



Potential denitrification stimulated by water-soluble organic carbon from plant residues during initial decomposition

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ABSTRACT

Denitrification usually takes place under anoxic conditions and over short periods of time, and depends on readily available nitrate and carbon sources. Variations in CO₂ and N₂O emissions associated with plant residues have mainly been explained by differences in their decomposability. A factor rarely considered so far is water-extractable organic matter (WEOM) released to the soil during residue decomposition. Here, we examined the potential effect of plant residues on denitrification with special emphasis on WEOM. A range of fresh and leached plant residues was characterized by elemental analyses, ¹³C-NMR spectroscopy, and extraction with ultrapure water. The obtained solutions were analyzed for the concentrations of organic carbon (OC) and organic nitrogen (ON), and by UV-VIS spectroscopy. To test the potential denitrification induced by plant residues or three different OM solutions, these carbon sources were added to soil suspensions and incubated for 24 h at 20 °C in the dark under anoxic conditions; KNO₃ was added to ensure unlimited nitrate supply. Evolving N₂O and CO₂ were analyzed by gas chromatography, and acetylene inhibition was used to determine denitrification and its product ratio. The production of all gases, as well as the molar (N₂O + N₂)-N/CO₂-C ratio, was directly related to the water-extractable OC (WEOC) content of the plant residues, and the WEOC increased with carboxylic/carbonyl C and decreasing OC/ON ratio of the plant residues. Incubation of OM solutions revealed that the molar (N₂O + N₂)-N/CO₂-C ratio and share of N₂O are influenced by the WEOM's chemical composition. In conclusion, our results emphasize the potential of WEOM in largely undecomposed plant residues to support short-term denitrification activity in a typical 'hot spot-hot moment' situation.

1. Introduction

Denitrification, the microbial reduction of nitrate (NO₃⁻) via a series of enzymatic steps to NO₂⁻, NO, N₂O, and finally N₂ (Philippot et al., 2007), results in net ecosystem losses of N and the release of climate-relevant N₂O and CO₂. Consequently, understanding the factors controlling denitrification and its N₂O/N₂ product ratio is crucial for developing strategies to minimize emissions of greenhouse gases.

In soil, denitrification mainly occurs in anoxic microhabitats ('hot spots') where sufficient nitrogenous oxides (NO₃⁻, NO₂⁻) as alternative electron acceptors and organic carbon (OC) as electron donor are bioavailable (e.g., Groffman et al., 2009). Those microsites are mostly

associated with particulate organic matter derived from farmyard manure or plant residues (e.g., Parkin, 1987; Parry et al., 2000). When comparing different plant residues, variations in mineralization rates and gas emissions were mostly explained by their chemical properties, such as C/N and lignin/N ratios (Aulakh et al., 1991; Vanlauwe et al., 1996; Chantigny et al., 2002; Lynch et al., 2016). A few studies, however, hinted at the possible role of soluble C compounds in the denitrification of residue-amended soils (e.g., deCatanzaro and Beauchamp, 1985; deCatanzaro et al., 1987). Soluble C is considered most effective in fueling denitrification over a period of a few days (e.g., Bremner and Shaw, 1958; Burford and Bremner, 1975). Despite evidence of large differences in release, chemical composition, and biodegradability of

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water-extractable organic matter (WEOM) from different litter materials (Kalbitz et al., 2003; Don and Kalbitz, 2005; Mastný et al., 2018), most previous studies only compared well-defined C compounds such as glucose, glycerol, and organic acids, for their potential to promote emissions of CO₂ and nitrogenous gases (e.g., Valera and Alexander, 1961; Jacobson and Alexander, 1980; Akunna et al., 1993). While WEOM leached from fresh aboveground plant residues is rich in easily degradable, low molecular-weight C compounds, such as simple sugars, amino acids, and proteins, WEOM derived from partly decomposed plant material and roots is enriched in less biodegradable high-molecular-weight and phenolic compounds (e.g., Marschner and Kalbitz, 2003; Clemente et al., 2013). Although leaching and subsequent degradation of soluble compounds have been recognized as initial steps of litter decomposition (Berg and McClaugherty, 2014) that may cause formation of anoxic microsites in soil (Kravchenko et al., 2017), possible effects of WEOM from different plant residues on potential denitrification have not been evaluated so far.

We hypothesized that plant residues of different composition and particle size provide differing amounts of water-extractable OC (WEOC) that fuel denitrifying organisms during short-term anoxic conditions. To address this hypothesis, we examined the effects of residues of various agriculturally important plant species (ryegrass, maize, and alfalfa) on potential denitrification and CO₂ production; including plant parts (roots, stems, and leaves) with a wide range in C/N ratios and varying in molecular composition (estimated by ¹³C nuclear magnetic resonance spectroscopy). In addition, we compared two different particle sizes and three leaching stages of ryegrass residues. Further, we expected that potential denitrification, its product ratio, and CO₂ production are not only controlled by the WEOC content but also by the quality, i.e. chemical composition, of WEOM. This was studied using WEOM solutions of equivalent C concentrations prepared from maize straw and ryegrass leaves, as well as maize root exudates.

2. Materials and methods

2.1. Test soil

In summer 2016, we collected topsoil (0–20 cm) material of a C-poor arable Haplic Luvisol with a silty loam texture (19% sand, 71% silt, and 10% clay) and a pH (CaCl₂) of 6.7 from a long-term trial site at Höhere Landbauschule Rotthalmünster, approximately 150 km east of Munich, Germany (latitude N48°21', longitude E13°11', elevation 360 m a.s.l.; Yamashita et al., 2006). The mean annual precipitation and temperature at the site is 890 mm and 8.2 °C, respectively (Ellerbrock and Kaiser, 2005). The soil was air-dried, and then sieved to <2 mm to remove coarse plant debris and stones. The test soil had 13.4 g OC kg⁻¹, 1.5 g organic nitrogen (ON) kg⁻¹, an OC/ON ratio of 9.0, 219.4 mg WEOC kg⁻¹, and 26.8 mg water-extractable ON (WEON) kg⁻¹, as well as 18.5 mg NO₃⁻-N kg⁻¹ and 2.0 mg NH₄⁺-N kg⁻¹ (extracted with 0.1 M KCl). To reactivate the microbial community of the soil, subsamples were rewetted to 50% water holding capacity and aerobically pre-incubated for 2 weeks at 20 °C in the dark before anoxic incubation.

