are reflected in the structural composition of the soil microbial community.

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Maize plants were grown in a greenhouse to produce root and shoot litter. To realize differences in soil N_{min} concentration at harvest, two N fertilizer regimes (low vs. high) were applied. We then set up a laboratory incubation experiment with fresh maize root or root and shoot litter under fully controlled conditions and determined hourly CO₂ and N₂O fluxes for 22 days. Soil samples were taken in regular intervals and analyzed for soil mineral N and water-extractable organic C (WEOC) concentrations. At the end of the incubation experiment, soil microbial community structures were analyzed to identify adaptations to litter input.

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2 Material and Methods

2.1 Preparation of plants and soils prior to incubation experiment

The soil for the experiment was collected 10 km south of Göttingen, Germany at the experimental farm Reinshof of the University of Göttingen (51.484°N, 9.923°E). Soil was classified as Gleyic Fluvisol (21 % clay, 68 % silt, 11 % sand) containing 1.5 % C and 2.81 % humus, with a pH (CaCl₂) = 7.44.

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Prior to the incubation experiment, maize plants were cultivated to obtain shoot and root biomass. For maize cultivation, Mitscherlich pots were filled with 5 kg air dried and sieved (2 mm) soil previously mixed with fertilizers (0.2 g N kg⁻¹ as NH₄NO₃, 0.14 g P kg⁻¹ as Ca(H₂PO₄)₂, 0.2 g K kg⁻¹ as K₂SO₄ and 0.04 g Mg kg⁻¹ as MgSO₄ * 7 H₂O including 0.135 g S kg⁻¹). Soil moisture was adjusted to 25 Vol. % and volumetric water content (VWC) sensors (EC-5, Decagon Devices, Pullman, USA) were used to monitor soil water content. Six maize plants (*Zea mays* L. var. Ronaldinio) were sown

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per pot and cultivated in a greenhouse with 16 h light and 8 h dark cycles. Pots were randomized in regular intervals to avoid microclimatic effects in the greenhouse.

To get different soil mineral N concentrations in soil, a second N fertilizer dose (0.2 g N kg^{-1} as $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{ H}_2\text{O}$) was applied to half of the pots six weeks after sowing. Soil with one N dose is referred to as N1 (0.2 g N kg^{-1}) and soil with two N doses is referred to as N2 ($2 \times 0.2 \text{ g N kg}^{-1}$). Plants were harvested 8 weeks after sowing: Maize plants were cut above the soil surface and roots were removed from soil by sieving and handpicking. Fresh roots were shaken and slightly brushed to remove adhering soil.

A subsample of aboveground maize biomass and maize roots was dried at 60°C to determine dry matter contents. To determine water-extractable C and N concentrations, subsamples were extracted with $\text{H}_2\text{O}_{\text{bidest}}$ (maize root 1:1000 w/v, maize shoot 1:10000 w/v) for 16h and analyzed using a multi N/C® Analyzer (Model 3100, Analytik Jena, Jena, Germany). Another subsample was analyzed for the sum of structural components following established feedstuff analysis protocols based on the method proposed by Goering and Van Soest (1970), namely ash free neutral detergent fiber aNDFom, (VDLUFA, 2012a) acid detergent fiber ADFom, (VDLUFA, 2011) and acid detergent lignin ADL, (VDLUFA, 2012b). According to the definitions, hemicellulose, cellulose and lignin contents were calculated as following: Hemicellulose = aNDFom - ADFom; Cellulose = ADFom - ADL; Lignin = ADL. Another subsample was milled using a ball mill and total carbon and nitrogen concentrations were analyzed using a C/N analyzer (Model 1110, Carlo Erba, Milano, Italy).

2.2 Incubation experiment

For the incubation experiment, shoot litter was taken from N2 plants only, and cut to a size of 2 cm. Roots of all plants (N1+N2) were mixed and also cut to 2 cm. Within each N level, all soil was mixed to ensure homogeneous initial conditions. Subsamples of both soils were taken for analysis of mineral N, water extractable C_{org} concentration, and total soil C. C remaining from rhizodeposition, root hairs and small root fragments was calculated as the difference in soil C concentration before and after maize growth. The incubation experiment consisted of a two-factorial setup comprising two N levels (N1 and N2) and three litter levels (Control = Cn, Root = Rt, Root+Shoot = RS) (see Table 1 and Figure 1 for details). Each treatment was replicated four times. Soil mineral N concentrations were 0.93 and $1.97 \text{ mg N kg}^{-1}$ for N1 and N2, respectively. Control soils (N1-Cn and N2-Cn) did not receive plant biomass, yet they contained C input from rhizodeposition of the previous maize growth. For the root treatment, 100 g fresh root biomass was added per kg dry soil (N1-Rt and N2-Rt), and in the root and shoot treatment, 100 g fresh root and 100 g fresh shoot biomass was added per kg dry soil (N1-RS, N2-RS). Plant litter was homogeneously mixed with the soil, simulating residue incorporation and tillage. PVC pots with a diameter of 20 cm and a total volume of 6.8 L were filled with fresh soil equivalent to 3.5 kg dry weight previously mixed with plant litter. Soil was compacted in a stepwise mode by filling a 2cm -layer of soil in pots and compacting it with a plunger. To ensure continuity between soil layers, the surface of the compacted layer was gently



scratched before adding the next soil layer. Due to high litter input, target bulk density was 1.1 g cm^{-3} . Actual bulk density was determined by measuring headspace height, and these values were used for calculations.

To adjust soil moisture of all pots to 70% WHC, equivalent to 49% WFPS, water was dripped on the soil surface through hollow needles (outer diameter 0.9 mm). Pots were covered with lids to minimize evaporation from the soil surface. The incubation experiment was carried out under controlled temperature and light conditions (16 h day at 25°C , 8 h night at 19°C) for 22 days. Volumetric water content (VWC) sensors (EC-5, Decagon Devices, Pullman, USA) were used to monitor soil water content.

2.3 Gas sampling and analysis

Gas fluxes were measured using the closed chamber method (Hutchinson and Mosier, 1981). Gas samples were taken every 12 hours (morning and evening) for the first 15 days and every 24 hours (midday) for the remaining 7 days. Due to technical issues, gas samples taken in the morning of day 10 to day 15 had to be discarded. Before gas sampling, all pots were opened for ventilation to ensure homogenous ambient air background conditions. Pots were closed with gastight PVC lids and 30 ml gas samples were taken from each pot 0, 20, and 40 minutes after closure and filled into pre-evacuated 12 ml Exetainer glass bottles (Labco, High Wycombe, UK). Samples were analyzed on a Bruker gas chromatograph (456-GC, Bruker, Billerica, USA) deploying an electron capture detector (ECD) for N_2O and a thermal conductivity detector (TCD) for CO_2 . Samples were introduced using a Gilson Autosampler (Gilson Inc., Middleton, WI, USA). Data processing was performed using CompassCDS software. The analytical precision was determined by repeated measurements of standard gases (2500 and 550 ppm CO_2 , 307, 760, and 6110 ppm N_2O) and was consistently $< 2 \%$.

2.4 Soil analyses

Soil samples were taken from the pots using a soil auger of 16 mm diameter on 5, 9, 14 and 22 DAO (days after onset of experiment). Holes were closed with glass tubes to avoid variation in the soil surface. Fresh subsamples were analyzed for water extractable C_{org} concentration (WEOC), and a subsample was frozen at -20°C for soil mineral N analysis.

Total soil carbon and nitrogen concentrations were analyzed using a C/N analyzer (Model 1110, Carlo Erba, Milano, Italy). For determination of soil mineral N content, frozen samples were extracted with a 0.0125 M CaCl_2 solution (1:5 w/v) for 60 min on an overhead shaker (85 rpm). The extracts were filtered with 615 $\frac{1}{4}$ filter paper (Macherey - Nagel GmbH & Co. KG, Düren, Germany) and stored at -20°C . The extracts were analyzed colorimetrically for the concentrations of NO_3^- and NH_4^+ using the San++Continuous-Flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands). Soil water content was determined with a parallel set of samples. Net N mineralization was calculated as the difference between the $\text{NH}_4^+-\text{N} + \text{NO}_3^-$ N concentrations at the start and end of the incubation period plus N lost as N_2O (Eq. 1).

$$\text{Net mineralization} = (\text{NO}_3^- + \text{NH}_4^+)_{\text{end}} - (\text{NO}_3^- + \text{NH}_4^+)_{\text{start}} + \text{N}_2\text{O} - \text{N} \quad (1)$$



WEOC was determined according to Chantigny et al. (2007). Briefly, fresh soil was homogenized with deionized water (1:2 w/v), samples were centrifuged and filtered with 0.45 µm polyether sulfone syringe filters (Labso-
lute, Renningen, Germany) and stored at -20°C. The extracts were analyzed using a multi N/C® Analyzer (Analytik Jena, Jena, Germany).

