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Residue-C effects on denitrification vary with soil depth

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ABSTRACT

A stable isotope (^{13}C-residue, ^{15}N-NO_3^- fertiliser) approach combined with measurements of soil pore space gas concentrations was used to investigate spatial and temporal mechanisms of residue carbon (C) affecting denitrification. Whilst relationships between residue addition and N_2O fluxes have previously been well characterised, the influence of residues on production and reduction of N_2O at depth is less well understood. Here we investigated the relationship between residue-^{13}C addition (0, 1 and 2 mg C g^{-1} soil) and denitrification (^{15}N-N_2O and ^{15}N-N_2 production) at 2, 5 and 8 cm soil depths and also fluxes from the soil surface. Hydrophobic probes that equilibrate with the soil gas phase were used to extract gases at soil depth, followed by analysis for ^{15}N-N_2O, ^{15}N-N_2, ^{13}C-CO_2 and O_2 concentrations. ^{15}N-N_2O and CO_2 surface fluxes peaked one day after $^{14}NH_4^{15}NO_3$ application (1 mg N g^{-1} soil), with residue application resulting in a more than 20-fold greater ^{15}N-N_2O emission rate compared to the non-amended control. Eight days after N application ^{15}N-N_2O pore space concentrations had significantly increased 20-fold at 8 cm depth below the residue layer compared to no residue application. However, simultaneous increases in ^{15}N-N_2 surface fluxes and profile concentrations showed efficient reduction of the N_2O at shallow depth (3–10 cm depth) resulting in surface emission of N_2 rather than N_2O. Our results have implications for management to lower emissions as denitrifier activity at greater depth, and the greater reduction of N_2O to N_2, appeared to be indirectly driven by residue addition via the depletion of O_2 during aerobic heterotrophic respiration in the surface layer. In contrast, net surface fluxes of N_2O were more directly related to the residue addition through substrate provision for denitrification.

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1. Introduction

Emissions of the potent greenhouse gas N_2O generally increase following application of organic residues to agricultural soils (Chen et al., 2013; Muhammad et al., 2010). Residues supply C and N to the soil, enhancing mineralisation and stimulating heterotrophic microbial activity. Whereas mineralisation supplies ammonium for the first step of nitrification, the associated increased respiration may lower concentrations of soil O_2 to create conditions conducive for denitrification (Baggs et al., 2000; Huang et al., 2004; Li et al., 2016). Despite the large body of literature on residue quality and quantity effects on net emissions of the greenhouse gas N_2O, relatively little is known on the role of residue-C in the reduction of N_2O to N_2 during denitrification, especially with regard to depth within arable topsoil. A greater understanding of the relative importance of indirect and direct residue effects on N_2O reduction could make important contributions to the design of management strategies that target decreased emission of N_2O from arable soil.

The quantity and chemical composition of residues has been demonstrated to have a large impact on N_2O emissions following addition to agricultural soils (Chen et al., 2013; Miller et al., 2012; Thangarajan et al., 2013). Low C:N ratio residues have been shown to increase net N_2O emissions compared to high C:N ratio residues (Chen et al., 2013). With low C:N ratio residues microbial activity and growth are stimulated and N availability can subsequently increase through increased mineralisation and nitrification of residue N and soil organic matter N. The resulting available nitrate can be utilized during denitrification and can also regulate the reduction of N_2O to N_2 especially when C availability is low (Miller et al., 2008; Senbayram et al., 2012). Individual C compounds have been shown to exert different influences on the $N_2O/(N_2O+N_2)$

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product ratio of denitrification (Morley et al., 2014), and so the chemical composition of the residue has a direct influence on the product ratio and net N_2O emission (Miller et al., 2008; Gillam et al., 2008) with, for example, residue polyphenol and lignin contents being negatively related to net N_2O emissions (Frimpong and Baggs, 2010; Millar and Baggs, 2005, 2004).

In order to understand the effects of residue on nitrate reduction we however need to dig a bit deeper than just measuring surface fluxes of N_2O. Considerable microbial activity occurs below the soil surface and surface fluxes can be a poor predictor of denitrification dynamics especially when considering the complete denitrification pathway (Ball, 2013). The decrease in O_2 concentrations with depth, emphasised by impeded gas diffusivity at high water-filled pore space, can drive both the production and reduction of N_2O (Jarecke et al., 2016; McCarty et al., 1999). Jahangir et al. (2012) explored denitrification over a range of soil depths following substrate amendment, but the amendments were mobile liquids (glucose or DOC) so would not create a substrate hotspot and the diffusion of secondary compounds that would be found for solid residue. The oxygen status of arable topsoil can however, be altered as a consequence of organic inputs through increased heterotrophic respiration and C/N mineralisation. Zhu et al. (2015) showed that O_2 depletion below a layer of decomposing manure can alter net N_2O surface emissions most likely by creating conditions conducive to N_2O formation in denitrification. On a much smaller scale, but following a similar rationale, Højberg et al. (1994) found that the consumption of O_2 by mineralisation of residue-C on a soil aggregate surface can enhance the production and reduction of N_2O in the centre of an aggregate.

Residue addition can both directly drive denitrification via quality and quantity of input but also indirectly through oxygen depletion, influencing the magnitude of net N_2O surface fluxes. However, the effects of residue-C addition on the spatial location of denitrifier N_2O production and reduction hotspots have yet to be addressed. This study was performed to determine the influence of residue-C in driving production and reduction of N_2O within and throughout an arable topsoil, following surface application of two rates of ^{13}C-labelled barley residue. It combined surface flux measurements of ^{15}N-N_2O and ^{15}N-N_2 and residue- and SOM-derived CO_2 with the same measurements taken at 3 depths. We hypothesised i) a positive relationship of N_2O and N_2 with residue-derived ^{13}C-CO_2 and ii) greater reduction of N_2O to N_2 at greater depth within the soil core related to decreasing O_2 availability.

2. Materials and methods

2.1. Experimental design and set-up

To enable gas sampling at depth a novel microcosm setup was developed (Fig. 1). It consisted of a 5.3 cm diameter x 10 cm depth soil core with a sectioned gas permeable sampling tube inserted into the centre. The sampling tube was manufactured from sections of stainless steel tube (1.54 cm inner diameter) and hydrophobic, gas permeable tubing (1.4 cm inner diameter x 1.6 cm outer diameter, ePTFE at a density of 0.9 g cm^{-3}, fibre porosity of 60%, Markel Corporation, Plymouth Meeting, USA). A 1 cm section of the gas permeable tube was fixed at depths of either 1.1–2.1 cm, 4.4–5.4 cm or 7.8–8.8 cm with sections of stainless steel at either end to create a 10 cm length tube. For simplicity the sampling depths of the soil gas phase are referred to as 2 cm, 5 cm and 8 cm. The top of the tube was permanently sealed with a Suba seal septum (Suba Seal no. 29), permitting periodical gas sampling with a needle and syringe. The equilibration time of the soil sampler with the atmosphere was below 80 s as tested by measuring atmospheric O_2 diffusion into the tube.

The soil was field moist at 65% water-filled pore space (WFPS) after packing around the gas sampling tube to a bulk density of 1.2 g soil cm^{-3}. This resembled field conditions at the site and resulted in 213 g soil per tube. In order to obtain the same bulk density throughout the core, packing was done in six steps of soil addition and compaction. A platen that covered the entire area of the soil surface, with a hole cut in the centre, was used to ensure even packing.

