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

Pauline Sophie Rummel¹, Birgit Pfeiffer^{1,2}, Johanna Pausch³, Reinhard Well⁴, Dominik Schneider², Klaus Dittert¹

5 ¹Division of Plant Nutrition and Crop Physiology, Department of Crop Science, University of Göttingen, Germany ²Institute of Microbiology and Genetics, Dept. of Genomic and Applied Microbiology, University of Göttingen, Germany ³Agroecology, Faculty for Biology, Chemistry, and Earth Sciences, University of Bayreuth, Germany ⁴Thünen Institute, Climate-Smart Agriculture, Braunschweig, Germany

Correspondence to: Pauline Sophie Rummel (pauline.rummel@uni-goettingen.de)

- 10 Abstract. Chemical composition of root and shoot litter controls decomposition and, subsequently, C availability for biological nitrogen transformation processes in soils. While above ground plant residues have been proven to increase N_2O 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.

20

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 NO_3^- and WEOC availability and decomposition related CO_2 emissions. Emissions induced by maize root litter remained low, while high bioavailability of maize shoot litter strongly increased CO_2 and N_2O 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
- 25 microsites. The interdependency of C and N availability was validated by analyses of regression. Moreover, there was a strong positive interaction between soil NO_3^- and WEOC concentration resulting in much higher N_2O 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 30 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)

- 35 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).
- 40 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
- 50 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
- 55 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 *Bacteriodetes* are described as
- 60 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
- 65 (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. 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 homogenously 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).
- 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 extend 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 differences in N₂O emissions between treatments can be related to degradability of maize litter with easier
- 80 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 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 adaptions to litter input.

90 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.

95 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

100 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 $(0.2 \text{ g N kg}^{-1} \text{ as } Ca(NO_3)_2 * 4 \text{ H}_2\text{O})$ was applied to half of the pots six weeks after sowing. Soil with one N dose is referred to as N1 $(0.2 \text{ g N kg}^{-1})$ 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

105 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 H_2O_{bidest} (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.

- 110 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
- 115 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 (Control = Cn, Root = Rt, Root+Shoot = RS) (see Table 1 and Figure 1 for details). To allow comparison of litter treatments

- 120 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 small root fragments was calculated as the difference in soil C concentration before and after maize growth. For the root
- 125 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 analysis of mineral N, water extractable C_{org} concentration, and total soil C. Soil mineral N concentrations were 0.93 and

130 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,

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

- 135 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 at 25 °C, 8 h night at 19 °C) for 22 days. Volumetric water content (VWC) sensors (EC-5, Decagon Devices, Pullman, USA) 140 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 145 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
- 150 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 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 155 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, 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 160 determined with a parallel set of samples. Net N mineralization was calculated as the difference between the NH_4^+ -N + NO_3^- N concentrations at the start and end of the incubation period plus N lost as N₂O-N (Eq. 1).

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

WEOC was determined according to Chantigny et al. (2007). Briefly, fresh soil was homogenized with deionized water

(1)

165 (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

- 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
- 185 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.

190 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

- 195 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
- 200 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 wth Hellinger transformed data (Legendre and Gallagher, 2001), ASV's with a relative abundance lower than 0.1 % in any sample were removed. Correlations of
- 205 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
- 210 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

215 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 N_2O emissions including the factors cumulative CO_2 emissions, initial soil NO_3^- concentration, and net N mineralization during incubation period. For cumulative CO_2 emissions, regression models included the factors total C input, water-extractable C input, hemicellulose fraction, cellulose fraction, and lignin fraction from all litter

230 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

235 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

255 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 \leq

 $0.04 \text{ pg N}_2\text{O-N g}^{-1} \text{ s}^{-1}$, 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,

260 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 (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 N_2O emissions by roughly 100-times compared to

(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 NO_3^- , NH_4^+ 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) 270 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, Table 4) during the incubation experiment. In treatments with litter, soil NO₃⁻ concentrations decreased 275 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 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 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 1.10 280 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.