2.5 Analysis of bacterial community structures

2.5.1 DNA isolation and 16S rRNA gene amplification

To analyze the soil inhabiting bacterial communities, DNA was extracted from 0.5 g (fresh weight) soil sample taken at the end of the incubation experiment (22 DAO) using the DNA extraction protocol described by Griffiths et al. (2000). Plant litter was removed from samples prior to extraction. In brief, cells were mechanically disrupted using bead beating and nucleic acids were extracted using phenol:chloroform:isoamyl alcohol (25:24:1; Carl Roth, Karlsruhe, Germany). Nucleic acids were then precipitated using polyethylene glycol (Carl Roth, Karlsruhe, Germany) and washed with 70% ice-cold ethanol (VWR, Radnor, Pennsylvania, USA). Subsequently, RNA was removed by RNase A digestion (Thermo Fischer Scientific, Waltham, Massachusetts, USA) as described by the manufacturer. The RNA-free DNA was used for amplification of the V3 to V4 region of the 16S rRNA gene. We used the bacterial primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 targeting the V3-V4 region of the 16S rRNA gene described by Klindworth et al. (2013) with adapters for Illumina MiSeq sequencing. The PCR reaction mixture contained 5-fold Phusion GC buffer, 200 µM of each of the four deoxynucleoside triphosphates, 5% DMSO, 0.4 µM of each primer, 1 U of Phusion HF DNA polymerase (Fisher Scientific GmbH, Schwerte, Germany), and 25 ng of RNA-free DNA as template. The following cycling scheme was used for DNA amplification: initial denaturation at 98 °C for 5 min and 25 cycles of denaturation at 98 °C for 45 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. For each sample, PCR reactions were performed in triplicate. Resulting PCR products were pooled in equimolar amounts and purified using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. Quantification of the PCR products was performed using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer as described by the manufacturer (Invitrogen GmbH, Karlsruhe, Germany). Indexing of the PCR products was performed by the Göttingen Genomics Lab (G2L, Göttingen, Germany) using the Nextera XT Index kit as recommended by the supplier (Illumina, San Diego, CA, USA) and sequencing of 16S rRNA amplicons was performed using the dual index paired-end approach (2 × 300 bp) with v3 chemistry for the Illumina MiSeq platform.

2.5.2 Sequence processing

All bioinformatic processing of sequence data was done using Linux based software packages. Adapter removal and quality filtering of raw paired-end sequences was done using fastp v0.19.6 (Chen et al., 2018), with base correction in overlapped regions, a qualified quality phred of 20, size exclusion of sequences shorter than 50 bp and per read trimming by quality (phred 20). Merging of quality filtered paired-end reads was done by PEAR v0.9.11 (64 bit) with default parameters (Zhang



et al., 2014). Primer removal was conducted using cutadapt v1.18 (Martin, 2013). Subsequently, dereplication, denoising, as well as chimera detection and removal (denovo followed by reference based against the SILVA 132 SSU database), was performed with VSEARCH v2.13.0 (64 bit) (Rognes et al., 2016). Taxonomic classification of the amplicons sequence variants (ASVs, 100% sequence identity) was performed with BLAST+ v2.7.1 against the SILVA 132 SSU reference database (Quast et al., 2013). Subsequently, extrinsic domain ASVs and chloroplasts were removed from the dataset. Sample comparisons were performed at the same surveying effort of 50.000 sequences. Statistical analyses were done using ASVs in R version 3.5.3 (R Core Team, 2019). All graphs were prepared using the R package *ampvis2* v2.4.7 (Andersen et al., 2018). Species richness, alpha diversity estimates, and rarefaction curves were determined using *ampvis2* v2.4.7 as well. To visualize the multivariate constrained dispersion Canonical Correspondence Analysis (CCA) was conducted with Hellinger transformed data (Legendre and Gallagher, 2001), ASV's with a relative abundance lower than 0.1% in any sample were removed. Correlations of environmental parameters to the bacterial communities were analyzed using the *envfit* function of the *vegan* package v2.5-4 (Oksanen et al., 2015) and projected into the ordination with arrows with a p-value cutoff of 0.005. For further statistical analysis of the microbial community composition (on phyla, order and genus level) and diversity (Shannon, Simpson and PD index) multivariate generalized linear models (MGLM; with N level and litter addition as factors) as implemented in the *mvabund* R package v4.0.1 were employed with adjusted p-values (Wang et al., 2019). For the generalized linear model analysis of variance (MGLM-ANOVA) tests, p-values < 0.05 were considered to be significant. In addition, we attempted to analyze the soil inhabiting fungal community using the fungal specific primer set ITS3_KYO2 and ITS4 (Toju et al., 2012), but were not able to amplify them.

2.6 Calculations and statistical analyses

All statistical analyses were performed using the statistical software R version 3.5.2 (R Core Team, 2018). Arithmetic means and standard error of the four replicates were calculated for hourly CO₂ and N₂O fluxes. Cumulative gas emissions were calculated by linear interpolation between measured fluxes. To account for different C input in treatments, cumulative CO₂ and N₂O emissions were standardized against the C input per treatment (see Table 1 for details on C input). Tukey's HSD test was used after analysis of variance to test for treatment effects (i.e., N level and litter addition) on cumulative CO₂ emissions. An interaction was identified between N level and litter addition on cumulative N₂O emissions using interaction plots from the package *HH* v3.1-35 (Heiberger, 2018). A linear model using generalized least squares (gls) was fitted between cumulative N₂O as response variable and N level, litter addition, and their interaction as fixed effects. Additionally, the model was fitted to account for inhomogeneous within-class variances. Estimated marginal means were then computed to analyze treatment effects using the R package *emmeans* v1.3.4 (Lenth, 2018). Several regression models were tested to analyze the effect of maize litter on cumulative N₂O emissions including the factors cumulative CO₂ emissions, initial soil NO₃⁻ concentration, and net N mineralization during incubation period. For cumulative CO₂ emissions, regression models



included the factors total C input from litter, water-extractable C input from litter, hemicellulose fraction from litter input, cellulose fraction from litter input, and lignin fraction from litter input.

To evaluate effects of soil environmental variables on hourly N_2O and CO_2 fluxes, a linear mixed effect model (lme) was fitted between N_2O fluxes (ln transformed), soil NO_3^- -N and WEOC concentrations using the *lme* function from the package *nlme* v3.1-131 (Pinheiro et al., 2017). Pseudo- R^2 for lme was calculated using *r.squaredGLMM* from the package *MuMIn* v1.42.1 (Barton, 2018). Soil NO_3^- -N and WEOC concentrations between sampling dates were estimated by linear interpolation. Only evening/midday gas measurements were included in model calculations. To account for repeated measurements, incubation vessel and sampling day were set as random effects. Models were compared using maximum likelihood (ML), selected using AIC (Akaike's information criterion), and fitted using restricted maximum likelihood (REML).

All plots were made using the statistical software R version 3.5.2 (R Core Team, 2018) including the packages *plotrix* v3.7.4 (Lemon, 2006), *plot3D* v1.1.1 (Soetaert, 2017), and *viridisLite* v0.3.0 (Garnier, 2018).

3 Results

3.1 Chemical analyses of maize litter

Maize root and shoot litter differed in their chemical compositions (Table 2). Dry matter content of maize roots was much higher compared to shoot as roots had not been washed prior to analyses, so some soil adhering to roots was included in dry matter determinations. Thus, we calculated water-extractable concentrations in relation to total C instead of dry matter. Maize shoot litter was characterized by higher concentrations of water soluble C and N, and a higher share of easily degradable compounds like hemicellulose and cellulose compared to maize roots.

3.2 Hourly and cumulative CO_2 and N_2O fluxes

Addition of maize litter increased hourly CO_2 fluxes compared to Control (Fig. 2), where addition of root and shoot litter (N1-RS, N2-RS) resulted in much higher fluxes compared to roots only (N1-Rt, N2-Rt). While absolute emission rates were strongly affected by litter input, time courses were similar in all litter treatments without visible differences between N1 and N2. CO_2 fluxes stayed on a similar level for the first ten days after onset of incubation showing fluctuations between morning and evening sampling times, and then constantly decreased until the end of the experiment.

