

# Low N<sub>2</sub>O emissions induced by root-derived residues compared to aboveground residues of red clover or grass mixed into soil

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## ABSTRACT

The default The Intergovernmental Panel on Climate Change (IPCC) guidelines assume a constant N<sub>2</sub>O emission factor (EF<sub>N2O</sub>) for both belowground crop residues (BGR) and aboveground residues (AGR), and that ~70 % of total N<sub>2</sub>O emissions following renewal of temporary grasslands come from BGR. However, empirical evidence is lacking, which motivated this study. BGR-free and BGR-rich clay loam collected in grass or red clover leys were incubated alone or mixed with AGR and different doses of nitrate over 107 days. The average EF<sub>N2O</sub> of BGR was around 18 % of that of AGR, and remained low even when soil nitrate concentration was very high, whereas EF<sub>N2O</sub> of AGR varied largely and rocketed even with a small increase in soil nitrate. The decomposition of the carbon present in crop residues was critical for N<sub>2</sub>O emissions. Lower EF<sub>N2O</sub> of BGR relative to AGR were related to slower C decomposition, which was not predicted by the biochemical characteristics. It is also likely that BGR were less conducive than AGR to develop into hotspots for N<sub>2</sub>O emission because of the roots' finer distribution and closer contact with soil particles. Differences in EF<sub>N2O</sub> among AGR were mostly linked to the availability of N, either derived from residue mineralization or present in the soil. In conclusion, N<sub>2</sub>O accountings based on present IPCC default methodology likely overestimate the contribution by crops' BGR.

## 1. Introduction

Crop residues are critical for sustaining soil fertility and maintaining soil carbon levels. However, they are a major source of direct nitrous oxide (N<sub>2</sub>O) emissions from agricultural soils (Syakila and Kroeze, 2011). Thus, the net greenhouse gas (GHG) balance of crop residue management depends on the balance between contributions to soil carbon sequestration and N<sub>2</sub>O emissions, and a judicious management is needed to maintain N<sub>2</sub>O emissions from crop residues low. However, our knowledge of the total amounts of nonremovable residues and their effects on N<sub>2</sub>O emissions is scarce.

National GHG inventories use the amount of nitrogen (N) in crop residues to estimate N<sub>2</sub>O emissions. The refined guidelines for GHG inventories of The Intergovernmental Panel on Climate Change (IPCC) stipulate a standard emission factor (EF<sub>N2O</sub>) of 0.005 or 0.006 (CV: 50 %) N<sub>2</sub>O-N per kg N in crop residues in dry and wet climates, respectively (IPCC, 2019). This factor for direct N<sub>2</sub>O emissions is applied equally to both aboveground (AGR) and belowground (BGR) residues.

In general, the amount and quality of BGR are poorly documented (see Thiébeau et al., 2021). Furthermore, N<sub>2</sub>O emissions related to BGR

and stubble are commonly included in the reference treatment used to estimate the effect of removable residues and are therefore usually included in the background emissions. There is little empirical evidence for the present default methodology of the IPCC guidelines, according to which roots contribute approximately to 30–70 % of N<sub>2</sub>O emissions from agricultural crop residues, with the largest relative contribution from termination of grasslands for ley renewal or crop rotation. Abalos et al. (2022) did not find observations of the specific effect of BGR and stubble (nonremovable AGR below cutting height of harvest machinery) that could be included a recent meta-analysis of N<sub>2</sub>O emissions from crop residues. Thus, there is a need for improved knowledge on emissions from BGR and the importance of their quality for N<sub>2</sub>O emissions (Olesen et al., 2023).

Concurrent carbon (C) and N transformations are critical for N<sub>2</sub>O emissions from crop residues. Labile C provides anoxic spots at the microsite level through enhanced microbial respiration, and serves as an energy substrate for denitrifiers, thus stimulating N<sub>2</sub>O emissions (Hesselsøe et al., 2001; Surey et al., 2020), but it can also reduce N<sub>2</sub>O emissions through mineral N immobilization (Chen et al., 2013). This 'C effect' on N<sub>2</sub>O emission is contingent on the supply of mineral N, thus

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the ultimate effect of plant residues on N<sub>2</sub>O emissions depends on their C and N interacting with mineral N in the soil. Recent laboratory screening of AGR residues (Janz et al., 2021; Lashermes et al., 2022) and a meta-analysis of field studies (Abalos et al., 2022) concluded that the immature AGR, characterised by high total N concentration and low cellulose, hemicellulose and lignin fractions, and which are often green in colour (as herbage of leys and green manure), strongly stimulated N<sub>2</sub>O emissions, whereas mature residues with opposite characteristics (such as cereal straw) had marginal effects on N<sub>2</sub>O emissions. This is in line with the fact that in annual crops non-structural compounds are largely translocated to the seeds during ripening (Masclaux-Daubresse et al., 2010; Shibbles et al., 1975), and therefore their BGR and AGR are likely devoid of labile C and N compounds. In contrast, BGR and AGR of perennial grassland plants can be rich in labile C and N compounds in physiologically active and storage organs. Thus, a higher EF<sub>N2O</sub> can be expected from AGR and BGR of leys and cover crops terminated while plants are still alive, than from those of mature annual crops.

Roots generally decompose slower than shoot residues (Rasse et al., 2005). It has been shown that the separation of roots from the soil increases their decomposition rate compared to roots incubated *in situ*, i.e., keeping the roots nearly undisturbed in the soil matrix (Rasse et al., 2005). This is probably partly due to chemical and physical protection from the activity of decomposers, e.g., by soil particles in close contact and soil aggregates surrounding thin roots. This suggests that although roots can also be rich in N and non-structural C, lower EF<sub>N2O</sub> can be expected for BGR than for AGR, in contrast to what is stipulated in the IPCC (2019) guidelines.

To our knowledge, published studies on the specific effect of BGR and other nonremovable residues on N<sub>2</sub>O emissions are few. Such studies either involved a large degree of residue manipulation (Begum et al., 2014; Lou et al., 2007; Pal et al., 2013) or started more than a month after the harvest of annual crops (Machado et al., 2021; Van Vleck et al., 2017), which might be too late for observing the main N<sub>2</sub>O emission events. To gain insight into the contribution of removable and nonremovable residues to N<sub>2</sub>O emissions after ploughing, we ran a field study with grass and clover leys (Bleken et al., 2022). However, the effects of BGR and stubble, namely the nonremovable AGR below harvest height, were confounded. Thus, to separate the effect of BGR from that of the stubble, we conducted a laboratory incubation with fresh specimens taken from the field experiment. Care was taken to keep BGR (mainly roots) '*in situ*', in the sense that they were maintained as intact as possible within the soil aggregates where they had grown.

Our hypotheses were: (H1) *in situ* BGR of both grass and red clover will decompose slower than AGR (stubble and/or herbage); (H2) EF<sub>N2O</sub> will increase with the C decomposition rate of the residues and the availability of mineral N; (H3) due to a lower C:N ratio, BGR, stubble and herbage of clover residues will decompose faster and thus have a higher EF<sub>N2O</sub> than the corresponding grass residues.

## 2. Materials and methods

### 2.1. Treatment outline and experimental plan

We planned a fractional factorial design to compare emissions from soils with incorporated fresh residues of grass or red clover to emissions from control soil without fresh plant residues. Grass and red clover residues consisted of three types: belowground residues (BGR) contained in the soil where they had grown, and aboveground residues (AGR) which were separated in stubble and herbage. Stubble and herbage or both were blended with control soil or soil containing BGR, though never mixing clover with grass residues. Some treatments were also combined with KNO<sub>3</sub> at low dose (1.5 g N m<sup>-2</sup>), resembling excess mineral N remaining in the soil at the end of the growing season, and at high dose (15 g N m<sup>-2</sup>), resembling an N fertilizer application. In the case of control soils, an even higher dose (30 g N m<sup>-2</sup>) was also used. This led to treatments with N additions up to ~ 30 g N m<sup>-2</sup> provided in

different forms: clover residues, grass residues + KNO<sub>3</sub>, or KNO<sub>3</sub> only (Table 1).

An experiment was started in the autumn. Due to the early failure of one incubation cabinet, many treatments were lost. Therefore, a second batch was run in the following spring. As the timing of BGR collection could affect the results, two treatments with soil containing grass BGR were repeated in both batches (G1 and G2, G0G1 and G0G2). Red clover in pure stands was used in the first batch only. Unfortunately, we did not have the capacity to complete the intended fractional factorial design. However, we still had sufficient treatments for comparing the effect of BGR versus AGR on N<sub>2</sub>O emissions at several levels of NO<sub>3</sub><sup>-</sup> concentration in the soil.

The following rule was used in the treatments' acronyms: the first letter indicates the soil with or without fresh BGR (grass: G, clover: C and black fallow: K), the second position indicates the stubble (G, or C, or nothing: 0), the third letter indicates the herbage (G or C). An H is inserted when high pH soil was used. When KNO<sub>3</sub> was added, the amount of N in g m<sup>-2</sup> is given (1.5, 15, 30), followed by N. Finally, 1 or 2 indicates the batch (Table 1).

