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Denitrification potential in subsoils: a mechanism to reduce nitrate leaching to groundwater

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Abstract

Understanding subsurface denitrification potential will give greater insights into landscape nitrate (NO_3^-) delivery to groundwater and indirect nitrous oxide (N_2O) emissions to the atmosphere. Potential denitrification rates and ratios of $N_2O/(N_2O+N_2)$ were investigated in intact soil cores collected from 0-0.10, 0.45-0.55 and 1.20-1.30 m depths representing A, B and C soil horizons, respectively, under intensively managed grazed grassland in south eastern Ireland. The soil was moderately well drained with textures ranging from loam to clay loam (gleysol) in the A to C horizon. An experiment was carried out by amending soils from each horizon with (i) 90 mg NO_3^- -N as KNO_3, (ii) - (i) + 150 mg glucose-C, (iii) - (i) + 150 mg DOC (dissolved organic carbon, prepared from top soil layer in intensively managed grassland) kg^{-1} dry soil. An automated laboratory incubation system was used to simultaneously measure N_2O and N_2, at 15°C, with the moisture content raised by 3% above the moisture content at field capacity, giving a water-filled pore space of 80, 85 and 88% in the A, B and C horizons, respectively. There was a significant effect (p<0.01) of soil horizon and added carbon on cumulative N_2O emissions. N_2O emissions were higher from the A than the B and C horizons and were significantly lower from soils that received only nitrate than soils that received NO_3^- + either of the C sources. The two c sources were similar in N_2O emissions. The N_2 fluxes differed significantly (p<0.05) only between the A and C horizons. During a 17-day incubation, total denitrification losses of the added N significantly (p<0.01) decreased with soil depth and were increased by the addition of either C source. The amounts of added N lost for each horizon were A: 25, 61, 45%; B: 12, 29, 28.5% and C: 4, 20, 18% for nitrate, nitrate + glucose-C and nitrate + DOC, respectively. The ratios of N_2O to N_2O+N_2 differed significantly (p<0.05) only between soil horizons, being higher in the A (0.58 – 0.75) than in the deeper horizons (0.10 –

0.36 in B and 0.06 – 0.24 in C), clearly indicating the potential of subsoils for a more complete reduction of N_2O to N_2. Stepwise multiple regression analysis revealed that N_2O flux increased with total organic C and total N but decreased with NO_3^--N which together explained 88% of the variance ($p<0.001$). The N_2 flux was best explained ($R^2 = 0.45$, $p<0.01$) by total organic N (positive) and with NO_3^--N (negative). A better fit model obtained by stepwise multiple regression showed that total denitrification rates were positively related to total C and negatively related to NO_3^--N with $R^2 = 0.76$ and $p<0.001$. The results suggest that without C addition potential denitrification below the root zone was low. Therefore, added C sources in subsoils can satisfactorily increase nitrate depletion via denitrification with decreased N_2O mole fraction as N_2O would further be reduced to N_2 while passing through soil profile to soil surface or to groundwater. Subsoil denitrification could be manipulated either through introducing C directly into permeable reactive barriers and/or indirectly by dirty water irrigation and manipulating agricultural plant composition and diversity.

Keywords**:** denitrification potential, N_2O mole fractions, subsoil, greenhouse gas, nitrate leaching, grassland

1. Introduction

An excess of N in the environment is viewed as an escalating global threat, due to its impacts on groundwater quality and the atmosphere (Stark and Richards, 2008). Soils under grazed grassland often have high concentrations of nitrate (NO_3^-), arising from the application of mineral fertilizers, slurries, animal excreta and from the native soil organic matter (Foster, 2000). Large amounts of N transferred within the soil system increase the potential and the opportunities for NO_3^- losses (Davies, 2000). The average

leaching losses of NO_3^- from terrestrial ecosystems in central Europe is 15 kg N ha^{-1} y^{-1} (Werner, 1994). Nitrate transformation in the root zone is well documented (Ibendahl and Fleming, 2007), but its movement and transformations in prevailing geochemical conditions below the root zone are less well understood (Jarvis and Hatch, 1994). The added NO_3^- can be transported through percolating water and transformed to gaseous forms, thereby leaving agricultural systems, or may be lost through leaching and runoff (Clough et al., 2005). Substantial quantities of dissolved inorganic N, particularly NO_3^-, are exported through low order streams (Alexander et al., 2000). Nitrate contamination of surface water and groundwater is common in watersheds dominated by agricultural activities (Townsend et al., 2003), primarily because of diffuse pollution from intensive farming (Foster and Young, 1980). Denitrification is one of the most important processes that can control the quantity of nitrate available for leaching from soil to water (Jarvis, 2000).

