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Evaluating the potential of different carbon sources to promote denitrification

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Abstract

Organic carbon (C) plays an essential role in the denitrification process as it supplies energy for N_2O, N_2 and CO_2 producing reactions. The objectives of this study were to: (i) rank the reactivity of different C compounds found in manures based on their availability for denitrification and (ii) explore C-quality in different C sources based on their capacity to promote denitrification. Evaluation of different C-sources in promoting denitrification was conducted based on the molar ratio of CO_2 production to NO_3^- reduction after incubation. Results of the first experiment (a 12-day investigation) showed that glucose and glucosamine were highly reactive C compounds with all applied NO_3^- being exhausted by day 3, and glucosamine had significantly high amount of NH_4^+-N present at end of the experiment. The glucose and glucosamine treatments resulted in significantly greater cumulative CO_2 production, compared to the other treatments. In the second experiment (a 9-day investigation), all NO_3^- had been depleted by day 6 and 9 from acetic acid and glucose, respectively, and the greatest cumulative CO_2 production was from acetic acid. The CO_2 appearance to NO_3^- molar ratios revealed that glucose and glucosamine were compounds with highly available C in the first experiment. In the second experiment, the pig slurry and acetic acid were found to be C-sources that promoted potential denitrification. The application of slurry to soil results in the promotion of denitrification and this depends on the availability of the C compounds it contains. Understanding the relationship between C availability and denitrification potential is useful for developing denitrification mitigation strategies for organic soil amendments.

Introduction

Reducing greenhouse gas emissions from agricultural systems is vital in developing sustainable food production practices (Gagnon *et al.*, 2016). Intensively managed grassland systems recycle considerable amounts of nitrogen (N) (Deenen, 1994) and retain inherent amounts of labile organic carbon (C) (Baggs *et al.*, 2000; Jérôme *et al.*, 2014). Both N and C are mainly sourced from fertilizer, excreta and mineralization of soil organic matter (OM) (Franzluebbers *et al.*, 2000; Soussana and Lemaire, 2014). Globally, grasslands cover about 26% of the ice-free areas (Steinfeld *et al.*, 2009). Because of their geographical positioning, grasslands are often subjected to high soil moisture conditions, which reduces soil aerobicity, thus increasing the potential for denitrification (Jarvis *et al.*, 1994; Steinfeld *et al.*, 2009). Denitrification is a bacterially mediated process whereby nitrate (NO_3^-) is transformed to NO_2^-, NO, N_2O and finally to N_2, under limited oxygen (O_2) as most denitrifying bacteria are facultative anaerobes (Robertson and Groffman, 2007). Most denitrifying bacteria couple NO_3^- reduction with organic C oxidation to gain energy, making a supply of readily available C a usual requirement for denitrification to occur, a process which further produces CO_2 (Knowles, 1982; Beauchamp *et al.*, 1989).

Livestock manures are returned to the land to recycle nutrients for plant growth (Watson *et al.*, 2002; Tittonell *et al.*, 2010). Studies on the effects of manure applications on the N and C cycles are found in the literature, especially related to soil processes such as denitrification (Morley and Baggs, 2010; Soussana and Lemaire, 2014). The source and concentration of C in relation to NO_3^- and O_2 have been documented to control denitrification rates (Beauchamp *et al.*, 1989). Early studies demonstrated the effect of cattle manure applications on denitrification rates and explanations for the resulting increase were found to be related to the appearance of anaerobic microsites for the denitrification process (Guenzi *et al.*, 1978; Beauchamp *et al.*, 1989).

Manure mainly comprises undigested materials, which are not always easily decomposable (Rufino *et al.*, 2006; Gómez-Brandón *et al.*, 2013). Carbon and N dynamics are linked during

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the decomposition of animal manure (Chantigny *et al.*, 2001). Animal slurries undergo decomposition processes, and the products are mainly volatile fatty acids, which are available C sources for the soil micro-organisms (Chantigny *et al.*, 2002; Hossain *et al.*, 2017). Among these, acetic, propionic and butyric acid are the most common (Mathur *et al.*, 1993; Zhu and Jacobson, 1999). Grazed grassland soils may also contain a wide range of C compounds, mainly sourced from plant material and animal excreta (Chen *et al.*, 2003). These may include cellulose, glucose (Chen *et al.*, 2003), glucosamine (Sradnick *et al.*, 2013); vanillin (Yamamoto *et al.*, 2008), benzoic acid (Dijkstra *et al.*, 2013), stearic acid (Hristov *et al.*, 2009) and phytic acid (Burkholder *et al.*, 2004). Based purely on water solubility, it is expected that glucose (Gunina and Kuzyakov, 2015) and glucosamine (Roberts *et al.*, 2007) are more available to the micro-organisms in the soil, than vanillin and cellulose (Brown *et al.*, 1988), which are less soluble (Table 1).

Published data on the composition of organic manures such as pig slurry show that the majority of the components are readily decomposable, and that cellulose and lignin are only minor components (Dendooven *et al.*, 1998*b*). The NO_3^- content in animal slurries is usually very low due to the anaerobic environment, with almost all of the inorganic nitrogen in the ammonium form, and organic N representing between 40 and 60% of total N in slurries (Chadwick *et al.*, 2000*a*).

A large amount of C compounds contained in biomass are released through complex decomposition processes and used in denitrification (Chen *et al.*, 2014). The availability of C substrates for microbial processes is linked to their decomposability and ability to support microbial growth (Tusneem, 1970). Denitrification is carried out by facultative anaerobes and free energy, N_2 and CO_2, which are produced as a result of electron transfer between NO_3^- and C (Tusneem, 1970; Hume *et al.*, 2002). This process is highly dependent upon the supply of C and accounts for about 37% of the CO_2 produced in the soil respiration system (Ingersoll and Baker, 1998; Rastogi *et al.*, 2002).

