

Authors' response to minor revision

I kindly ask you to upload a final revised manuscript, for which you have carefully checked the consistency of all units used (e.g. the units for N₂O fluxes are currently not consistent between Table 5, Fig. 3 and Fig. 5). I furthermore suggest you to add, both to the conclusions section and the abstract, a concluding sentence highlighting the broader implications of your study.

We updated Table 1, 3, 4, and 5, as well as Figures 4 and 5 to present all results per g soil. For the methods section, we'll still refer to kg soil as we did the experiment with several kg soil per incubation vessel.

In addition, we included a general sentence in Abstract and Conclusions stating that "litter quality is a major driver of N₂O and CO₂ emissions from crop residues, especially when soil mineral N is limited" (Line 32 and line 489).

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

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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 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 CO₂ and N₂O fluxes, analyzed soil mineral N and water extractable organic C (WEOC) concentrations, and determined quality parameters of maize litter. Bacterial community structures were analyzed using 16S rRNA gene sequencing.

Maize litter quality controlled NO₃⁻ and WEOC availability and decomposition related CO₂ emissions. Emissions induced by maize root litter remained low, while high bioavailability of maize shoot litter strongly increased CO₂ and N₂O emissions when both root and shoot litter were added. 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 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 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*. In conclusion, litter quality is a major driver of N₂O and CO₂ emissions from crop residues, especially when soil mineral N is limited.

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 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 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., 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 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 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 (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

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.

70 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
75 (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).

The aims of this study ~~was~~^{were} 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
80 hypothesize that differences in N₂O emissions between treatments can be related to degradability of maize litter with easier degradable shoot litter leading to higher N₂O formation. We further expect that differences in litter chemical quality are reflected in the structural composition of the soil microbial community with higher availability of N and C leading to a more specialized community.

Maize plants were grown in a greenhouse to produce root and shoot litter. As in many European countries law prohibits
85 addition of mineral N with incorporation of crop residues or catch crops, we applied two N fertilizer regimes (low vs. high) to realize differences in soil N_{min} concentration at harvest. We then set up a laboratory incubation experiment with fresh maize root or root and shoot litter under fully controlled conditions and determined 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
90 adaptations to litter input.

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)
95 containing 1.5 % C and 2.81 % humus, with a pH (CaCl₂) = 7.44.

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
 100 Devices, Pullman, USA) were used to monitor soil water content. Six maize plants (*Zea mays* L. var. Ronaldinio) were sown
 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 Ca(NO₃)₂ * 4 H₂O) 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
 105 N doses is referred to as N2 (2 x 0.2 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 and milled
 to a particle size <1mm. To determine water-extractable C and N concentrations, subsamples were extracted with H₂O_{bidest}
 110 (maize root 1:1000 w/v, maize shoot 1:10000 w/v) for 16 h 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
 115 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

The incubation experiment consisted of a two-factorial setup comprising two N levels (N1 and N2) and three litter levels
 120 (Control = Cn, Root = Rt, Root+Shoot = RS) (see Table 1 and Figure 1 for details). To allow comparison of litter treatments
 over soil conditions, the same litter types for both soil N levels were used. As N2 plants had produced greater and healthier
 biomass during pre-experimental growth phase, only N2 shoots were used for both soils. Roots from N1 and N2 plants were
 mixed to ensure sufficient amounts for all replicates. Control soils (N1-Cn and N2-Cn) did not receive plant biomass, yet
 they contained C input from rhizodeposition of the previous maize growth. C remaining from rhizodeposition, root hairs and
 125 small root fragments was calculated as the difference in soil C concentration before and after 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). Each treatment was replicated four
 times.

Within each N level, soil was homogenized to ensure similar starting conditions. Subsamples of both soils were taken for
 130 analysis of mineral N, water extractable C_{org} concentration, and total soil C. Soil mineral N concentrations were 0.93 and
 1.97 mg N kg⁻¹ for N1 and N2, respectively. Plant litter was cut to a size of 2 cm and homogeneously mixed with the soil,

135 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 2 cm-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 PVC lids to minimize evaporation from the soil and to
incubate samples in the dark. The incubation experiment was carried out under controlled temperature conditions (16 h day
140 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
145 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 N₂O and a thermal conductivity detector (TCD) for CO₂. Samples
150 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 CO₂, 307, 760, and 6110 ppm N₂O) 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
155 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 CaCl₂ solution (1:5 w/v) for 60 min
on an overhead shaker (85 rpm). The extracts were filtered with 615 ¼ filter paper (Macherey - Nagel GmbH & Co. KG,
160 Düren, Germany) and stored at -20 °C. The extracts were analyzed colorimetrically for the concentrations of NO₃⁻ and NH₄⁺
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 NH₄⁺-N + NO₃⁻-
N concentrations at the start and end of the incubation period plus N lost as N₂O-N (Eq. 1).

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$$Net\ mineralization = (NO_3^- + NH_4^+)_{end} - (NO_3^- + NH_4^+)_{start} + N_2O \quad (1)$$

165 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 (Labsolute, 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

170 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
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190 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

195 (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 amplicon 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 61200 sequences. Statistical analyses were done using ASVs in R version 3.5.3 (R Core Team, 2019). The R package *ampvis2* v2.4.7 (Andersen et al., 2018) was used to determine species richness, alpha diversity estimates, rarefaction curves, and to prepare all graphs. 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, core microbiomes and respective responders were analyzed on genus level, grouped by either the applied litter treatment or N fertilizer levels using *ampvis2* v2.4.7.

For one replicate of N2-Rt, DNA concentration was very low and the 16S rRNA gene could not be amplified. Thus, we only evaluated the remaining three replicates of this treatment. 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 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, water-extractable C input, hemicellulose fraction, cellulose fraction, and lignin fraction from all litter treatments (-Cn, -Rt, -RS, n=24).

To evaluate effects of soil environmental variables on N₂O and CO₂ fluxes, a linear mixed effect model (lme) was fitted between ~~hourly~~ N₂O fluxes (ln transformed), soil NO₃⁻-N and WEOC concentrations using the *lme* function from the package *nlme* v3.1-131 (Pinheiro et al., 2017). Pseudo-R² for lme was calculated using *r.squaredGLMM* from the package *MuMIn* v1.42.1 (Barton, 2018). Soil NO₃⁻-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 CO₂ and N₂O fluxes and cumulative emissions

Addition of maize litter increased CO₂ 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. CO₂ 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, N₂O emissions increased in all litter treatments compared to control treatments (Fig. 3 a+b). Highest fluxes were measured in N2-RS, reaching 7.8 pg N₂O-N g⁻¹ s⁻¹ on day 5. Fluxes stayed on a similar level from day 7 to day 15, and then declined until the end of the experiment. N₂O fluxes from root (N1-Rt, N2-Rt) and control treatments (N1-Cn, N2-Cn) remained on a low level during the whole incubation period (≤ 0.59 pg and ≤

0.04 pg N₂O-N g⁻¹ s⁻¹, for -Rt and -Cn respectively). N₂O fluxes from N1 were slightly lower than from N2 in both litter treatments. Over all treatments and sampling dates, CO₂ and N₂O fluxes were positively correlated (R²=0.5993, p<0.001, data not shown).

To account for different C inputs in treatments, cumulative CO₂ and N₂O emissions were standardized against the C input per treatment (Table 1). Still, cumulative CO₂ emissions were almost twice as high in -Rt and about four times higher in -RS compared to -Cn (p<0.05), indicating that differences between litter treatments cannot simply be explained by differences in C input. Addition of maize root and shoot litter increased cumulative N₂O emissions by roughly 100-times compared to control treatments (p<0.05). In contrast, root litter increased cumulative N₂O emissions only by a factor of 5.4 (N1-Rt) and 7 (N2-Rt) compared to the respective controls (p<0.05).