### 2.2. Soil and plant material

The soil was a clay loam (31 % clay, 22 % sand, organic carbon: 2.81 %, C:N ratio 11, bulk density 1.18 kg L<sup>-1</sup>) collected from a forage sward experiment on an artificially drained Umbric Epistagnic Albeluvisol (Siltic) (WRB classification, IUSS, 2015; NIBIO Kilden, 1991) at NMBU, Ås, Norway (59°39'47"N, 10°45'42"E). Plots with a grass mixture (*Lolium perenne* L.; *Phleum pratense* L.; *Schedonorus pratensis* (Huds.) P. Beauv. and *Schedonorus arundinaceus* (Schreb.) Dumort) and plots with red clover (*Trifolium pratense* L.) in pure stands, sown in 2015, were used (Bleken et al., 2022). During the following three production years the grass mixture received a normal N fertilization (270 kg N ha

**Table 1**

Treatments overview. Acronym of the treatment, type of plant residues, amount of KNO<sub>3</sub> added, amount of dry matter and total N in clover and grass residues per area of jar section. Batch 1 and 2 refer to the autumn and spring experiment, respectively.

Batch	Name	Ley residue	Plant parts	Added KNO <sub>3</sub>	Dry matter	Total N in residues
		Red clover (c); Grass (g)	Root (R); Stubble (S); Herbage (H)	N g m <sup>-2</sup>	g m <sup>-2</sup>	g m <sup>-2</sup>
1	KH1 <sup>a</sup>	None	None	0	0	0.00
2	K2	None	None	0	0	0.00
2	K15 N2	None	None	15	0	0.00
2	K30N2	None	None	30	0	0.00
2	K0C2	c	H	0	400	9.93
1	K0C11 <sup>b</sup>	c	H	0	400	9.93
2	K0G2	g	H	0	400	8.57
1	C1	c	R	0	230	5.97
1	CC1	c	R+S	0	657	16.57
1	CCC1	c	R+S+H	0	1057	26.50
1,2	G1,2	g	R	0	240	4.25
1	GH1 <sup>a</sup>	g	R	0	240	4.25
1	GG1	g	R+S	0	654	9.96
1,2	G0G1,2	g	R+H	0	640	12.82
1	G0GH1 <sup>a</sup>	g	R+H	0	640	12.82
1	GGG1	g	R+S+H	0	1054	18.53
1	G1_5 N1	g	R	1.5	240	4.25
1	G0G1_5 N1	g	R+H	1.5	640	12.82
1	GG1_5 N1	g	R+S	1.5	654	9.96
2	G15 N2	g	R	15	240	4.25
2	G0G15 N2	g	R+H	15	640	12.82
2	K0G15N2	g	H	15	400	8.57

<sup>a</sup> Higher pH soil

<sup>b</sup> Inverted soil layers

$y^{-1}$ ), while the red clover plots received none. The sward treatments were factorially combined with low and high soil pH ( $pH_{H_2O} \sim 5.5$  and  $pH_{H_2O} \sim 6.2$ ) treatments, the latter was established by liming in 2014 (Bleken and Rittl, 2022). In the present laboratory experiment we used mainly low pH soil.

As control soil, we used unfertilized black fallow subplots (established in early spring 2018, Bleken et al., 2022) in the autumn and an adjacent field kept as black fallow in 2018 with the same low pH ( $pH_{H_2O} \sim 5.1$ ) in the spring batch, since the former subplots were no longer available. Control soils were collected in advance and sieved through a large mesh sieve (8 mm), while crumble-moist and kept moist and well aerated at ambient temperature ( $\sim 15^\circ\text{C}$ ) before sample preparation. See Supplemental Table S1 for the timing of sample preparation.

Harvest-mature herbage of pure stands of ryegrass (*Lolium perenne* L., cv. Figgio, third harvest) and red clover (cv. Lea, spring harvest) was collected in adjacent field experiments, dried under strong ventilation at  $35\text{--}40^\circ\text{C}$ , chopped to  $\sim 1.5$  cm length and portioned in advance. This was done to avoid decomposition of the herbage prior to the start of the experiment. Each red clover portion contained aliquots of leaves, petioles and stems proportional to the original contribution.

The day before preparing the incubation jars, shortly after the last forage harvest at the end of the third production year (10–11 September 2018, autumn batch), and at the start of the new growing season (15 May 2019, spring batch), the stubble was carefully removed by cutting with a knife between  $-0.5$  and  $0$  cm depth. Only stubble collected in the autumn was used, because overwintered stubble had largely decomposed under the snow cover. Then bulk soil samples containing BGR were collected in the upper  $0\text{--}15$  cm soil layer from four field plots per BGR x soil pH combination. This soil layer contained 85 % or more of the total BGR to  $30$  cm depth, mainly as roots but also as crowns, which join roots to shoots (Bleken et al., 2022). Stubble and soils with BGR were stored overnight at  $4^\circ\text{C}$ .

All plant residues were analysed for total N, ammonium, nitrate, water-soluble C, neutral detergent soluble fraction (ND-soluble), and proxy for hemicellulose, cellulose as described by Bleken et al. (2022) and are reported in Table S2 and Fig. S1. The C:N ratio of red clover was approximately the same ( $\sim 18$ ) in all organs, including roots. The C:N of the grasses was largest in the stubble ( $\sim 33$ ) and lower in roots ( $\sim 26$ ) and herbage ( $\sim 21$ ).

The amount of AGR was chosen to represent a realistic value under field conditions (approximately  $400\text{ g DM m}^{-2}$ , DM: dry matter), while the amount of roots plus crowns (about  $235\text{ g DM m}^{-2}$ ) was given by their density in the soil in the top  $0\text{--}15$  cm soil layer and was determined in separate soil samples (Bleken et al., 2022), (Table 1 and Table S2).

### 2.3. Jar preparation and incubation

Two sets of cylindrical jars were used: glass jars ( $226\text{ ml}$ , circular section  $28.27\text{ cm}^2$ , four replicates) for gas sampling, and narrower plastic jars ( $164\text{ ml}$ , circular section  $21.24\text{ cm}^2$ , three replicates) for soil mineral N extraction. Both types of jars were filled with soil to  $8$  cm height and target soil bulk density  $1.15\text{ kg L}^{-1}$ , close to bulk density observed in the field (Bleken et al., 2022). The amount of soil used was  $130\text{ g}$  and  $94\text{ g}$  dry weight, for gas sampling and mineral N extraction, respectively, divided into two equally high ( $4$  cm) layers, with the treatment in the lower layer covered by a layer of control soil only. This was done to resemble conditions after ploughing. A treatment with inverted soil layers was included (FOCI1). Control treatments contained control soil without plant residues in any of the layers.

All incubation jars were prepared the day after the collection of the stubble (autumn batch only) and soil with fresh BGR. The soil moisture was good for soil crumbling, about  $28\%$ . The soils were coarsely homogenized by manually cutting the larger clods with a knife, taking care to maintain the structure of fine and medium soil aggregates. The crowns and larger clover tap roots were cut to approximately  $1\text{--}2$  cm thick pieces without removing them from the soil (photographs

available in dataset Bleken and Rittl, 2023). The fresh stubble was cut to  $1.5\text{--}2$  cm, and the pre-cut dry herbage was rewetted to  $20\%$  dry matter, which is approximately the DM content of fresh specimens. Portioned soil was blended in a bowl, alone or with fresh stubble and/or herbage, and  $\text{KNO}_3$  when relevant, and filled into the lower part of the incubation jar. During filling it was gradually compacted to target soil bulk density and soil height. When necessary, water was gently added after each soil compaction to reach  $60\%$  water-filled pore space (WFPS). An equal amount of the corresponding control soil (same batch and pH) was added on top, compacted and moistened in the same way.

The jars were kept aerobically in the dark at  $15^\circ\text{C}$  in thermostatically controlled incubators (Termaks KB 8182 and KBP 6151, Norway). This temperature is approximately the average soil temperature at  $10$  cm depth in July, at the site where the soil was sampled. To retard water evaporation, jars for gas measurement were covered with perforated parafilm and the lid of the nitrogen extraction jars was loosely placed. The moisture of the soil was kept at  $60\%$  WFPS by compensating the lost moisture weight with distilled water, when necessary.