It has been proposed that the electron supply per mole of C from various substrates can affect the efficiency of denitrification (Beauchamp *et al.*, 1989). A positive correlation has been observed between denitrification and water-soluble organic C (Burford and Bremner, 1975). Sainju *et al.* (2010) found peak CO_2 fluxes immediately after substantial precipitation events (above 10 mm), which further highlights the role of denitrifying conditions on CO_2 production. Furthermore, Paul and Beauchamp (1989) reported a strong correlation between CO_2 produced under anaerobic conditions and total denitrification.

Carbon sources supply the electrons for these processes in the presence of glucose, as shown in the below reaction:

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$$

This reaction indicates a molar ratio of NO_3^- reduction to CO_2 production of 0.8. Differences in this value can be attributed to the presence of other electron acceptors or other factors such as sources of CO_2. Ratios of ~0.7 have been attributed to labile compounds, while ratios lower than 0.2 to non-labile compounds (Beauchamp *et al.*, 1989; Kumar and Sarma, 2018).

The majority of research on N losses from agricultural soils has overlooked the role and ranking of different C compounds found in manure for potential denitrification; thus, there exists a knowledge gap, to understand the availability of varying C compounds found in manure in promoting denitrification. The aim of this study was to evaluate the availability of different C compounds in manures and their role in promoting denitrification by (i) establishing the reactivity of varying C compounds found in cattle and pig slurry through their availability for denitrification and (ii) exploring C-quality effects in promoting denitrification.

Materials and methods

Site description

Soil samples were collected from an experimental site at Rothamsted Research, North Wyke, Devon, UK (50°46′10″N, 3°54′05″E). This area is situated at an altitude ranging between 157 and 177 m above sea level, receives a mean annual precipitation of 1040 mm, and has a 30-year mean annual temperature of 10.1°C (Orr *et al.*, 2016). The soils are classified as a clayey pelostagnogley of the Hallsworth series (Clayden and Hollis, 1985), or an FAO dystric gleysol (FAO, 2006). The top 10 cm are characterized by 36.6% clay, 47.7% silt and 13.9% fine sand and 1.8% coarse sand in the inorganic fraction. The soil pH was 5.7, and organic C was 5.3% (Armstrong and Garwood, 1991; Scholefield *et al.*, 1997; Harrod and Hogan, 2008). The experimental site is part of the Rowden Drainage Experiment at North Wyke, which consists of 1 ha paddocks divided into ten equal sections, which were either only grazed, only cut or grazed and/or cut and were used for pasture production. Soil samples for this experiment were collected from two plots receiving no-fertilizer N and without tile drains, which were also grazed during the summer season. Samples were collected in a W-pattern, mixed to form a composite sample and then split into different vessels for treatment application.

Experimental design

Two experiments were carried out to rank different C compounds based on their capability to promote denitrification: (i) experiment 1: incubation of eight different standard C compounds found in manures to rank their availability for potential denitrification and (ii) experiment 2: cattle and pig slurry treatments were included in the incubation and their C availability for denitrification compared to the four highest-ranked C compounds from experiment 1. Changes in time were measured for soil NO_3^- and NH_4^+ and the production of CO_2. The ranking was based on the molar ratio of CO_2 evolved to NO_3^- reduced after incubation using a low N and C soil medium. The ratio was calculated by dividing CO_2 evolved by NO_3^- reduced between days 1 and 3 of the experiment. The determination of NH_4^+ was used to help in the interpretation of the results, particularly in the changes in NO_3^-.

Experiment 1

A range of carbon compounds was selected to provide a variety of molecular weights and structures that may be typically found in animal manure (Dendooven *et al.*, 1998*a*; Bertora *et al.*, 2008; Velthof and Mosquera, 2011). These compounds were individually applied as dry materials in combination with NO_3^- to a soil/sand mixture. Thus, the treatments were: Control – No N or C (CO), N only (CO + N), N + glucose-C (GLU), N + glucosamine-C (GLU-INE), N + cellulose-C (CELL), N + stearic acid-C (STEA), N + benzoic acid-C (BEN), N + lignin-C (LIG), N + vanillin-C (VAN) and N + phytic acid-C (PHY).

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Table 1. Examples of slurry characteristics, as reported in the literature and the slurries from this study

Slurry	Pig slurry Dendooven *et al.* (1998*a*)	Pig slurry Dendooven *et al.* (1998*b*)	Cattle slurry 1 Chadwick *et al.* (2000*b*)	Cattle slurry 2 Chadwick *et al.* (2000*b*)	Pig slurry Chadwick *et al.* (2000*b*)	Cattle slurry Bertora *et al.* (2008)	Cattle slurry 1 Velthof and Mosquera (2011)	Cattle slurry 2 Velthof and Mosquera (2011)	Pig slurry, this study	Cattle slurry, this study
DM						2.9	11.4	1.9	3.15	4.6
Cellulose + hemicellulose (%)	2	6								
Lignin + ash (%)	6	16								
Readily decomposable fraction (%)	92	78								
Total C content (g/kg dry soil)	263	263	351–355	384	288–383		51	7	39	37.7
Organic N (g/kg dry soil)	27.3	27.3	22.9–35.9	25.6	28.6–42.6					
NH_4^+-N (g/kg dry soil)	52.4	52.4	1–8	1	1	0.74	1.7			
NO_3^--N (g/kg dry soil)	0.09	0.09								
Kjeldahl N (%)						0.39			2.9	3.5
OM (%)						1				
Organic C (%)						0.52				
C: N			9.9–15.3	15	7.8–12.9	1.3	14	7	13.2	10.9
Total N (g/kg dry soil)							5.3	2.5		
Labile fraction (%)	92	78								
Non- labile fraction (%)	8	22								
Total	100	100								

The bottom two values for labile and non-labile fractions are the sum (respectively) of the reported results Dendooven *et al.* (1998*a*) and Dendooven *et al.* (1998*b*).