The soil was sampled from NPK fertilised pasture and hay plots from the long-term fertilisation experiment at Woodlands Field, Craibstone Estate, Aberdeen in May 2014. Field moist soil was bulked, mixed and sieved to ≤2 mm and stored at 4 °C for approximately two weeks before the experiment. The soil is a loamy sand with 68% sand, 24% silt and 8% clay, an average pH_{H2O} of 5.5 and a total organic carbon content of 5.5%. The soil had received fertiliser rates of 80 kg N ha^{-1} as NH_4NO_3, 26 kg P ha^{-1} as triple superphosphate and 58 kg K ha^{-1} as muriate of potash in early March 2014. The soil contained 1.7 μg NH_4^+-N g^{-1} soil, 39.3 μg NO_3^--N g^{-1} soil and 81.7 μg C g^{-1} soil dissolved organic carbon at the beginning of the experiment.

Ground barley residue (<2 mm particle size, see below) was added to the soil at 0 mg, 1 mg or 2 mg C g^{-1} soil. The C addition was calculated for the total soil content of the soil core, but addition was concentrated to the top 3 cm of the soil core to mimic superficial residue incorporation. Each combination of sampling depth with residue treatment was replicated 4 times (3 residue treatments x 3 depths x 4 replicates) resulting in 4 replicates per residue treatment for the soil gas phase at each of the 3 depths but, as the headspace flux was determined for all soil cores, independent of the gas sampler inserted, 12 replicates per treatment for the surface gas fluxes. Additional 9 soil cores (3 residue treatments x 3 replicates) were set up for destructive sampling of the soil N and C pools (described in Section 2.4) on days 0, 1, 3, 8, 15 while on the last day of the experiment, day 23, a triplicate of the gas sampling cores were used.

To provide conditions favourable for denitrification in this soil (Ball, 2013) the water content was adjusted to 85% WFPS by pipetting N fertiliser solution onto the soil surface of all soil cores. The N fertiliser addition of 4.77 ml of $NH_4^{15}NO_3$ solution (25 atom% ^{15}N) to the soil surface marked the beginning of the experiment. The application rate corresponded to 120 kg N ha^{-1} or 0.99 mg N g^{-1} soil, therefore 210.9 mg N were added to each soil core. The soil cores were kept on the lab bench and WFPS was maintained by water addition, when required, on a mass basis.

2.2. ^{13}C-labelling of barley plant material

Barley (*Hordeum vulgare*, ssp. Belgravia) was grown at 30 °C in the glasshouse in February 2014 for a period of 52 days prior to ^{13}C-labelling. Two trays of approximately 40 plants were transferred to a gas-tight glove box and enriched with ^{13}C-CO_2. Enrichment of the atmosphere was done by volatilizing 99 atom% $NaH^{13}CO_3$ using 0.1 M HCl solution on a magnetic stirrer within the glove box. After 7 h the above-ground plant material was cut and immediately dried at 40 °C until no further weight loss occurred. It was milled to <2 mm particle size using a hammer mill. The milled and bulked plant material had a carbon content of 44.2% and a C/N ratio of 10.8. The bulked plant material was 1.98 atom% excess ^{13}C as determined on a 20/20 isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK).

Due to using pulse labelling, the barley plant material was not homogeneously enriched in ^{13}C and thus fractionated into the hot-water soluble C content and the non-soluble residue. This was done by extracting the plant material for 30 min at 60 °C on a magnetic stirrer and filtering it through a 20 μm membrane (Soong et al., 2014). The water soluble fraction was freeze-dried, the residue

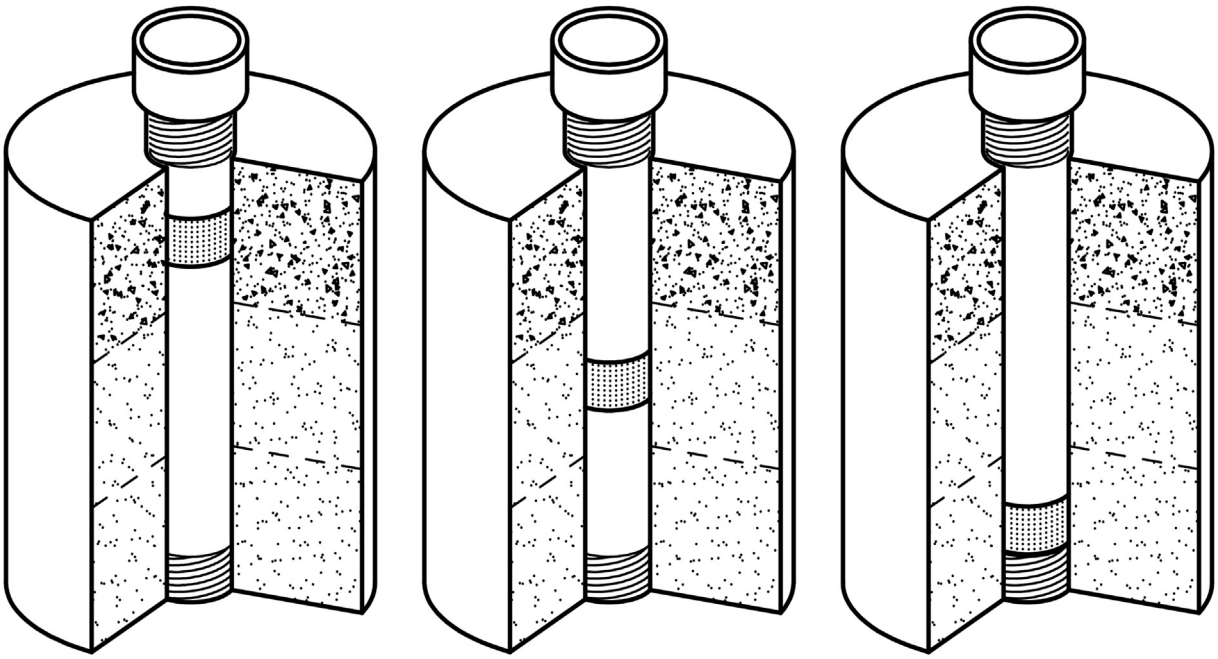

Fig. 1. Schematic representation of the soil core set-up, with residue added to the top 3.3 cm of the soil and the soil sampler inserted into the centre with the gas permeable membrane replacing the steel tube at 2, 5 or 8 cm depth.

oven-dried at 40 °C and both isotopically characterised by isotope ratio mass spectrometry. Of the total C 79.15% and 20.85% was recovered in the residue and the hot-water extract, respectively, with isotopic enrichments of 1.60 atom% excess ^{13}C and 3.47 atom% excess ^{13}C, respectively.

2.3. Gas sampling and analyses

On days 0, 1, 3, 8, 15 and 23 the soil cores were placed in 1 L Kilner jars fitted with two-way valves in the lid for 180 min. Samples for initial concentrations of N_2O and CO_2 were taken immediately after jar closure and after a period of 180 min headspace gas was sampled. Preliminary tests showed linear accumulation of N_2O and CO_2 during that period of time. Using a gas-tight syringe, 5 ml of gas was taken and stored in pre-evacuated 3 ml LabCo exetainer vials for analysis for CO_2 and N_2O by gas chromatography. Furthermore, a 15 ml headspace sample was taken and stored in pre-evacuated 12 ml LabCo gas vials for ^{13}C-CO_2 analysis as well as 150 ml of gas stored in He-flushed, pre-evacuated, 120 ml amber bottles prior to analysis for ^{15}N-N_2O and ^{15}N-N_2 concentrations on the isotope ratio mass spectrometer. The cores were removed from the Kilner jars after headspace sampling.