After a short lag phase right after onset of experiment, hourly N_2O emissions increased in all litter treatments compared to control treatments (Fig. 3 a+b). Highest hourly fluxes were measured in N2-RS, reaching $28 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$ on day 5. Fluxes stayed on a similar level from day 7 to day 15, and declined until the end of the experiment. Hourly N_2O fluxes from root (N1-Rt, N2-Rt) and control treatments (N1-Cn, N2-Cn) remained on a low level during the whole incubation period ($\leq 1.11 \mu\text{g}$ and $\leq 0.1 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$, for -Rt and -Cn respectively). N_2O fluxes from N1 were slightly lower than from N2 in



both litter treatments. Over all treatments and sampling dates, hourly CO_2 and N_2O fluxes were positively correlated ($R^2=0.5993$, $p<0.001$, data not shown).

To account for different C inputs in treatments, cumulative CO_2 and N_2O emissions were standardized against the C input per treatment (Table 1). Still, cumulative CO_2 emissions were almost twice as high in -Rt and about four times higher in -RS compared to -Cn, indicating that differences between litter treatments cannot simply be explained by differences in C input. Addition of maize root and shoot litter increased cumulative N_2O emissions by roughly 100-times compared to control treatments. In contrast, root litter increased cumulative N_2O emissions only by a factor of 5.4 (N1-Rt) and 7 (N2-Rt) compared to the respective controls.

3.3 Soil NO_3^- , NH_4^+ and water-extractable Corg concentrations

Addition of maize litter affected the time course of soil NO_3^- , NH_4^+ and WEOC concentrations (Fig. 4 a-c). In control treatments, initial soil NO_3^- concentrations of 0.93 (N1-Cn) and 1.97 mg $\text{NO}_3^- \text{N kg}^{-1}$ dry soil (N2-Cn) continuously increased until the end of the experiment reaching concentrations of 8.24 mg N kg^{-1} (N1-Cn) and 11.74 mg N kg^{-1} (N2-Cn) respectively. Soil NH_4^+ concentrations showed variations on a low level only. Soil NO_3^- concentrations were continuously higher in N2 than in N1 and differences in soil NH_4^+ concentration were small. Higher fertilization in N2 during previous plant growth led to higher residual organic N and higher net N mineralization (7.61 and 10.08 mg N kg^{-1} for N1-Cn and N2-Cn, respectively) during the incubation experiment. In treatments with litter, soil NO_3^- concentrations decreased after an initial increase. In root treatments, soil NO_3^- concentrations continuously decreased until the end of the incubation experiment to 1.9 (N1-Rt) and 2.5 mg N kg^{-1} (N2-Rt), while in root plus shoot treatments soil NO_3^- concentrations increased again until the end of the experiment, reaching concentrations of 9.46 (N1-RS) and 9.52 mg N kg^{-1} (N2-RS). During the whole incubation period, soil NO_3^- concentrations in -RS were higher than in -Rt. Soil NH_4^+ concentrations only marginally increased for -Rt. Contrary to -Rt and -Cn, soil NH_4^+ concentrations increased until the end of the incubation experiment to 1.68 (N1-RS) and 1.52 mg N kg^{-1} (N2-RS) in root and shoot treatments. Net N mineralization was 1.44 (N1-Rt) and 1.10 mg N kg^{-1} (N2-Rt) in root treatments, and 14.32 (N1-RS) and 14.14 mg N kg^{-1} (N2-RS) in root and shoot treatments. Maize root litter did not affect WEOC, as concentrations were similar to Control throughout the incubation period. However in -RS treatments, WEOC increased after onset of incubation, reaching highest values (45.32 mg C kg^{-1}) for N1-RS at day 9, after which they decreased until the end of the experiment.

3.4 Relations between N_2O emissions and C and N parameters of plant litter and soil

To identify the effect of N and C availability on hourly N_2O fluxes, a linear mixed effect model was applied. The best model included a significant interaction between soil NO_3^- and WEOC ($p<0.0394$, Pseudo- $R^2=0.8888$, Table 4), and incubation vessel and sampling time as random parameters. Predictions of N_2O fluxes based on this model are shown in (Fig. 5).

Linear regression analyses were used to identify relations between cumulative CO_2 and N_2O emissions, litter quality and N parameters. Either hemicellulose + cellulose fraction or water-extractable C fraction of plant litter explained more than 96%



of variance of total cumulative CO₂ emissions ($p < 2.2 \times 10^{-16}$) (Table 5). Regression analyses of the relationships between total
285 cumulative N₂O emissions and influencing factors identified a strong positive relationship between total cumulative N₂O
emissions and total cumulative CO₂ emissions ($R^2 = 0.9362$, $p < 7.632 \times 10^{-15}$) (Table 6), and between cumulative N₂O emissions
and mineralized N ($R^2 = 0.5791$, $p < 9.551 \times 10^{-6}$), while initial soil NO₃⁻ concentration did not explain any variance.

3.5 Bacterial community structure

The comparison over all maize litter treatments revealed that the bacterial diversity was slightly higher in N1 than in N2 soil
290 as shown by a higher number of amplicon sequence variants (ASVs, $R^2 = 0.1195$, $p = 0.059$, Fig. S1). In addition, the alpha
diversity indices Shannon ($R^2 = 0.1844$, $p = 0.023$) and Simpson ($R^2 = 0.1131$, $p = 0.065$), as well as Faith's phylogenetic
diversity (PD; $R^2 = 0.1844$, $p = 0.059$) were higher for N1 than for N2 samples (Table S4).

The canonical correspondence analysis revealed a significant correlation ($p < 0.001$) of the bacterial community composition
with total CO₂ ($R^2 = 0.6758$) and N₂O ($R^2 = 0.6179$) emissions, and the litter level, expressed by a clear separation of the N1-
295 RS and N2-RS samples of all other samples (Fig. 6). With increasing C input, N2 samples cluster more closely than N1
samples. No significant correlation of litter level and microbial diversity was observed, PD index increased in N1 samples
with increasing C input, while the opposite was found for N2 samples. Comparison of N1-C and N1-RS revealed no
difference in diversity indices (Shannon and Simpson), while N1-R showed lower Shannon and Simpson diversity indices
(Table S4). The Shannon diversity index was lowest in N2-R comparing all N2 treatments, while the Simpson index was
300 lowest for N2-RS.

Overall, the soil bacterial communities were dominated by *Actinobacteria*, *Proteobacteria* and *Chloroflexi* accounting for 15
to 31% (Fig. S2). The highest relative abundance of *Actinobacteria* and *Chloroflexi* was found in N2-R and of
Proteobacteria in N1-R. Among these phyla, the order *Gaiellales* (*Actinobacteria*), *Sphingomonadales* (*Proteobacteria*) and
Thermomicrobiales (*Chloroflexi*) showed the highest relative abundance, especially in N2-R (9.3%), N1-R (7.5%) and N2-
305 RS (9%), respectively. Nevertheless, the phyla *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Gemmatimonadetes*,
Firmicutes, *Patescibacteria* and *Bacteroidetes* were also detected ($>1\%$) (Fig. 7). In detail, *Bacteroidetes* and
Gemmatimonadetes decreased (with a negative slope, but not significant) with increasing N level, while the abundance of
Firmicutes increased significantly ($p = 0.038$). In addition, although present only in low relative abundance, the
Cyanobacteria decreased significantly ($p = 0.003$) with increasing N levels. At the genus level, *Pseudomonas*,
310 *Altererythrobacter*, *Gaiella*, *Nocardioides*, *Agromyces*, *Bacillus*, and *Lysobacter* were most abundant accounting for up to
5.7 % of all ASVs. The genera *Bacillus*, *Gaiella*, *Altererythrobacter*, *Blastococcus*, and *Pseudomonas* showed highest
abundance in N2 samples, while *Lysobacter*, and *Sphingomonas* were more abundant in N1 samples (Fig. S3). The most
abundant classified species found were *Agromyces* sp., *Bacillus* sp. and *Sphingomonas* sp. Nevertheless, species such as
Pseudomonas sp., *Nitrosospira* sp., *Nitrosospira briensis*, *Alcaligenes* sp. and *Mesorhizobium* sp. were also identified.
315 Overall, the bacterial community composition was significantly influenced by N-level ($p = 0.005$) and maize litter treatment
($p = 0.033$).



4 Discussion

4.1 Decomposability of maize litter

Maize root and shoot litter quality controlled NO_3^- and WEOC availability and decomposition related CO_2 emissions during the initial phase of maize litter decomposition. Harvest of plants, removal of roots and mixing of soil fostered mineralization and nitrification, as reflected by gradually increasing soil NO_3^- concentrations. The absence of changes in soil NH_4^+ concentrations in control treatments without litter addition (N1-Cn, N2-Cn) indicate that all NH_4^+ was directly nitrified. Also in controls, available C was low as indicated by low CO_2 emissions and decreasing WEOC concentrations.