285 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<0.0394, Pseudo-R²=0.8888, Table 5), and incubation vessel and sampling time as random parameters. Predictions of N₂O fluxes based on this model are shown in (Fig. 5).

Linear regression analyses were used to identify relations between cumulative CO2 and N2O emissions, litter quality, and N

290 parameters. Either hemicellulose + cellulose fraction or water-extractable C fraction of plant litter explained more than 96 % of variance of total cumulative CO₂ emissions ($p<2.2e^{-16}$) (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 ($R^2=0.9362$, $p<7.632 e^{-15}$) (Table 7), and between cumulative N₂O emissions and mineralized N ($R^2=0.5791$, $p<9.551 e^{-06}$), while initial soil NO₃⁻ concentration did not explain any variance.

295 **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).

- 300 The canonical correspondence analysis revealed a significant correlation (p<0.001) of the bacterial community composition with total CO₂ (R²=0.6758) and N₂O (R²=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
- 305 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

- 310 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
- 315 *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 micorbiome (Tables S6 and S8). Overall, 80 genera represented the core microbiome, when grouped by N levels, while 21 genera and 6 genera were
- 320 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

Devosia for N1 and *Conexibacter* for N2 samples (Table S. 8). 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). When grouped by litter treatment, the core microbiome comprised 77 genera accounting

325 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 *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

330 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 identified. Overall, the bacterial community composition was significantly influenced by N-level (p=0.005) and maize litter treatment (p=0.033).

335

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 NO₃⁻ concentrations. Availability of easily degradable compounds was also reflected by strongly increased CO₂ 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

350 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 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 NO3⁻ concentrations especially when C:N ratios are low (Li et al., 2013;

- 355 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
- 360 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 (R²=0.966, p< 2.2*e⁻¹⁶) (Table 6).

365 4.2 N₂O 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 N₂O and CO₂ 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). CO₂ fluxes increased directly with onset of incubation and started to decline after day 10, thus mostly C

- 370 compounds with a short turnover time, i.e. sugars, proteins, starch, and hemicellulose were decomposed and contributed to CO₂ fluxes. Availability of easily degradable C compounds stimulates microbial respiration, limiting O₂ at the microsite level and thus increasing N₂O emissions from denitrification (Azam et al., 2002; Chen et al., 2013; Miller et al., 2008). Accordingly, N₂O fluxes increased after a lag phase of two days. The strong positive correlation (R²=0.9362, p \leq 7.632 e⁻¹⁵) between cumulative CO₂ and N₂O emissions (Table 7) further supports our hypothesis that litter quality, in particular
- 375 degradability 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 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, N₂O is mainly produced in the interface of aerobic and anaerobic

- 380 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 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 (R²=0.5791, p<9.551 e⁻⁰⁶) 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 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 (Kuzyakov 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 405 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).
- 410 The interdependency of C and N availability was further validated by analyses of regression highlighting a strong positive interaction between soil NO_3^- and WEOC concentrations resulting in much higher N_2O emissions only when both NO_3^- 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 N_2O formation.

Variation in N₂O emissions is often related to quality parameters of plant residues, mostly the C:N ratio (Baggs et al., 2000;

- 415 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
- 420 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, CO_2 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 -

- 425 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
 420 between N₂O formation pathways
- 430 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
- 450 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).

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

- 455 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 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*
- 460 (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 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 under conditions of very low mineral nitrogen availability in soil. This was further validated as *Nitrospirae* (*Nitrospirae*), known to oxidize nitrite (Koch et al., 2015), was identified as a responder for N1 and -RS. The reduction of nitrate has been

- 470 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 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
- 475 microbiome but were more abundant in N2 samples. The genus *Opitutus* was identified as responder to -Cn and comprises the bacterium *Optitutus 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 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.