### 2.4. Gas flux measurements and calculations

Fluxes of  $\text{N}_2\text{O}$ ,  $\text{CO}_2$ ,  $\text{O}_2$ , and  $\text{CH}_4$  were measured 1 (omitted in the autumn), 2, 5, and 8 days after incubation start, and gradually less frequently until 107 (132 in the autumn) incubation days. The measurements were halted when there were no more substantial emissions of  $\text{N}_2\text{O}$ . At each sampling, two gas samples were taken with 60 minutes deployment time, after closing the glass jars with gas-tight lid provided with a butyl rubber septum. Each time a  $15\text{ ml}$  gas sample was drawn by a syringe thorough the septum, after plunging the syringe twice, and transferred into a  $12\text{ ml}$  pre-evacuated glass vial. To avoid under-pressure,  $15\text{ ml}$  of ultrapure helium was injected into the jar before the second sample. The gas samples were analysed using a gas chromatograph (Agilent 7890 A, Agilent, Santa Clara, CA, US) equipped with an electron capture detector (ECD), thermal conductivity detector (TCD) and flame ionization detector (FID) (Molstad et al., 2007). Fluxes were calculated from the concentration difference between the first and second samples, taking into account the headspace volume, the gas molar volume at the working temperature and the dilution effect of the added He (which was checked by the  $\text{N}_2$  partial pressure, also measured by the chromatograph). Cumulative emissions were calculated per jar by linear interpolation between consecutive sampling dates.

The marginal emission factor  $EF_q$  of either  $\text{N}_2\text{O}$  or  $\text{CO}_2$  for each type of plant residue  $q$  (either roots, stubble or herbage of either clover or grass) was calculated per jar replicate  $i$  as:

$$EF_{qi} = (F_{jq_i} - \bar{F}_j) / S_q$$

Where  $F_j$  and  $F_{jq}$  are the cumulative  $\text{N}_2\text{O-N}$  or  $\text{CO}_2\text{-C}$  fluxes of treatments that differ only with respect to  $q$  and are otherwise equal and from the same batch, and  $S_q$  is the amount of N or C contained in the specific residue  $q$ . The EF of BGR was calculated by subtracting the gas emissions of the corresponding control fallow treatment. In batch 1, the low pH control treatment (K1) was lost due to the mentioned incubator failure. It was therefore replaced by the high pH fallow soil treatment (KH1), supported by the fact that up to 13 days of incubation K1 and KH1 were very similar and non-significantly different. The presented EF values are used to compare the relative effect of different residues and are not intended for direct use in GHG inventories.

The apparent first-order substrate's C decay rates were calculated from the slope of  $\ln(Z_{qt})$  versus time ( $t$ ), where ( $Z_{qt}$ ) is the share of substrate carbon ( $S_q$ ) not recovered as  $\text{CO}_2$  calculated as:

$$Z_{qt} = (S_q - F_{jt} - F_{jq_t}) / S_q$$

where  $F_{jt}$  and  $F_{jq_t}$  are cumulative  $\text{CO}_2\text{-C}$  emissions of pairwise treatments at time  $t$ .

2.5. Soil mineral nitrogen (N<sub>min</sub>)

On days 1, 4, 7, 15, 28, 62 and 107 after incubation start, the two soil layers of the plastic jars were separated, each layer was blended, and a

35 g subsample per layer and replicate was frozen (-20 °C) until extraction with 60 ml of 1 M KCl, then analysed for NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> contents as described in Bleken et al. (2022). No NO<sub>2</sub><sup>-</sup> was detected in the soil samples. Due to working capacity and time

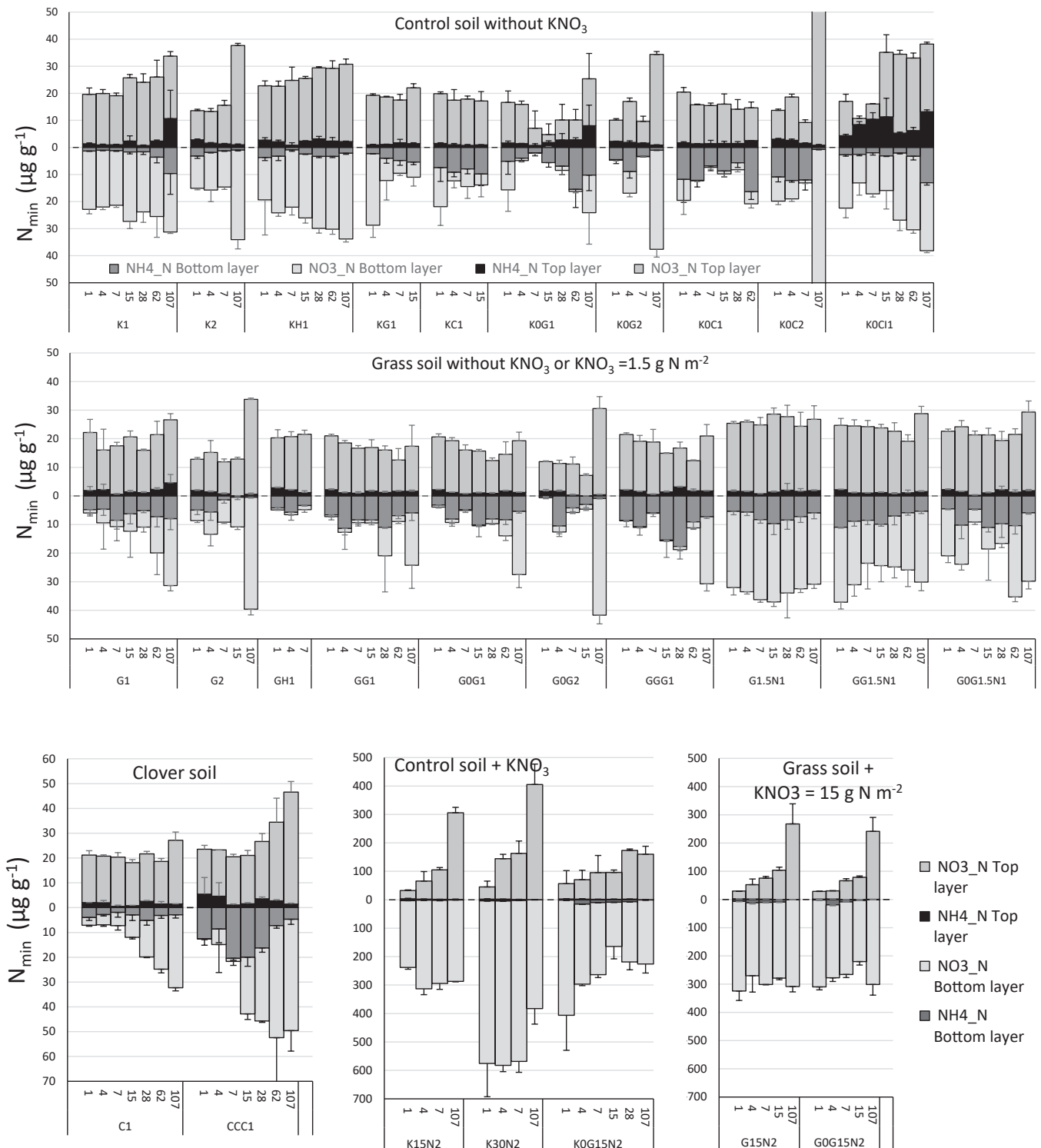


Fig. 1. Mineral N concentration (NH<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, µg N g<sup>-1</sup> soil DW) in the top and bottom soil layers (the bottom layer axis is in reverse order), on selected days after experiment start (1, 4, 7, 15, 28, 62 and 107); note that for many treatments only a few dates are available. Soil bulk density was 1.15, thus 10 µg N g<sup>-1</sup> soil corresponds to 0.46 g m<sup>-2</sup> in a 4 cm layer. Treatments are grouped by absence of BGR (control soil) or type of BGR (grass or clover soil). For treatment description see Table 1; results are also presented for the control soil alone (K1) and the control soil plus grass herbage (KOG1) for which gas measurements are missing. Bars are one-side SD, n=3. Note different y-axis scales.

limitations, for some treatments, jars were only prepared for a limited number of sampling dates and none were prepared for the CC treatment. The last N<sub>min</sub> determination used the soil of the jars for gas sampling (Day 132 in batch 1, and 107 in batch 2).

## 2.6. Statistical analysis

Based on the results of preliminary Box–Cox transformations, approximation of normal distribution of the cumulative emissions of N<sub>2</sub>O and CO<sub>2</sub> end EF<sub>N<sub>2</sub>O</sub> were obtained through transformation to natural logarithm, and in the case of EF<sub>CO<sub>2</sub></sub> to its square root. Treatment effects were tested in a one-way analysis of variance; equal treatment combination in separate batches (G1 and G2, G0G1 and G0G2) were considered separate treatments. In this article  $p < 0.05$  is, for brevity, referred to as significant. For all response variables considered the effect of treatment was significant. Thus, treatment means were compared using the least significant difference test (LSD), the Tukey's studentized range test and the Ryan-Einot-Gabriel-Welsch multiple range of the transformed data. As all tests essentially individuated the same major grouping, we report only the results of the LSD test ( $p \leq 0.05$ ). All statistical analyses were performed using SAS 9.4 (Statistical Analysis System; SAS Institute Inc, Cary NC, USA 2016). Two replicates of treatment CCC showed extremely high decomposition rates. However, we decided to keep all replicates in the analysis since we found no reason to doubt about the treatment preparation.