The potential for mineralization in soil is known to be high after tillage (Höper, 2002) and positive net mineralization has been reported in control soil without litter addition (Machinet et al., 2009; Velthof et al., 2002), and in the fallow period after rice harvest (Aulakh et al., 2001).

Maize shoot litter was characterized by a high share of easily degradable compounds. High percentages of water-soluble N and water-soluble C_{org} from maize shoot litter strongly increased soil WEOC and NO_3^- concentrations. Availability of easily degradable compounds was also reflected by strongly increased hourly and cumulative CO_2 fluxes from N1-RS and N2-RS. While net mineralization in -RS was similar to -Cn, it was very small in -Rt indicating that N from mineralization was immobilized by soil microorganisms to decompose root C compounds (Robertson and Groffman, 2015). Cumulative CO_2 emissions in litter treatments were clearly higher than in control, but hourly CO_2 fluxes continuously decreased after onset of incubation, as easily degradable C was consumed. This is in accordance with results of Hu et al. (2016), who reported that maize fine root input initially increased CO_2 fluxes, which then decreased during the first 20 days of incubation.

Mineralization of plant litter may increase soil NO_3^- concentrations especially when C:N ratios are low (Li et al., 2013; Millar and Baggs, 2004). However, net N immobilization has been reported after addition of roots of maize (Machinet et al., 2009; Mary et al., 1993; Velthof et al., 2002), wheat (Jin et al., 2008; Velthof et al., 2002), barley and sugar beet (Velthof et al., 2002), reaching a maximum around day 21 (Mary et al., 1993). Chemical composition has been proven to be the primary controller of decomposition rates of both roots (Birouste et al., 2012; Redin et al., 2014; Silver and Miya, 2001) and aboveground plant litter (Jensen et al., 2005; Zhang et al., 2008) of many different species. Slower decomposition of roots compared to leaves and stems was related to differences in chemical composition of plant organs (Jenkinson, 1965; Johnson et al., 2007). Accordingly, decomposition of roots from 16 maize genotypes was controlled by soluble residue components in the short term whereas lignin and the interconnections between cell wall polymers were important in the long-term (Machinet et al., 2011). In our study, regression analyses identified a strong positive relationship between cumulative CO_2 emissions and water-extractable C fraction of plant litter ($R^2=0.966$, $p<2.2\cdot10^{-16}$) (Table 5).

4.2 N_2O emissions as affected by biodegradability of maize litter and soil N level

Denitrification in soil is largely controlled by the supply of readily decomposable organic matter (Azam et al., 2002; Burford and Bremner, 1975; Loecke and Robertson, 2009). Availability of easily degradable C compounds stimulates microbial



respiration, limiting O₂ at the microsite level and increasing N₂O emissions (Azam et al., 2002; Chen et al., 2013; Miller et al., 2008) leading to significant correlations between hourly and cumulative N₂O and CO₂ emissions (Azam et al., 2002; Fiedler et al., 2017; Frimpong and Baggs, 2010; Huang et al., 2004; Millar and Baggs, 2004, 2005). We identified a strong positive correlation ($R^2=0.9362$, $p \leq 7.632 \times 10^{-15}$) between cumulative CO₂ and N₂O emissions (Table 6), supporting our hypothesis that litter quality, especially quality of C compounds, affects N₂O fluxes from denitrification by creating plant litter associated microsites with low O₂ concentrations.

High mineralization in -RS treatments may have especially favored coupled nitrification-denitrification where NO₂⁻ and NO₃⁻ are produced by nitrifiers in aerobic habitats and subsequently denitrified by denitrifiers in close-by anaerobic habitats (Butterbach-Bahl et al., 2013; Wrage et al., 2001). Here, N₂O is mainly produced in the interface of aerobic and anaerobic zones, which are typically found in plant litter associated hotspots (Kravchenko et al., 2017). In addition, N₂O can also be produced aerobically during heterotrophic and autotrophic nitrification (Anderson et al., 1993; Van Groenigen et al., 2015; Wrage et al., 2001; Zhang et al., 2015). In both processes, N₂O can be formed as byproduct from chemical hydroxylamine oxidation (Butterbach-Bahl et al., 2013; Van Groenigen et al., 2015). Nitrifier denitrification as a pathway of autotrophic nitrification has been reported mostly under soil conditions differing from our study, namely high NO₂⁻, NH₃ or urea concentrations, and low organic C availability (Wrage-Mönnig et al., 2018; Wrage et al., 2001). In contrast, with high availability of organic C and N compounds, high N₂O emissions from heterotrophic nitrification have been reported (Anderson et al., 1993; Hu et al., 2016; Papen et al., 1989; Wrage et al., 2001). Zhang et al. (2015) reported 72-77% of N₂O being produced by heterotrophic nitrification with an arable soil under incubation conditions similar to our study. However, Li et al. (2016) estimated that denitrification was the dominant source of N₂O in residue-amended soil at 40-60 % WFPS. High correlation of cumulative N₂O emissions and mineralized N during the incubation period ($R^2=0.5791$, $p < 9.551 \times 10^{-6}$) indicates that heterotrophic nitrification may have contributed to N₂O production in our study. To differentiate further between processes contributing to N₂O production, stable isotope methods can be used (Baggs, 2008; Butterbach-Bahl et al., 2013; Van Groenigen et al., 2015; Wrage-Mönnig et al., 2018).

Another aim of this study was to investigate the effect of residual mineral N on plant litter induced N₂O emissions. To this end, we included two N levels that were obtained by different N fertilization during the pre-experimental plant growth phase (N1: 0.2 g N kg⁻¹, N2: 2 x 0.2 g N kg⁻¹). At the onset of the incubation experiment, soil mineral N concentration was twice as high in N2 compared to N1, but generally very low (0.93 and 1.97 mg NO₃⁻-N kg⁻¹ dry soil for N1 and N2, respectively). Higher N fertilizer input in N2 during plant growth led to lower C input from rhizodeposition (Tab. 1), which is consistent with literature findings (Kuzakov and Domanski, 2000; Paterson and Sim, 1999). Cumulative N₂O emissions were in tendency higher in N2 than in N1, suggesting that NO₃⁻ was limited, especially in -RS treatments where C availability was highest. In addition, litter chemical quality strongly affected N availability. The interdependency of C and N availability was validated by analyses of regression highlighting a strong positive interaction between soil NO₃⁻ and WEOC concentrations resulting in much higher N₂O emissions only when both NO₃⁻ and WEOC were available. This further supports our findings



that high bioavailability of maize shoot litter increased microbial respiration by heterotrophic microorganisms resulting in plant litter associated hotspots with high N₂O formation.

Variation in N₂O emissions is often related to quality parameters of plant residues, mostly the C:N ratio (Baggs et al., 2000; Chen et al., 2013; Millar and Baggs, 2004; Novoa and Tejeda, 2006). Especially easily degradable fractions, such as water-soluble C (Burford and Bremner, 1975) or the holocellulose fraction (hemicelluloses + cellulose) (Jensen et al., 2005), explained a large share of variability of C mineralization and N₂O emissions, while lignin content was not relevant (Redin et al., 2014; Silver and Miya, 2001). Comparing 28 laboratory and field studies, Chen et al. (2013) reported that microbial growth-induced microsite anaerobicity could be the major driver for the dynamic change in soil N₂O emissions following residue amendment and Kravchenko et al. (2017) showed that water absorption by plant residues further enhances formation of plant-litter associated anaerobic hotspots. In the initial phase of decomposition, water-soluble compounds (sugars, amino acids) are leached from litter providing easily degradable compounds for microbial metabolism. After litter addition, CO₂ fluxes increased immediately due to increased respiration, rapidly reducing pO₂, and creating anaerobic microsites. We anticipate that formation of such hotspots was further enhanced by the amount of litter addition, as litter input was higher in RS than in -Rt, and higher compared to other studies (Chen et al., 2013).

4.3 Bacterial community response to maize litter input and soil N level

After litter addition, the bacterial community adapts within a few days to substrate availability (Pascault et al., 2013). The canonical correspondence analysis (CCA) showed a clear correlation of the soil inhabiting bacterial community, litter input and total CO₂ and N₂O emissions. As shown by the CCA, the bacterial community structure in N1-RS and N2-RS was distinct from those in the control samples and soil with addition of root residues. Combined addition of root and shoot litter affected the soil bacterial community leading to a less diverse and more specialized community structure, which was also shown by the alpha diversity indices (see supplemental Table S1). A significant reduction of soil bacterial diversity was induced by different N levels, as previously shown by Zeng et al. (2016). In addition, Rousk and Bååth (2007) observed a negative correlation between mineral N addition and bacterial growth, while the addition of barley straw and alfalfa correlated positively. The phylogenetic diversity (PD) supports these findings by showing a more complex picture. While PD in N1 samples increased with increasing C input, it decreased in N2 samples with increasing C input, indicating a shift of the influencing factors from the C input to the N level.