3.3 Soil NO₃⁻, NH₄⁺ and water-extractable Corg concentrations

Addition of maize litter affected the time course of soil NO₃⁻, NH₄⁺ and WEOC concentrations (Fig. 4 a-c). In control treatments, initial soil NO₃⁻ concentrations of 0.93 (N1-Cn) and 1.97 mg NO₃⁻-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 NH₄⁺ concentrations showed variations on a low level only. Soil NO₃⁻ concentrations were continuously higher in N2 than in N1 and differences in soil NH₄⁺ 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, Table 4) during the incubation experiment. In treatments with litter, soil NO₃⁻ concentrations decreased after an initial increase. In root treatments, soil NO₃⁻ 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 NO₃⁻ 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 NO₃⁻ concentrations in -RS were higher than in -Rt. Soil NH₄⁺ concentrations only marginally increased for -Rt. Contrary to -Rt and -Cn, soil NH₄⁺ 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 (Table 4). Maize root litter did not affect WEOC, as concentrations were similar to -Cn 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 N₂O emissions and C and N parameters of plant litter and soil

To identify the effect of N and C availability on N₂O fluxes, a linear mixed effect model was applied. The best model included a significant interaction between soil NO₃⁻ and WEOC (p<0.0024394, Pseudo-R²=0.8882, Table 5), and incubation vessel and sampling time as random parameters. Predictions of N₂O fluxes based on this model are shown in (Fig. 5).

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Linear regression analyses were used to identify relations between cumulative CO₂ and N₂O 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 6). Regression analyses of the relationships between total 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 7), 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 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-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-Cn and N1-RS revealed no difference in diversity indices (Shannon and Simpson), while N1-Rt showed lower Shannon and Simpson diversity indices (Table S4). The Shannon diversity index was lowest in N2-Rt comparing all N2 treatments, while the Simpson index was 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-Rt 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-Rt (9.3 %), N1-Rt (7.5 %) and N2-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*, *Altererythrobacter*, *Gaiella*, *Nocardioides*, *Agromyces*, *Bacillus*, and *Lysobacter* were most abundant accounting for up to 5.7 % of all ASVs. Accordingly, these were also most abundant genera attributed to the core microbiome (Tables S6 and S8). Overall, 80 genera represented the core microbiome, when grouped by N levels, while 21 genera and 6 genera were identified as responders to N1 and N2, respectively (Fig. S5). In detail, the classified responders to the applied N treatments were the genera *Chthonibacter*, *Luteimonas*, *Sphingobium*, *Novosphingobium*, *Adhaeribacter*, *Nitrospira*, *Gemmata*, and

325 *Devosia* for N1 and *Conexibacter* for N2 samples (Table S. 8). The genera *Bacillus*, *Gaiella*, *Altererythrobacter*,
330 *Blastococcus*, and *Pseudomonas* showed highest abundance in N2 samples, while *Lysobacter*, and *Sphingomonas* were more
abundant in N1 samples (Fig. S3). When grouped by litter treatment, the core microbiome comprised 77 genera accounting
for 73 % of the relative abundance, while 9, 3 and 10 genera were identified as responders to the applied litter treatments
Control, Root and Root+Shoot, respectively (Fig. S5). *Nonomuraea*, *Fluviicola*, and *Nitrospira* responded to the Root+Shoot
treatment, while the genera *Lapillicoccus* and *Adhaeribacter* responded to the Root treatment (Table S7). The genera
335 *Litorilinea*, *Gemmata*, *Novosphingobium*, and *Opitutus* were identified as responders to the Control treatment. For N levels
and litter treatments respectively, 833 and 838 genera were identified as non-core microbiomes, accounting for 20 % and
19.5 % of relative abundance (Fig. S5).

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
335 identified. 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

340 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
345 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
350 degradable compounds was also reflected by strongly increased CO_2 fluxes and cumulative emission 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 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
355 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 6).

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), leading to significant correlations between both N_2O and CO_2 fluxes and cumulative emissions (Azam et al., 2002; Fiedler et al., 2017; Frimpong and Baggs, 2010; Huang et al., 2004; Millar and Baggs, 2004, 2005). CO_2 fluxes increased directly with onset of incubation and started to decline after day 10, thus mostly C compounds with a short turnover time, i.e. sugars, proteins, starch, and hemicellulose were decomposed and contributed to CO_2 fluxes. Availability of easily degradable C compounds stimulates microbial respiration, limiting O_2 at the microsite level and thus increasing N_2O emissions from denitrification (Azam et al., 2002; Chen et al., 2013; Miller et al., 2008). Accordingly, N_2O fluxes increased after a lag phase of two days. The strong positive correlation ($R^2=0.9362$, $p\leq7.632\cdot10^{-15}$) between cumulative CO_2 and N_2O emissions (Table 7) further supports our hypothesis that litter quality, in particular degradability of C compounds, affects N_2O fluxes from denitrification by creating plant litter associated microsites with low O_2 concentrations.

High mineralization in -RS treatments may have especially favored coupled nitrification-denitrification where NO_2^- and NO_3^- 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, N_2O 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, N_2O 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, N_2O 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 NO_2^- , NH_3 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 N_2O 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 from 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, in addition to denitrification, heterotrophic nitrification may have contributed to N₂O production in our study. However, to further differentiate between processes contributing to N₂O production, stable isotope methods need to 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 $\mu\text{g N kg}^{-1}$, N2: 2 x 0.2 $\mu\text{g N kg}^{-1}$). 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 $\mu\text{g NO}_3^- \text{N kg}^{-1}$ 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.

Under N limiting conditions, a higher portion of N is recovered in soil microbial biomass in relation to litter N input (Bending and Turner, 1999; Troung and Marschner, 2018). When N is abundant relative to C availability, excess N is released by soil microorganisms and can be lost as N₂O. In -Rt, where N availability was low, N was immobilized by soil microorganisms and N₂O emission were low. When more easily degradable N was added with maize shoots, N released from decomposition of maize shoots presumably fostered decomposition of maize roots (Robertson and Groffman, 2015) and denitrification of excess N leading to strongly increased CO₂ and N₂O emissions in -RS. To estimate the contribution of plant litter N to mineralization, immobilization, and denitrification, ¹⁵N labeled litter together with analysis of microbial biomass N and ¹⁵N₂O emissions could be used (e.g. Frimpong and Baggs, 2010; Ladd et al., 1981).

The interdependency of C and N availability was further 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).

In addition to soil mineral N concentration and plant litter, soil type and soil moisture may have influenced our results (e.g. Aulakh et al., 1991). Increasing soil moisture leads to increasing N₂O emissions, but relative contribution of nitrification and denitrification to N₂O formation may change with increasing soil moisture (Bateman and Baggs, 2005; Baral et al., 2016; Li et al., 2016). Therefore, future experiments with different soil moisture contents should include methods to differentiate between N₂O formation pathways.

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. Accordingly, the increase of N₂O emissions from N2 compared to N1 was smaller in -RS where C availability was highest indicating that N was limited here.

The most abundant phyla in our soil samples were the *Actinobacteria*, *Proteobacteria*, and *Chloroflexi*. Among these phyla, the genera *Pseudomonas* (*Proteobacteria*) and *Gaiella* (*Actinobacteria*) were also affiliated to the core microbiomes. *Thermomicrobiales* (*Chloroflexi*) showed the highest abundance in 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 *Thermomicrobiales* increased with N and C input, indicating favorable growth conditions for this genus (Fig. 7).

455 We further identified several genera involved in C cycling including members of *Agromyces*, *Bacillus*, and *Micromonospora*, which were also affiliated to the core microbiome. *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 and cellulases (Lyngwi and Joshi, 2014). Among the

460 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-Cn 2 %, N2-Rt 1.1 % and N2-RS 0.8 %) and *Micromonospora* (N2-R 1.9 %, N2-RS 1 %) decreased with increasing input of water-extractable C indicating that cellulose

465 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*, 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 competitive

470 under conditions of very low mineral nitrogen availability in soil. This was further validated as *Nitrospira* (*Nitrospirae*), known to oxidize nitrite (Koch et al., 2015), was identified as a responder for N1 and -RS. 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*), which was affiliated to the core microbiomes, are also known to reduce nitrate (Zgurskaya et al., 2008). In addition, species capable

475 of denitrification under anaerobic, O₂-limited and aerobic conditions can be found in the genera *Bacillus* and *Micromonospora*, as well as *Pseudomonas* and *Rhodococcus* (Verbaendert et al., 2011) that were affiliated to the core microbiome but were more abundant in N2 samples. The genus *Opitutus* was identified as responder to -Cn and comprises the bacterium *Opitutus terrae* that was only found in anoxic habitats in soils (Chin et al., 2001).