Before each sampling for the soil gas phase from the soil sampler, an O_2 fixed needle type sensor (OXF500PT, PyroScience, Munich, Germany) coupled to an oxygen meter (FireStingO2, PyroScience, Munich, Germany) was inserted through the surface facing septum to obtain O_2 concentrations representing the pore space of the bulk soil at 2, 5 and 8 cm depth. The O_2 sensor was calibrated with two points, i.e. 0% O_2 in He as well as atmospheric O_2.

The soil gas phase from the depths of 2, 5 and 8 cm was sampled directly into exetainer vials using a three-way valve to prevent injecting the needle more than once. One ml of gas was sampled into 3 ml He-filled vials for quantification of CO_2 and N_2O using gas chromatography, and 2 ml were removed each for ^{15}N and ^{13}C analysis and transferred to He-filled 12 ml exetainer vials. In total, 5 ml of gas were removed from the soil gas sampler (12 ml volume) on each sampling occasion in the monitoring sequence.

Concentrations of CO_2 and N_2O were analysed on a gas chromatograph (GC) (Agilent 6890, Santa Clara, USA) equipped with an electron capture detector at 300 °C and a methaniser and flame ionisation detector at 250 °C. Gases were separated on a Haysep Q column at 45 °C, the sample volume injected by the autosampler (HT29748, HTA, Italy) was 500 μl. The GC was calibrated for CO_2 and N_2O using standards of known concentrations. Isotope ratios for N_2O, N_2 and CO_2 were determined on the isotope ratio mass spectrometer after cryofocusing the gas sample in an ANCA TGII trace gas preparation module. The minimum concentration of N_2O in the exetainer vials sampled from 5 and 8 cm depth after dilution in He was 0.5 ppmv. However, in the case of gas samples obtained from 2 cm depth, the concentrations were not sufficient to reliably quantify ^{15}N enrichment in both N_2O and N_2 and were therefore not presented. The natural abundance in air was used as a reference for N isotope determination and in 12 ml exetainer vials the average atom% ^{15}N value of atmospheric concentrations of N_2O was 0.3735 atom% ^{15}N with a standard deviation of 0.0304 atom% ^{15}N. Acidified $NaCO_3$ calibrated versus the international standard (IAEA NBS-18) was used as a reference for carbon isotope determination.

2.4. Biochemical soil analyses

Soil samples were taken at three depth intervals (0–3.3 cm, 3.3–6.6 cm and 6.6–10 cm from the core surface) by inserting a soil corer of 10 mm ID into the respective depths, first to 3.3 cm, then to 6.6 and 10 cm depth, and making a composite sample of at least 40 g fresh soil from each depth. For the sake of simplicity, the depths are referred to as 2 cm, 5 cm and 8 cm depth when describing extraction data and match those of the gas samples.

Nitrate concentrations were measured in 1 M KCl soil extracts which had been shaken for 1 h and subsequently filtered (Whatman No.1). Extracts were stored at −18 °C until colorimetric analysis by a flow injection analyser (FIAstar 5000 Analyser, Foss Tecator, Hillerød, Denmark). To analyse for ^{15}N-NO_3 the diffusion method as described by Stark and Hart (1996) was used. The ^{15}N-

enrichment of NO_3^- was determined on the isotope ratio mass spectrometer.

Standard chloroform fumigation extraction procedures were followed to determine microbial biomass carbon (Vance et al., 1987). A 5 g subsample of fresh soil was extracted or exposed to chloroform saturated air for 24 h before extraction with 0.5 M K_2SO_4. The extracts were analysed for total organic carbon (LABTOC analyser, Pollution & Process Monitoring Ltd., Sevenoaks, UK). Total organic carbon extracted from non-fumigated soil is referred to as dissolved organic C (DOC). Microbial biomass carbon was calculated as the difference between fumigated and non-fumigated soil multiplied by a correction factor of 0.45 (Joergensen, 1996).

2.5. Calculations and statistical analyses

The proportion of the residue-derived CO_2 flux as the fraction of the $^{12+13}C$-CO_2 fluxes and the soil gas phase was calculated according to,

$$P_{barley} = \left(atom\%\,^{13}C_{sample} - atom\%\,^{13}C_{SOM}\right) \Big/ \left(atom\%\,^{13}C_{barley} - atom\%\,^{13}C_{SOM}\right), \quad (1)$$

where P_{barley} is the proportion of the total CO_2 in flux and soil gas that was barley derived, atom% $^{13}C_{sample}$ represents the ^{13}C of the CO_2 sample, atom% $^{13}C_{SOM}$ is the average ^{13}C–CO_2 of the soil that received no barley C and atom% $^{13}C_{barley}$ is the atom% ^{13}C measured in the hot-water extract of the barley plant material as this most likely represented the utilised fraction of residue-C (Trinsoutrot et al., 2000).

The proportion of N gas (total denitrification products in the following referred to as ^{15}N-N_2O and ^{15}N-N_2) in the headspace or samplers that was derived from the applied ^{15}N-NO_3^- and ^{14}N-NO_3^- was calculated accounting for the isotopic enrichment of the NO_3^- pool according to

$$\text{Fraction nitrate derived } N_2O \text{ or } N_2 = atom\%\,^{15}N \text{ excess } N_2O \text{ or } N_2 \Big/ atom\%\,^{15}N \text{ excess } NO_3^-, \quad (2)$$

where atom% ^{15}N excess for gas samples was calculated by subtraction of 14/15 N ratios obtained from headspaces of triplicate non-^{15}N enriched control soils and for the NO_3^- pool by subtracting natural abundance of ^{15}N from the atom % value for NO_3^- derived by the diffusion method.

Cumulative values for gaseous emissions were calculated from measured fluxes using the trapezoidal method.

All statistical analyses were performed using R (R Development Core Team, 2015). Effects of the sampling day and barley C amendment rate on fluxes and concentrations of N_2O, N_2 and CO_2 were compared using either a linear model or a linear mixed model accounting for repeated measurement if that improved the model fit based on model comparison. Regression analysis was performed using the same approach. Variables were log-transformed to achieve normal distribution and homogeneity of variances where necessary.

3. Results

3.1. Cumulative gaseous emissions

The total CO_2 emitted over 23 days more than tripled with an amendment of 2 mg residue-C compared to non-C amended soil ($P < 0.001$) (Table 1). Overall, significantly more $^{14+15}N$-N_2O and ^{15}N-N_2O was emitted from the surface of both residue amended soils compared to the non-C amended control ($P < 0.01$) (Table 1) but emissions between the two residue-C treatments did not differ. Fertiliser nitrate derived ^{15}N-N_2O accounted for 45.4%, 81.8% and 73.5% of total N_2O surface emission in the 0 mg, 1 mg and 2 mg residue-C treatments, respectively. Compared to the non-C amended control, soils that received residue-C emitted significantly more ^{15}N-N_2 over 23 days ($P < 0.05$) with no significant difference between amendment rates. The ^{15}N-(N_2O/(N_2O+N_2)) ratio of the surface emissions over 23 days was not significantly different between residue treatments, but was greater than the ratio calculated for the non-C amended control (Table 1).