The most abundant phyla in our soil samples were the *Actinobacteria*, *Proteobacteria* and *Chloroflexi*. Among these phyla, the genera *Pseudomonas* (*Proteobacteria*), *Gaiella* (*Actinobacteria*) and *Thermomicrobiales* (*Chloroflexi*) showed the highest abundance in the N2 samples, indicating their involvement in N-cycling. *Pseudomonas* species such as *Pseudomonas aeruginosa*, *P. stutzeri*, and *P. denitrificans* are known to reduce NO₃⁻ and to contribute to N₂O and N₂ emissions (Carlson and Ingraham, 1983). *Gaiella occulta*, belonging to the *Actinobacteria*, is also known for the reduction of NO₃⁻ to NO₂⁻ (Albuquerque et al., 2011). The genus *Thermomicrobiales* comprises species which can grow on nitrate, ammonia and



alanine as sole nitrogen sources and are able to hydrolyze cellulose or starch (Houghton et al., 2015). Relative abundance of
415 *Thermomicrobiales* increased with N and C input, indicating favorable growth conditions for this genus (Fig. 7).

We identified several genera involved in C cycling including members of *Agromyces*, *Bacillus*, and *Micromonospora*.
Agromyces ulmi was present in low abundance in our samples and it is known to contribute to C cycling in soils through
xylanolytic activity (Rivas et al., 2004). Members of the genus *Bacillus* (*Firmicutes*) have been reported to play a crucial role
in carbon cycling in a wide range of environments by functions such as plant growth promotion or production of amylases
420 and cellulases (Lyngwi and Joshi, 2014). Among the genus *Bacillus*, we found one species, *Bacillus* sp. KSM-N252, in
relatively high abundance (1-2%) in N2 samples. This species encodes an alkaline endoglucanase, which can hydrolyze
cellulose (Endo et al., 2001). Similarly, *Micromonospora* (*Actinobacteria*) are known to produce hydrolytic enzymes
showing cellulolytic and xylanolytic activity (Carro et al., 2018; de Menezes et al., 2012). Abundance of *Bacillus* sp. KSM-
N252 (N2-C 2%, N2-R 1.1% and N2-RS 0.8%) and *Micromonospora* (N2-R 1.9%, N2-RS 1%) decreased with increasing
425 input of water-extractable C indicating that cellulose was only decomposed when no easily degradable C was available.

Culture-independent sequence techniques have revealed that members of the phyla *Actinobacteria*, *Chloroflexi*, *Firmicutes*,
Bacteroidetes and *Nitrospirae* possess *nirK* or *nirS* genes, and can reduce nitrite to nitric oxide (Cantera and Stein, 2007;
Nolan et al., 2009). In our treatments, *Actinobacteria*, *Chloroflexi*, and *Firmicutes* were more abundant in N2 samples,
whereas *Bacteroidetes*, and *Nitrospirae* were more abundant in N1 samples which may indicate that the latter are more
430 competitive under conditions of very low mineral nitrogen availability in soil. The reduction of nitrate has been shown for
Mesorhizobium sp. (Okada et al., 2005) and *Rhizobium* sp. (Daniel et al., 1982). Although only in low abundance, we found
these species predominantly in N2 samples. Species belonging to the genus *Agromyces* (*Actinobacteria*) are also known to
reduce nitrate (Zgurskaya et al., 2008). In addition, species capable of denitrification under anaerobic, O₂-limited and aerobic
conditions can be found in the genera *Bacillus* and *Micromonospora*, as well as *Pseudomonas* and *Rhodococcus*
435 (Verbaendert et al., 2011) that were more abundant in N2 samples.

The higher abundances of C cycling and N reducing bacteria in N2 samples reflects the tendency of increased N₂O emissions
with increasing N level and further supports our hypothesis that C and N availability from plant litter were the main drivers
of N₂O emissions in our study.

5 Conclusions

440 We examined CO₂ and N₂O emissions after simulated post-harvest incorporation of maize root or root plus shoot litter in a
laboratory incubation study. High bioavailability of maize shoot litter strongly increased microbial respiration in plant litter
associated hotspots leading to increased N₂O emissions when both C and NO₃⁻ were available. Coupled nitrification-
denitrification and heterotrophic nitrification presumably contributed to N₂O formation. Maize root litter was characterized
by a higher share of slowly degradable C compounds and lower concentrations of water soluble N, hence formation of
445 anaerobic hotspots was limited and microbial N immobilization restricted N₂O emissions. Bacterial community structures



reflected degradability of maize litter types. Its diversity decreased with increasing C and N availability, favoring fast growing C cycling and N reducing bacteria, namely *Actinobacteria*, *Chloroflexi*, *Firmicutes* and *Proteobacteria*.

Data availability: The 16S rRNA gene sequences were deposited in the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under bioproject number PRJNA557843. Data from measurements are available upon request from the corresponding author.

Supplement: The supporting information related to this study will be published online.

Author contributions: PSR, RW and KD designed the experiments and PSR carried them out. BP and DS carried out microbial analyses, sequence processing and provided figures. JP, RW and KD contributed to interpretation of results. PSR prepared the manuscript with contributions from all co-authors.

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Table 1: Two-factorial setup of the incubation experiment. Soil N_{min} concentrations were measured directly before onset of the incubation experiment. C input in Control is from rhizodeposition (RD) only, C input in Root is from rhizodeposition and roots, C input in Root + Shoot is from rhizodeposition, roots and shoot biomass. N input is from root and shoot biomass, respectively.

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N level	N_{min} [mg NO_3^- -N kg ⁻¹ dry soil]	Treatment	Biomass input [g FM kg ⁻¹ dry soil]	C input [g C kg ⁻¹ dry soil]	N input [g N kg ⁻¹ dry soil]
N1	0.93	Control	RD	3.47	n.d.
		Root	RD + 100	3.47 + 4.18 = 7.65	0.25
		Root + Shoot	RD + 100 + 100	3.47 + 4.18 + 6.16 = 13.80	0.25 + 0.27 = 0.52
N2	1.97	Control	RD	2.74	n.d.
		Root	RD + 100	2.74 + 4.18 = 6.92	0.25
		Root + Shoot	RD + 100 + 100	2.74 + 4.18 + 6.16 = 13.07	0.25 + 0.27 = 0.52



Table 2: Chemical characteristics of maize root and shoot litter used in the incubation experiment. Hemicellulose and cellulose are expressed relative to lignin content.

	Root	Shoot
Dry matter [%]	62.9	14.7
C:N ratio	17.0	23.2
Lignin:N ratio	2.82	1.44
Water soluble C _{org} [% of total C]	11.6	23.4
Water soluble N [% of total N]	8.8	25.8
Hemicellulose (relative content)	3.36	9.08
Cellulose (relative content)	3.18	11.5
Lignin (relative content)	1	1



Table 3: Absolute cumulative N₂O and CO₂ emissions and relative to C input and N₂O / CO₂ ratio of 22-day incubation experiment with two pre-incubation-N-levels (N1, N2) and three litter addition treatments (Control = no litter input, Root = 100 g root FM kg⁻¹ dry soil, Root+Shoot = 100 g root FM + 100g shoot FM kg⁻¹ dry soil).

N level	Treatment	N ₂ O [μg N ₂ O-N kg ⁻¹]		N ₂ O [μg N ₂ O-N kg ⁻¹ g ⁻¹ C input]		CO ₂ [mg CO ₂ -C kg ⁻¹]		CO ₂ [mg CO ₂ -C kg ⁻¹ g ⁻¹ C input]		N ₂ O/CO ₂ ratio [μg N mg ⁻¹ C]
N1	Control	10.21 ± 4.23	a	2.95 ± 1.22	a	141.89 ± 29.74	a	40.94 ± 8.58	a	0.07
	Root	120.91 ± 24.09	b	15.81 ± 3.15	b	533.51 ± 83.19	b	69.78 ± 10.88	b	0.23
	Root+Shoot	4337.31 ± 424.98	c	314.25 ± 30.95	c	2287.23 ± 289.48	c	165.72 ± 20.97	c	1.91
N2	Control	11.35 ± 6.75	a	4.15 ± 2.47	a	129.44 ± 47.47	a	47.30 ± 17.35	a	0.08
	Root	201.14 ± 105.62	ab	29.08 ± 15.27	ab	647.48 ± 196.13	ab	93.61 ± 28.36	b	0.31
	Root+Shoot	5357.87 ± 1193.50	c	409.82 ± 91.30	c	2361.19 ± 287.20	c	180.63 ± 21.97	c	2.25

Values represent means (n=4) ± standard deviation. Different letters in the same column indicate a significant difference according to the Tukey's HSD post-hoc tests

715 at p ≤ 0.05.