2.2. Plant residues

In 2016, ryegrass plants (*Lolium perenne* L.), i.e., stems + leaves and roots, as well as maize straw (*Zea mays* L.) were collected from experimental fields (Reinshof and Relliehausen, respectively) of the University of Göttingen, Germany; alfalfa (*Medicago sativa* L.) stems and leaves were collected near the field trial Ewiger Roggenbau in Halle (Saale), Germany. Ryegrass roots were washed with ultrapure water to remove adherent soil before air-drying. Maize roots were obtained from plants grown for root exudate sampling (see below). Pre-leached, double, and triple-leached rye grass residues were produced by multiple extractions with water (see below), followed by air-drying. All plant residues were ground to <1 mm using an impact mill (Rekord A, Gebr. Jehmlich

GmbH, Nossen, Germany). A separate portion of non-leached ryegrass (stems + leaves) was additionally ground to <3 mm (in the following referred to as coarse ryegrass), to determine the possible effect of particle size.

Solid plant residue samples were analyzed for total C (assuming total C = OC) and total nitrogen (TN) using a Vario Max Cube (Elementar Analysensysteme GmbH, Langensfeld, Germany). In addition, the residues' contents of WEOC and water-extractable total N (WETN) were determined. Briefly, 50 mg of ground plant residues were suspended in 25 ml of ultrapure water and shaken horizontally for 1 h. The supernatants were passed through 0.45-μm membrane filters (Supor®-450, Pall Cooperation, New York, NY, USA). The extracts were analyzed for OC and TN, using a multi N/C® 3100 analyzer (Analytik Jena AG, Jena, Germany). Concentrations of N_{min} (NO₃⁻ and NH₄⁺) were determined using a Continuous Flow Analyzer (ScanPlus, Skalar Analytical B.V., Breda, The Netherlands). WEON (or ON) was calculated by: WEON = (WE)TN - N_{min}. All reported (WE)OC and (WE)ON contents refer to dry mass determined at 105 °C. The specific UV absorbance at 280 nm, as an estimate of the aromaticity of WEOM (Chin et al., 1994), was determined using a photometer (SPECORD® 210 PLUS, Analytik Jena AG).

2.3. Root exudates and water-extractable organic matter

Maize plants (*Zea mays* L. var. Ronaldinio) were grown in nutrient solution (Mengutay et al., 2013) for 5–6 weeks in a greenhouse. To sample root exudates, the dipping method described by Neumann and Römhild (2007) was used. Maize roots were soaked in deionized water for 3 × 5 min to remove nutrient solution from the root surfaces. Then, roots were submerged in the trap solution (aerated distilled water) for 2 h. The exudate solution was filtered through a 4-μm-filter paper (MN615 1/4 Ø 90 mm, Macherey-Nagel GmbH, Düren, Germany), flash frozen in liquid nitrogen, freeze-dried, and stored at -20 °C prior to the experiments. For analysis and incubation, freeze-dried root exudates were re-dissolved in ultrapure water in a sonication bath for 15 min.

To obtain sufficiently large and reproducible amounts of plant-derived WEOM for incubation experiments, either 50 g ryegrass (stems + leaves; cut to <10 cm) or 25 g maize straw (cut to <10 cm) were soaked in 500 ml ultrapure water for 18 h at ~20 °C in the dark. The parent residue materials were removed by passing the suspensions first through 0.7-μm-glass microfiber filters (13440-130—K, Sartorius Stedim Biotech GmbH, Göttingen, Germany) using a pressure filter holder with barrel (16274, Sartorius Stedim Biotech GmbH), and then, through 0.45-μm membrane filters (Supor®-450). The resulting filtrates, containing the WEOM, were used in the incubation experiments.

Re-dissolved root exudates and WEOM from ryegrass and maize straw were analyzed for pH (SenTix® 41, WTW, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany), OC, ON, and specific UV absorbance at 280 nm as detailed above.

2.4. Solid-state ¹³C-NMR spectroscopy

Plant residues were ground with a vibratory disc mill (RS 100, Retsch, Haan, Germany) before analysis by solid-state ¹³C cross-polarization magic angle spinning NMR spectroscopy (¹³C-CPMAS NMR spectroscopy) with an Avance III 200 spectrometer (Bruker Bio-Spin GmbH, Karlsruhe, Germany). Samples were placed into a 7-mm zirconia rotor that was spun at 6.8 kHz around a 'magic angle' of 54.74°. Contact time was 1 ms and the recycle delay time was set to 0.4 s. The spectra were processed with 100 Hz line broadening, phase adjusted, and baseline corrected; no spinning side bands appeared in the spectra. Peaks were assigned to four integration areas: -10–45 ppm (alkyl C), 45–110 ppm (O/N-alkyl C), 110–160 ppm (aromatic C), and 160–220 ppm (carboxylic/carbonyl C); spectra are shown in the Supplementary Material (Fig. S1).

2.5. Incubation and gas measurements

Anoxic incubations were carried out at 20 °C in the dark for 24 h, to assess potential short-term effects in a typical 'hot spot-hot moment' situation (McClain et al., 2003). Homogeneous soil suspensions were prepared by filling 10 g (dry mass) of pre-incubated soil material into 250-ml glass infusion bottles and mixing with amounts of different residues equivalent to 2 g C kg⁻¹ dry soil, and then adding 50 ml of KNO₃ solution (50 mg NO₃⁻-N kg⁻¹ dry soil; soil/water ratio = 1/5 w/v), to avoid NO₃⁻ limitation during at least the first 8 h of incubation. As indicated by the WEON/total N ratios of plant residues (average 34%), a significant portion of ON is structurally bound (e.g., as proteins) and would require depolymerization within the experimental period to yield NH₄⁺. Since the oxidation of NH₄⁺ to NO₃⁻ is unlikely under anoxic conditions and the input amount of residue-NH₄⁺ is negligible (only for alfalfa residues; <1.0 g kg⁻¹ dry matter), no significant effect of the initially present NH₄⁺ on determined gas productions was expected. In soil incubations with WEOM and root exudates, KNO₃ was added with the 50 ml of respective solutions. Again, the C addition amounted to 2 g C kg⁻¹ dry soil.