A mixture of inert sand and sieved soil (<2 mm) was used as a matrix for the incubation to provide a low N and C content medium. For each vessel (250 ml), the soil/sand mixture comprised of 60 g of dry sand (acid washed) with 40 g of field moist soil, and water was added to achieve 80% water-filled pore space (WFPS) from the initial 30% WFPS. Nitrate (15 mg, equivalent to 150 mg N/kg soil) and 30 mg of C (equivalent to 1000 mg C/kg soil) were added to each vessel and mixed with a spatula in each of the incubation vessels. After that, the soil in each vessel was then pressed to a bulk density of 1 g/cm^3. Thereafter, 15 replicates of each treatment were incubated under standard temperature conditions of 15° C for 12 days. CO_2 production, NO_3^- depletion and NH_4^+ appearance were measured from each vessel on days 1, 3, 6, 9 and 12 after incubation, using the methods described below.

Experiment 2

Based on the results of the previous experiment, we selected a series of C compounds to study the effect of C quality in promoting denitrification. Pig and cattle slurry treatments were included, and their C availability compared with four standard C compounds; glucose, acetic acid, vanillin and cellulose. Thereafter, the treatments were: Control – No N or C (CO), N only (CO + N), N + glucose-C (GLU), N + cellulose-C (CELL), N + acetic acid-C (ACETIC), N + vanillin-C (VAN), N + cattle slurry-C (CS) and N + pig slurry-C(PS) (Table 2).

The procedure for soil medium preparation was the same as for the first experiment. Slurries were obtained from commercial farms and applied at the equal C loading of 30 mg C per vessel (the equivalent of 1000 mg C/kg soil). The same incubation procedures were followed as in the first experiment, with the exception that twice as much NO_3^- (30 mg per vessel, the equivalent of 300 mg N/kg soil) was applied to each vessel than in the first experiment to ensure an adequate N-source for the entire 9-day incubation period. Slurries were added to the soil/sand medium at the same C loading rate as the C compounds. Gravimetric moisture content was determined, and water was added to achieve 80% WFPS. The moisture content of the slurry was accounted for when adjusting the water content to 80% WFPS. Additionally, CO_2 production and NO_3^- depletion were measured from each vessel on days 1, 3 and 9 after application, using the methods described below.

Carbon dioxide production measurements

The amount of CO_2 produced during the incubation period was determined by CO_2 adsorption into sodium hydroxide (NaOH). Immediately after adding the C and NO_3^- amendments, open top 25 ml vials containing 10 ml of 0.5 M NaOH solution were placed in each vessel before sealing the vessels with a screw cap. The experiments were designed with sufficient replication to allow three replicates of each treatment to be destructively sampled on days 1, 3, 6 and 9 (for experiment 2), and additionally on day 12 (for experiment 1) after amendment application. During each destructive sampling, the vial of NaOH was carefully removed, and a 5 ml aliquot was added to 5 ml of 10% $BaCl_2$ in a flask and three drops of 1% phenolphthalein were added. This was then titrated against 0.2 M HCl, and the volume of HCl recorded when the solution became colourless. The amount of CO_2 respired during the period was then calculated using the below equation:

$$CO_2\ (\text{mg/kg}) = \frac{(B - V)N22}{w} \quad (1)$$

where B = standard HCl used to titrate NaOH in the blank (ml), V = the standard HCl used to titrate NaOH in treatment (mL), N = normality of HCl (1.00 N), 22 = equivalent weight of CO_2 and W = dry weigh of soil per vessel (mg).

Table 2. Percentage of added carbon (C) lost as carbon dioxide (CO_2) following C and slurry additions in experiment 2

	Days after incubation			
C source	Day 1	Day 3	Day 6	Day 9
Acetic acid	3.7 ± 1.4‡	22.4 ± 2.8	52.8 ± 3.9	61.3 ± 1.9
Glucose	19.4 ± 4.0	16.1 ± 0.55	37.1 ± 4.0	42.6 ± 2.5
Pig slurry	7.2 ± 2.4	13.1 ± 1.9	26.7 ± 0.78	24.4 ± 1.2
Vanillin	4.3 ± 1.9	11.7 ± 0.9	14.9 ± 0.5	20.8 ± 2.3
Cattle slurry	7.1 ± 0.54	8.7 ± 0.94	9.8 ± 1.4	12.0 ± 1.1
Cellulose	3.2 ± 1.6	2.7 ± 0.5	1.1 ± 1.6	4.8 ± 1.9

‡All values are mean ± standard error (S.E.) (n = 3).
No observations were made in the control treatment; hence was omitted.

Soil nitrate and ammonium analysis

At the end of each CO_2 trapping period, the soil in each vessel was analysed for inorganic N. This was done by adding 200 ml of 2 M KCl to each vessel and mixing for 1 h employing a rotary shaker. The contents of each vessel were then filtered through Whatman no. 4, and the filtrate was analysed for NO_3^- and NH_4^+ using a colorimetric automated flow injection technique after Kamphake *et al.* (1967) and Searle (1984), respectively.

Statistical analysis

To estimate the ratios between nitrate depletion and CO_2 production, we used the change in concentration and flux, respectively, between days 1 and 3 for experiment 1 and days 1 and 6 for experiment 2. This was because NO_3^- had been completely depleted in some treatments at days 3 and 6 for experiments 1 and 2, respectively. Data were tested for significant effects ($P < 0.05$) of C sources on NO_3^- reduction, CO_2 production, NH_4^+ appearance, percentage of C evolved as CO_2 and molar ratios of CO_2 produced : NO_3^- reduced. This was performed by a two-way analysis of variance followed by the Tukey's post-hoc test using the Statistical Analysis System (Version 9.4, Cary, North Carolina, USA). All values in results and discussion are presented as means (n = 3) and graphs were prepared using SigmaPlot (Version 14, Systat Software Inc., CA, USA).