3.2. CO_2 fluxes and concentrations at different depths

The $^{12+13}C$-CO_2 fluxes peaked one day following N fertilisation and residue amendment. This was similar for both residue-C treatments and the control where no C but only N had been added (Fig. 2A). The response of residue-derived ^{13}C-CO_2 to residue treatment was dependent on the day of measurement (significant interaction between Day and C, $P < 0.001$) (Fig. 2D). For the 2 mg residue-C amendment we observed greatest residue-derived surface fluxes of 453 μg ^{13}C-CO_2 $core^{-1}$ h^{-1} immediately after application of N and residue, followed by a steep decline in surface fluxes between days 0 and 3. Rates still decreased steadily after day 3 until the end of the experiment, but the differences between days were much smaller.

Except for day 0 when residue-derived C accounted for 64% and 32% of the total CO_2 flux in the 2 and 1 mg residue-C treatments, respectively, the fraction of residue-derived CO_2 was similar between soils amended with either 1 or 2 mg residue-C. It decreased from ~30% on day 1 to about 10% at the end of the experiment, and from day 3 SOM-C mineralisation was the dominating C source for microbial respiration.

At 5 cm depth the effect of residue amendment rate on $^{12+13}C$-CO_2 concentrations differed between days ($P < 0.01$). In soils amended with 1 mg residue-C, $^{12+13}C$-CO_2 concentrations on days 3, 8 and 23 exceeded the concentrations in control soils that did not receive residue. $^{12+13}C$-CO_2 concentrations in the 2 mg residue-C treated soil were similar to the control on all days except day 0 (Fig. 2B). At 8 cm depth $^{12+13}C$-CO_2 concentrations were significantly different between days ($P < 0.001$) but not between C treatments. Significantly higher concentrations were measured on all days compared to day 0 with a steady increase from day 1–15 and a significant decrease in $^{12+13}C$-CO_2 concentrations from day 15–23 (Fig. 2C).

At both soil depths the % residue-derived CO_2 of the total CO_2 concentration was greatest on day 0 (data not shown). Immediately after application of 1 mg residue-C, the % residue-derived CO_2 was 20% and 9% of the total CO_2 concentration at 5 and 8 cm depth, respectively. The contribution of ^{13}C-CO_2 to the total CO_2 in the 2 mg residue-C treatment was 48% and 11% at 5 and 8 cm depth, respectively. By day 3, the % residue-derived CO_2 had decreased to below 10% of the total CO_2 concentration at both soil depths for both residue amendment rates.

3.3. N_2O fluxes and concentrations at different soil depths

Residue amendment greatly affected headspace N_2O emission. The ^{15}N-N_2O surface fluxes peaked on day 1 when soil amended with 1 mg residue-C emitted significantly more ^{15}N-N_2O (21.31 μg ^{15}N-N_2O $core^{-1}$ h^{-1}) than soils that received no C (0.14 μg ^{15}N-N_2O $core^{-1}$ h^{-1}) or 2 mg residue-C (7.31 μg ^{15}N-N_2O $core^{-1}$ h^{-1}) (significant interaction between Day and C, $P < 0.001$) (Fig. 3A). ^{15}N-N_2O fluxes did not differ between residue treatments on any other

Table 1
Mean total emissions of $^{14+15}N$-N_2O, ^{15}N-N_2O, ^{15}N-N_2 and CO_2 and mean ^{15}N-(N_2O/(N_2O+ N_2)) ratio over the 23 day experimental period after addition of residue (n = 12).

Carbon	CO_2 mg C core^{-1}	$^{14+15}N$-N_2O mg N core^{-1}	^{15}N-N_2O mg N core^{-1}	^{15}N-N_2 mg N core^{-1}	^{15}N-(N_2O/(N_2O+N_2))
0 mg residue-C (control)	42.48 (2.04)c	0.42 (0.14)b	0.26 (0.13)b	2.07 (0.39)b	0.08 (0.03)
1 mg residue-C	81.84 (4.11)b	1.67 (0.28)a	1.58 (0.33)a	7.35 (2.59)a	0.23 (0.05)
2 mg residue-C	150.59 (4.43)a	1.91 (0.55)a	1.65 (0.53)a	6.93 (2.30)a	0.22 (0.05)

Different letters indicate significantly different means across residue treatments ($P < 0.05$).
Values in parentheses are ± one SEM.