All incubations were carried out in triplicate, with the bottles sealed with a bromine-butyl-rubber stopper and crimped with an aluminum cap (32 mm; Chroma Globe GbR, Kreuznach, Germany). An O₂-free atmosphere was obtained by evacuating (<250 mbar), and then flushing the bottles including the suspensions with He gas (99.999%, Air Liquide, Düsseldorf, Germany) three times; the final pressure was about 1025 mbar. Soil suspensions were homogenized by horizontal shaking during incubation. After 1 h of incubation, the first (t₀) gas sample (18 ml) was taken with a gastight syringe (25 ml, 25MDR-LL-GT; SGE Analytical Science Pty. Ltd., Ringwood, VIC, Australia), equipped with a push button valve (Luer Lock; SGE Analytical Science) and a 0.7-mm ID cannula (Sterican G26, 25 mm; B. Braun AG, Melsungen, Germany), and transferred to pre-evacuated (90 mbar residual pressure, rinsed with He) 12-ml Exetainer® vials, which were sealed with a double septum cap (IVAVC329; IVA Analysentechnik e.k., Meerbusch, Germany). This resulted in an overpressure of >200 mbar in the Exetainer® vials, which was necessary to avoid contamination with air during storage and for measuring gas concentrations with the gas chromatography system described below. To avoid low pressure in the incubation bottles, 18 ml He at ~1 bar were injected after gas sampling, resulting in constant absolute pressure of ~1025 mbar during incubation. The absolute pressure in the bottles was measured before and after gas sampling as well as after He injection, using a GMSD 2 BA-K31-L01 pressure sensor coupled with a Greisinger GMH 3151 reader (GSG Geologie-Service GmbH, Würzburg, Germany). Gas samples were taken after 0, 2, 4, 6, 8, and 24 h.

All gas samples were analyzed on a custom-tailored gas chromatography system by Chromtech (Bad Camberg, Germany), using an Agilent HP 7890B GC as basis. The samples were introduced into the injector by an autosampler (PAL GC-xt; CTC Analytics AG, Zwingen, Switzerland), using an open needle syringe. Calibration was done online, using standard gas cups connected to bottles with certified calibration gases with known concentrations of CO₂ and N₂O in He (Linde Gas AG, Pullach, Germany). After injecting the sample at a liner temperature of 150 °C, it was transferred to a Shin Carbon chromatographic column (2 m, 0.53 mm ID, Restek GmbH, Bad Homburg, Germany) using He as carrier gas (purity 99.9999%) and directly transferred to a He ionization detector (Vici AG International, Schenk, Switzerland) run at 180 °C. The GC oven was set to a starting temperature of 60 °C, kept constant for 3 min, increased to 110 °C at a rate of 10 °C min⁻¹, kept for 1 min, and finally increased to 220 °C at a rate of 50 °C min⁻¹, kept for 3 min. The limit of detection for both gases was calculated using 10 blank samples that were drawn and measured in the same way as all other samples. The limit of detection was calculated as 0.1 ppb and 11.76 ppm for N₂O and CO₂, respectively. Precision and accuracy were analyzed injecting a reference standard every ten samples. On average, precision

was 4.8 and 0.5% relative standard error for N₂O and CO₂, respectively, while accuracy was 3.3 and 1.5% offset from the specified concentration for N₂O and CO₂, respectively.

Cumulative emissions of gases represent the sum of produced amounts, i.e., the detected gas mass in the headspace plus estimated gas mass in the suspension at time point t_x minus the total gas mass at time point t_{x-1}, considering the removed gas amount during each gas sampling. Gas masses in the suspension were calculated by using the respective Henry's law constant, volume of solution, headspace pressure and gas concentration; for different CO₂ species the pH was also taken into account. For the acetylene inhibition technique (Yoshinari and Knowles, 1976), a second line of incubations as described above was carried out with additional injection of 30 ml of C₂H₂ (99.6%; Air Liquide) in exchange for 30 ml He, resulting in an initial C₂H₂ concentration of ~10% (v/v). An C₂H₂ concentration of >5% (v/v) was expected to be maintained during the 24 h of incubation (Yeomans and Beauchamp, 1978; Terry and Duxbury, 1985). Therefore, the amount of N₂O in presence of C₂H₂ was assumed to represent total denitrification (N₂O + N₂). Consequently, the difference between the N₂O amounts of incubations with and without C₂H₂ addition was an estimate of the N₂ production, while the ratio of the two N₂O species was used to determine the molar N₂O-N/(N₂O + N₂)-N ratio. Cumulative amounts of N₂O in presence of C₂H₂ and CO₂ in incubations without C₂H₂ were used to determine the molar (N₂O + N₂)-N/CO₂-C ratio, which indicates the contribution of denitrification to total CO₂ production. Based on the stoichiometry of complete and incomplete denitrification (Ottow, 2011), ratios between 0.8 and 1.0 indicate that the CO₂ production is exclusively linked to denitrification reactions. Main potential problems in application of the acetylene inhibition technique, i.e., underestimated N₂ production due to acetylene inhibition of nitrification and incomplete inhibition of N₂O reduction caused by low NO₃⁻ concentrations or acetylene diffusion effects (Knowles, 1990; Almaraz et al., 2020), can be excluded for our incubation approach (anoxic, excess NO₃⁻, soil suspension). Alleviation of acetylene inhibition by sulfide compounds potentially released from plant materials is unlikely, given that the reported effect typically shows a delay of 2–3 days (Yeomans and Beauchamp, 1982; Knowles, 1990), which beyond the time frame of our 24-h experiments.

2.6. Statistical evaluation

Basic statistical analyses were performed using Sigma Plot 11.0 (Systat Software Inc., Erkrath, Germany). One-way ANOVA with type of OM addition as independent variable followed by the Tukey HSD test was used for testing for differences in cumulative emissions of CO₂, N₂O, and N₂O + N₂, and respective ratios. Linear regression analyses were used to test for relationships between chemical properties of residues or their water extracts and resulting gas emissions, or respective ratios after confirming the normal distribution of data by the Shapiro-Wilk test. In some cases, regression analyses were additionally carried out under exclusion of ryegrass roots because, unlike the other residues, they were washed to remove adherent soil, while alfalfa residues were partly excluded for being depleted in NO₃⁻ at the end of incubation.