## 3. Results

### 3.1. Soil mineral N (N<sub>min</sub>)

Differences in soil N<sub>min</sub> between treatments and changes over time were most evident in the lower layer, where plant residues were present. Nitrate was often the dominant form and when its concentration was depleted or increased in the bottom layer this also occurred in the top layer (Fig. 1), reflecting the higher mobility of NO<sub>3</sub><sup>-</sup> compared to NH<sub>4</sub><sup>+</sup>. The added KNO<sub>3</sub> in the bottom soil layer was generally completely recovered as soil NO<sub>3</sub><sup>-</sup>, and part of it gradually diffused to the upper soil layers.

The initial N<sub>min</sub> content of the control soils (K1, KH1, K2) was moderate,  $\leq 23$  mg N g<sup>-1</sup> ( $\sim 1$  g N m<sup>-2</sup> in the 4 cm thick layer), mainly as NO<sub>3</sub><sup>-</sup>, and it increased by  $\sim 0.4$  g N m<sup>-2</sup> by the end of the experiment. Grass and clover soils had lower initial N<sub>min</sub> content (0.2–0.4 g N m<sup>-2</sup> in the bottom layer), and despite a slightly greater net N mineralization, the N<sub>min</sub> content in BGR treatments (G1, GH1, G2 and C1) remained generally lower than in controls, but with a higher NH<sub>4</sub><sup>+</sup> content. Grass stubble and herbage decreased the initial NO<sub>3</sub>-N content of control soil to  $< 1$  μg g<sup>-1</sup>, and when they were added to grass soil the nitrate content remained mostly below 2 μg g<sup>-1</sup> also when net mineralization took over, usually after one or two weeks. This immobilization effect of grass herbage and, though slightly weaker, stubble (C:N ratios of  $\sim 21$  and  $\sim 24$ , respectively) was £ 0.9 g N m<sup>-2</sup> for 40 g AGR m<sup>-2</sup>, and it was not detected in presence of a large dose of KNO<sub>3</sub> (15 g N m<sup>-2</sup>).

Clover AGR strongly increased NH<sub>4</sub>-N, although it also reduced the initial amount of NO<sub>3</sub><sup>-</sup>. The largest net mineralization, nearly 3.2 g N m<sup>-3</sup> including the top layer (15 % of N in AGR), was observed in CCC (calculated as the final N<sub>min</sub> content in CCC above the initial N<sub>min</sub> content in C1).

### 3.2. Carbon decomposition rate

All trials containing residues had two periods in common (up to 19 days and from day 30 to end of experiment), when the apparent decomposition rate could be well approximated by first-order decay. During both periods, the apparent decomposition rates ( $k$ ) of BGR were lower, roughly  $< 1/3$  of the  $k$  value of AGR in the first period and  $\sim 2/3$  in the second period. On average,  $k$  was  $6.5 \times 10^{-3}$  d<sup>-1</sup> and  $2.1 \times 10^{-3}$

d<sup>-1</sup> for the early and late period of BGR, and  $22 \times 10^{-3}$  d<sup>-1</sup> and  $3.1 \times 10^{-3}$  d<sup>-1</sup> for AGR, all values for treatments without KNO<sub>3</sub> (Table S3). For the treatments including KNO<sub>3</sub>, the difference between the decomposition rate of BGR and AGR was larger.

### 3.3. Cumulative N<sub>2</sub>O and CO<sub>2</sub> emissions

N<sub>2</sub>O fluxes peaked on the first sampling day or within the first incubation week and declined rapidly thereafter (Fig. S2). On average, excluding the treatments with the addition of KNO<sub>3</sub>, 68 % of the total N<sub>2</sub>O emissions occurred during the first 30 days, whereas the contribution during the last period (62–107 days) was on average only 14 %. However, some treatments showed smaller late peaks, and in some low-emission treatments (C1 and GG1), the last period accounted for more than 50 % of the total emissions (Fig. 2 and Table S4).

The treatments varied largely in cumulative N<sub>2</sub>O and CO<sub>2</sub> emissions (107 days). In the control treatments without plant residues, emissions were low (N<sub>2</sub>O: 3–11 mg N m<sup>-2</sup>, CO<sub>2</sub>: 19–29 g C m<sup>-2</sup>) and did not respond to the application of 15 g N m<sup>-2</sup> as KNO<sub>3</sub>; only when a very large dose (30 g N m<sup>-2</sup> as KNO<sub>3</sub>) was added a statistically significant but trivial increase of N<sub>2</sub>O emissions to 20 mg N m<sup>-2</sup> was observed. This was accompanied by a decrease in CO<sub>2</sub> emissions (Fig. 2, Table S4).

In soils with BGR only (C1, G1, G2, GH1), N<sub>2</sub>O emissions remained low ( $\leq 22$  mg N m<sup>-2</sup>, highest for clover roots but not significantly different from grass), and mostly non-significantly different from the controls. A small dose of KNO<sub>3</sub> (1.5 g N m<sup>-2</sup>) added to grass roots did not increase N<sub>2</sub>O emissions, and the effect of a large dose (15 g N m<sup>-2</sup>) was significant but small. CO<sub>2</sub> emissions were higher than in the controls, but still low ( $< 67$  g C m<sup>-2</sup>). Interestingly, the addition of KNO<sub>3</sub> decreased CO<sub>2</sub> emissions in soils with only BGR, similarly to what observed in the control soil, which can be due to a positive effect of N supply on the microbial growth yield.

In contrast to BGR, AGR residues increased CO<sub>2</sub> respiration several-fold compared to controls ( $> \sim 100$  g C m<sup>-2</sup>). In the case of clover residues, N<sub>2</sub>O emissions were always large (160–430 mg N m<sup>-2</sup>), even without KNO<sub>3</sub> additions, while in the case of grass AGR, N<sub>2</sub>O emissions varied from very low in the first batch to moderate in the second batch in treatments without KNO<sub>3</sub> (10–102 mg N m<sup>-2</sup>). However, in presence of grass AGR even a small dose of 1.5 g N m<sup>-2</sup> as KNO<sub>3</sub> tripled the cumulative emissions and 15 g KNO<sub>3</sub> m<sup>-2</sup> increased the emissions by one order of magnitude to 401–513 mg N m<sup>-2</sup>.

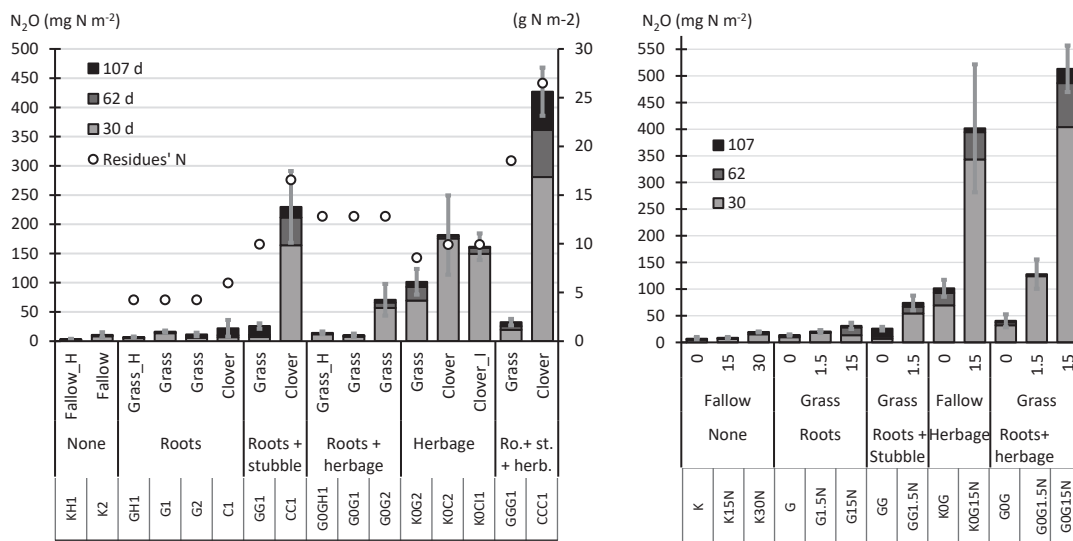
The low-high pH pair treatments left that survived a cabinet failure (G1 and GH1, K1 and KH1, G0G1 and G0GH1) had similar low cumulative N<sub>2</sub>O emissions (including the first 13 incubation days of K1 and KH1, after which K1 was lost, data not shown).

There were no substantial interactions enhancing N<sub>2</sub>O emissions when BGR and AGR were mixed in the same treatment.

### 3.4. EF<sub>N<sub>2</sub>O</sub> and EF<sub>CO<sub>2</sub></sub> over 107 incubation days

Given the lack of evident interaction between AGR and BGR, the emission factors of AGR were calculated from treatment pairs in combination with BGR as well for AGR in control soil (Section 2.4). Notice that the biomass and nitrogen amount of BGR were approximately 50–60 % of those in AGR (Table 1, Table S2).