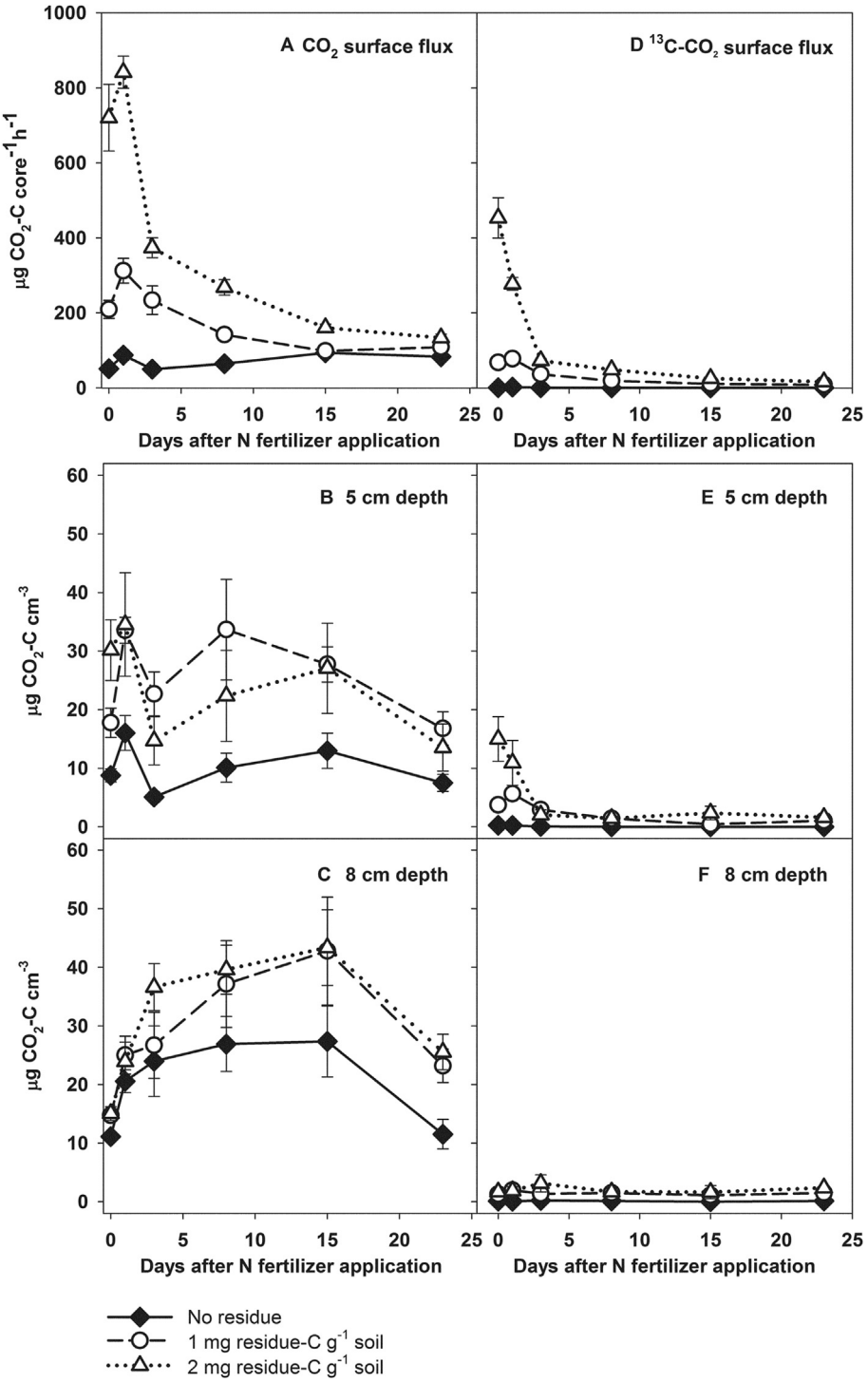

Fig. 2. Mean surface fluxes of $^{12+13}C$-CO_2 (A) and ^{13}C-CO_2 (D) (µg C core^{-1} h^{-1}), $^{12+13}C$-CO_2 and ^{13}C-CO_2 concentrations (µg C cm^{-3}) at 5 cm (B, E) and 8 cm (C, F) depth over the experimental period of 23 days. Error bars represent ± one SEM.

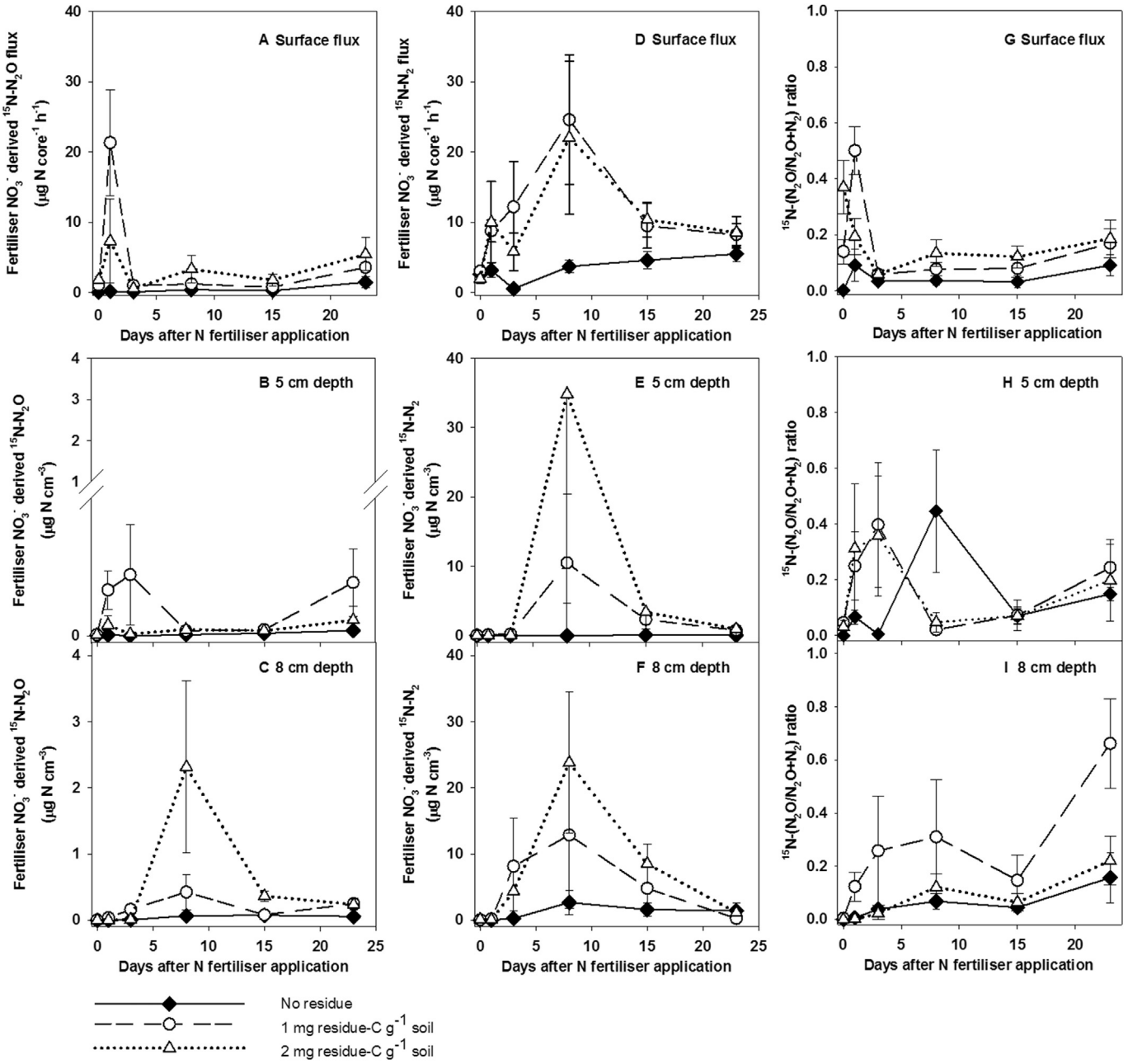

Fig. 3. Mean surface fluxes of ^{15}N-N_2O (A) and ^{15}N-N_2 (D) (μg N core^{-1} h^{-1}) and ^{15}N-(N_2O/(N_2O+N_2) ratio of the surface flux (G). ^{15}N-N_2O and ^{15}N-N_2 concentrations (μg N cm^{-3} gas) and ratios at 5 cm (B, E, H) and 8 cm (C, F, I) from the soil surface over the experimental period of 23 days. Error bars represent ± one SEM.

day.

At 5 cm depth ^{15}N-N_2O concentrations differed significantly between residue treatments ($P < 0.01$). Amendment with 1 mg residue-C resulted in greater ^{15}N-N_2O concentrations compared to the control and the 2 mg residue-C treatment ($P < 0.05$) (Fig. 3B). The effect of residue on ^{15}N-N_2O concentration at 8 cm depth depended on the day of measurement (significant interaction between Day x C, $P < 0.01$) (Fig. 3C). Concentrations differed from the control on day 8 when amended with 2 mg residue-C and a peak concentration of 2.32 μg ^{15}N-N_2O cm^{-3} was measured. When comparing across depths on days of significant surface peaks of fertiliser derived emissions (i.e. day 1 for ^{15}N-N_2O and day 8 for ^{15}N-N_2, see section 2.4) we found no differences in ^{15}N-N_2O concentrations between depths on day 1. On day 8, ^{15}N-N_2O concentrations tended to be greater at 8 cm depth with an average concentration of 0.94 μg compared to 0.01 μg ^{15}N-N_2O cm^{-3} soil gas at 5 cm depth ($P = 0.053$).

3.4. N_2 fluxes and profile concentrations

The average daily flux of ^{15}N-N_2 at the soil surface was greater with 1 mg C amendment compared to the control ($P < 0.05$). Interestingly, 2 mg residue-C amendment had a smaller impact on ^{15}N-N_2 fluxes, with emissions differing from neither the control nor from the lower C amended soil. Averaged over residue treatments, fluxes of N_2 were consistently greater on day 8 and onwards compared to day 0 of the experiment ($P < 0.001$) (Fig. 3D).