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Results

Experiment 1. Ranking of the different carbon compounds

Soil mineral nitrogen

The soil NO_3^- reduction followed a zero kinetics order described by Swerts *et al.* (1996*a*), with NO_3^- concentrations decreasing rapidly, reaching almost zero in all treatments at day 6 after incubation (Table 3). NO_3^- reduction ranged from 276.3 ± 6.59 to 337.6 ± 1.24, below the detection limit (bdl) to 171.6 ± 9.29, and bdl to 6.43 ± 7.12 mg/kg during the 1st, 3rd and 6th days after incubation, respectively. Rapid NO_3^- reduction was associated with GLU and GLU-INE treatments, with all NO_3^- having been utilized on the 3rd day after incubation. Nitrate had disappeared in all treatments on the 6th day after incubation, except for the CO + N treatment, which still contained 6.43 ± 7.2 mg/kg of soil.

Ammonium evolution was detected from day 1 after incubation (Table 3), and all treatments had an increase until day 6. The largest amount of NH_4^+ was observed during day 6 in all treatments ranging from 101.1 ± 7.5 to 272.2 ± 10.1 mg/kg. The GLU-INE treatment resulted in significantly ($P < 0.05$) greater NH_4^+ compared to the other treatments at each sampling date. Correlations between disappeared NO_3 and evolved NH_4^+ were found to be positive and significant on day 1 ($r = 0.13$; $P = 0.01$); negative and significant on day 3 ($r = -0.51$; $P = 0.02$) and negative and not significant on day 6 ($r = -0.35$; ns).

Carbon dioxide production

There were significant differences in cumulative CO_2 production (between days 1 and 6) by the different C compounds on different days (Table 3). On most days, GLU-INE resulted in the greatest CO_2 production, but they were not significantly different from GLU and BEN ($P > 0.05$). On day 6, BEN had the greatest cumulative CO_2 production (944.8 ± 0.0 mg C-CO_2/kg), but this was not significantly different from the GLU and GLU-INE treatments ($P > 0.05$). On days 9 and 12, cumulative CO_2 production followed the same trend as day 6, and no significant differences were observed between the highest treatments; GLU, GLU-INE and BEN.

The CO_2 production during the 12-day incubation represented about 60% of the C added in the GLU-INE treatment (Table 3). The treatments had the following order, in terms of the CO_2 production as a percentage of added C:

$$\text{GLU-INE}\ (61.7 \pm 7.7\%) > \text{GLU}\ (50.7 \pm 3.8\%) > \text{BEN}\ (46.7 \pm 1.8\%) > \text{PHY}\ (32.8 \pm 4.24\%) \sim \text{VAN}\ (30.9 \pm 6.9\%) > \text{LIG}\ (8.0 \pm 2.6\%) \sim \text{CELL}\ (4.5 \pm 3.1\%) \sim \text{STEA}\ (0.3 \pm 3.8\%).$$

Carbon dioxide : nitrate molar ratios

Glucosamine (1.17 ± 0.04) and GLU (1.07 ± 0.04) had a significantly higher reactivity compared to the other treatments ($P < 0.05$), but they were not necessarily significantly different from each other (Fig. 1). We grouped the compounds according to the mean differences, and they fall into three reactivity groups based on the values obtained:

(i) 'high' CO_2 production/'fast' NO_3^- reduction; GLU, GLU-INE;
(ii) 'intermediate' CO_2 production/'intermediate' NO_3^- reduction; BEN, VAN, LIG;
(iii) 'low' CO_2 production/'slow' NO_3^- reduction; STEA, CELL.

Experiment 2. Carbon quality effect in promoting denitrification

Slurry composition

The main properties of the slurries selected for the experiment are summarized in Table 1. The CS had a slightly higher dry matter and total N content than the PS, while the total C was marginally lower in the CS (Table 1). The total C and C : N ratio of the slurries used in the current experiment were similar to slurries reported by Risberg *et al.* (2017), and Velthof *et al.* (2003), for the pig and cattle slurry, respectively.

Soil nitrate

The highest rate of NO_3^- reduction was observed from the ACETIC and GLU treatments (Fig. 2). Indeed, all of the NO_3^- had been depleted in the ACETIC treatment by day 6, and by day 9 in the GLU treatment. The apparent rate of NO_3^- removal from the PS and CS treatments was similar to that of the CO + N treatment.

Carbon dioxide production

There were significant differences between treatments for cumulative CO_2 and % C produced as CO_2 from days 1 to 9 (Table 2 and Fig. 3). Cumulative CO_2 production ranged from 267.6 ± 18.6 to 920.9 ± 18.6 mg/kg, with the ACETIC treatment (920.9 ± 18.6 mg/kg), resulting in the highest CO_2 output from all the treatments ($P < 0.05$) (Fig. 3). The % of C evolved as CO_2 ranged from 4.8 ± 1.9 to 61.3 ± 1.9%, with the ACETIC treatment (61.3 ± 1.93%) resulting in the highest value ($P < 0.05$) followed by GLU (42.6 ± 2.5%) (Table 2). The PS (24.4 ± 1.2%) resulted in a higher percentage of CO_2 production than the CS treatment (12.0 ± 1.1%).

Carbon dioxide : nitrate molar ratios

Based on the molar ratios, the PS (0.79 ± 0.11) ranked significantly higher ($P < 0.05$) as a C-source for promoting denitrification compared to other treatments, except for ACETIC treatment which had a molar ratio of 0.72 ± 0.23.