In line with what reported for the cumulative emissions, over 107 days the EF<sub>N<sub>2</sub>O</sub> of BGR were low (0.02–0.32 %, SE 0.04–0.24) (Table 2, Fig. S3). A plot of EF<sub>N<sub>2</sub>O</sub> versus EF<sub>CO<sub>2</sub></sub> (Fig. 4) illustrates that low EF<sub>N<sub>2</sub>O</sub> of BGR compared to AGR were largely related to lower EF<sub>CO<sub>2</sub></sub>. In contrast to BGR, the EF<sub>N<sub>2</sub>O</sub> of AGR varied largely and were much higher for clover (1.5–2.0 %, SE £0.7) than for grass, for which emissions diverged depending on the treatment combination and batch time, from nearly nil ( $-0.07$  % and 0.11 %, SE £0.04) in the autumn batch to 1 % (SE 0.26, comparison K0G-K) in the spring batch, all in treatments without KNO<sub>3</sub> supplement. The EF<sub>CO<sub>2</sub></sub> for BGR were 9 % for clover roots and £36 % for grass roots, significantly lower than for any AGR of grass and clover. The



**Fig. 2.** Cumulative  $N_2O$  emission ( $mg\ N\ m^{-2}$ ) over 107 incubation days, divided by time intervals (0–30, 31–62 and 63–107 days) and organized by type of plant residue. Left: treatments without addition of  $KNO_3$ . Open circles indicate the amount of total N in plant residues (scale on the right vertical axis). Grass, Clover and Fallow indicate the soil and thus BGR used (fallow soil was the control soil). Right: treatments with addition of  $KNO_3$  (0, 1.5, 15 or  $30\ g\ N\ m^{-2}$ ), here only grass residues were used and nil  $KNO_3$  treatments are averages of both batches when relevant. The bottom row in the legend lists the treatments, see Table 1. Error bars show  $\pm SE$  of the cumulative 107-days emission,  $n=4$  except when  $n=8$  for treatments pooled together as mentioned above. See Supplemental Table S4 for LSD grouping.

**Table 2**

$EF_{N_2O}$  and  $EF_{CO_2}$  (%) for residues of grass (g) or red clover (c) calculated separately for BGR (R), stubble (S) and herbage (H) or S and H incubated together (SH), over a 107 day incubation period in a clay loam at  $15\ ^\circ C$  and 60 % water filled pore space and treatment pair used to calculate EF. See Table 1 for treatment description. SE: standard error,  $n=4$ . Letters indicate *post hoc* Tukey grouping (two ways,  $p=0.05$ ), calculated on transformed data to normalize residuals distribution. These data are also visualized in Supplemental Fig. S3.

$KNO_3$ ( $g\ N\ m^{-2}$ )	Residue substrate	Treatment comparison	$EF_{CO_2}$ (%)			$EF_{N_2O}$ (%)			
			Batch	Mean	SE	Mean	SE		
0	cR	C - KH	1	9.3	3.7	o	0.313	0.24	fghl
		G - KH	1	36.0	4.4	hlm	0.304	0.04	ghl
	gR	G - K	2	32.5	5.2	lmn	0.020	0.07	mn
		GH - KH	1	28.0	2.1	mn	0.084	0.02	lm
	cS	CC - C	1	82.2	14.3	a	1.961	0.58	bc
	gS	GG - G	1	24.3	0.7	n	0.166	0.08	ghl
	cH	KOC - K	2	51.0	0.9	cde	1.520	0.23	c
		KOCI - KH	1	73.0	7.3	ab	1.795	0.68	bc
	gH	KOG - K	2	52.5	1.2	cde	1.061	0.26	cde
		GOG - G	1	37.5	3.1	hlm	-0.071	0.03	n
	GOG - G	2	54.6	3.7	cd	0.692	0.32	def	
	GOGH - GH	1	46.6	1.5	deh	0.084	0.03	lm	
cSH	CCC - C	1	54.1	5.0	cd	1.972	0.20	bc	
gSH	GGG - G	1	37.5	2.0	hlm	0.114	0.04	hlm	
1.5	gR	G1.5 N - K1.5 N	1	23.5	3.2	n	0.402	0.09	efgh
	gS	GG1.5 N - G1.5 N	1	40.4	3.3	ehl	0.940	0.34	cdef
15	gH	GOG1.5 N - G1.5 N	1	60.8	4.2	bc	1.256	0.45	cd
	gR	G15N - K15N	2	14.2	1.6	o	0.527	0.20	defg
15	gH	KOG15N - K15N	2	43.7	0.8	deh	4.587	1.95	ab
		GOG15N - G15N	2	53.3	1.4	cd	5.628	0.71	a

$EF_{CO_2}$  of AGR varied less than  $EF_{N_2O}$ ; on average, they were roughly  $< 50\%$  for grass and  $\sim 60\%$  for clover.

We also explored how the specific effect of crop residues varied under different levels of  $NO_3^-$  in the soil (Table 2 and Fig. 4). Note that we calculated the  $EF_{N_2O}$  for emissions induced by plant residues above those induced by the application of nitrate alone, except in the case of BGR and  $1.5\ g\ KNO_3-N\ m^{-2}$  for which an appropriate control was missing, and we then used the comparison G1.5 N-K.  $EF_{N_2O}$  of grass AGR strongly increased in the presence of even a moderate  $KNO_3$  dose ( $1.5\ g\ KNO_3-N\ m^{-2}$ ) to 0.9–1.2 % (SE  $\pm 0.45$ ) in the autumn batch (contrast GG1.5 N-G1.5 N and GOG1.5 N-G1.5 N), and a dose equivalent to an abundant N fertiliser application ( $15\ g\ N\ m^{-2}$ ) boosted the  $EF_{N_2O}$  of

grass herbage to 4.6–5.6 % (SE 2 and 0.7); this was in striking contrast to the  $EF_{N_2O}$  of BGR, which remained low (0.52 %, SE = 0.14 %) even with a high dose of nitrate ( $15\ g\ N\ m^{-2}$ ) (Table 2 and Fig. 4). Red clover treatments received no  $KNO_3$  supplementation.

### 3.5. Duration of the experiment

Over the entire 107-day incubation period and including all treatments, the ratio (BGR  $EF_{N_2O}$ ):(AGR  $EF_{N_2O}$ ) was 0.18. This ratio increased from  $\sim 0.15$  in the first 62 days to  $\sim 0.27$  for the remaining incubation period (63–107 days), indicating that the difference between the effects of BGR and AGR attenuated during the incubation period.

Clover BGR showed an emission peak in the second half of the incubation period and was excluded from the late period; when included the ratio for the period 63–107 days increases to  $\sim 0.59$  (Table 3).

### 3.6. Residue quality

The remarkable differences between AGR and BGR in  $EF_{N_2O}$  and, to a lesser extent,  $EF_{CO_2}$  were not related to differences in the common biochemical parameters used to predict decomposition, as C:N ratio and ND-soluble fraction (neutral detergent soluble fraction, as opposed to the hemicellulose, cellulose and lignin fractions). On the contrary, all clover residues had the same low C:N ratio ( $\sim 18$ ), grass BGR had a lower C:N ratio than stubble (26 and 33, respectively) and both clover and grass BGR had a much larger ND-soluble fraction than AGR (Table S2). Even the ADL (mainly lignin) content, which is often linked to slow decomposition (Bonanomi et al., 2021), was not related to the differences in decomposition rate between BGR and AGR.

Exclusively considering AGR, there were six quality combinations: stubble, herbage, or both in the same treatment of either clover or grass. The ND-soluble fraction and total N content were the properties that correlated closest with C decomposition and  $EF_{N_2O}$  (Table S5).

## 4. Discussion

### 4.1. Carbon decomposition rate

As expected (H1) the apparent decomposition rate of BGR was markedly slower than that AGR incorporated in the soil, in agreement with observations in previous studies (e.g., Balesdent and Balabane, 1996; Kätterer et al., 2011; Poeplau et al., 2021; Puget and Drinkwater, 2001; Rasse et al., 2005). This has been attributed to a different biochemical composition and to the fact that roots establish close contact with soil particles and microaggregates, which can provide both physical and chemical protection. However, differently from AGR, the lower decomposition rate of BGR could not be related to measured biochemical characteristics in the residues. For example, the C:N ratio of roots was similar to that of AGR, and the ND-soluble fraction was larger. Other characteristics may have played a role. Important factors retarding decomposition of fine roots are chemical and physical protection (Rasse et al., 2005), which play an important role on decomposition, especially on clayey texture soils (Abiven et al., 2005; Frøseth and Bleken, 2015; Poeplau et al., 2021; Thomsen et al., 1996). However, neither biochemical quality nor soil mineral protection can explain the very low short-term decomposition rate of clover BGR, which included clearly visible tap roots. It is possible that although clipped, tap roots did not start to rot before late in the incubation, an assumption that is also supported by the relatively high  $CO_2$  emissions in the later period.