At both 5 and 8 cm depth, the effects of residue amendment on

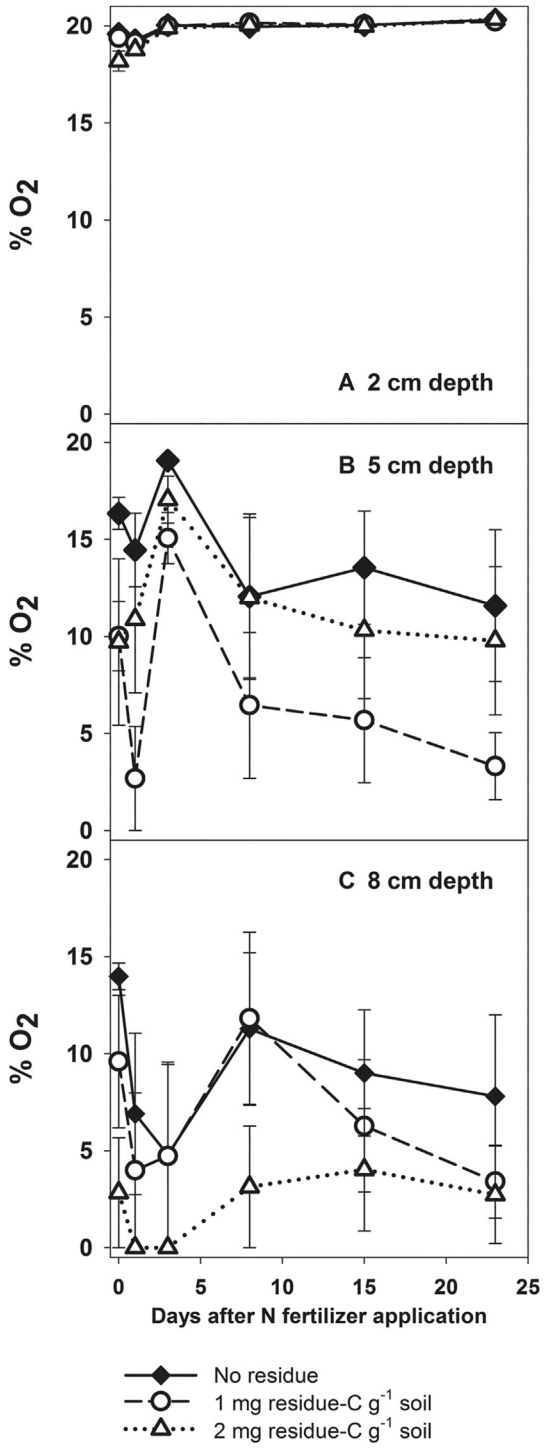

Fig. 4. Oxygen concentrations at 2 cm (A), 5 cm (B) and 8 cm (C) depth of the soil cores amended with 0, 1 or 2 mg residue-C g^{-1}. Error bars represent ± one SEM.

^{15}N-N_2 concentrations were not significant. However, we observed differences between days with significantly increased ^{15}N-N_2 concentrations at both 5 and 8 cm depths on days 8 and 15 when compared to ^{15}N-N_2 concentrations at the start of the experiment ($P = 0.001$ and $P < 0.001$, respectively) (Fig. 3E,F). Average concentrations on day 1, when surface ^{15}N-N_2O concentrations peaked, were greater at 8 cm compared to 5 cm depth ($P = 0.001$). There was no difference in ^{15}N-N_2 concentrations between depths on day 8 when the ^{15}N-N_2 surface flux peaked.

The ^{15}N-(N_2O/(N_2O+N_2)) ratio of fluxes was significantly different between residue treatments depending on day (significant interaction between Day x C, $P < 0.001$) (Fig. 3G). It was significantly greater on day 0 when amended with 2 mg residue-C g^{-1} soil and on day 1 when amended with 1 mg residue-C g^{-1} soil compared to the control soil on the respective days ($P < 0.001$). At 5 cm depth the ^{15}N-(N_2O-to-N_2) ratio was neither significantly different between C treatments nor between days (Fig. 3H) with no significant interaction between the factors. At 8 cm depth, the ratio was greater on day 23 compared to all other days ($P < 0.001$) (Fig. 3I).

3.5. O_2 profile concentrations

Oxygen concentrations decreased with soil depth with an average concentration of 11.1 ± 0.8% at 5 cm depth and 5.9 ± 0.8% at 8 cm depth of the soil core. At 5 cm depth we measured a significantly greater O_2 concentration three days after residue-C and N application compared to all other days ($P < 0.001$) but residue treatments did not affect O_2 at that depth (Fig. 4B). At 8 cm depth, O_2 concentration differed between residue-C treatments ($P < 0.001$) with an average of 6.8% less O_2 when 2 mg of residue-C were amended compared to the control and the 1 mg residue-C treatment (Fig. 4C). Interaction terms between the factors Day and C were not significant at 5 and 8 cm depth.

3.6. Biochemical soil properties

In the topsoil, nitrate concentrations were significantly different between days depending on residue treatment (significant interaction between C x Day, $P < 0.05$). Nitrate increased significantly from day 0 to day 23 with 2 mg of residue-C amended and from day 0–15 with 1 mg residue-C amended (Fig. 5A) ($P < 0.05$). After the surface application of N fertiliser, NO_3^--N concentrations at 5 and 8 cm depth increased throughout the experiment (Fig. 5B and C). Maximum concentrations of nitrate-N at 2 cm, 5 cm and 8 cm depth were 238, 138 and 82 μg N g^{-1} soil averaged across residue-C treatments, respectively (data not shown). Interaction effects between C and Day on ^{15}N-NO_3^- concentrations were significant at all three soil depths (all $P < 0.001$). However, multiple comparisons of ^{15}N-NO_3^- concentrations for each soil depth showed no significant differences between residue-C treatments on any of the extraction days (Fig. 5D, E, F).

Microbial biomass at 2 cm depth differed greatly between residue treatments and days of measurement with significant interaction between the factors ($P < 0.001$). At 5 and 8 cm depths microbial biomass remained at concentrations similar to the initial biomass of 291.6 μg C g^{-1} soil (Supplementary Fig. 1). Similarly, the interaction between factors was significant for DOC concentrations at 2 cm depth ($P < 0.001$), and only different between days at 5 or 8 cm depth ($P < 0.001$ for both) (Supplementary Fig. 1).

3.7. Relationships between variables

On day 1 when ^{15}N-N_2O fluxes peaked, the fertiliser nitrate derived denitrification surface flux from the carbon amended soils was negatively related to total CO_2 ($R^2 = 0.45$, $P < 0.001$), ^{12}C-CO_2 ($R^2 = 0.56$, $P < 0.001$) and more weakly related to ^{13}C-CO_2 ($R^2 = 0.18$, $P < 0.05$) surface fluxes (Fig. 6). When divided into residue-C treatments the relationship between ^{12}C-CO_2 and total denitrification (rate of N_2O+N_2) was stronger with 2 mg residue-C ($R^2 = 0.50$, $P < 0.05$) than with 1 mg residue-C treatment ($R^2 = 0.30$, $P < 0.05$). For both residue-C treatments separately, the relationship between ^{13}C-CO_2 and total denitrification was not significant.