480 **5 Conclusions**

We examined CO_2 and N_2O 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 N_2O emissions when both C and NO_3^- were available. Coupled nitrificationdenitrification and heterotrophic nitrification presumably contributed to N_2O formation. Maize root litter was characterized

- 485 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*.
- 490 **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.

495

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.

500 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.

505 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
- 510 *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
 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

- 520 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.
- 525 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

- 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
- 535 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

- 540 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.
- 545 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., Nouioui, I., Sangal, V., Meier-Kolthoff, J. P., Trujillo, M. E., Montero-Calasanz, M. D. C., Sahin, N., Smith, D.

550 L., Kim, K. E., Peluso, P., Deshpande, S., Woyke, T., Shapiro, N., Kyrpides, 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.
- 555 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

- 560 '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 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 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–
- 575 1364, doi:10.1890/05-1839, 2016.

565

- 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.
- 580 Garnier, S.: viridisLite: Default Color Maps from "matplotlib" (Lite Version), R package version 0.3.0, https://cran.rproject.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

- 585 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., Kuyper, 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
 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, 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.,
- 600

605

615

- 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 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.
- 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

- 620 Res., 41(1), 1–11, doi:10.1093/nar/gks808, 2013.
 - Koch, H., Lücker, 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 gens *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,

625 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
- 630 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 Earginal Means, aka Least-Squares Means. R package version 1.3.4, https://cran.rproject.org/package=emmeans, 2018.

- 640
 - 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.
- 645 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.
- 650 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
- 655 genetic variations affecting cell wall quality, Eur. J. Soil Sci., 60(2), 176–185, doi:10.1111/j.1365-2389.2008.01109.x, 2009.
 - 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.
- 660 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.

- 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.
 - 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.
 - 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., Ovchinikova, 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.

- 685 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.
 - 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.

- Pascault, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., Mathieu, O., Lévêque, J., Mougel, C.,
- 695 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.
- 700 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.
- 705 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
- 710 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.
 - 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.

720

- 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.
- 725 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.
- 730 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.

- 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.
 - Verbaendert, 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.
- 740 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 denitrication in the production of nitrous oxide revisited, Soil Biol. Biochem., 123, A3–A16, doi:10.1016/j.soilbio.2018.03.020, 2018.
- 745 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.
- 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.

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.

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.

- 755 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.
 - 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.

Table 1: Two-factorial setup of the incubation experiment. Soil Nmin concentrations were measured directly before onset of the incubation experiment. C input in Control is765from 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} [mg NO ₃ ⁻ -N kg ⁻¹ dry soil]	Treatment	Litter 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

	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 2: Chemical characteristics of maize root and shoot litter used in the incubation experiment. Hemicellulose and770cellulose are expressed relative to lignin content.

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 + 100 g shoot FM kg⁻¹ dry soil).

N level	Treatment	N₂O [µg N₂O-N kg⁻¹]		N₂O [µg N₂O-N kg⁻¹ g⁻¹ C i	input]	CO ₂ [mg CO ₂ -C kg ⁻¹]		CO ₂ [mg CO ₂ -C kg ⁻¹ g ⁻¹	¹ C input]	N ₂ O/CO ₂ ratio [µg N mg ⁻¹ C]
N1	Control	10.21 ± 4.23	а	2.95 ± 1.22	а	141.89 ± 29.74	а	40.94 ± 8.58	а	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	С	314.25 ± 30.95	С	2287.23 ± 289.48	С	165.72 ± 20.97	С	1.91
N2	Control	11.35 ± 6.75	а	4.15 ± 2.47	а	129.44 ± 47.47	а	47.30 ± 17.35	а	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	С	409.82 ± 91.30	С	2361.19 ± 287.20	С	180.63 ± 21.97	С	2.25

Values represent means (n=4) \pm standard deviation. Different letters in the same column indicate a significant difference according to the Tukey's HSD post-hoc tests at p ≤ 0.05 .