Discussion

Ranking the reactivity of carbon compounds

Nitrate had disappeared entirely from the GLU and GLU-INE treatments by day 3 of the incubation period (Table 3). The rapid NO_3^- reduction upon incubation with GLU and GLU-INE C compounds was likely due to their high solubility and immediate availability to provide electrons for NO_3^- reduction by soil micro-organisms (Robertson and Groffman, 2007; Gunina and Kuzyakov, 2015). The results of the current study are in agreement with reports by Beauchamp *et al.* (1989) and Geisseler *et al.* (2010), that concluded that glucose is an immediately available electron source for microbial utilization of NO_3^- in the soil. Also, an immediate reduction of N in the presence of elevated glucosamine in grazed grassland soils was reported by Roberts and Jones (2012).

Significant amounts of NH_4^+ were observed in all treatments, including the CO (Table 3) treatment where no NO_3^- and/or C amendments were applied, which could have been because of

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Table 3. Soil nitrate (NO_3^-) reduction, ammonium (NH_4^+) appearance, cumulative carbon dioxide (CO_2) and % C evolved as CO_2 at days 1, 3, 6, 9 and 12 after incubation in experiment 1

		C-Source									
Parameter	Day	CO	CO + NI	GLU	GLU-INE	CELL	STEA	BEN	LIG	VAN	PHY
NO_3^- (mg/kg dry soil)	1	0 ± 0.0[‡]	329 ± 9.6	276.3 ± 6.6	312.4 ± 7.2	337.6 ± 1.3	333.4 ± 2.5	337.3 ± 0.25	337.5 ± 0.73	328.7 ± 3.31	314.8 ± 6.4
	3	0 ± 0.0	123.1 ± 25.3	bdl[§]	bdl	149.4 ± 2.4	147.1 ± 4.8	44.7 ± 12.4	171.6 ± 9.3	48.3 ± 25.5	7.74 ± 0.53
	6	0 ± 0.0	6.43 ± 7.12	bdl	bdl	0.15 ± 1.3	0.9 ± 1.5	0.3 ± 1.6	bdl	bdl	bdl
NH_4^+ (mg/kg dry soil)	1	23.7 ± 3.4	23.4 ± 0.22	10.1 ± 3.4	50.5 ± 2.1	26.7 ± 5.3	29.4 ± 2.7	18.3 ± 2.8	27.0 ± 2.8	20.9 ± 0.53	59.1 ± 3.2
	3	76.8 ± 5.6	59.9 ± 10.5	89.2 ± 11.6	223.4 ± 8.2	76.2 ± 4.2	76.1 ± 4.2	77.7 ± 13.6	68.6 ± 3.8	100.6 ± 11.5	147.6 ± 9.1
	6	128.5 ± 5.85	106.3 ± 7.5	139.2 ± 8.5	272.2 ± 10.1	119 ± 10.4	126.3 ± 12.5	97.8 ± 3.3	111.6 ± 4.5	101.1 ± 7.5	212.6 ± 8.0
CO_2 (mg C-CO_2/kg dry soil)	1	26.7 ± 3.81	25.4 ± 2.5	33.0 ± 2.5	38.1 ± 0.0	22.9 ± 0.0	38.1 ± 0.0	38.1 ± 0.0	22.9 ± 0.0	35.6 ± 2.5	22.9 ± 0.0
	3	154.9 ± 9.17	167.6 ± 8.8	355.6 ± 11.1	363.2 ± 14.2	157.5 ± 6.7	160 ± 8.8	251.4 ± 22.9	132.1 ± 20.8	238.7 ± 44.6	221 ± 0.0
	6	289.5 ± 4.4	342.9 ± 37.7	665.4 ± 12.7	685.7 ± 42.0	365.7 ± 8.8	368.3 ± 17.8	944.8 ± 10.0	350.5 ± 30.8	477.5 ± 16.7	480 ± 4.4
C evolved as CO_2 (%)	1	0 ± 0.0	0 ± 0.0	0.8 ± 0.28	1.3 ± 0.0	−0.27 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	−0.27 ± 0.0	1.1 ± 0.27	−0.27 ± 0.0
	3	0 ± 0.0	0 ± 0.0	20.5 ± 1.2	20.5 ± 1.5	−1.1 ± 0.7	−0.84 ± 0.97	8.8 ± 2.4	−3.7 ± 2.2	7.5 ± 4.7	5.6 ± 0.46
	6	0 ± 0.0	0 ± 0.0	35.2 ± 1.4	36 ± 4.4	2.4 ± 0.9	2.8 ± 1.9	0 ± 0.0	0.8 ± 3.2	14.1 ± 1.8	14.4 ± 0.46
	9	0 ± 0.0	0 ± 0.0	41.5 ± 1.5	50.7 ± 5.7	−5.33 ± 2.9	6.2 ± 1.5	33.1 ± 1.1	0.0 ± 4.7	18.1 ± 1.6	20.3 ± 2.7
	12	0 ± 0.0	0 ± 0.0	50.7 ± 3.8	61.7 ± 7.7	4.5 ± 3.1	0.3 ± 3.8	46.7 ± 1.8	8.0 ± 2.6	30.9 ± 6.9	32.8 ± 4.24

Treatment description: CO (Control – No N or C), CO + N (N only), GLU (N + glucose-C), GLU-INE (N + glucosamine-C), CELL (N + cellulose-C), STEA (N + stearic acid-C), BEN (N + benzoic acid-C), LIG (N + lignin-C), VAN (N + vanillin-C) and PHY (N + phytic acid-C). [‡]All values are mean ± standard error (S.E.) ($n = 3$).
[§]bdl = below the detection limit, and NO_3^- and NH_4^+ were not determined at day 9 and 12.