Altogether, the higher relative abundances of C cycling and N reducing bacteria in N2 samples and their affiliation with the

480 core microbiomes reflect 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

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

485 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 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*.

Hence, litter quality is a major driver of N₂O and CO₂ emissions from crop residues, especially when soil mineral N is limited.

Formatiert: Englisch (USA)

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.

Competing interests: The authors declare no competing interests.

Acknowledgments: The authors thank Jakob Streuber, Simone Urstadt, and Finn Malinowski for gas sampling and laboratory analyses, as well as Alexander Silbersdorff (ZfS Statistical Consulting) and Oliver Caré for advice on data handling and statistical analysis.

This study was funded by the Deutsche Forschungsgemeinschaft through the research unit DFG-FOR 2337: Denitrification in Agricultural Soils: Integrated Control and Modelling at Various Scales (DASIM).

References

Albuquerque, L., França, L., Rainey, F. A., Schumann, P., Nobre, M. F., and Da Costa, M. S.: *Gaiella occulta* gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class *Actinobacteria* and proposal of *Gaiellaceae* fam. nov. and *Gaiellales* ord. nov., Syst. Appl. Microbiol., 34(8), 595–599, doi:10.1016/j.syapm.2011.07.001, 2011.

Andersen, K. S., Kirkegaard, R. H., Karst, S. M., and Albertsen, M.: ampvis2: an R package to analyse and visualise 16S rRNA amplicon data, bioRxiv, 299537, doi:10.1101/299537, 2018.

Anderson, I. C., Poth, M., Homstead, J., and Burdige, D.: A comparison of NO and N₂O production by the autotrophic
520 nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*, Appl. Environ. Microbiol., 59(11),
3525–3533, 1993.

Aulakh, M. S., Doran, J. W., Walters, D.T., and Power, J.F.: Legume residue and soil water effects on denitrification in soils
of different textures, Soil. Biol. Biochem, 23, 1161–1167, doi:10.1016/0038-0717(91)90029-J, 1991.

Aulakh, M. S., Khera, T. S., Doran, J. W., and Bronson, K. F.: Denitrification, N₂O and CO₂ fluxes in rice-wheat cropping
525 system as affected by crop residues, fertilizer N and legume green manure, Biol. Fertil. Soils, 34, 375–389,
doi:10.1007/s003740100420, 2001.

Azam, F., Müller, C., Weiske, A., Benckiser, G., and Ottow, J. C. G.: Nitrification and denitrification as sources of
atmospheric nitrous oxide - Role of oxidizable carbon and applied nitrogen, Biol. Fertil. Soils, 35(1), 54–61,
doi:10.1007/s00374-001-0441-5, 2002.

530 Baggs, E. M.: A review of stable isotope techniques for N₂O source partitioning in soils: Recent progress, remaining
challenges and future considerations, Rapid Commun. Mass Spectrom., 22(11), 1664–1672, doi:10.1002/rcm.3456, 2008.

Baggs, E. M., Rees, R. M., Smith, K. A., and Vinten, A. J. A.: Nitrous oxide emission from soils after incorporating crop
residues, Soil Use Manag., 16, 82–87, doi:10.1111/j.1475-2743.2000.tb00179.x, 2000.

Baral, K. R., Arthur, E., Olesen, J. E., and Petersen, S. P.: Predicting nitrous oxide emissions from manure properties and
535 soil moisture: An incubation experiment, Soil. Biol. Biochem., 97, 112–120, doi:10.1016/j.soilbio.2016.03.005, 2016.

Barton, K.: MuMIn: Multi-Model Inference, R package version 1.43.6, <https://cran.r-project.org/package=MuMIn>, 2018.

Bateman, E. J. and Baggs, E. M.: Contributions of nitrification and denitrification to N₂O emissions from soils at different
water-filled pore space, Biol. Fertil. Soils, 41, 379–388, doi:10.1007/s00374-005-0858-3, 2005.

Bending, G. D. and Turner, M. K.: Interaction of biochemical quality and particle size of crop residues and its effect on the
540 microbial biomass and nitrogen dynamics following incorporation into soil, Biol. Fertil. Soils, 29, 319-327,
doi:10.1007/s003740050559, 1999.

Birouste, M., Kazakou, E., Blanchard, A., and Roumet, C.: Plant traits and decomposition: Are the relationships for roots
comparable to those for leaves?, Ann. Bot., 109(2), 463–472, doi:10.1093/aob/mcr297, 2012.

Burford, J. R. and Bremner, J. M.: Relationships between the denitrification capacities of soils and total, water-soluble and
545 readily decomposable soil organic matter, Soil Biol. Biochem., 7(6), 389–394, doi:10.1016/0038-0717(75)90055-3,
1975.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S.: Nitrous oxide emissions
from soils : how well do we understand the processes and their controls ?, Philos. Trans. R. Soc. B, 368, 1–13,
doi:10.1098/rstb.2013.0122, 2013.

550 Cantera, J. J. L. and Stein, L. Y.: Molecular diversity of nitrite reductase genes (nirK) in nitrifying bacteria, Environ.
Microbiol., 9(3), 765–776, doi:10.1111/j.1462-2920.2006.01198.x, 2007.

- Carlson, C. A. and Ingraham, J. L.: Comparison of denitrification by *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, and *Paracoccus denitrificans*, Appl. Environ. Microbiol., 45(4), 1247–1253, 1983.
- Carro, L., Nouiou, I., Sangal, V., Meier-Kolthoff, J. P., Trujillo, M. E., Montero-Calasanz, M. D. C., Sahin, N., Smith, D.
555 L., Kim, K. E., Peluso, P., Deshpande, S., Woyke, T., Shapiro, N., Kypides, N. C., Klenk, H. P., Göker, M., and Goodfellow, M.: Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential, Sci. Rep., 8(1), 1–23, doi:10.1038/s41598-017-17392-0, 2018.
- Chantigny, M. H., Angers, D. A., Kaiser, K., and Kalbitz, K.: Extraction and Characterization of Dissolved Organic Matter, in Soil Sampling and Methods of Analysis, 2007.
- 560 Chen, H., Li, X., Hu, F., and Shi, W.: Soil nitrous oxide emissions following crop residue addition: a meta-analysis, Glob. Chang. Biol., 19(10), 2956–2964, doi:10.1111/gcb.12274, 2013.
- Chen, S., Zhou, Y., Chen, Y., and Gu, J.: Fastp: An ultra-fast all-in-one FASTQ preprocessor, Bioinformatics, 34(17), i884–i890, doi:10.1093/bioinformatics/bty560, 2018.
- Chin, K.-J., Liesack, W., and Janssen, P. H.: *Opitutus terrae* gen. nov., sp. nov., to accommodate novel strains of the division
565 ‘*Verrucomicrobia*’ isolated from rice paddy soil, Int. J. Syst. Evol. Microbiol., 51, 1965–1968, doi:10.1099/00207713-51-6-1965, 2001.
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R. B., Piao, S., and Thornton, P.: Carbon and Other Biogeochemical Cycles, in Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the
570 Intergovernmental Panel on Climate Change, pp. 465–570, 2013.
- Daniel, R. M., Limmer, A. W., Stelle, K. W., and Smith, I. M.: Anaerobic Growth, Nitrate Reduction and Denitrification in 46 Rhizobium Strains, J. Gen. Microbiol. 128, 1811–1815, doi:10.1099/00221287-128-8-1811, 1982.
- Endo, K., Hakamada, Y., Takizawa, S., Kubota, H., Sumitomo, N., Kobayashi, T., and Ito, S.: A novel alkaline endoglucanase from an alkaliphilic *Bacillus* isolate: Enzymatic properties, and nucleotide and deduced amino acid
575 sequences, Appl. Microbiol. Biotechnol., 57(1–2), 109–116, doi:10.1007/s002530100744, 2001.
- Fiedler, S. R., Augustin, J., Wrage-Mönnig, N., Jurasinski, G., Gusovius, B., and Glatzel, S.: Potential short-term losses of N₂O and N₂ from high concentrations of biogas digestate in arable soils, SOIL, 3, 161–176, doi:10.5194/soil-3-161-2017, 2017.
- Fierer, N., Bradford, M. A., and Jackson, R. B.: Toward an Ecological Classification of Soil Bacteria, Ecology, 88(6), 1354–
580 1364, doi:10.1890/05-1839, 2016.
- Frimpong, K. A. and Baggs, E. M.: Do combined applications of crop residues and inorganic fertilizer lower emission of N₂O from soil?, Soil Use Manag., 26(4), 412–424, doi:10.1111/j.1475-2743.2010.00293.x, 2010.
- Gamble, T. N., Betlach, M. R., and Tiedje, J. M.: Numerically dominant denitrifying bacteria from world soils, Appl. Environ. Microbiol., 33(4), 926–939, 1977.