Table 4: Significance of fixed effects of soil NO_3^- -N (mg NO_3^- -N kg^{-1}), water extractable organic C (WEOC, mg C kg^{-1}) and first-order interaction on N_2O fluxes ($\mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$; \ln transformed) using linear mixed effect model.

	Estimate	Standard error	p value
Intercept	-0.4237	0.2074	0.0416
NO_3^- -N	0.0152	0.0248	0.5401
WEOC	0.0210	0.0077	0.0065
NO_3^- -N : WEOC	0.0023	0.0011	0.0394



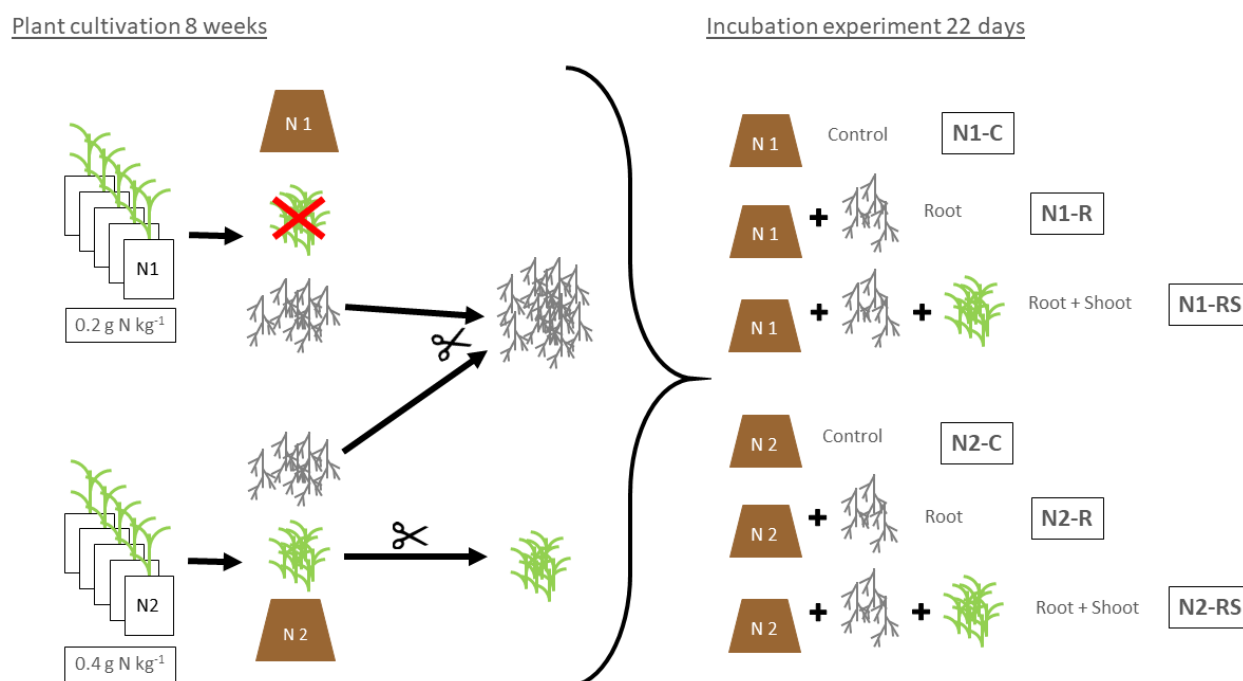
720 **Table 5: Results of regression analyses of the relationship between total cumulative CO₂ emissions and C quality parameters of plant litter (AICc = Akaike's information criterion).**

Regression model	residual standard error	degrees of freedom	adjusted R ²	p value	AICc
CO ₂ ~ total litter C input	274.5	22	0.9213	7.65*e ⁻¹⁴	342.73
CO ₂ ~ water-soluble C input	181.9	22	0.9655	< 2.2*e ⁻¹⁶	322.98
CO ₂ ~ Hemicellulose	272.4	22	0.9225	6.497*e ⁻¹⁴	342.38
CO ₂ ~ Cellulose	221.1	22	0.9489	6.478*e ⁻¹⁶	332.35
CO ₂ ~ Lignin	496.6	22	0.7425	3.873*e ⁻⁰⁸	371.19
CO ₂ ~ Hemicellulose + Cellulose	180.2	21	0.9661	< 2.2*e ⁻¹⁶	324.32



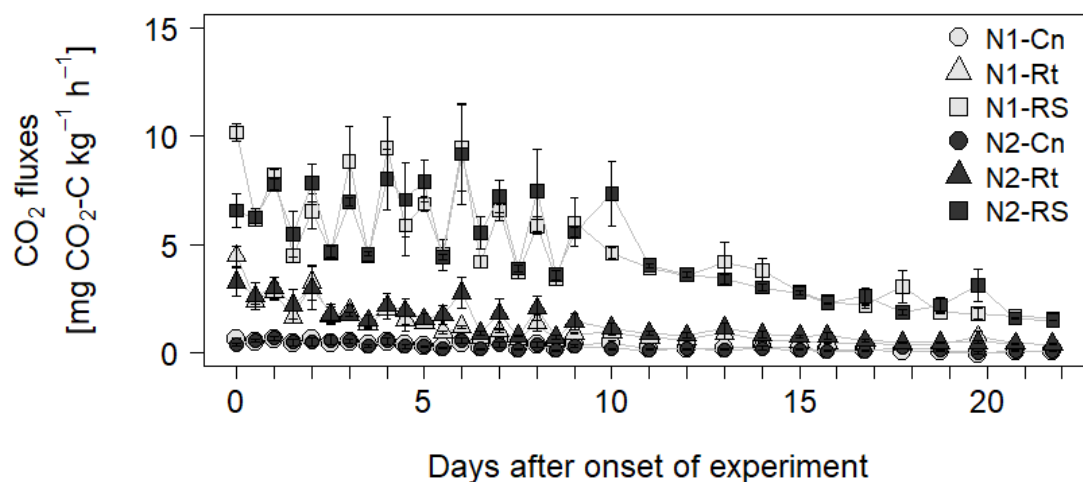
Table 6: Results of regression analyses of the relationship between total cumulative N₂O emissions, total cumulative CO₂ emissions and N parameters of plant litter and soil (AIC = Akaike's information criterion).

Regression model	residual standard error	degrees of freedom	adjusted R ²	p value	AICc
N ₂ O ~ CO ₂	593.9	22	0.9366	7.073*e ⁻¹⁵	379.78
N ₂ O ~ initial soil NO ₃ ⁻	2404	22	-0.03885	0.7119	446.89
N ₂ O ~ Mineralized N	2191	22	0.5791	9.551*e ⁻⁰⁶	425.21