At depth, fertiliser nitrate derived denitrification (the sum of $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$ concentrations) was positively related to $^{12+13}C\text{-}CO_2$ ($R^2 = 0.52$, $P < 0.001$) and when separated into depths, this relationship was stronger at 8 cm depth ($R^2 = 0.59$, $P < 0.001$) compared to 5 cm depth ($R^2 = 0.36$, $P < 0.001$) (Fig. 7). While the relationship of the profile concentrations of fertiliser nitrate derived denitrification with $^{13}C\text{-}CO_2$ was not significant, it was significant and positive with SOM-derived $^{12}C\text{-}CO_2$ ($R^2 = 0.57$, $P < 0.001$). As for $^{12+13}C\text{-}CO_2$, this positive relationship was stronger at 8 cm ($R^2 = 0.54$, $P < 0.001$) compared to 5 cm depth ($R^2 = 0.42$, $P < 0.001$).

Fertiliser nitrate derived denitrification was negatively related to O_2 concentrations in the whole profile ($R^2 = 0.31$, $P < 0.001$), at 5 cm depth ($R^2 = 0.39$, $P < 0.001$) and at 8 cm depth ($R^2 = 0.17$, $P < 0.001$). Nitrate concentrations were not significantly related to fertiliser nitrate derived denitrification within the soil core.

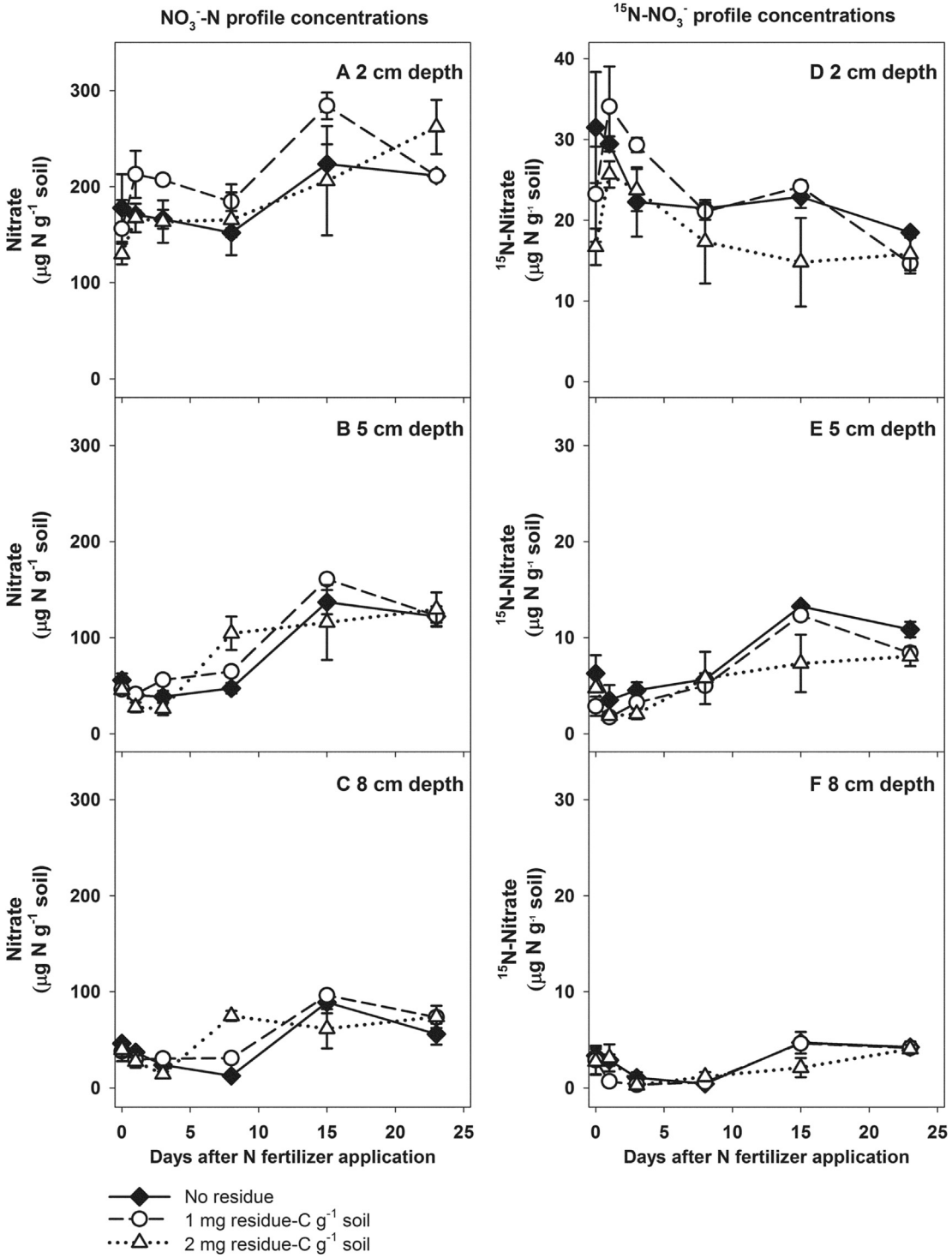

Fig. 5. $^{15+14}N\text{-}NO_3^-$ (A, B, C) and $^{15}N\text{-}NO_3^-$ (µg N g^{-1} soil) (D, E, F) concentrations at 2 cm, 5 cm and 8 cm depth for soil amended with 0, 1 or 2 mg residue-C g^{-1} soil in the top 3 cm. Error bars represent ± one SEM.

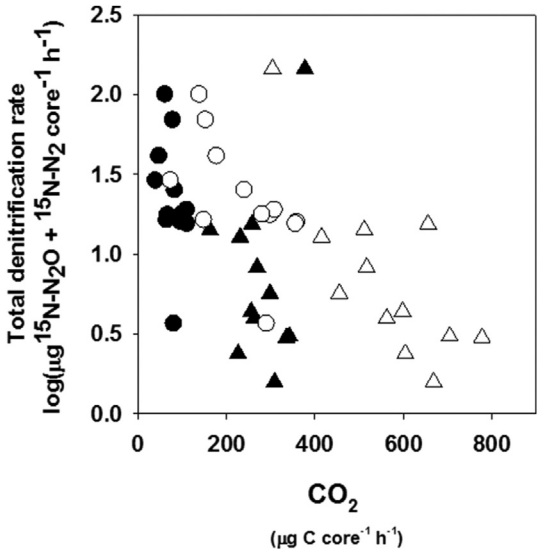

Fig. 6. Relationship between total denitrification rate and source-partitioned CO_2 emitted on day 1 from the surface of soils amended with 1 mg residue-C (circles) and 2 mg residue-C (triangles). Filled and open symbols represent ^{13}C-CO_2 and ^{12}C-CO_2 fluxes, respectively.

4. Discussion

Using a stable isotope approach combined with soil pore space measurements of gas concentrations at depth we showed two spatially and temporally distinct mechanisms of residue-C affecting denitrification. Next to an immediate stimulatory effect at the soil surface, we measured a second, residue-driven increase of denitrifier activity at depth. Residue-C respiration and oxygen consumption close to the soil surface stimulated denitrification at greater depth indirectly, and resulted in an increased surface flux of N_2 rather than N_2O due to efficient reduction within the soil core.