585 Garnier, S.: viridisLite: Default Color Maps from “matplotlib” (Lite Version), R package version 0.3.0, <https://cran.r-project.org/package=viridisLite>, 2018.

Goering, H. K. and Van Soest, P. J.: Forage fiber analyses, Agric. Handb. No. 379, (379), 12–20, 1970.

Griffiths, R. I., Whiteley, A. S., Anthony, G., Donnell, O., Bailey, M. J., Griffiths, R. I., Whiteley, A. S., and Donnell, A. G. O.: Rapid Method for Coextraction of DNA and RNA from Natural Environments for Analysis of Ribosomal DNA- and
590 rRNA-Based Microbial Community Composition, Appl. Environ. Microbiol. 66(12), 1–5, doi:10.1128/AEM.66.12.5488-5491.2000.Updated, 2000.

Van Groenigen, J. W., Huygens, D., Boeckx, P., Kuypers, T. W., Lubbers, I. M., Rütting, T., and Groffman, P. M.: The soil N cycle: New insights and key challenges, Soil, 1(1), 235–256, doi:10.5194/soil-1-235-2015, 2015.

Hayatsu, M., Tago, K., and Saito, M.: Various players in the nitrogen cycle: Diversity and functions of the microorganisms
595 involved in nitrification and denitrification, Soil Sci. Plant Nutr., 54(1), 33–45, doi:10.1111/j.1747-0765.2007.00195.x, 2008.

Heiberger, R. M.: HH: Statistical Analysis and Data Display: Heiberger and Holland, R package version 3.1-35, <https://cran.r-project.org/package=HH>, 2018.

Höper, H.: Carbon and nitrogen mineralisation rates of fens in Germany used for agriculture, in Wetlands in Central Europe,
600 edited by Broll G., M. W., and P. EM., pp. 149–164, Springer, Berlin, Heidelberg., 2002.

Houghton, K. M., Morgan, X. C., Lagutin, K., Mackenzie, A. D., Vyssotskii, M., Mitchell, K. A., McDonald, I. R., Morgan, H. W., Power, J. F., Moreau, J. W., Hanssen, E., and Stott, M. B.: *Thermorudis pharmacophila* sp. Nov., a novel member of the class *Thermomicrobia* isolated from geothermal soil, and emended descriptions of *Thermomicrobium roseum*, *Thermomicrobium carboxidum*, *Thermorudis peleae* and *Sphaerobacter thermophilus*, Int. J. Syst. Evol. Microbiol.,
605 65(12), 4479–4487, doi:10.1099/ijsem.0.000598, 2015.

Hu, X., Liu, L., Zhu, B., Du, E., Hu, X., Li, P., Zhou, Z., Ji, C., Zhu, J., Shen, H., and Fang, J.: Asynchronous responses of soil carbon dioxide, nitrous oxide emissions and net nitrogen mineralization to enhanced fine root input, Soil Biol. Biochem., 92, 67–78, doi:10.1016/j.soilbio.2015.09.019, 2016.

Huang, Y., Zou, J., Zheng, X., Wang, Y., and Xu, X.: Nitrous oxide emissions as influenced by amendment of plant residues
610 with different C:N ratios, Soil Biol. Biochem., 36(6), 973–981, doi:10.1016/j.soilbio.2004.02.009, 2004.

Hutchinson, G. and Mosier, A.: Improved soil cover method for field measurement of nitrous oxide fluxes, Soil Sci. Soc. Am. J., 45, 311–316, 1981.

Jenkinson, D. S.: Studies on the Decomposition Of Plant Material in Soil. I. Losses of Carbon from ¹⁴C labelled Ryegrass incubated with soil in the field, J. Soil Sci., 16(1), 104–115, doi:10.1111/j.1365-2389.1965.tb01424.x, 1965.

615 Jensen, L. S., Salo, T., Palmason, F., Breland, T. A., Henriksen, T. M., Stenberg, B., Pedersen, A., Lundström, C., and Esala, M.: Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil, Plant Soil, 273(1–2), 307–326, doi:10.1007/s11104-004-8128-y, 2005.

Jin, K., Sleutel, S., De Neve, S., Gabriels, D., Cai, D., Jin, J., and Hofman, G.: Nitrogen and carbon mineralization of surface-applied and incorporated winter wheat and peanut residues, *Biol. Fertil. Soils*, 44(4), 661–665, doi:10.1007/s00374-008-0267-5, 2008.

Johnson, J. M.-F., Barbour, N. W., and Weyers, S. L.: Chemical Composition of Crop Biomass Impacts Its Decomposition, *Soil Sci. Soc. Am. J.*, 71(1), 155, doi:10.2136/sssaj2005.0419, 2007.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F. O.: Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies., *Nucleic Acids Res.*, 41(1), 1–11, doi:10.1093/nar/gks808, 2013.

Koch, H., Lückner, S., Albertsen, M., Kitzinger, K., Herbold, C., Spieck, E., Nielsen, P. H., Wagner, M., and Daims, H.: Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus *Nitrospira*, *PNAS*, 112, 11371–11376, doi:10.1073/pnas.1506533112, 2015.

Kögel-Knabner, I.: The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter, *Soil Biol. Biochem.*, 34, 139–162, doi:10.1016/S0038-0717(01)00158-4, 2002.

Kravchenko, A. N., Toosi, E. R., Guber, A. K., Ostrom, N. E., Yu, J., Azeem, K., Rivers, M. L., and Robertson, G. P.: Hotspots of soil N₂O emission enhanced through water absorption by plant residue, *Nat. Geosci.*, 10, 496–500, doi:10.1038/NGEO2963, 2017.

Kravchenko, A. N., Fry, J. E., and Guber, A. K.: Water absorption capacity of soil-incorporated plant leaves can affect N₂O emissions and soil inorganic N concentrations, *Soil Biol. Biochem.*, 121, 113–119, doi:10.1016/j.soilbio.2018.03.013, 2018.

Kuzyakov, Y. and Domanski, G.: Carbon input by plants into the soil. Review, *J. Plant Nutr. Soil Sci.*, 163(4), 421–431, doi:10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R, 2000.

Ladd, J. N., Oades, J. M., and Amato, M.: Microbial biomass formed from ¹⁴C, ¹⁵N-labelled plant material decomposing in soils in the field, *Soil. Biol. Biochem.*, 13, 119–162, doi:10.1016/0038-0717(81)90007-9, 1981.

Legendre, P. and Gallagher, E. D.: Ecologically meaningful transformations for ordination of species data, *Oecologia*, 129(2), 271–280, doi:10.1007/s004420100716, 2001.

Lemon, J.: Plotrix: a package in the red light district of R, *R-News*, 6(4), 8–12, 2006.

Lenth, R.: Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.4, <https://cran.r-project.org/package=emmeans>, 2018.

Li, X., Sørensen, P., Olesen, J. E., and Petersen, S. O.: Evidence for denitrification as main source of N₂O emission from residue-amended soil, *Soil Biol. Biochem.*, 92, 153–160, doi:10.1016/j.soilbio.2015.10.008, 2016.

Li, X., Hu, F., and Shi, W.: Plant material addition affects soil nitrous oxide production differently between aerobic and oxygen-limited conditions, *Appl. Soil Ecol.*, 64, 91–98, doi:10.1016/j.apsoil.2012.10.003, 2013.