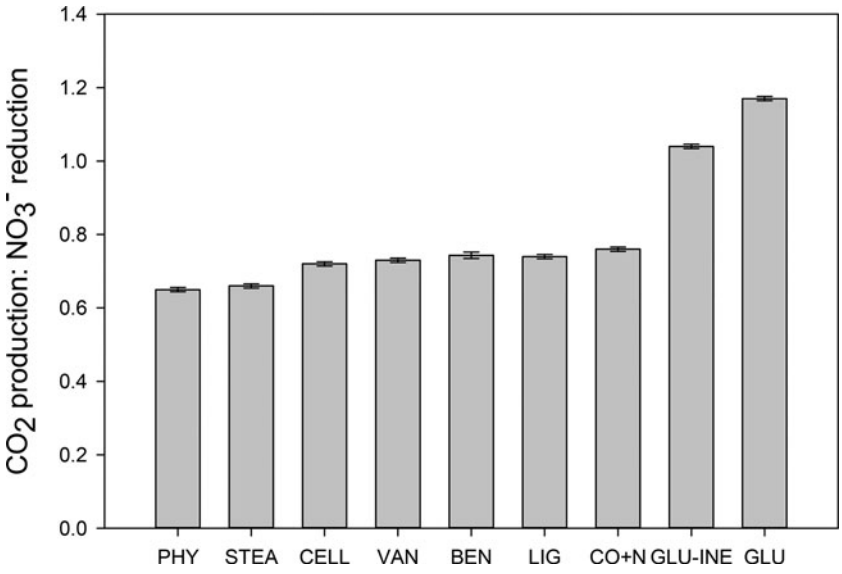


Fig. 1. Ranking of C reactivity based on the ratio of CO_2 produced : NO_3^- reduced in experiment 1. Treatment description: PHY (N + phytic acid-C), STEA (N + stearic acid-C), CELL (N + cellulose-C), VAN (N + vanillin-C), BEN (N + benzoic acid-C), LIG (N + lignin-C) CO + N (N only), GLU-INE (N + glucosamine-C) and GLU (N + glucose-C). Vertical lines represent standard error of each treatment mean ($n = 3$). No reactions were observed in the CO treatment; hence it was omitted.

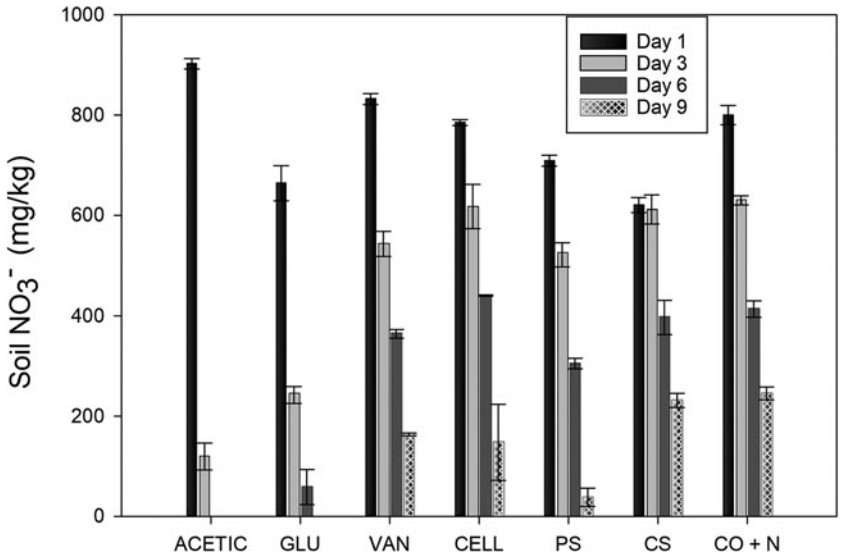


Fig. 2. Average soil NO_3^- reduction following C and slurry additions in experiment 2. Treatment description: ACETIC (N + acetic acid-C), GLU (N + glucose-C), VAN (N + vanillin-C), CELL (N + cellulose-C), PS (N + pig slurry-C), CS (N + cattle slurry-C) and CO + N (N only). Vertical lines represent standard error of each treatment mean ($n = 3$). No reactions were observed in the CO treatment; hence it was omitted.

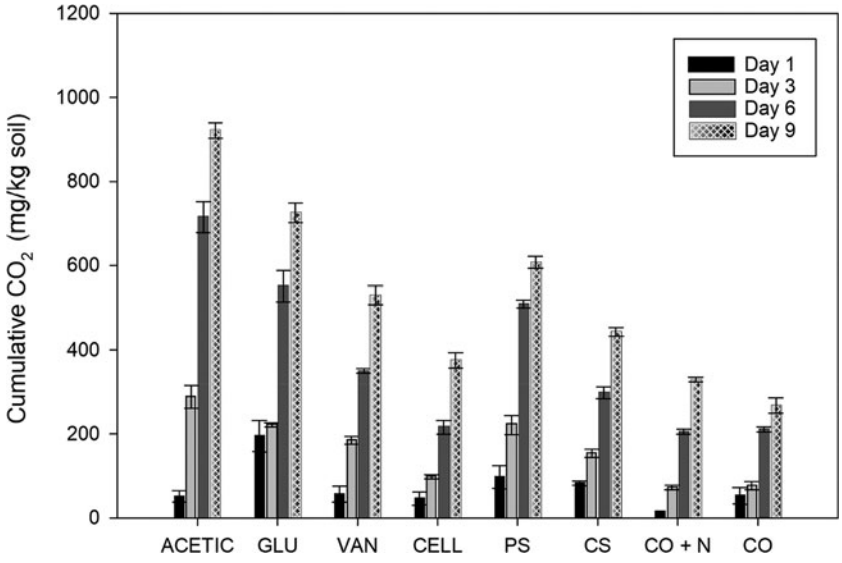


Fig. 3. Average cumulative CO_2 production after incubation following C and slurry additions in experiment 2. Treatment description: ACETIC (N + acetic acid-C), GLU (N + glucose-C), VAN (N + vanillin-C), CELL (N + cellulose-C), PS (N + pig slurry-C), CS (N + cattle slurry-C), CO + N (N only) and CO (Control-No N or C). Vertical lines represent standard error of each treatment mean ($n = 3$).