3. Results

3.1. Characterization of plant residues, root exudates, and water-extractable organic matter

The OC/ON ratios of plant-derived residue types ranged from 9.0 (alfalfa leaves) to 83.7 (ryegrass roots). Multiple leaching of ryegrass with ultrapure water resulted in an increasing OC/ON ratio (Table 1). Triple-leached ryegrass was enriched in O/N-alkyl C and aryl C but proportions of alkyl C and carboxylic/carbonyl C were reduced (Table 2). Root residues contained relatively less (O/N)-alkyl C than respective aboveground residues while proportions of aryl C and

Table 1

Contents of total (OC and ON) and water-extractable organic C (WEOC) and N (WEON) as well as the OC/ON and WEOC/WEON ratios of residue types. The specific UV absorbance at 280 nm ($SUVA_{280}$) was measured on WEOM solutions.

Residue type	OC [g kg ⁻¹]	ON [g kg ⁻¹]	OC/ON ratio	WEOC [g kg ⁻¹]	WEON [g kg ⁻¹]	WEOC/WEON ratio	$SUVA_{280}$ [l mg ⁻¹ C cm ⁻¹]
Ryegrass	400.8 D ^a	11.7 CD	34.3 E	52.6 C	4.7 D	11.3 B	0.013 E
Coarse ryegrass	400.8 D	11.7 CD	34.3 E	46.3 D	4.3 D	10.7 B	0.012 E
Pre-leached ryegrass	405.5 CD	10.9 D	37.2 DE	17.7 F	1.8 EF	9.9 BC	0.017 C
Double-leached ryegrass	413.3 AB	10.7 D	38.6 D	10.4 G	1.6 F	6.5 D	0.020 B
Triple-leached ryegrass	418.9 A	9.3 DE	45.0 C	5.2 H	1.3 F	4.1 E	0.023 A
Ryegrass roots	411.3 BC	4.9 F	83.7 A	28.6 E	0.9 F	30.5 A	0.006 G
Maize straw	400.7 D	7.3 EF	54.9 B	21.3 F	2.5 E	8.6 C	0.016 D
Maize roots	409.9 BC	14.1 C	29.1 F	56.1 C	6.4 C	8.8 C	0.009 F
Alfalfa stems	390.8 E	19.8 B	19.7 G	72.2 B	13.9 B	5.2 DE	0.005 G
Alfalfa leaves	409.2 BC	45.6 A	9.0 H	126.9 A	21.0 A	6.0 D	0.011 E

^a Values followed by the same letters within a column are not significantly different ($p < 0.05$) based on a one-way ANOVA test followed by the Tukey HSD test. Values represent means ($n = 3$).

Table 2

Distribution of C species in different plant residue types. Values represent the percentage contribution of the different integrated chemical shift regions determined by ¹³C-CPMAS NMR spectroscopy.

Residue type	Alkyl C [%]	O/N-alkyl C [%]	Aryl C [%]	Carboxylic/carbonyl C [%]
Ryegrass	12	79	6	3
Triple-leached ryegrass	7	85	7	1
Ryegrass roots	3	78	14	5
Maize straw	6	80	11	3
Maize roots	4	78	12	6
Alfalfa stems	10	72	9	9
Alfalfa leaves	21	44	18	17

carboxylic C were higher. The WEOC contents of not pre-leached residues varied between 21.3 g kg⁻¹ (maize straw) and 126.9 g kg⁻¹ (alfalfa leaves) (Table 1). Triple-leaching of ryegrass caused a decrease in WEOC and WEOC/WEON ratio by 90% and 64%, respectively, while the specific UV absorbance (at 280 nm) of the respective WEOM increased by 77%. The WEOC content of the residues was significantly related to the residues' OC/ON ratio ($r = -0.67$, $p < 0.05$, $n = 10$; and $r = -0.89$, $p < 0.001$, $n = 9$, when ryegrass roots were excluded; Fig. 1a), the residues' proportion of carboxylic/carbonyl C determined by ¹³C-NMR spectroscopy ($r = 0.94$, $p < 0.01$, $n = 7$; Fig. 1b), and specific UV absorbance (at 280 nm) of respective WEOM solutions ($r = -0.56$, $p < 0.001$, $n = 10$;

and $r = -0.97$, $p < 0.001$, $n = 8$, when ryegrass roots and alfalfa leaves were excluded).

Root exudates and WEOM solutions used for incubation experiments differed in their chemical composition (Table 3). Maize straw-derived WEOM and root exudates had lower OC/ON ratios but higher specific UV absorbances (at 280 nm) than ryegrass WEOM.

3.2. Effect of residues on potential denitrification and CO₂ production

The addition of different residues to the soil resulted in a wide range of cumulative N₂O + N₂, N₂O, and CO₂ production rates (Fig. 2a–c). Soil amended with alfalfa stems or leaves reached the highest cumulative amounts of N₂O + N₂ and CO₂ over the entire incubation period (Fig. 2a

Table 3

Chemical properties and composition of plant residue-derived water-extractable organic matter (WEOM) solutions and the maize root exudate solution. Values represent the pH, organic nitrogen (ON) concentration, the ratio of organic C (OC) to ON of solutions with an OC concentration of 400 mg l⁻¹ used for the incubation experiments. $SUVA_{280}$ refers to the specific UV absorbance at 280 nm.