Loecke, T. D. and Robertson, G. P.: Soil resource heterogeneity in terms of litter aggregation promotes nitrous oxide fluxes and slows decomposition, *Soil Biol. Biochem.*, 41(2), 228–235, doi:10.1016/j.soilbio.2008.10.017, 2009.

- Lou, Y., Ren, L., Li, Z., Zhang, T., and Inubushi, K.: Effect of Rice Residues on Carbon Dioxide and Nitrous Oxide Emissions from a Paddy Soil of Subtropical China, *Water. Air. Soil Pollut.*, 178, 157–168, doi:10.1007/s11270-006-9187-x, 2007.
- 655 Ludovici, K. H. and Kress, L. W.: Decomposition and nutrient release from fresh and dried pine roots under two fertilizer regimes, *Can. J. For. Res.*, 36(1), 105–111, doi:10.1139/X05-227, 2006.
- Lyngwi, N. A. and Joshi, S.: Economically important *Bacillus* and related genera: a mini review, in *Biology of Useful Plants and Microbes*, 33–43, 2014.
- Machinet, G. E., Bertrand, I., Chabbert, B., and Recous, S.: Decomposition in soil and chemical changes of maize roots with genetic variations affecting cell wall quality, *Eur. J. Soil Sci.*, 60(2), 176–185, doi:10.1111/j.1365-2389.2008.01109.x, 2009.
- 660 Machinet, G. E., Bertrand, I., Barrière, Y., Chabbert, B., and Recous, S.: Impact of plant cell wall network on biodegradation in soil: Role of lignin composition and phenolic acids in roots from 16 maize genotypes, *Soil Biol. Biochem.*, 43(7), 1544–1552, doi:10.1016/j.soilbio.2011.04.002, 2011.
- 665 Martin, M.: Cutadapt removes adapter sequences from high-throughput sequencing reads, *EMBnet.journal*, 17(1), 10–12, doi:10.14806/ej.17.1.200, 2013.
- Mary, B., Fresneau, C., Morel, J. L., and Mariotti, A.: C and N cycling during decomposition of root mucilage, roots and glucose in soil, *Soil Biol. Biochem.*, 25(8), 1005–1014, doi:10.1016/0038-0717(93)90147-4, 1993.
- Megonigal, J. P., Hines, M. E., and Visscher, P. T.: Anaerobic Metabolism: Linkages to Trace Gases and Aerobic Processes, in *Treatise on Geochemistry: Second Edition*, vol. 10, 273–359., 2013.
- 670 de Menezes, A. B., McDonald, J. E., Allison, H. E., and McCarthy, A. J.: Importance of *Micromonospora* spp. as colonizers of cellulose in freshwater lakes as demonstrated by quantitative reverse transcriptase PCR of 16s rRNA, *Appl. Environ. Microbiol.*, 78(9), 3495–3499, doi:10.1128/AEM.07314-11, 2012.
- Millar, N. and Baggs, E. M.: Chemical composition, or quality, of agroforestry residues influences N₂O emissions after their addition to soil, *Soil Biol. Biochem.*, 36, 935–943, doi:10.1016/j.soilbio.2004.02.008, 2004.
- 675 Millar, N. and Baggs, E. M.: Relationships between N₂O emissions and water-soluble C and N contents of agroforestry residues after their addition to soil, *Soil Biol. Biochem.*, 37, 605–608, doi:10.1016/j.soilbio.2004.08.016, 2005.
- Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C., and Trevors, J. T.: Crop residue influence on denitrification, N₂O emissions and denitrifier community abundance in soil, *Soil Biol. Biochem.*, 40(10), 2553–2562, doi:10.1016/j.soilbio.2008.06.024, 2008.
- 680 Müller, C. and Clough, T. J.: Advances in understanding nitrogen flows and transformations: Gaps and research pathways, *J. Agric. Sci.*, 152, S34–S44, doi:10.1017/S0021859613000610, 2014.
- Nolan, M., Tindall, B. J., Pomrenke, H., Lapidus, A., Copeland, A., Del Rio, T. G., Lucas, S., Chen, F., Tice, H., Chen, J.-F., Saunders, E., Han, C., Bruce, D., Goodwin, L., Chain, P., Pitluck, S., Ovchinnikova, G., Pati, A., Ivanova, N., Mavromatis, K., Chen, A., Palaniappan, K., Land, M., Hauser, L., Chang, Y.-J., Jeffries, C. D., Brettin, T., Göker, M.,

Bristow, J., Eisen, J. A., Markowitz, V., Hugenholtz, P., Kyrpides, N. C., Klenk, H.-P., and Detter, J. C.: Complete genome sequence of *Rhodothermus marinus*, Stand. Genomic Sci., 1, 283–291, doi:10.4056.sigs46736, 2009.

Novoa, R. S. A. and Tejeda, H. R.: Evaluation of the N₂O emissions from N in plant residues as affected by environmental and management factors, Nutr. Cycl. Agroecosystems, 75(1–3), 29–46, doi:10.1007/s10705-006-9009-y, 2006.

690 Okada, N., Nomura, N., Nakajima-Kambe, T., and Uchiyama, H.: Characterization of the Aerobic Denitrification in *Mesorhizobium* sp. Strain NH-14 in Comparison with that in Related Rhizobia, Microbes Environ., 20(4), 208–215, doi: 10.1264/jsme2.20.208, 2005.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., and Wagner, H.: vegan: Community Ecology Package, R Package version 2.3-2, <https://cran.r-project.org/web/packages/vegan/index.html>, 2015.

695 Papen, H., von Berg, R., Hinkel, I., Thoene, B., and Rennenberg, H.: Heterotrophic Nitrification by *Alcaligenes faecalis*: NO₂⁻, NO₃⁻, N₂O, and NO Production in Exponentially Growing Cultures, Appl. Environ. Microbiol., 55(8), 2068–2072, 1989.

Pascual, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., Mathieu, O., Lévêque, J., Mougél, C., 700 Henault, C., Lemanceau, P., Péan, M., Boiry, S., Fontaine, S., and Maron, P. A.: Stimulation of Different Functional Groups of Bacteria by Various Plant Residues as a Driver of Soil Priming Effect, Ecosystems, 16(5), 810–822, doi:10.1007/s10021-013-9650-7, 2013.

Paterson, E. and Sim, A.: Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation, Plant Soil, 216(1–2), 155–164, doi:10.1023/A:1004789407065, 1999.

705 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team: nlme: Linear and Nonlinear Mixed Effects Models, R package version 3.1-140, <https://cran.r-project.org/package=nlme>, 2017.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools, Nucleic Acids Res., 41(D1), 590–596, doi:10.1093/nar/gks1219, 2013.

710 R Core Team: R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>, 2018.

R Core Team: R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>, 2019.

Redin, M., Guénon, R., Recous, S., Schmatz, R., de Freitas, L. L., Aita, C., and Giacomini, S. J.: Carbon mineralization in soil of roots from twenty crop species, as affected by their chemical composition and botanical family, Plant Soil, 378, 205–214, doi:10.1007/s11104-013-2021-5, 2014.

715 Rivas, R., Trujillo, M. E., Mateos, P. F., Martínez-Molina, E., and Velázquez, E.: *Agromyces ulmi* sp. nov., xylanolytic bacterium isolated from *Ulmus nigra* in Spain, Int. J. Syst. Evol. Microbiol., 54(6), 1987–1990, doi:10.1099/ijs.0.63058-0, 2004.