### 4.2. Nitrogen and carbon effects on $EF_{N_2O}$

Addition of nitrate had a smaller effect on EF of BGR compared to those of AGR. The fact that even a small dose of  $KNO_3$  strongly enhanced the  $EF_{N_2O}$  of AGR, while in the case of BGR a dose ten times as large

caused only a moderate increase, reveals a basic dissimilarity of AGR and BGR, linked to C decomposition rather than supply of mineral N. This indicates that in treatments with BGR as the only plant residue,  $N_2O$  emissions were limited by lack of carbon substrate (respiration  $\leq 70$  g  $CO_2-C\ m^{-2}$  over 107 days), regardless of  $NO_3$  concentration in the soil, whereas in certain treatments with grass AGR and  $CO_2$  respiration above  $\sim 100$  g  $C\ m^{-2}$ ,  $N_2O$  emissions were limited by lack of mineral N, as indicated by the effect of  $KNO_3$  (Fig. 3). This is in agreement with our hypothesis that C decomposition and availability of mineral N together affect  $EF_{N_2O}$  of crop residues (H2). Notice also the diverse effect of  $KNO_3$  supplement on  $EF_{CO_2}$  from grass stubble compared to BGR. In the case of stubble, which had a low  $EF_{CO_2}$  similar to that of BGR,  $EF_{CO_2}$  increased which can indicate that residue decomposition was limited by lack of N, while in the case of BGR  $EF_{CO_2}$  decreased, which could indicate that microbial growth yield rather than net residue decomposition benefited of more mineral N available (Table 2, Fig. 4 A).

The treatments were incubated aerobically. Local anaerobic sites were facilitated by the soil moisture (60 % WFSP) in a range of moisture labile to denitrification (Davidson et al., 2000). Denitrification has often been pointed out as the main process contributing to high  $N_2O$  emissions from cultivated soils, even under relatively low soil moisture conditions (Li et al., 2016). In our study, denitrification was undoubtedly involved when  $KNO_3$  was added in treatments with grass AGR (GG1.5N1, GOG1.5N1, KOG15N2, GOG15N2). Heterotrophic denitrification was also confirmed by the fact that large doses of  $KNO_3$  added to treatments with low C-mineralization rate (control soil and soil with grass roots) caused minimal increase in  $N_2O$  production, evidently limited by lack of labile carbon compounds, as indicated by the low soil respiration. The higher decomposition rate of AGR likely created hotspots for denitrification close to the residues (Kravchenko et al., 2017; Kuzyakov and Blagodatskaya, 2015). Whereas in the case of BGR both the lower decomposition rate and the finer distribution of roots compared to AGR can have reduced the occurrence of anaerobic hotspots.

Partial anaerobicity can also enhance  $N_2O$  emissions from nitrification. Tightly coupled nitrification-denitrification (Nadeem et al., 2020; Wrage et al., 2001) probably played an important role when the initial concentration of  $NO_3-N$  in the soil was low ( $0-6\ \mu g\ g^{-1}$  soil), as in the treatments with grass herbage in the spring batch (KOG2 and GOG2). The case of grass AGR with no  $KNO_3$  addition was peculiar, because emissions depended on the batch (Table A2, Fig. S3), as discussed later.

The reason why we found no indication of a soil pH effect was that only treatments with low  $N_2O$  emissions were also tested with higher pH soil. Low soil pH-soil enhances  $N_2O$  production from denitrification, since acidic conditions hamper  $N_2O$ -reductase activity, which in turn leads to a high  $N_2O/(N_2O+N_2)$  ratio of the denitrification products (Liu et al., 2014). In a parallel field study in the leys from which our material was taken, higher soil pH halved  $N_2O$  emissions following autumn ploughing (Bleken and Rittl, 2022). We interpreted this as an indication that denitrification was the main source of  $N_2O$  and the beneficial effect on a more complete denitrification exceeded any  $N_2O$  increase due to enhanced N mineralization of the crop residues at the higher soil pH level (Bleken and Rittl, 2022).

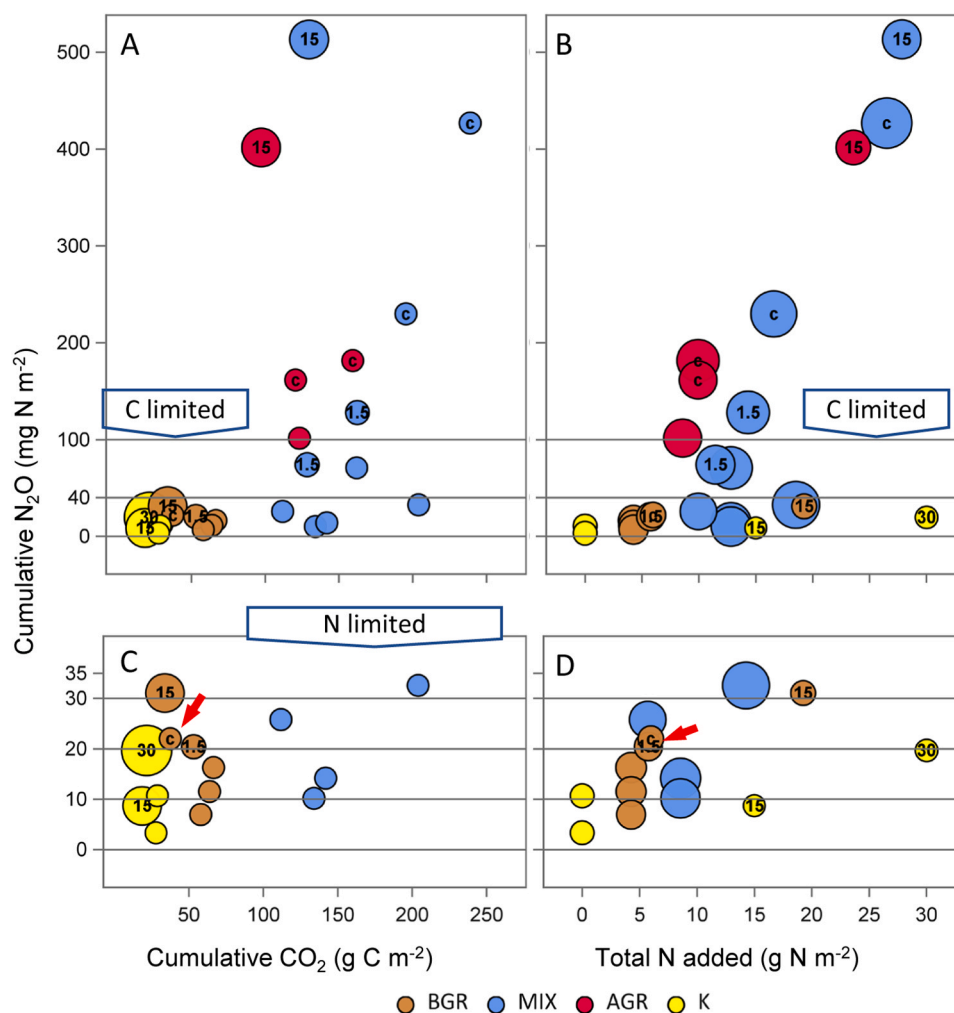
**Table 3**

Ratios of  $EF_{N_2O}$  and  $EF_{CO_2}$  related to BGR and AGR, calculated from the average of all BGR estimates above the average of all AGR estimates, or using only treatments with  $KNO_3$  or only treatments without  $KNO_3$ . Ratios are given for increasing periods (30, 62 and 107 days) since incubation start, and for the interval day 63 to day 107.

Observation interval (days)	$EF_{N_2O}$				$EF_{CO_2}$			
	0-30	0-62	0-107	63-107	0-30	0-62	0-107	63-107
Ratio BGR/AGR all	0.13	0.15	0.18	0.27 <sup>a</sup>	0.35	0.43	0.47	0.90
Ratio BGR/AGR without $KNO_3$	0.14	0.14	0.19	0.25 <sup>b</sup>	0.39	0.48	0.52	0.95
Ratio BGR/AGR with $KNO_3$	0.11	0.15	0.15	0.26	0.27	0.32	0.38	0.91

<sup>a</sup> without clover BGR, if clover is included: 0.59

<sup>b</sup> without clover BGR, if clover is included: 0.81



**Fig. 3.** Cumulative  $\text{N}_2\text{O}$  fluxes ( $\text{mg N m}^{-2}$ ) during 107 incubation days, plotted versus (A & C) cumulative  $\text{CO}_2$  emission ( $\text{g C m}^{-2}$ ) or (B & D) total N ( $\text{g N m}^{-2}$ ) in plant residues and  $\text{KNO}_3$ . The whole  $\text{N}_2\text{O}$  emission range is shown in panels A and B, while in C and D the Y-axis is limited to the range of BGR only. In panels A and C the size of the symbols is proportional to the total N added, while in B and D it is proportional to the  $\text{CO}_2$  emission. Colours identified type of residues (K: no plant residues, BGR, AGR, MIX: both AGR and BGR). When  $\text{KNO}_3$  was added, the amount in  $\text{g N m}^{-2}$  is shown with numbers inside the symbols. Treatments with clover plants are identified with a 'c' in the symbol. The treatment with clover roots only (C1) is indicated by a red arrow in panels C and D. For error values of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions see Supplemental Table S4, and Fig. 2 for treatment identification.