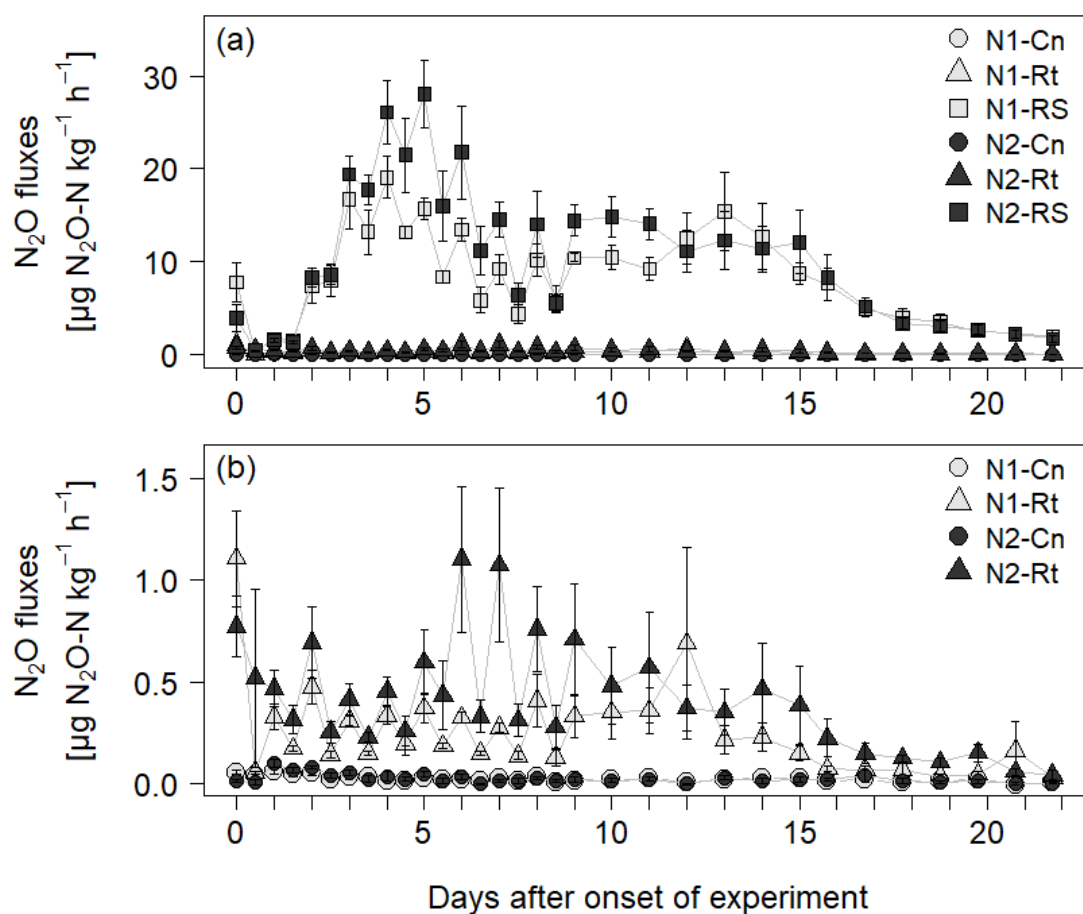


725 **Figure 1: Preparation and experimental setup of the incubation experiment. N1 (0.2 g N kg^{-1}) and N2 ($2 \times 0.2 \text{ g N kg}^{-1}$)**
 referring to the N levels during plant growth. Control soil (N1-C and N2-C) without addition of plant biomass. Root
 treatment with addition of 100g fresh root biomass per kg dry soil (N1-R and N2-R) and Root + Shoot treatment with
 addition of 100 g root and 100 g shoot biomass per kg dry soil (N1-RS, N2-RS).

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735 **Figure 2: Hourly CO₂ emissions from soils with two N levels (N1, N2) after incorporation of maize root litter (-Rt), maize root + shoot litter (-RS) and control (-Cn) without litter. Error bars show standard error of mean values (n = 4). When not visible, error bars are smaller than the symbols.**



740 **Figure 3 a+b:** Hourly N_2O emissions from soils with two N levels (N1, N2) after incorporation of maize root litter (-Rt), maize root + shoot litter (-RS) and control (-Cn) without litter. Error bars show standard error of mean values (n = 4). When not visible, error bars are smaller than the symbols. Note: data of figure 3 b are excerpt from 3 a, and are shown with different scaling.

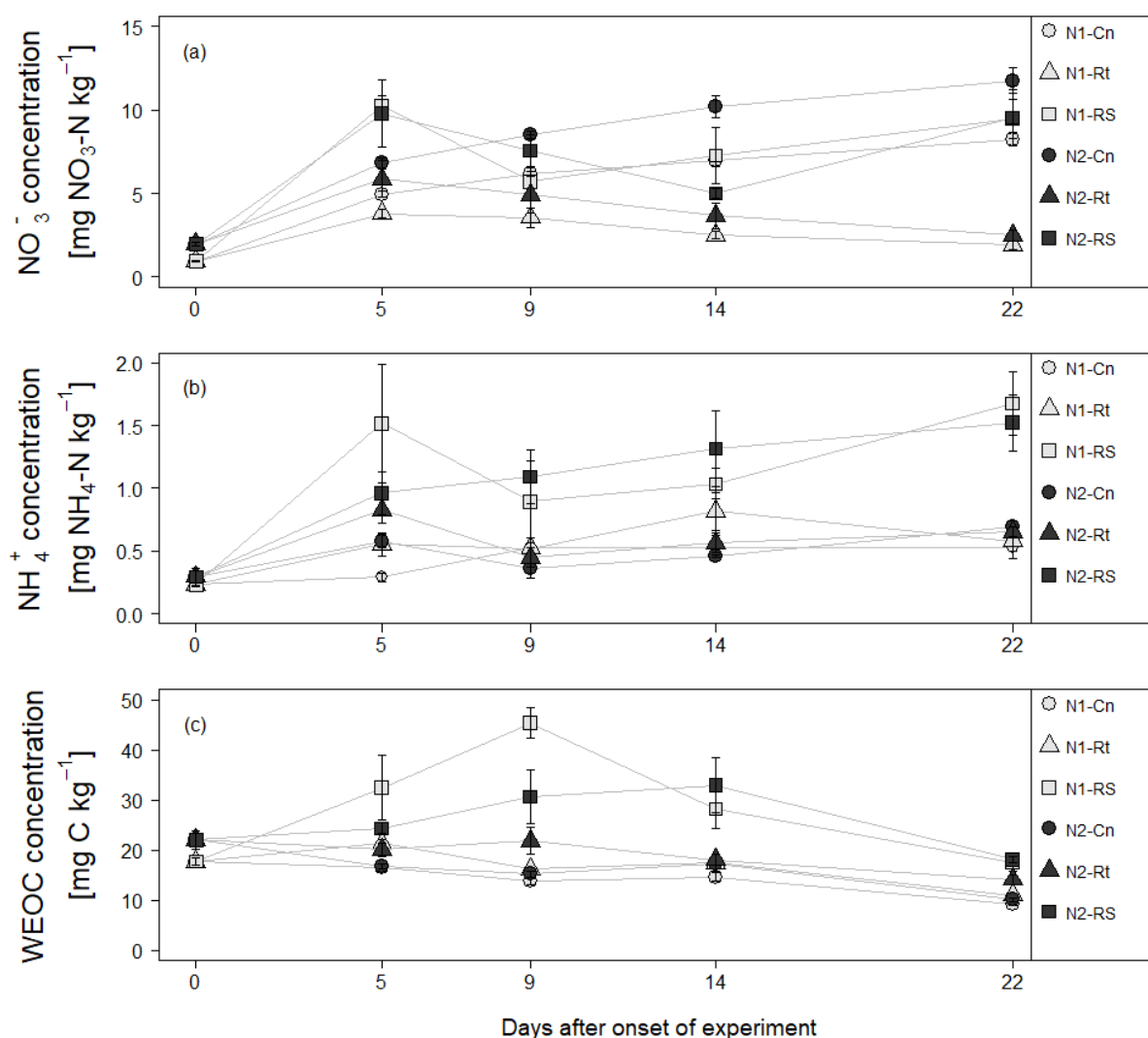
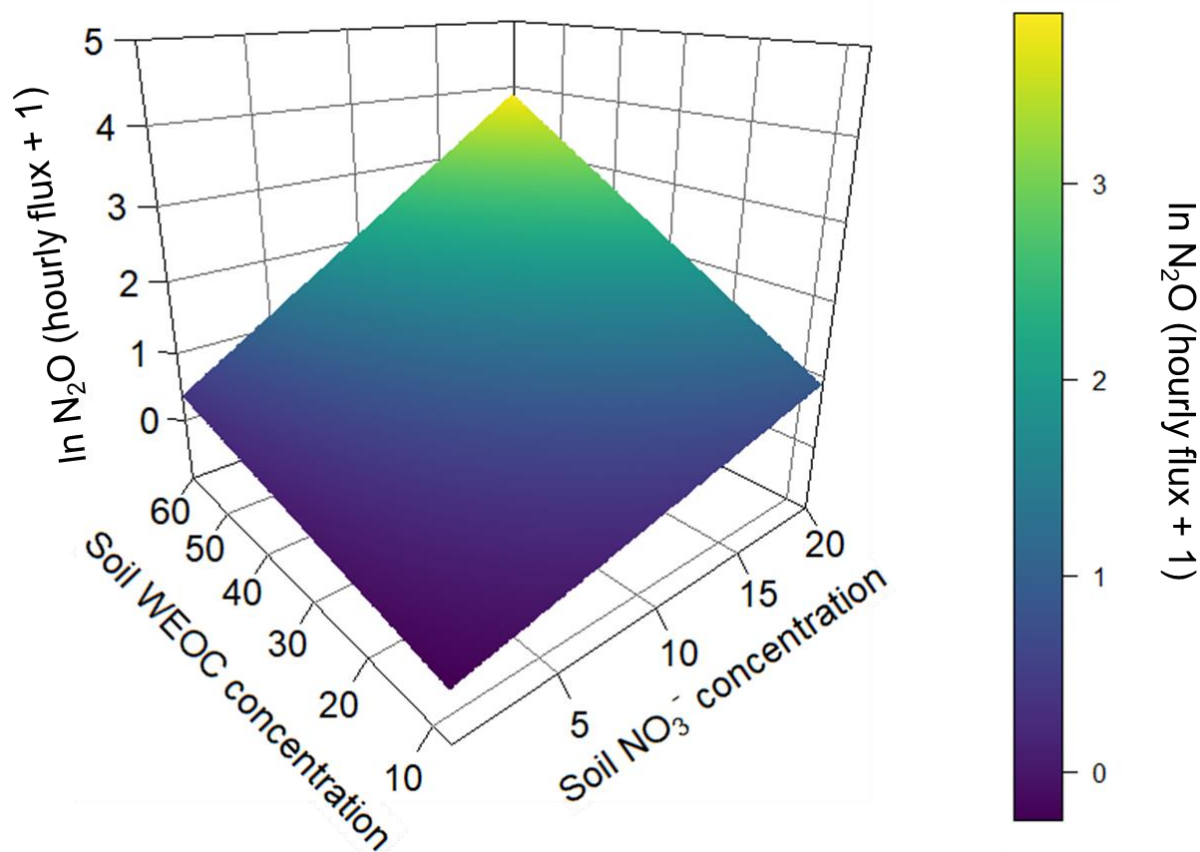