- Robertson, G. P. and Groffman, P. M.: Nitrogen Transformations, in *Soil microbiology, ecology and biochemistry*, edited by E. A. Paul, pp. 421–446, Academic Press, Burlington, Massachusetts, USA., 2015.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F.: VSEARCH: a versatile open source tool for metagenomics, *PeerJ*, 4, e2584, doi:10.7717/peerj.2584, 2016.
- Rousk, J. and Bååth, E.: Fungal and bacterial growth in soil with plant materials of different C/N ratios, *FEMS Microbiol. Ecol.*, 62(3), 258–267, doi:10.1111/j.1574-6941.2007.00398.x, 2007.
- Silver, W. L. and Miya, R. K.: Global patterns in root decomposition: Comparisons of climate and litter quality effects, *Oecologia*, 129(3), 407–419, doi:10.1007/s004420100740, 2001.
- Soetaert, K.: plot3D: Plotting Multi-Dimensional Data, R package version 1.1.1, <https://cran.r-project.org/package=plot3D>, 2017.
- Toju, H., Tanabe, A. S., Yamamoto, S., and Sato, H.: High-coverage ITS primers for the DNA-based identification of Ascomycetes and Basidiomycetes in environmental samples, *PLoS One*, 7(7), doi:10.1371/journal.pone.0040863, 2012.
- Truong, T. H. H. and Marschner, P.: Respiration, available N and microbial biomass N in soil amended with mixes of organic materials differing in C/N ratio and decomposition stage, *Geoderma*, 319, 167-174, doi:10.1016/j.geoderma.2018.01.012, 2018.
- VDLUFA, Ed.: 6.5.2 Bestimmung der Säure-Detergentien-Faser (ADF), in *Methodenbuch Band III Die chemische Untersuchung von Futtermitteln*, pp. 1–4, VDLUFA-Verlag, Darmstadt., 2011.
- VDLUFA, Ed.: 6.5.1 Bestimmung der neutral-Detergentien-Faser (NDF), in *Methodenbuch Band III Die chemische Untersuchung von Futtermitteln*, pp. 1–4, VDLUFA-Verlag, Darmstadt., 2012a.
- VDLUFA, Ed.: 6.5.3 Bestimmung des Säure-Detergentien-Lignings (“Rohlignin”), in *Methodenbuch Band III Die chemische Untersuchung von Futtermitteln*, pp. 1–3, VDLUFA-Verlag, Darmstadt., 2012b.
- Velthof, G. L., Kuikman, P. J., and Oenema, O.: Nitrous oxide emission from soils amended with crop residues, *Nutr. Cycl. Agroecosystems*, 62, 249–261, doi:10.1023/A:1021259107244, 2002.
- Verbaandert, I., De Vos, P., Boon, N., and Heylen, K.: Denitrification in Gram-positive bacteria: an underexplored trait, *Biochem. Soc. Trans.*, 39(1), 254–258, doi:10.1042/bst0390254, 2011.
- Wang, Y., Naumann, U., Eddelbuettel, E., Wilshire, J., Warton, D., Byrnes, J., dos Santos Silva, R., Niku, J., Renner, I., and Wright, S.: mvabund: Statistical Methods for Analysing Multivariate Abundance Data, R package version 4.0.1, <https://cran.r-project.org/web/packages/mvabund/index.html>, 2019.
- Wrage-Mönnig, N., Horn, M. A., Well, R., Müller, C., Velthof, G., and Oenema, O.: The role of nitrifier denitrification in the production of nitrous oxide revisited, *Soil Biol. Biochem.*, 123, A3–A16, doi:10.1016/j.soilbio.2018.03.020, 2018.
- Wrage, N., Velthof, G. L., van Beusichem, M. L., and Oenema, O.: The role of nitrifier denitrification in the production of nitrous oxide, *Soil Biol. Biochem.*, 33, 1723–1732, doi:10.1016/S0038-0717(01)00096-7, 2001.

- Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., and Chu, H.: Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition, *Soil Biol. Biochem.*, 92, 41–49, doi:10.1016/j.soilbio.2015.09.018, 2016.
- 755 Zgurskaya, H. I., Evtushenko, L. I., Akimov, V. N., Voyevoda, H. V., Dobrovolskaya, T. G., Lysak, L. V., and Kalakoutskii, L. V.: Emended Description of the Genus *Agromyces* and Description of *Agromyces cerinus* subsp. *cerinus* sp. nov., subsp. nov., *Agromyces cerinus* subsp. *nitratus* sp. nov., subsp. nov., *Agromyces fucosus* subsp. *fucosus* sp. nov., subsp. nov., and *Agromyces fucosus* subsp. *hippuratus* sp. nov., subsp. nov., *Int. J. Syst. Bacteriol.*, 42(4), 635–641, doi:10.1099/00207713-42-4-635, 1992.
- 760 Zhang, D., Hui, D., Luo, Y., and Zhou, G.: Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors, *J. Plant Ecol.*, 1(2), 85–93, doi:10.1093/jpe/rtn002, 2008.
- Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A.: PEAR: A fast and accurate Illumina Paired-End reAd mergeR, *Bioinformatics*, 30(5), 614–620, doi:10.1093/bioinformatics/btt593, 2014.
- Zhang, J., Müller, C., and Cai, Z.: Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils, *Soil Biol. Biochem.*, 84, 199–209, doi:10.1016/j.soilbio.2015.02.028, 2015.
- 765 Zhang, X. and Wang, W.: The decomposition of fine and coarse roots: Their global patterns and controlling factors, *Sci. Rep.*, 5, 1–10, doi:10.1038/srep09940, 2015.
- Zumft, W.: Cell biology and molecular basis of denitrification, *Microbiol. Molecular Biol. Rev.*, 61(4), 533–616, 1997.

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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 (FM = fresh matter).

N level	N_{\min}	Treatment	Litter input	C input	N input
	$[\mu\text{g NO}_3^- \text{-N kg}^{-1} \text{ dry soil}]$		$[\text{mg FM kg}^{-1} \text{ dry soil}]$	$[\text{mg C kg}^{-1} \text{ dry soil}]$	$[\text{mg N kg}^{-1} \text{ dry soil}]$
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

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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 μg root FM kg^{-1} dry soil, Root+Shoot = 100 μg root FM + 100 μg shoot FM kg^{-1} dry soil).

N level	Treatment	N ₂ O		N ₂ O		CO ₂		CO ₂		N ₂ O/CO ₂ ratio
		[μg N ₂ O-N kg^{-1} dry soil]		[μg N ₂ O-N mg^{-1} C input]		[μg CO ₂ -C kg^{-1} dry soil]		[μg CO ₂ -C mg^{-1} C input]		
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 at $p \leq 0.05$.

Formatiert: Nicht Hochgestellt/ Tiefgestellt

Formatiert: Nicht Hochgestellt/ Tiefgestellt

Table 4: N mineralization during the incubation period.

N level	Treatment	N mineralized during incubation [µmg N kg ⁻¹ dry soil]	
N1	Control	7.61 ± 0.98	b
	Root	1.44 ± 0.72	a
	Root+Shoot	14.32 ± 2.66	c
N2	Control	10.08 ± 1.76	b
	Root	1.10 ± 0.68	a
	Root+Shoot	14.14 ± 4.83	c

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 at $p \leq 0.05$.

Table 5: Significance of fixed effects of soil NO₃⁻-N (μg NO₃⁻-N kg⁻¹), water extractable organic C (WEOC, μg C kg⁻¹) and first-order interaction on N₂O fluxes (μg N₂O-N kg⁻¹ h⁻¹; ln transformed) using linear mixed effect model.

	Estimate	Standard error	p value
Intercept	-0.21814237	0.12682074	0.0416860
NO ₃ ⁻ -N	-0.0043152	0.0165248	0.79305401
WEOC	0.0094240	0.005377	0.0770965
NO ₃ ⁻ -N : WEOC	0.0023	0.000811	0.0024394

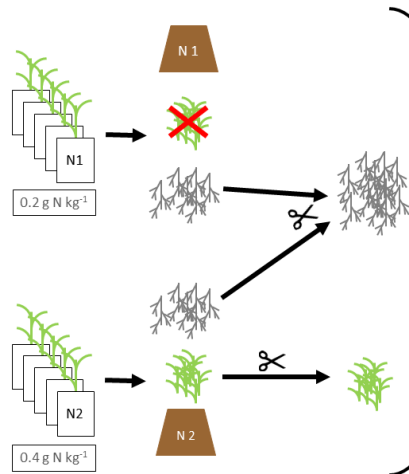
Table 6: Results of regression analyses of the relationship between total cumulative CO₂ emissions and C quality parameters of plant litter (AICc = Akaike’s information criterion).