#### 4.3. Residue quality

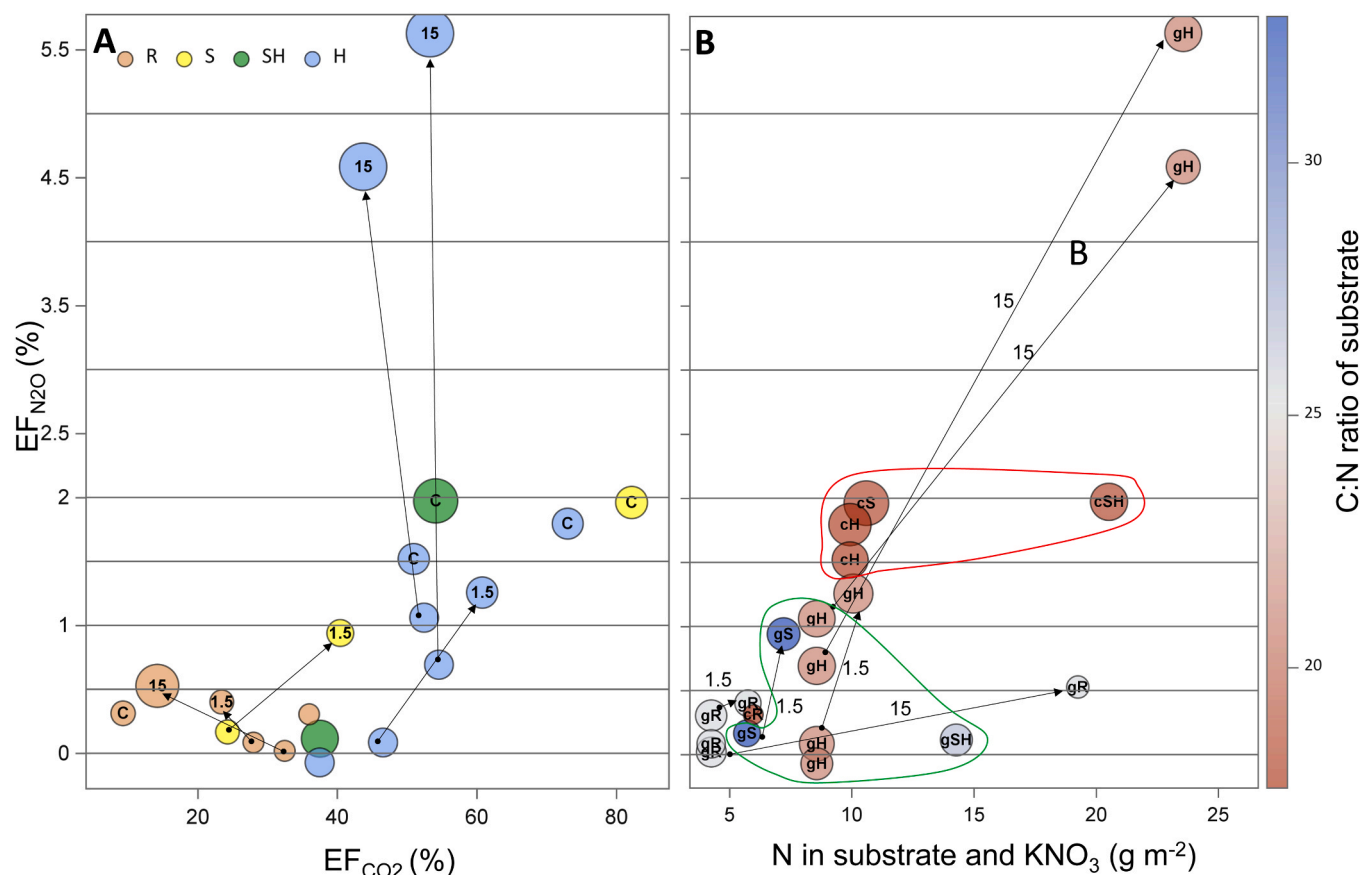
All belowground and aboveground residues were from living perennial forage plants, with a large share of physiologically active tissues and thus with a low or moderate C:N ratio (range 18–33) and a large share of easily degradable compounds (ND-soluble range 35–20 %) and were therefore expected to elicit  $\text{N}_2\text{O}$  emissions upon incorporation in the soil. However, as already discussed, other factors than those measured by typical digestibility quality traits could have curbed the decomposition of BGR carbon, and thus kept  $\text{EF}_{\text{N}_2\text{O}}$  low.

Exclusively considering AGR, there were six residue combinations: stubble, herbage, or both in the same treatment of either clover or grass. The ND-soluble fraction and total N content were the properties that correlated closest with C decomposition and  $\text{EF}_{\text{N}_2\text{O}}$  (Table S5), in accordance with our hypothesis (H3) and similarly to what was observed by Lashermes et al. (2022), who screened a range of AGR of widely different quality, including our clover herbage. Lashermes et al. (2022) reported that most N was found in the ND-soluble fraction. The optimization of residue decomposition models versus empirical incubation results has also identified the ND-soluble fraction as the one that correlates best with the readily decomposable litter C and N pools of AGR (Borgen et al., 2011; Henriksen and Breland, 1999). There was no

correlation in our data between  $\text{EF}_{\text{N}_2\text{O}}$  and water-soluble C, contrary to Surey et al. (2020), who attributed early  $\text{N}_2\text{O}$  peak fluxes after residue incorporation to water-soluble carbohydrates. Plant respiration in fresh residue likely consumes the soluble carbohydrates prior to heterotrophic decomposition.

It has been reported that a C:N ratio below  $\sim 30$  generally promotes  $\text{N}_2\text{O}$  emissions and that this effect is related to net N mineralization (Chen et al., 2013; Lashermes et al., 2022; Zhang et al., 2015) and the presence of more recalcitrant compounds at a high C:N ratio (Abalos et al., 2013). A higher C:N ratio can enhance N immobilization and thus restricts N availability to denitrification. In the present study, the C:N ratio of grass stubble and herbage was 33 and 21, respectively, and both kind of residues slightly reduced  $\text{N}_{\text{min}}$  content in the soil. However, when no  $\text{KNO}_3$  was added, grass AGR supported very low  $\text{N}_2\text{O}$  emissions in the autumn batch but substantial emission and  $\text{EF}_{\text{N}_2\text{O}}$  in the spring. The difference in effect between spring and autumn batches cannot be explained by a different  $\text{N}_{\text{min}}$  content in the soil, since it was similar and initially low ( $< 5 \mu\text{g g}^{-1}$ , see K0G2, G0G1 and G0G2 in Fig. 1) in both batches. As mentioned, the low soil pH favoured  $\text{N}_2\text{O}$  emissions from denitrification in the field, which in the autumn batch was limited by lack of nitrate, as shown by the response to  $\text{KNO}_3$  (see GG1 and G0G1 versus GG1.5N1 and G0G1.5N1). Likely,  $\text{N}_2\text{O}$  production from coupled





**Fig. 4.**  $EF_{N_2O}$  (% of N in residue) versus **A:**  $EF_{CO_2}$  (% of C in residue), or **B:** the sum of the amount of N in the residue substrate for which EF is calculated plus  $KNO_3$ . Values for 107-day incubation. Arrows join pairs of treatments in the same batch without or with  $KNO_3$ . In panel **A** the symbol size is proportional to the amount of N in the residue substrate plus  $KNO_3$ , while the colour indicates the type of substrate (BGR: R, Stubble: S, Herbage: H, Stubble and Herbage: SH), a 'C' inside the symbol indicates clover residues, while 1.5 and 15 indicate the amount ( $g\ N\ m^{-2}$ ) of  $KNO_3$  added to grass residues. In panel **B** the symbol size is proportional to  $EF_{CO_2}$ , the colour indicates the C:N ratio of the residue (scale to the RHS), and the letters inside the symbols indicate the substrate: grass (g) or clover (c) plant parts as BGR (R), stubble (S), herbage (H) or both stubble and herbage (SH), and the amounts ( $g\ N\ m^{-2}$ ) of  $KNO_3$  are shown next to the arrows. Shapes join EF of AGR of clover (red) or grass (green) without  $KNO_3$  supplement. For identification of the treatments and standard errors, see [Table 2](#) and [Supplemental Fig. S3](#).

nitrification-denitrification was present in the spring more than in the autumn. Several treatments underscore this difference: GG1, GOG1 and GGG1 in the autumn, GOG2 and KOG2 in the spring. Soils used in the autumn batch were taken after a dry summer and when  $N_2O$  emissions were about nil (Bleken and Rittl, 2022), while the soils of the spring batch were collected when field  $N_2O$  emissions were high (Bleken et al., 2022). This indicates a different activity of the soil microbial community when the incubation started. Summing up, our results indicate that when the initial  $N_{min}$  content in the soil is low ( $<10\ mg\ N\ g^{-1}$ ), the effect on  $N_2O$  emissions of plant AGR with a moderate C:N ratio ( $<30$ ) is hard to predict, and it increases several-fold if nitrate is available in the soil ( $\sim 30\ \mu g\ g^{-1}$  in our study). Thus, it is important to minimize residual mineral N in the soil and fertilization when AGR residues are incorporated in the soil (Taghizadeh-Toosi et al., 2021).