WEOM type	pH	ON [mg l ⁻¹]	OC/ON ratio	$SUVA_{280}$ [l mg ⁻¹ C cm ⁻¹]
Ryegrass WEOM	6.1	30.5	13.1	0.007
Maize straw WEOM	7.3	36.5	10.9	0.013
Maize root exudates	9.4	39.9	10.0	0.014

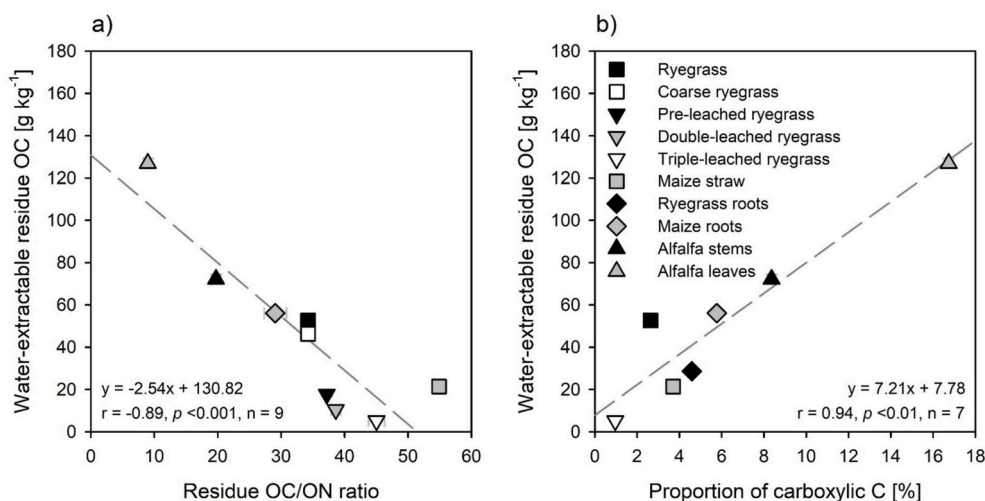


Fig. 1. Relationship between the content of water-extractable OC and (a) the OC/ON ratio of different plant residue types (except for ryegrass roots) as well as (b) the proportion of carboxylic C in residues determined by ¹³C-NMR spectroscopy. Error bars represent standard deviation of means ($n = 3$).

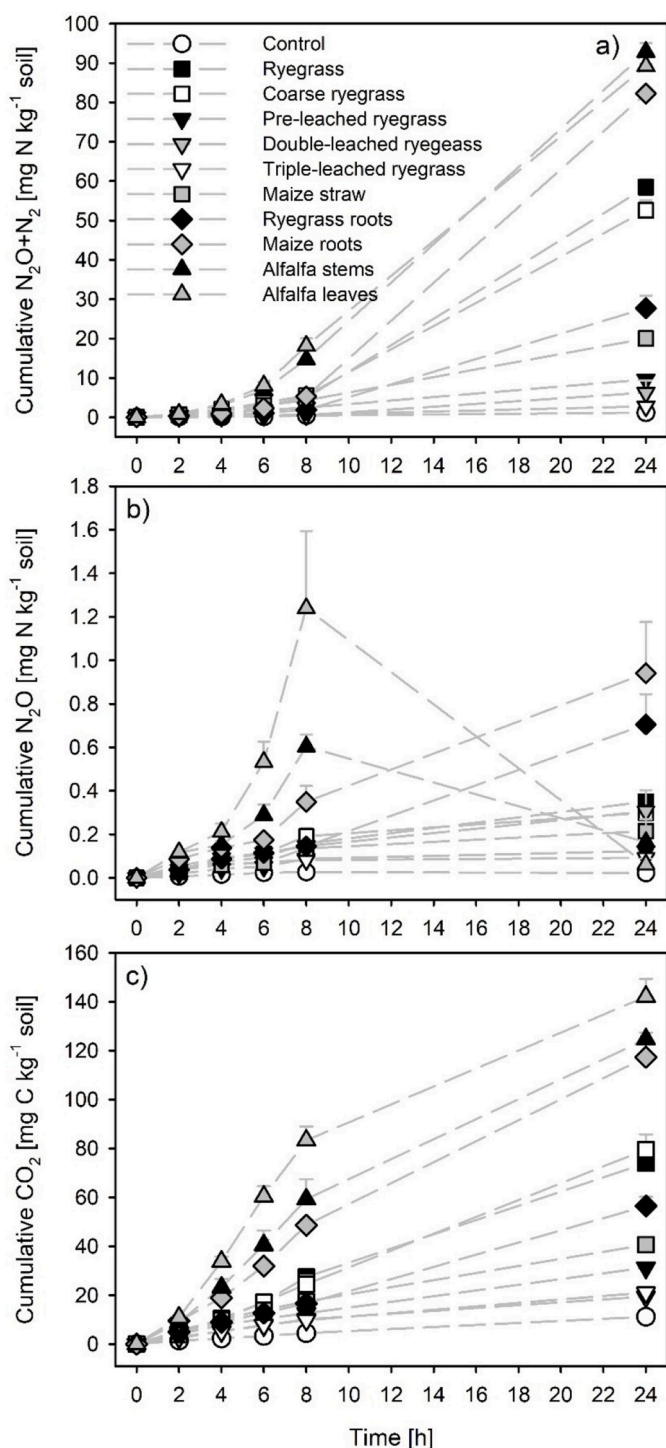


Fig. 2. Cumulative (a) $\text{N}_2\text{O} + \text{N}_2$, (b) N_2O , and (c) CO_2 production during anoxic incubation at 20 °C of soil without (control) and with addition of different plant residues. Error bars represent standard deviation of means ($n = 3$). All incubations received initial KNO_3 additions of 50 $\text{mg NO}_3\text{-N kg}^{-1}$ dry soil. Total denitrification ($\text{N}_2\text{O} + \text{N}_2$) was determined by using the acetylene inhibition technique.

and c). Differences between maize roots and alfalfa residues were small by the end of incubation (82.2–92.8 mg N kg^{-1} and 117.3–142.1 mg C kg^{-1}). Lowest cumulative production of $\text{N}_2\text{O} + \text{N}_2$ and CO_2 after 24 h was determined for multiple leached ryegrass (about 5 mg N kg^{-1} and 20 mg C kg^{-1} , respectively). Pre-leaching caused substantial decreases in the potential denitrification (84%) and CO_2 production (58%) after

24 h; double- and triple-leaching reduced the release of $\text{N}_2\text{O} + \text{N}_2$ by 86 and 95%, respectively, and the CO_2 production by about 73% compared to not-leached ryegrass. Cumulative amounts of $\text{N}_2\text{O} + \text{N}_2$ after 24 h were closely related to WEOC contents of residues ($r = 0.98$, respectively, $p < 0.001$, $n = 9$, when excluding alfalfa leaves; Fig. 3a) and cumulative amounts of CO_2 ($r = 0.99$, $p < 0.001$, $n = 10$; Fig. 3b). Multiple leaching of ryegrass also resulted in decreasing molar ($\text{N}_2\text{O} + \text{N}_2$)- N/CO_2 -C ratios, which were significantly lower than those of the control (Table 4). The molar ($\text{N}_2\text{O} + \text{N}_2$)- N/CO_2 -C ratios after 24 h were positively related to contents of WEOC for all residues ($r = 0.94$, $p < 0.001$, $n = 8$, when excluding alfalfa residues).