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 7: 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

Plant cultivation 8 weeks



Incubation experiment 22 days

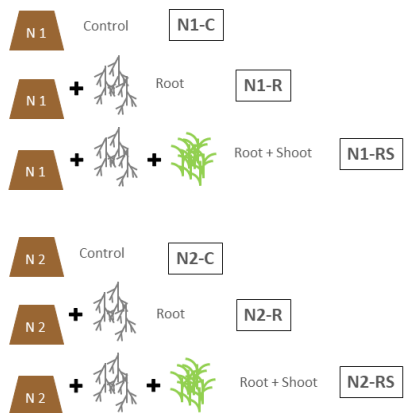


Figure 1: Preparation and experimental setup of the incubation experiment. N1 (0.2 g N kg⁻¹) and N2 (2 x 0.2 g N kg⁻¹) referring to the N levels during plant growth. Control soil (N1-C and N2-C) without addition of plant litter. Root treatment with addition of 100 g fresh root biomass per kg dry soil (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).

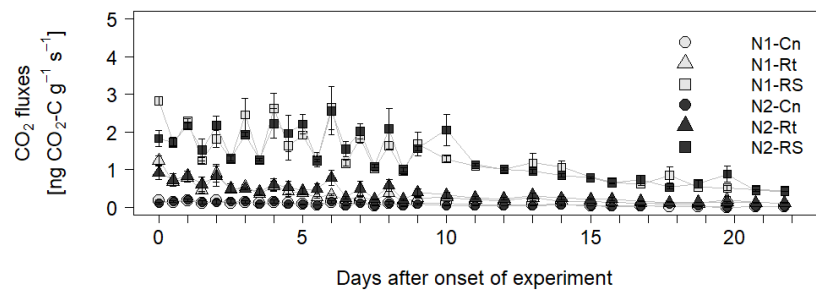
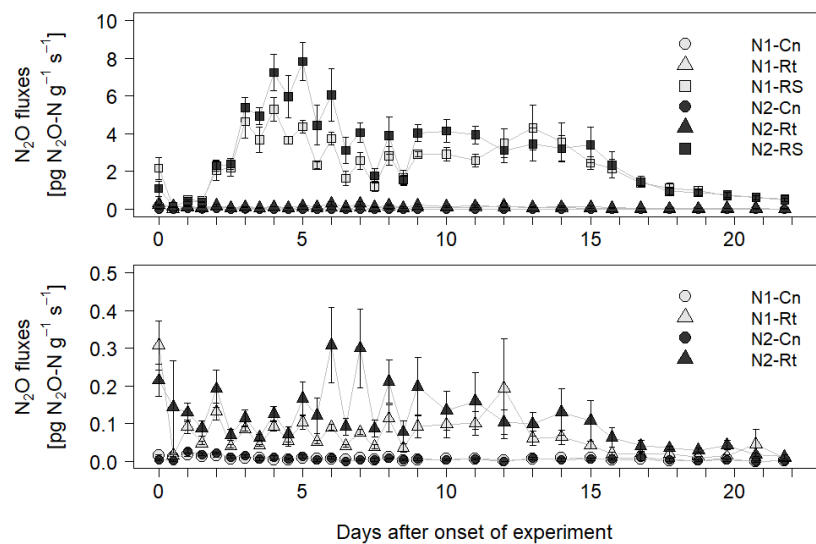
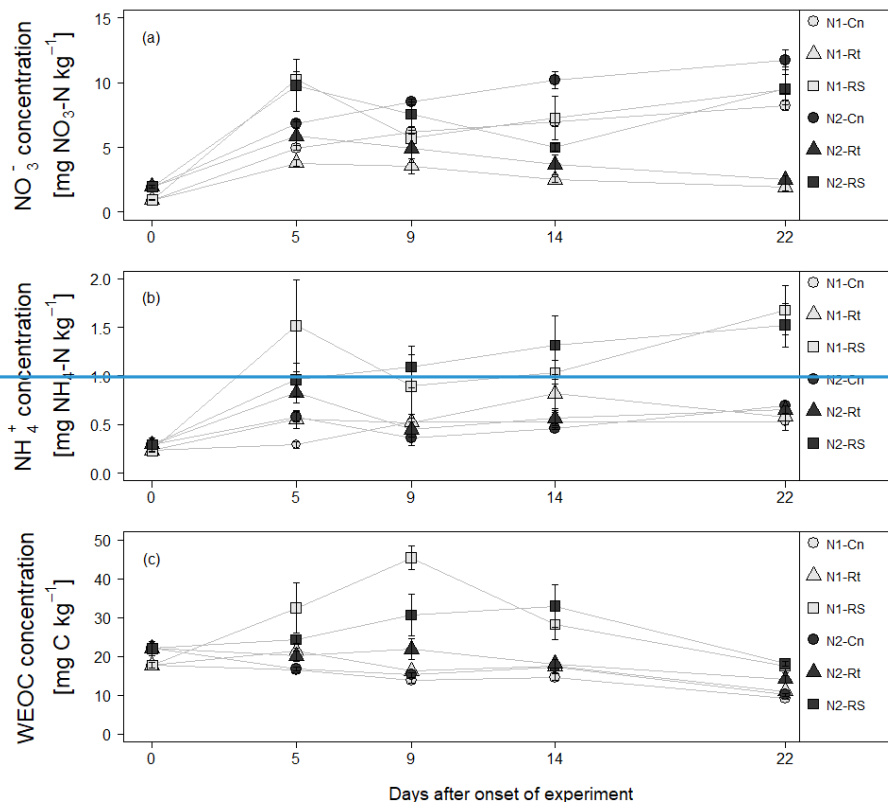


Figure 2: CO₂ fluxes from soils with two N levels (N1, N2) after incorporation of maize root litter (-Rt), maize root + shoot litter (-RS) and control (-Cn) without litter. Error bars show standard error of mean values (n = 4). When not visible, error bars are smaller than the symbols.



810 **Figure 3 a+b:** N₂O fluxes 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.

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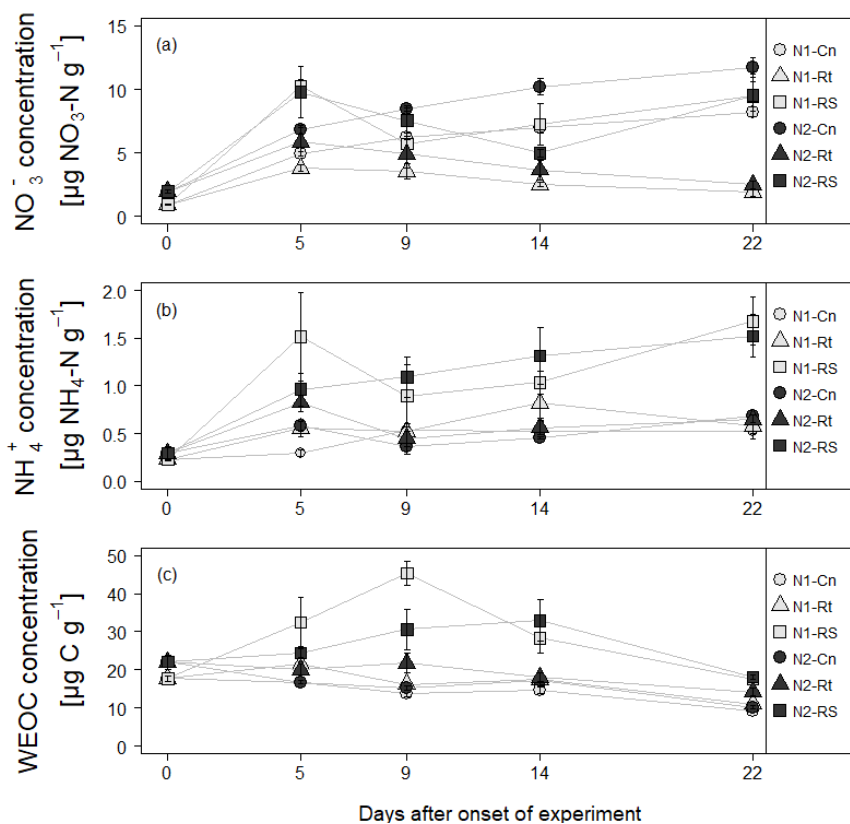
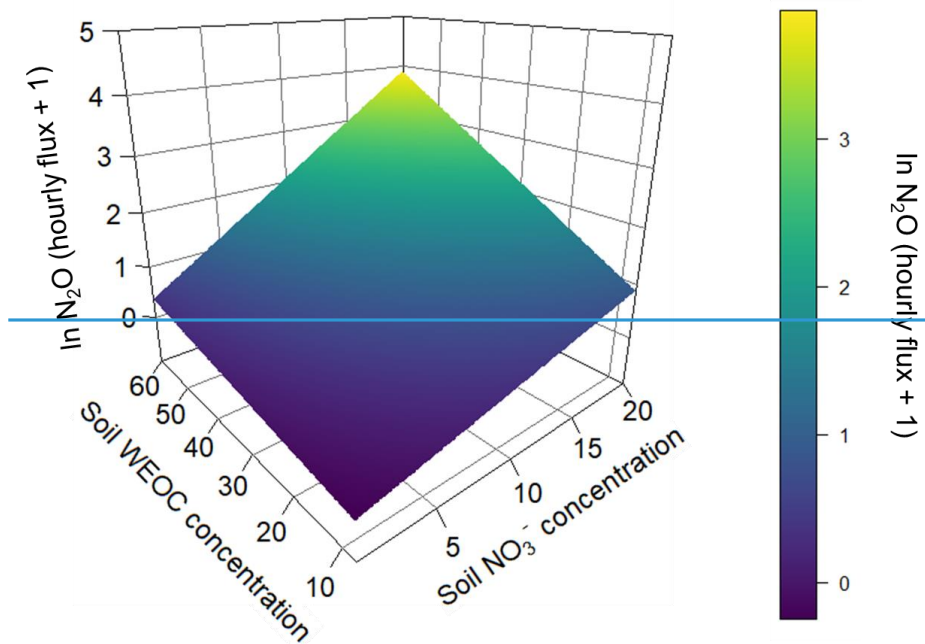