4.1. Residue addition and immediate emissions of N_2O – the immediate effect

One day after the addition of residue-C and N fertiliser we observed a peak surface flux of ^{15}N-N_2O. The N_2O peak only occurred in soils to which residue had been applied, likely driven by greater microbial biomass as well as an increased supply of electrons to the denitrification pathway (Giles et al., 2012; Smith and Tiedje, 1979).

The surface CO_2 flux indicated rapid mineralisation of the barley residue with greatest rates of CO_2 production within three days of its application. This pattern of mineralisation was similar to that generally observed when applying agricultural residues to soil (De Troyer et al., 2011; Redin et al., 2014). Here, this residue-C stimulated heterotrophic activity and growth in the soil layer where it was concentrated and made little to no contribution to C mineralisation in the soil below the top 3 cm. The ^{13}C-CO_2 concentrations at 5 cm depth in our case were most likely a result of gas diffusion as it has been shown in other studies that residue mineralisation is spatially limited to about 4 mm around the detritus (Gaillard et al., 1999; Nicolardot et al., 2007). In accordance with that, DOC or microbial biomass C did not differ between the control and the residue treated soils at both 5 and 8 cm soil depth in our study, suggesting a limited movement of residue below the layer of incorporation. Hence, on day 1 we did not observe significantly increased concentrations of ^{15}N-N_2O at 5 or 8 cm depth in the residue amended soil cores that could explain the peaking surface fluxes. Residue therefore facilitated, increased rates of N_2O production in close proximity to the soil surface possibly directly providing a source of C or by creating anaerobic microsites within the soil surface layer. However, we did not measure increased rates of N_2 surface emissions and the soil pore space concentrations of oxygen remained close to atmospheric. The conditions are likely to have promoted nitrate reduction while restricting the expression and activity of the O_2 sensitive N_2O reductase (Weier et al., 1993).

At the soil surface the initial peak of total denitrification, mainly being ^{15}N-N_2O, was negatively related to CO_2 fluxes from residue-amended soil. This disagrees with previous findings where CO_2 evolution was positively related to total denitrification (Burford and Bremner, 1975; Henderson et al., 2010; Miller et al., 2009; Walvoord et al., 2014). A possible explanation for this unexpected response could be out-competition of denitrifying organisms by the growth of other non-denitrifying heterotrophs at high available C. Immediate net N_2O fluxes after residue application might thus potentially be driven by interactions within the microbial community triggered by the quantity of newly available C and N.

4.2. Residue mineralisation and N_2O production and reduction – the indirect, delayed effect

With a delay of 1 week compared to the ^{15}N-N_2O emission peak in residue amended soils, the ^{15}N-N_2 surface fluxes peaked, irrespective of whether 1 or 2 mg residue-C g^{-1} soil had been added. Using the acetylene inhibition method Miller et al. (2008) have shown that reduction of N_2O may not occur immediately after

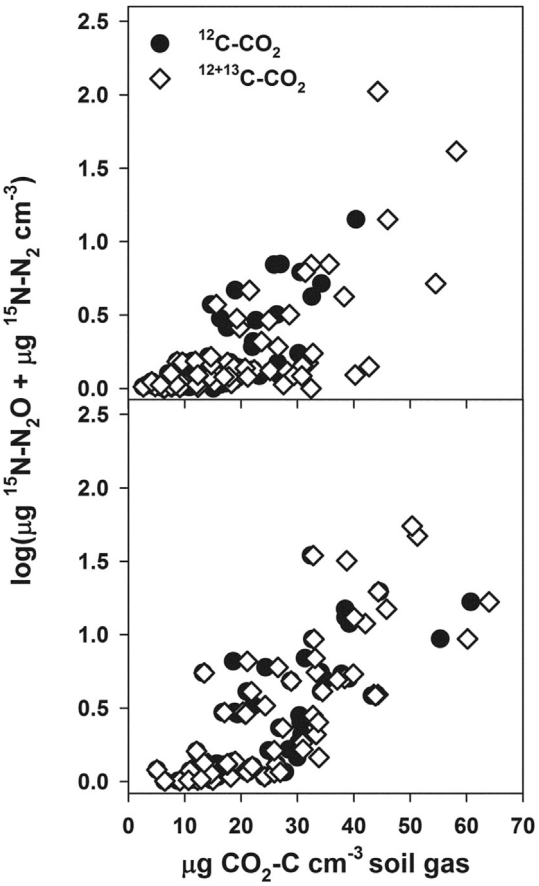

Fig. 7. Relationship between denitrification products and CO_2 derived from SOM-C (^{12}C-CO_2) and with total CO_2-C ($^{12+13}C$-CO_2) at 5 cm depth (top) and 8 cm depth (bottom).

residue-C and N fertiliser application. Accordingly, Morley and Baggs (2010) observed a lag of 65 h with which N_2O was reduced to N_2 following $^{15}N\text{-}NO_3^-$ addition to agricultural soil. This has often been associated with the time lag in synthesis of the enzyme N_2O reductase (Zheng and Doskey, 2016). However, our data provides an additional explanation for N_2 emission peaks at the surface which is related to the effect of residue addition within the soil profile.

We observed up to 50% residue-derived CO_2 only on the first day after application at 5 cm depth, whereas less than 10% of CO_2 in the soil gas was residue-derived throughout the whole experimental period at 8 cm depth. Yet, we observed that residue addition to the surface increased the concentrations of N_2O at depth. Corresponding to this increase, the concentration of N_2, the product of N_2O reduction, increased at both 8 cm and 5 cm depths. This points at efficient reduction of N_2O to N_2 in the location of its highest concentration and an upward movement finally resulting in surface emission of N_2 rather than N_2O.

This, in combination with the negative relationship between the concentrations of total denitrification products with O_2 at depth, suggests that the effects of residue addition were of indirect nature. Rather than being directly utilized by denitrifiers as an electron donor, residue-C mineralisation can indirectly deplete O_2 at the surface. As a result, the decreased O_2 concentrations in the bulk soil at greater depth facilitate the reduction of N_2O to N_2 which in turn lowers surface emission of N_2O. Further, the positive relationship of denitrification products with $^{12}C\text{-}CO_2$ at depth also highlights that soil organic matter derived C can serve as an electron donor for denitrification in soil. In accordance with our results, a layer of manure C led to low O_2 concentrations below its physical location in a study by Zhu et al. (2015). Anaerobic conditions developed below the manure layer within the first 12 h and created favourable conditions for denitrification at a distance of several cm from the mineralisation hotspot. Our findings are also in line with results obtained by Højberg et al. (1994) who measured denitrification on aggregate scale and observed rapid O_2 depletion at an aggregate surface following application of a piece of clover leaf that in turn increased N_2O production and reduction indirectly.

In the field, residue incorporation often occurs simultaneously with mechanical disturbance of the soil, which introduces O_2 into the topsoil. The conditions established in this experiment therefore resemble early time effects of surface residue incorporation such as with reduced or minimum tillage. It has previously been established that deeper N placement in reduced or no till systems can decrease net N_2O emissions (van Kessel et al., 2013) and our study contributes to the mechanistic understanding of this finding. Our results point to the need for targeted mitigation of hot-spot formation at the soil surface while fostering the completion of denitrification reduction of N_2O to N_2 within the soil profile where reduction efficiency is high. Agricultural management practices should thus consider combinations of residue quality and placement i.e. by tillage operations and/or deep fertiliser placement that maximises the reduction potential of the soil while ensuring optimal plant N uptake.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.09.012.

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