The cumulative N_2O production during the first 8 h (no NO_3^- limitation) was highest for maize roots and especially alfalfa residues (0.4–1.2 mg N kg^{-1}) (Fig. 2b). However, the soil suspensions with alfalfa were depleted in NO_3^- after 24 h, and therefore, N_2O decreased significantly. Application of ryegrass roots and especially maize roots resulted in increased denitrification rates after about 8 h, and thus, relatively high cumulative N_2O production (0.7–0.9 mg N kg^{-1}) by the end of incubation. Cumulative amounts of N_2O after 8 h were also most closely related to residue WEOC ($r = 0.93$, $p < 0.001$, $n = 10$). The molar $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratio was generally low after 2 h (0.16 on average) and decreased strongly over the entire incubation period for all residues (Table 4).

The particle size of ryegrass had only little effect on cumulative gas emissions (Fig. 2a–c) and respective ratios (Table 4).

3.3. Effect of different WEOM and root exudates on potential denitrification and CO_2 production

Differences between WEOM solutions and root exudates in promoting the production of nitrogenous gases and CO_2 were visible but showed no consistent patterns (Fig. 4a–c). Cumulative amounts of $\text{N}_2\text{O} + \text{N}_2$ differed only slightly within the first 8 h (Fig. 4a). After 24 h, the cumulative $\text{N}_2\text{O} + \text{N}_2$ production was significantly higher for maize root exudates than for ryegrass and maize straw WEOM. While the CO_2 production for ryegrass WEOM was lowest after 8 h (about 45 mg C kg^{-1} dry soil), it was highest (about 275 mg C kg^{-1} dry soil) after 24 h among all three WEOM types (Fig. 4b). The addition of ryegrass WEOM resulted in the highest molar ($\text{N}_2\text{O} + \text{N}_2$)- N/CO_2 -C ratio after 8 h (0.19). The maximum ratio after 24 h was observed for maize root exudates (0.55), being about 2.7 times higher compared to ryegrass and maize straw WEOM (Table 4).

The addition of maize root exudates caused the highest cumulative N_2O emission (2.2 mg N kg^{-1} dry soil) and molar $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratio (Table 4) during the first 8 h. Not only NO_3^- but also N_2O in the headspace was depleted by the end of incubation for all three OM solutions.

We found no distinct relationships between chemical properties of OM solutions (Table 3) and resulting gas emissions (Fig. 4a–c) or their respective ratios (Table 4).

4. Discussion

4.1. WEOC content of residues determines potential denitrification and CO_2 production

As hypothesized, and in agreement with previous studies (e.g., Bremner and Shaw, 1958), variations in cumulative gas emissions from soil amended with different plant residues were closely related to residue WEOC (Fig. 3a). Also, the molar ($\text{N}_2\text{O} + \text{N}_2$)- N/CO_2 -C ratios were related to residue WEOC contents. These findings suggest that during short-term anoxic conditions denitrifying organisms rely on soluble compounds from fresh residues as major C sources. Pre-leaching of ryegrass resulted in a strongly decreased production of CO_2 , N_2O , and N_2 (Fig. 2a and b), which additionally underlines that, at least during short anoxic periods, residue WEOC represents the most important C source for denitrifying organisms. This is consistent with Rummel et al. (2020),

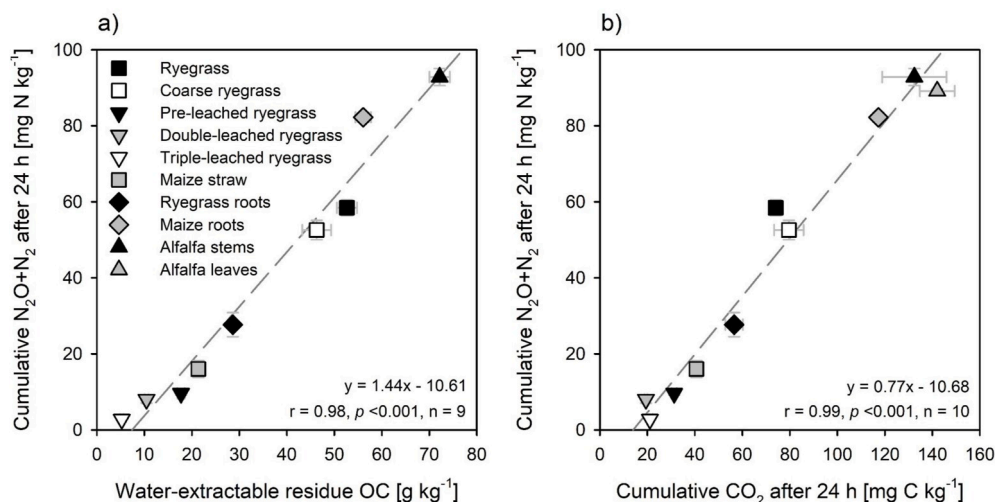


Fig. 3. Relationship between (a) water-extractable residue OC and cumulative $\text{N}_2\text{O} + \text{N}_2$ production after 24 h of anoxic incubation (except for alfalfa leaves because of NO_3^- depletion) as well as (b) between cumulative amounts of CO_2 and $\text{N}_2\text{O} + \text{N}_2$ at the end of incubation. Error bars represent standard deviation of means ($n = 3$).

Table 4

Molar $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratios and $(\text{N}_2\text{O} + \text{N}_2)\text{-N}/\text{CO}_2\text{-C}$ ratios after 2, 8, and 24 h of anoxic incubation at 20 °C of soil without (control) and with addition of different plant residues, WEOM solutions, and root exudates. All incubations received initial KNO_3 additions of 50 mg $\text{NO}_3^- \text{N kg}^{-1}$ dry soil.