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Table 4. Utilization of carbon (C, %) and carbon : nitrogen (C : N) ratios in slurries reported by some authors in the literature compared to the current study

Slurry type	Total C (%)	Utilization of added total C (%)	Total N (%)	C : N	References
PS	0.94	100	0.4	2.4	Bertora *et al.* (2008)
PS	40.0	31.5	5.3	7.5	Risberg *et al.* (2017)
PS	58.0	32.3	5.1	11.3	Risberg *et al.* (2017)
PS	37.2	27.7	5.6	6.6	Risberg *et al.* (2017)
PS	57.8	21.4	5.9	9.8	Risberg *et al.* (2017)
CS	51.0	ND	3.64	14.0	Velthof *et al.* (2003)
CS	44.0	ND	2.44	18.0	Velthof *et al.* (2003)
CS	44.0	ND	5.93	7.4	Velthof *et al.* (2003)
PS	37.7	24.4	3.47	10.9	This study
CS	39.0	12.0	2.95	13.2	This study

PS, pig slurry; CS, cattle slurry; ND, not determined.

residual N from the soil medium but most likely from mineralization of the soil OM especially after sieving at <2 mm (Cookson *et al.*, 2005). This was found in a study by Gill *et al.* (1995). In the case of the GLU-INE treatment, the largest amount of NH_4^+ recorded could have been due to its release from the glucosamine molecules (Tiedje, 1988; Currey *et al.*, 2010). This process occurs in anaerobic conditions; however, its importance in soils remains uncertain (Yonebayashi and Hattori, 1980). The significant correlations between NO_3^- reduction and NH_4^+ recovery are in agreement with findings by Page *et al.* (2003) and Tusneem (1970), that reported that NO_3^- is reduced to NH_4^+ under O_2-limited conditions, similarly to the current experiment. This process requires an electron source generally in the form of C; hence there are significant differences in NH_4^+ appearance and NO_3^- reduction as influenced by different C sources (Mohan and Cole, 2007; Soussana and Lemaire, 2014). Our findings are in agreement with the observations by Mohan and Cole (2007) at days 1 and 3 after incubation; however, the relationship became insignificant at day 6. This phenomenon might be the result of rapid microbial NO_3^- reduction in the presence of C electron sources than would naturally occur (Chantigny *et al.*, 2001). The amount of NO_3^- that disappeared was much larger than the amount of NH_4^+ produced (Table 3): a decrease in NO_3^- between 1.4 (for GLU-INE) to 4.2 (for BEN) times larger than the increase in NH_4^+. It seems that the reduction of NO_3^- could have been only due to denitrification. The relatively high soil moisture conditions of our experiment agree with denitrification as the dominant process occurring (Davidson *et al.*, 2000).

High CO_2 production from grassland soils was associated with the GLU and GLU-INE compounds (Table 3). The higher CO_2 production from GLU and GLU-INE treatments were in agreement with other authors, particularly Beauchamp *et al.* (1989), Roberts and Jones (2012) and Hossain *et al.* (2017), all reporting that higher soil respiration rates were associated with elevated glucose and glucosamine levels in soils.

GLU and GLU-INE were the most reactive C sources, whereas PHY and STEA were the less labile ones (Fig. 1). These results are in agreement with earlier findings by Swerts *et al.* (1996*a*) and Wang *et al.* (2013) where CO_2 to NO_3^- ratios above 0.7 were associated with highly labile C compounds. A study by Swerts *et al.* (1996*b*) showed a CO_2 to NO_3^- ratio of 0.80 when the C : N (as glucose and NO_3^-) application was 14 : 1. This ratio is smaller than the value from the current experiment, perhaps due to the lower amount of NO_3^- available (proportionally) in the current study compared to Swerts *et al.* (1996*b*). The ranking of the C compounds reactivity was based on the assumption that NO_3^- depletion was mostly due to NO_3^- reduction under anaerobic conditions (Ellis *et al.*, 1996), but other processes are reported to convert NO_3^- to NH_4^+ such as nitrate ammonification (DNRA) (Butterbach-Bahl *et al.*, 2013). Still, this is considered to be a small source when high rates of N are applied (Baggs, 2008). Additionally, in the relatively high soil moisture conditions the current experiment, it is not expected that nitrification will occur in soil (Davidson *et al.*, 2000).

Carbon quality effects on potential denitrification

The two slurries included in this study were relatively similar in overall composition (Table 4). However, it is possible there were differences in the proportions of labile *v.* non-labile C fractions that we did not assess in the current study. Dendooven *et al.* (1998*a*) and Dendooven *et al.* (1998*b*) reported pig slurry composition with labile fractions of the order of 78–92%. In cattle slurry, Fangueiro *et al.* (2017) reported labile C in the order of 50%, which was higher than observations of 35.5% by Köster *et al.* (2015). Therefore, it is possible that the PS in the current experiment contained greater quantities of labile C compared to the CS. This is reflected in the higher NO_3^- : CO_2 evolution in the PS treatment compared to the CS, which could have been due to the presence of carbonates in pig slurries as reported by other studies (Sommer and Husted, 1995). However, this was not measured in the experiments of the current study. Differences in the slurry composition are also found between years as Velthof and Mosquera (2011) report. These authors analysed N in pig and cattle slurries but did not report C contents.