Figure 4 a-c: NO_3^- , WEOC, and NH_4^+ concentration from soils with two N levels (N1, N2) after incorporation of maize root litter (-Rt), maize root + shoot litter (-RS) and control (-Cn) without litter. Error bars show standard error of mean values (n = 4) (day 0: n = 3). When not visible, error bars are smaller than the symbols.



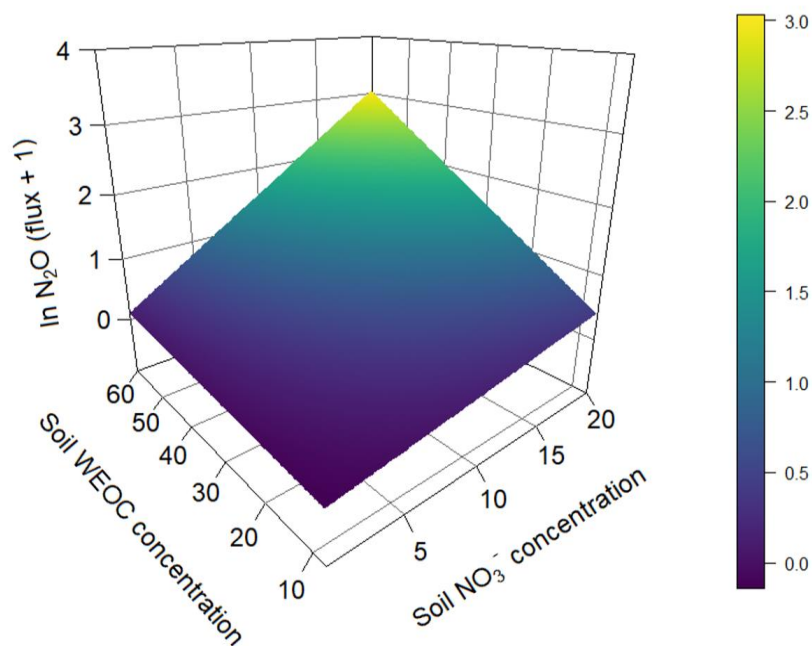


Figure 5: Prediction of N₂O fluxes ($\mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$) (ln transformed) based on soil NO₃⁻ ($\mu\text{g N g}^{-1}$) and water extractable C_{org} ($\mu\text{g C g}^{-1}$) concentrations based on linear mixed-effect model (Pseudo-R²=0.829).

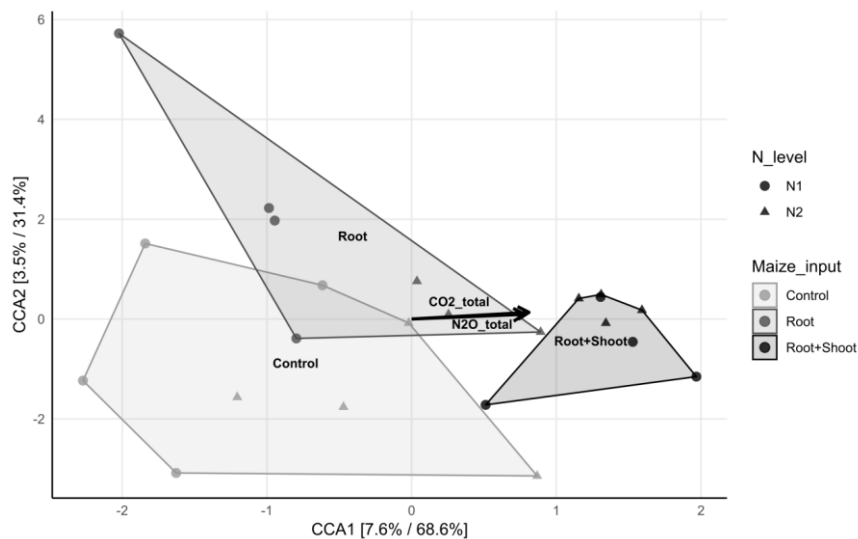


Figure 6: Canonical Correspondence Analysis (CCA) displaying the compositional distribution of the soil inhabiting bacterial communities between the control (N1-C and N2-C; n=4), root (N1-R and N2-R; n=4 and n=3) and root + shoot treatment (N1-RS and N2-RS; n=4). Significant correlations of total CO₂ 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.

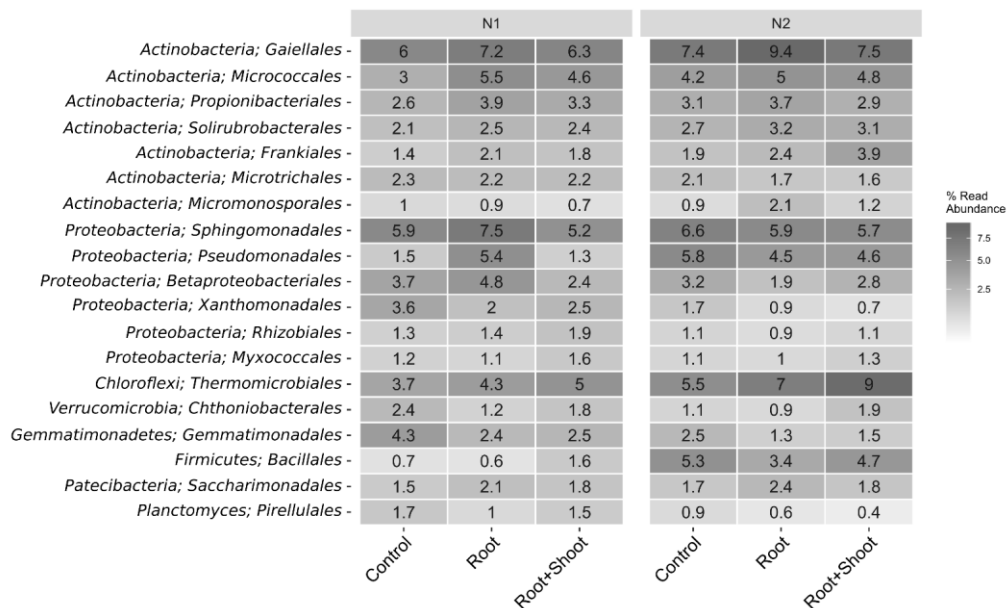


Figure 7: Heatmap of the 16 most abundant bacterial orders of the soil inhabiting bacterial community grouped by N levels and litter input (n=4, except for N2 Root: n=3).