Organic matter type	$\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratio			$(\text{N}_2\text{O} + \text{N}_2)\text{-N}/\text{CO}_2\text{-C}$ ratio		
	2 h	8 h	24 h	2 h	8 h	24 h
Control	0.03 E ^a	0.02 D	0.01 DEF	0.13 AB	0.22 A	0.26 DE
<i>Residue types</i>						
Ryegrass	0.07 DE	0.03 D	0.01 DEF	0.11 ABC	0.15 BC	0.68 A
Coarse ryegrass	0.04 E	0.03 D	0.01 EF	0.15 A	0.19 AB	0.57 B
Pre-leached ryegrass	0.04 E	0.04 D	0.01 DE	0.10 BCD	0.17 AB	0.26 DE
Double-leached ryegrass	0.63 A	0.21 B	0.04 A	0.03 GH	0.06 D	0.35 CD
Triple-leached ryegrass	0.23 BC	0.12 C	0.03 AB	0.05 EFGH	0.06 D	0.11 F
Ryegrass roots	0.11 CDE	0.08 CD	0.03 C	0.06 EFG	0.10 CD	0.42 C
Maize straw	0.06 DE	0.03 D	0.01 D	0.10 BCD	0.21 AB	0.34 C
Maize roots	0.19 CD	0.07 CD	0.01 DE	0.04 FGH	0.09 CD	0.60 AB
Alfalfa stems	0.12 CDE	0.04 D	0.00 F	0.08 CDE	0.22 AB	0.60 AB
Alfalfa leaves	0.14 CDE	0.07 CD	0.00 F	0.07 DEF	0.19 AB	0.54 B
<i>OM solutions</i>						
Ryegrass WEOM	0.05 E	0.04 D	0.00 F	0.13 AB	0.19 AB	0.19 EF
Maize straw WEOM	0.07 DE	0.04 D	0.00 F	0.04 FGH	0.09 D	0.22 E
Maize root exudates	0.35 B	0.30 A	0.00 F	0.02 H	0.07 D	0.55 B

^a Values followed by the same letters within a column are not significantly different ($p < 0.05$) based on a one-way ANOVA test followed by the Tukey HSD test. Values are means ($n = 3$).

who reported close relations between WEOC and N_2O and CO_2 emissions from a repacked topsoil (gleyic Fluvisol) amended with maize root and shoot litter under aerobic conditions. Our findings support their suggestion that litter can stimulate denitrification by creating plant litter-associated anaerobic microsites. Those 'hot spots' have a high potential to accelerate N losses but are only activated under certain

circumstances (Bernhardt et al., 2017). Especially in situations where soil moisture and NO_3^- concentrations are high, incorporated plant residues of varying age and freshness could substantially contribute to the emission of nitrogenous gases via denitrification. The WEOC content being a determining factor in plant residue-induced denitrification is well in line with studies showing that soluble low-weight compounds (e. g., organic acids and glycerol \gg glucose, methanol) are much more effective in promoting denitrification than insoluble polymers such as cellulose and especially lignin (e.g., Valera and Alexander, 1961; Rashid and Schaefer, 1988; Akunna et al., 1993).

Consistent with Hadas et al. (2004), the WEOC release from plant residues increased with lower OC/ON ratios. Similarly, Reinertsen et al. (1984) found that the initial microbial biomass production and decomposition rate of wheat straw with different OC/ON ratios are linked to the size of the soluble C pool. When testing residue effects on denitrification, Huang et al. (2004) found accumulation of DOC for residues with low OC/ON ratios under conditions with limited N supply, i.e., when no denitrification took place. This underlines the strong control of WEOC on denitrification and the link between residue OC/ON ratios and their WEOC contents. In addition, WEOC contents were positively related to proportions of carboxylic C (Fig. 1b), suggesting that acidic compounds are a major source of potentially soluble carbon. Leaching of ryegrass resulted in decreasing proportions of carboxylic C (Table 2), which underlines the water solubility of organic acids. Hence, the proportions of carboxylic C as well as the OC/ON ratios of plant residues can be used as proxy for their WEOC contents. The insignificant effect of the residue particle size on WEOC (Table 1) and gas emissions (Fig. 2a–c) apparently contrast some observations of varying decomposition and production rates of climate-relevant gases depending on the residue particle size (Angers and Recous, 1997; Shelp et al., 2000; Ambus et al., 2001). However, such effects may only become relevant for larger differences in the particle size and over time periods longer than those considered in our study.

Overall, the results suggest that plant residues have the potential to directly control denitrification rates and the share of N_2O by releasing soluble OM when environmental conditions promote the formation of 'hot spot-hot moment' situations.

4.2. Effect of soluble OM composition on potential denitrification and CO_2 production

We found that residues with low OC/ON ratios produced not only more WEOC, but also that specific UV absorbances at 280 nm were