The different C sources significantly influenced NO_3^- reduction in the current experiment (Fig. 2). Nitrate was depleted faster upon incubation with the ACETIC and GLU treatments, with all NO_3^- reduced at days 6 and 9 for the two C-sources, respectively. These results were consistent with findings by Takai and Kamura (1966) and Swerts *et al.* (1996*a*), indicating that glucose and acetic acid had increased availability as electron sources for soil microbial NO_3^- reduction reactions. On the other hand, the relatively slower NO_3^- depletion in the PS and CS treatments could be the

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Table 5. Molar ratio of carbon dioxide (CO_2) produced : nitrate (NO_3^-) reduction as a ranking for carbon (C) availability for potential denitrification for the first 6 days in experiment 2

C source	CO_2 evolved : NO_3^- reduction
Pig slurry	0.79 ± 0.11‡
Acetic acid	0.72 ± 0.23
Glucose	0.59 ± 0.1
Vanillin	0.61 ± 0.32
Cattle slurry	0.56 ± 0.21
Cellulose	0.44 ± 0.11
CO + N (N only)	0.40 ± 0.2

‡All values are mean ± standard error (S.E.) ($n = 3$).
No observations were made in the control treatment; hence was omitted.

result of their C being less decomposed (Rochette *et al.*, 2004) compared to the C found in the GLU and ACETIC treatments. Furthermore, the slow NO_3^- reduction in the CO + N treatment verifies the significance of C in NO_3^- reducing reactions since organic C has been well documented to stimulate such responses from soils (Sommer and Husted, 1995; Meijide *et al.*, 2007; Soussana and Lemaire, 2014).

The percentage of C evolved as CO_2 in slurries observed in the current study, was generally lower than that reported by other authors (Bertora *et al.*, 2008; Risberg *et al.*, 2017). The lower CO_2 evolved in the CS, PS, VAN and CELL treatments compared to the GLU and ACETIC treatments was likely the result of their C not being easily accessible during the denitrification process (Chantigny *et al.*, 2002; Rochette *et al.*, 2004; Hossain *et al.*, 2017); a significant CO_2 producing reaction. The coupling of N and C in the release of atmospheric CO_2 has been recorded by several studies (Bertora *et al.*, 2008; Morley and Baggs, 2010; Hossain *et al.*, 2017). The low CO_2 production from the CO + NI treatment in the current study agrees with the coupling effect of soil N and C in CO_2 production (Rastogi *et al.*, 2002; Van Groenigen *et al.*, 2011; Risberg *et al.*, 2017) (Table 5). During the denitrification process, bacteria utilize NO_3^- as a terminal electron acceptor in the absence of O_2 during respiration (Robertson and Groffman, 2007). Denitrifying organisms use C compounds as electron donors for energy; thus, denitrification is highly dependent on the amount and availability of C compounds (Beauchamp *et al.*, 1980; Aulakh *et al.*, 1992). The degradation and transformation of C contained slurries may result to several carbonates, and C compounds with varying availabilities and solubility (Rochette *et al.*, 2004); some of which may stimulate CO_2 production rates upon reaction with NO_3^- (Aulakh *et al.*, 1992; Rochette *et al.*, 2004).

Although the slurries did not have a rapid NO_3^- reduction and CO_2 production, the pig slurry emerged with the highest ($P < 0.05$) $CO_2 : NO_3^-$ molar ratio of 0.79 ± 0.11 (Table 5). This could be because the pig slurry might have contained more NH_4^+, which might have been nitrified, producing NO_3^- before inducing denitrifying conditions during the incubation. Table 1 shows some examples from other studies (Knowles, 1982; Skiba, 2008). The slurries would also provide NH_4^+ from their inorganic pool but because of the anaerobic soil conditions, it was not expected that this would have increased the NO_3^- pool due to nitrification. Subsequent denitrification of this additional NO_3^- under the favourable conditions of the experiment (temperature and moisture) would have masked the actual rate of reduction of the added NO_3^- (Jarvis *et al.*, 1994; Griffin *et al.*, 2002), but this was not the case in the current study.

Reactivity of slurries from livestock is complex not only because of the nature of the C they contain but also because they contribute with microbial populations themselves (Acea and Carballas, 1988; Clemens and Huschka, 2001). Changes in slurry composition during storage makes the original non-labile C available (Bertora *et al.*, 2008), increasing the potential for NO_3^- leaching, increased denitrification and production of N_2O. Studies to develop country-specific N_2O emission factors for dung and urine deposited during grazing, use freshly collected material that is preserved before use (Cardenas *et al.*, 2016; Thorman *et al.*, 2020). The results would likely differ if aged slurries were applied, and possibly emissions would be more substantial. Taylor *et al.* (1989) stated that the ratio of C to N (C : N) is useful as the first proxy of OM decomposability, with greater C : N generally leading to slower decomposition in slurries. Usually, labile compounds (i.e. soluble sugars and unshielded cellulose) are preferentially lost during the initial phase of decay, and then lignin progressively becomes the dominant constituent of decomposing OM (Berg, 2014). In the case of manure, due to the different quality, the dynamics of its chemical composition and its regulation on decomposition process may vary if compared to more standard materials as in the case of plants (Eldridge *et al.*, 2017). Markewich *et al.* (2010) and Bhogal *et al.* (2016) stated that manures with relatively low C : N ratios, generally mineralize rather than immobilize mineral N. It would be expected in the current study that, the CS would have more potential to mineralize compared to the PS due to its lower C : N ratio. This ratio has also been found to have a positive relationship with decomposition rate even when the C : N was relatively high and within a wide range (Chen *et al.*, 2019), so in the current study, the cattle slurry would decompose slower in agreement with the lower $CO_2 : NO_3^-$ ratio (Table 5).

Conclusions

The results of the molar ratio of CO_2 evolution to NO_3^- reduction ranked glucose and glucosamine as highly reactive C-compounds, and the pig slurry and acetic acid as good quality C-sources, to promote potential denitrification. The results of this study show the importance of characterization of the carbon quality of slurries and, if possible, for multiple years. These results could be useful for improving the accuracy of newly developed mitigation and emission factors and for feeding into models.

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Ethical standards. Not applicable.

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