Figure 4 a-c: NO₃⁻, WEOC, and NH₄⁺ concentration from soils with two N levels (N1, N2) after incorporation of maize root litter (-Rt), maize root + shoot litter (-RS) and control (-Cn) without litter. Error bars show standard error of mean values (n = 4) (day 0: n=3). When not visible, error bars are smaller than the symbols.



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Figure 5: Prediction of N_2O fluxes ($\mu g N_2O-N kg^{-1} h^{-1}$) (\ln transformed) based on soil NO_3^- and water extractable C_{org} concentrations based on linear mixed-effect model (Pseudo- $R^2=0.89$).

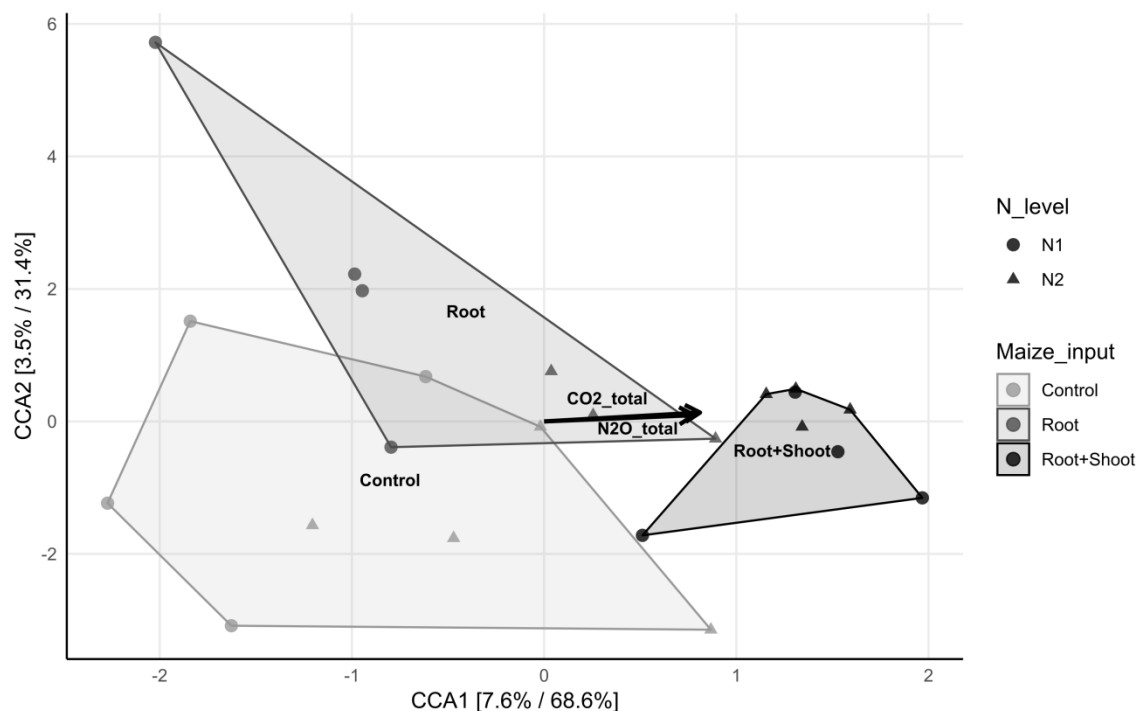
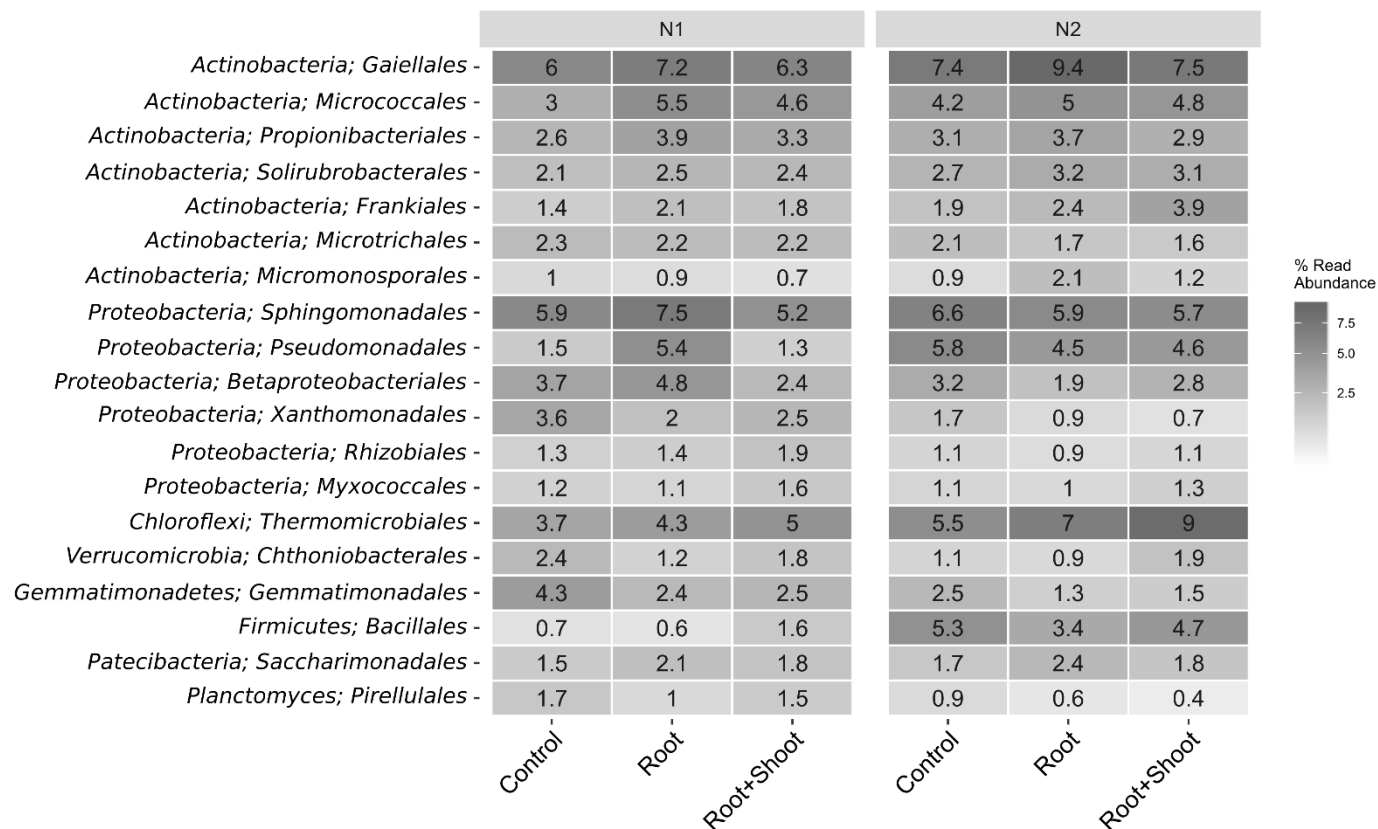


Figure 6: Canonical Correspondence Analysis (CCA) displaying the compositional distribution of the soil inhabiting bacterial communities between the control (N1-C and N2-C; n=4), root (N1-R and N2-R; n=4 and n=3) and root + shoot treatment (N1-RS and N2-RS; n=4). Significant correlations of total CO₂ and N₂O emissions are shown by black arrows ($p \leq 0.005$). The relative contribution (eigenvalue) of each axis to the total inertia in the data as well as to the constrained space only, respectively, are indicated in percent at the axis titles.



770 **Figure 7: Heatmap of the 16 most abundant bacterial orders in the analyzed soil samples grouped by N levels and litter input treatments (n=4, except for N2 Root: n=3)**