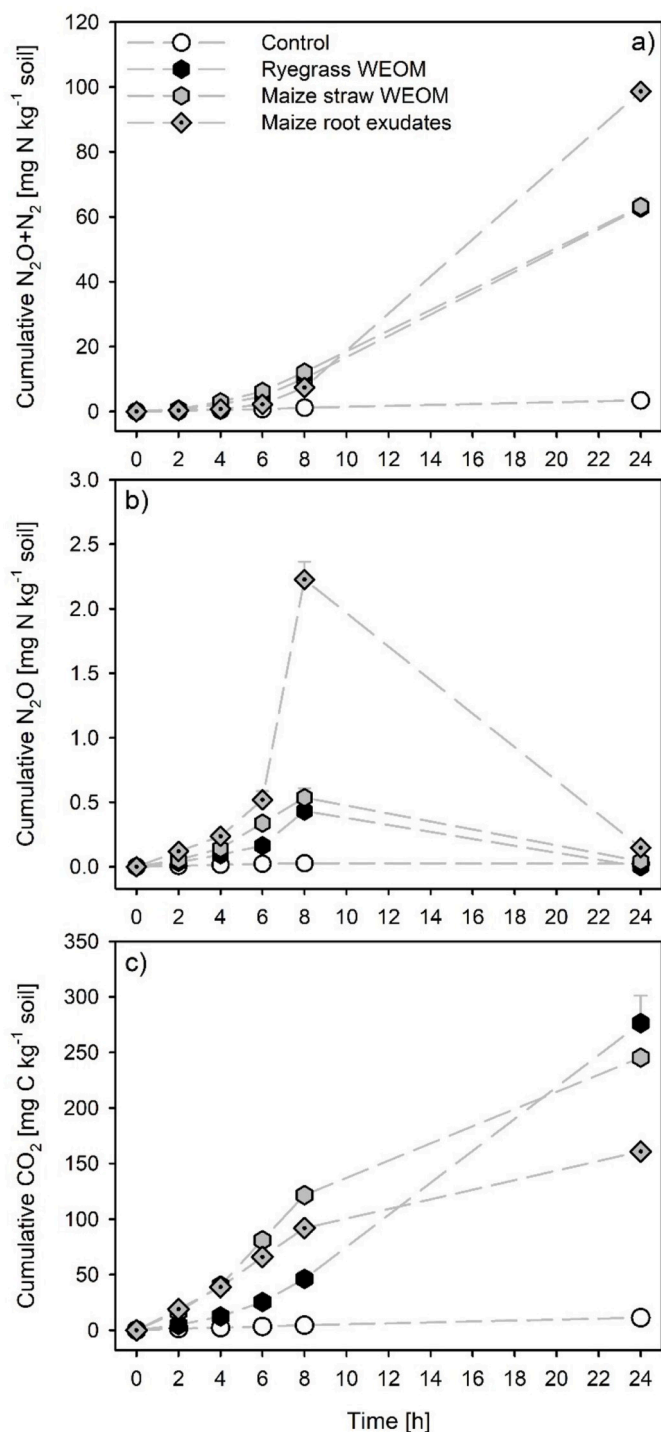


Fig. 4. Cumulative (a) $N_2O + N_2$, (b) N_2O , and (c) CO_2 production during anoxic incubation at 20 °C of soil without (control) and with addition of ryegrass- and maize straw-derived water-extractable organic matter (WEOM) and maize root exudates. Error bars represent standard deviation of means (n = 3). All incubations received initial KNO_3 additions of 50 mg NO_3^- -N kg⁻¹ dry soil. Total denitrification ($N_2O + N_2$) was determined by using the acetylene inhibition technique.

lower. According to [Chin et al. \(1994\)](#), this indicates lower aromaticity of WEOM. Since aromatic compounds are less decomposable than proteins and organic acids ([Marschner and Kalbitz, 2003](#)), the quality of WEOM derived from N-rich residues could be also higher. Hence, we assume that at similar concentrations, WEOC released from different residues might cause different productions of CO_2 , N_2O , and N_2 , depending on the chemical composition of WEOM.

Direct comparison revealed that the addition of maize straw WEOM resulted only in significantly higher CO_2 emissions than ryegrass-derived WEOM during the first 8 h, while differences in the N_2O and N_2 production were very small over the entire incubation period ([Fig. 4a–c](#)). Consequently, the molar ($N_2O + N_2$)-N/ CO_2 -C ratio ([Table 4](#)) was more affected by the WEOM composition than total denitrification. The small molar ($N_2O + N_2$)-N/ CO_2 -C ratios, especially during the first hours, indicate that the contribution of denitrification to total CO_2 production was initially minor and that other processes such as fermentation likely dominated.

The addition of maize root exudates resulted in a much higher cumulative production of $N_2O + N_2$ and also in the highest molar ($N_2O + N_2$)-N/ CO_2 -C ratio after 24 h ([Fig. 4a](#)), with NO_3^- becoming depleted towards the end of incubation for all three OM solutions. Based on mass balance calculations using the acetylene inhibition technique, about 40% of the initial NO_3^- was likely reduced to NH_4^+ in the WEOM incubations. This is supported by [Fazzolari et al. \(1998\)](#), who concluded that the dissimilatory nitrate reduction to ammonium (DNRA) is favored over denitrification at OC/ NO_3^- -N ratios above 4 (initial ratio of WEOM treatments was ~20). Thus, our findings suggest that NO_3^- distribution between denitrification and DNRA can also be affected by the chemical composition of readily available C compounds and is not only regulated by the total WEOC concentration ([Fazzolari et al., 1998](#)). [Akunna et al. \(1993\)](#) demonstrated that the addition of glucose and glycerol to anaerobic sludges resulted in a high share of DNRA and production of volatile fatty acids, particularly acetic acid, while no DNRA was observed for the application of acetic acid and lactic acid. Consequently, differences in effects between WEOM solutions and root exudates can be probably explained by differences in their molecular composition. Correspondingly, higher emissions of N_2O in the incubations with maize root exudates than in those with maize straw and ryegrass WEOM ([Fig. 4b](#)) could be due to smaller shares of sugars, such as glucose, and higher proportions of organic or amino acids ([Henry et al., 2008](#)).

In summary, the observed variations in molar ($N_2O + N_2$)-N/ CO_2 -C ratios and shares of N_2O with the soluble OM's molecular composition were likely due to anaerobic processes other than denitrification, such as fermentation and DNRA, becoming prominent at high WEOC availability.

5. Conclusions

As hypothesized, the potential denitrification of arable topsoils is – at least during the initial phase of residue decomposition – closely related to the release of WEOM from plant residues. The potential to release WEOM is closely linked to the plant residues' chemical composition, which, in turn, reflects the residues' source and degradation stage. Also as hypothesized, the chemical composition of soluble OM affects the extent and product ratio of denitrification, at least in situations where NO_3^- availability is not limited. In summary, the results of the study indicated the potential of WEOM in largely undecomposed plant residues to support the formation of anoxic microhabitats. Thus, WEOM is a very important factor for the denitrification potential of agricultural soils, especially in situations where soil moisture and NO_3^- concentrations are relatively high. This study aimed to improve basic mechanistic understanding of the effect of soluble OM for potential denitrification using incubation experiments under controlled conditions and additional studies need to address the relevance of observed effects under field conditions. Likewise, the results call for more efforts in the molecular characterization of readily available OM and their subsequent

transformation during denitrification.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2020.107841>.

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