N level	Treatment	N mineralized during incubation [mg N kg ⁻¹]				
N1	Control	7.61 ± 0.98	b			
	Root	1.44 ± 0.72	a			
	Root+Shoot	14.32 ± 2.66	с			
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) \pm standard deviation. Different letters in the same column indicate a significant 780 difference according to the Tukey's HSD post-hoc tests at p \leq 0.05.

	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
NO3 ⁻ -N : WEOC	0.0023	0.0011	0.0394

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

Regression model	residual standard error	degrees of freedom	adjusted R ²	p value	AICc
$CO_2 \sim total litter C input$	274.5	22	0.9213	7.65*e ⁻¹⁴	342.73
$CO_2 \sim$ 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
$CO_2 \sim$ 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 CO2 emissions and C quality785parameters of plant litter (AICc = Akaike's information criterion).

Regression model	residual standard error	degrees of freedom	f adjusted R ²	p value	AICc
$N_2O \sim CO_2$	593.9	22	0.9366	7.073*e ⁻¹⁵	379.78
$N_2O \sim initial \ soil \ NO_3^-$	2404	22	-0.03885	0.7119	446.89
N ₂ O ~ Mineralized N	2191	22	0.5791	9.551*e ⁻⁰⁶	425.21

Table 7: Results of regression analyses of the relationship between total cumulative N_2O emissions, total cumulative CO_2 emissions and N parameters of plant litter and soil (AIC = Akaike's information criterion).



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 100g fresh root biomass per kg dry soil (N1-R and N2-R) and Root + Shoot treatment with addition of 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.



Figure 3 a+b: N_2O 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.



810 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.



Figure 5: Prediction of N₂O fluxes (μ g N₂O-N kg⁻¹ h⁻¹) (In transformed) based on soil NO₃⁻ and water extractable C_{org} concentrations based on linear mixed-effect model (Pseudo-R²=0.89).



825 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 ≤ 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.

		N1			N2		
Actinobacteria; Gaiellales -	6	7.2	6.3	7.4	9.4	7.5	
Actinobacteria; Micrococcales -	3	5.5	4.6	4.2	5	4.8	
Actinobacteria; Propionibacteriales -	2.6	3.9	3.3	3.1	3.7	2.9	
Actinobacteria; Solirubrobacterales -	2.1	2.5	2.4	2.7	3.2	3.1	
Actinobacteria; Frankiales -	1.4	2.1	1.8	1.9	2.4	3.9	
Actinobacteria; Microtrichales -	2.3	2.2	2.2	2.1	1.7	1.6	
Actinobacteria; Micromonosporales -	1	0.9	0.7	0.9	2.1	1.2	% Read Abundance
Proteobacteria; Sphingomonadales -	5.9	7.5	5.2	6.6	5.9	5.7	7.5
Proteobacteria; Pseudomonadales -	1.5	5.4	1.3	5.8	4.5	4.6	5.0
Proteobacteria; Betaproteobacteriales -	3.7	4.8	2.4	3.2	1.9	2.8	- 2.5
Proteobacteria; Xanthomonadales -	3.6	2	2.5	1.7	0.9	0.7	
Proteobacteria; Rhizobiales -	1.3	1.4	1.9	1.1	0.9	1.1	
Proteobacteria; Myxococcales -	1.2	1.1	1.6	1.1	1	1.3	
Chloroflexi; Thermomicrobiales -	3.7	4.3	5	5.5	7	9	
Verrucomicrobia; Chthoniobacterales -	2.4	1.2	1.8	1.1	0.9	1.9	
Gemmatimonadetes; Gemmatimonadales -	4.3	2.4	2.5	2.5	1.3	1.5	
Firmicutes; Bacillales -	0.7	0.6	1.6	5.3	3.4	4.7	
Patecibacteria; Saccharimonadales -	1.5	2.1	1.8	1.7	2.4	1.8	
Planctomyces; Pirellulales -	1.7	1	1.5	0.9	0.6	0.4	
	control	800 ⁰	otshoot	control	Root	otshoot	

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).