These results are also in line with conclusions from a comprehensive meta-analysis (Chen et al., 2013), which stated that only residues with very large C:N ratios ( $> 100$ ) induced a consistent reduction in  $N_2O$  emissions compared to unamended controls. Hence, the role of crop residues on oxygen depletion and as energy substrate for denitrifiers often overrides temporary effects on N immobilization. Consequently, crop residues should be evaluated both for their N and C derived contributions to  $N_2O$  emissions (Olesen et al., 2023).

#### 4.4. Duration of the incubation period

We assumed that the  $EF_{N_2O}$  of BGR would decline more gradually

than the  $EF_{N_2O}$  of AGR, reflecting the above-mentioned slower decomposition rate of BGR. Indeed, difference between the EFs of BGR and AGR attenuated during the incubation period, as accentuated by the late emission peak of clover BGR (Table 3). Thus, a longer experimental period warrants better relevance for field conditions when studying BGR. Shorter incubation periods (e.g., 62 days as used by Janz et al. 2021 and Lashermes et al. 2022) are more suitable for a rapid screening of AGR.

#### 4.5. Simulated tillage and residue position in the soil

In our incubation study, the bottom soil layer was richer in plant residues than the topsoil layer. This resembles the residue distribution observed with shallow ploughing following harrowing on sod, which is a common practice in Norway for breaking turf and controlling certain perennial weeds (Thomsen et al., 2015). Carter et al. (2014) reported higher emissions with simulated ploughing (AGR layered at 15 cm depth) compared to simulated harrowing (mixing in the upper 5 cm of soil) of grass-clover green manure, and Taghizadeh-Toosi et al. (2021) reported higher emissions when residues were placed in a discrete layer rather than mixed in the upper soil layer. In a field study, Petersen et al. (2011) found lower  $N_2O$  emission when a fodder radish catch crop was incorporated by shallow tillage instead of by conventional tillage. The fact that we measured no effect of residues position is probably a consequence of the large variation between replicates of the treatments involved (FOC versus FOCl). In a preliminary study we observed higher

emissions with residues in the lower position (data not shown).

The contribution of BGR to N<sub>2</sub>O emissions remained low when blended with AGR, without indication of interactions). There was also no substantial evidence of interactions enhancing N<sub>2</sub>O emissions when stubble and herbage were added together in the same treatment. This makes sense in the case of BGR due to their recalcitrance to decomposition and fine distribution in the soil. We can assume that in our study the chopped AGR particles were sufficiently spaced to prevent N<sub>2</sub>O emission hotspots to interact with each other, thus maintaining the effects additive. Harrowing before ploughing may have a potential for reducing N<sub>2</sub>O emissions.

#### 4.6. Comparison with other studies and implications

We found very few studies of the specific effect of root residues, and none of them could be directly compared to ours. In the laboratory studies, roots were subjected to a high degree of manipulation, including extraction from soil, washing, and grinding or selection of only a fraction (Begum et al., 2014; Lou et al., 2007; Pal et al., 2013) and the results diverged, whereas field studies with <sup>15</sup>N labelled residues of annual crops (Machado et al., 2021; Van Vleck et al., 2017) reported very low emissions, but they excluded the first two months after harvest and therefore missed a potentially important period. There were thus no comparable study on the effect of BGR versus AGR on N<sub>2</sub>O emissions.

Reported EF<sub>N2O</sub> and analogous EF<sub>CO2</sub> are meant for comparison of residue effects and are not proposed as direct emission factors under field conditions. However, we explored to what extent the present laboratory results were consistent with parallel observations in the field experiment from which the material of the present study was taken (Bleken et al., 2022). In the field experiment, we selected treatments with clover-grass and grass leys, and estimated the effect of retaining the herbage at ploughing as the additional emissions compared to plots with nonremovable residue only. The effects of nonremovable residue, that is of BGR and the stubble left after the last harvest, were confounded, as it usually happens, and their combined effect was estimated by comparison with black fallow subplots or living leys, which gave similar results.

We can apply information from the present study to separate the field emissions factor of BGR from that of the stubble if we assumed that the ratio (EF-BGR:EF-AGR) was the same as in the laboratory incubation, ~ 0.19 for EF<sub>N2O</sub> over 107 days including clover and without KNO<sub>3</sub>. Using this approach, the EF<sub>N2O</sub> of BGR in the field from autumn to spring were approximately 0.11 % for both clover-grass and grass ley, which is lower, although within the uncertainty range, than the default IPCC value for wet regions (0.6 %, CV50 %, IPCC, 2019), whereas the EF<sub>N2O</sub> of stubble (both grass and clover) was about the same as observed for clover-grass herbage and practically the same as the IPCC default value (Table S5). Interestingly, incorporation of grass herbage in the autumn did not induce additional N<sub>2</sub>O emissions, similarly to what was observed in the first batch. Further, this implies that the stubble, which contained only ≤1/3 of the N, likely contributed more than 50 % to total N<sub>2</sub>O emission induced by nonremovable residue whereas BGR contributed to 37–47 %. This is considerably less than the range (69–73 %) estimated with default IPCC methodology (IPCC, 2019) which assume the same EF<sub>N2O</sub> for roots as for stubble.

Given the similar effect of stubble and herbage on N<sub>2</sub>O emissions (high EF<sub>N2O</sub> in the case of clover, strong interaction with soil N in the case of grass), it is evident that stubble should be included in empirical studies. Unfortunately in most field studies the effects of belowground residues and stubble are confounded with background emissions in the treatments used as control to estimate emissions from removable residues, and are usually not mentioned in review studies on the effect of crop residues (e.g. Abalos et al., 2022).

## 5. Conclusions

To our knowledge, this is the first study of the combined carbon and

nitrogen effects of roots and other belowground crop residues on N<sub>2</sub>O emissions, which also compares them to those of stubble (nonremovable aboveground residues) and harvestable herbage. Furthermore, in this incubation study, roots and other belowground residues were not separated from the soil matrix.

In the clayey loam used in this study, the EF<sub>N2O</sub> of fresh roots and other belowground residues was remarkably low, on average < 20 % the EF<sub>N2O</sub> of aboveground residues and remained amazingly low even when the soil NO<sub>3</sub>-N concentrations was high, whereas the EF<sub>N2O</sub> of aboveground residues always promptly increased when mineral nitrogen was provided either from residue decomposition or as soil mineral nitrogen. The recalcitrance of belowground residues to inducing N<sub>2</sub>O emissions was linked to their lower carbon decomposition rate, probably modulated by soil chemical and physical protection of thin roots rather than by classical quality parameters, as NDS-soluble fraction and C:N ratio. Also, roots thinness make them less prone to serve as ‘hotspots’ for denitrification. Stubble decomposed and contributed to N<sub>2</sub>O emissions more similarly to green herbage than to roots.

Our results highlight the importance of labile carbon in addition to nitrogen for eliciting N<sub>2</sub>O emissions from plant residues, and suggest that the present IPCC (2006), (2019) default methodology likely over-emphasizes the contribution of belowground residues to N<sub>2</sub>O emissions, while giving too little attention to the effect of labile carbon in non-removable aboveground residues (stubble) and their possible interaction with mineral N in the soil.

More studies of the specific effect of roots and stubble are needed, including green manure and cover crops which, like leys, are usually terminated while physiologically active (“green residues”), and a potentially a source of N<sub>2</sub>O emissions comparable to leys.

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## CRedit authorship contribution statement

**Shahid Nadeem:** Writing – review & editing, Validation, Investigation, Formal analysis. **Tatiana Francischinelli Rittl:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Marina Azzaroli Bleken:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## 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.

## Data Availability

A dataset has been established at Dataverse.no and is referred to in the article

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Statements and Declarations

## Author contributions

The study concept and design, the formal statistical analysis and the first draft of the manuscript were prepared by Marina A. Bleken. Material preparation and data collection were performed by Marina A. Bleken and Shahid Nadeem. Further curation of the data was done by Tatiana F. Rittl. All authors contributed to the writing of the final manuscript, read and approved it.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.still.2024.106309](https://doi.org/10.1016/j.still.2024.106309).

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