Denitrification is the mainly microbial reduction of NO_3^- to the gaseous products nitric oxide (NO), nitrous oxide (N_2O) or dinitrogen (N_2). This process is an important mechanism for nitrate removal in a variety of suboxic environments (Seitzinger et al., 2006). Some studies have shown that the highest rates of denitrification occur in the upper soil horizon (Clement et al., 2002; Cosandey et al., 2003; Kustermann et al., 2010), the extent of which depends on moisture levels (Khalil and Baggs, 2005). Recently, researchers have found microbial hot spots with significant denitrification activity in patches of organic rich subsoils at depths of several meters (Hill et al., 2004) and in urine treated subsoils (Dixon et al., 2010). Subsoil denitrification has been suggested as an important mechanism for the removal of excess NO_3^- before leaching to groundwater, transport within saturated subsoil zones or discharge to surface aquifers

via subsurface drainage (Fenton et al., 2009; Sotomayor and Rice, 1996). Denitrification not only serves as a natural pathway for the elimination of excess NO_3^- in soil and water (Ellis et al., 1975), but also contributes to the emissions of N_2O, a potent greenhouse gas (Knowles, 1982) and an indirect contributor to the depletion of ozone (O_3) in the stratosphere (Crutzen, 1970). An interesting feature of denitrification in subsurface soils is that it is likely to be overlooked as a contributor to global atmospheric N_2O concentrations, due to the possible further reduction of N_2O to N_2 during upward diffusion through the soil profile, under O_2 limited conditions, if adequate sources of organic carbon (C) are present (Elmi et al., 2003; Castle et al., 1998).

The beneficial effect to the environment of NO_3^- removal by denitrification depends on the partitioning of its end products into N_2O and N_2. Knowledge of the denitrification gaseous end-products and the $N_2O/(N_2O+N_2)$ ratio is necessary to assess accurately the environmental consequences of the denitrification process (Elmi et al., 2003), with emphasis on the subsoil environment (Bergsma et al., 2002). The lack of information on N_2 emissions from terrestrial ecosystems not only limits our understanding of its significance as a sink for reactive N, but also impedes the quantification of the process as a whole (Davidson and Seitzinger, 2006; Groffman et al., 2006) so that N budgets in biogeochemical models are incomplete (Boyer et al., 2006). To date, only a few estimates of denitrification in the subsoils of riparian wetlands and peat soils have been reported (Casey et al., 2001; Dhondt et al., 2004; Hill et al., 2000, 2004; Well et al., 2001). Depending on the environmental conditions, the mechanisms and magnitude of denitrification losses in subsoils of grazed grassland may however deviate considerably from those of other sites warranting further investigation under grassland ecosystems.

The relative importance of the denitrification process depends strongly on certain environmental conditions including O_2 concentration, NO_3^- content and C availability (Tiedje, 1988), though their influences on the mole fractions of N_2O and N_2 in agricultural soils are still under debate, with little consensus (Venterea et al., 2005). Where organic C is added, a significant denitrifying potential may be revealed at depths as great as 7 m (Jarvis and Hatch, 1994; McCarty and Bremner, 1992).

A lack of organic C to provide energy to denitrifiers is usually identified as the major factor limiting denitrification rates (Devito et al., 2000; Pabich et al., 2001). More precisely, the quality and quantity of the C source is most often more important than total organic C due to its variable availability to microbes (Ciarlo et al., 2007). The specific contribution of the different C sources available to denitrifying micro-organisms has not been defined (Beauchamp et al., 1989). Therefore, knowledge on the factors controlling denitrification and more specifically, the $N_2O/(N_2O+N_2)$ ratios are crucial to improve our understanding of the processes contributing to complete reduction of NO_3^- via denitrification in subsoil environments. Concerning health and environmental hazards of NO_3^- and the global warming potential of N_2O, we hypothesized that the addition of a readily available source of C (glucose) would enhance the reduction of N_2O to N_2 in subsoils, and show a lower $N_2O/(N_2O+N_2)$ ratio in amended soils than in unamended soils. The main objectives of this research were (a) to measure the potential denitrification rates in subsoils and (b) to relate soil parameters with the measured potential denitrification rates and ratios of end products, $(N_2O/(N_2O+N_2)$ in subsurface environments.

2. Materials and Methods

2.1 Study site characteristics

Soil samples were collected during January, 2008 (winter) from grazed grassland at the dairy farm of Teagasc Environment Research Centre, Johnstown Castle, Wexford, Ireland (152.3342^{o}N, -6.4575^{o}W). The soil textures of a profile up to 1.3 m depth varied from loam to clay loam (Brown Earth) overlying Ordovician sediments of sandstone and shale. Soil physical and chemical properties including the initial nitrate content of three horizons at the experimental site are presented in Table 1. The average groundwater table is below 1.2 m during winter and below 2.0 m during summer. On a yearly average, 24 cows graze the land for a total of 50 days and about 375 kg N ha^{-1} year^{-1} is harvested by both one silage cut and grazing animals. The total annual N inputs are about 450 kg N ha^{-1} from inorganic fertilizers, animal excrement and N deposition.

2.2 Soil sampling

Intact soil cores (45) were collected from three depths (0-0.10, 0.45-0.55 and 1.20-1.30 m), representing the A, B and C horizons, of the soil profile. Stainless steel cylinders (0.12 m x 0.15 m) were manually inserted using a percussion hammer into the soil after trimming off the swards to sample the surface/upper horizon (0-0.10 m) and then a hole was dug around the cylinder to assist removal giving each core size of 0.1 m x 0.15 m. The two other (deeper) horizons were sampled from the same locations by first removing the soil from the upper horizons. Fine mesh netting was placed over the top and bottom of the cylinders to contain the soil and kept in place using rubber bands at both ends. Soil samples were stored immediately after collection in a cold room at 4°C and transported to Roth Research, North Wyke, UK, in insulated boxes and then stored at 4°C until the commencement of experiments.

2.3 Soil core preparation and amendment

Three sets of 12 cores (3 horizons with 4 replications) were used where all of the soil cores were amended with nitrate (90 mg NO_3^--N kg^{-1} as KNO_3) and the treatments consist of (T_1) a control, (T_2) 150 mg glucose-C kg^{-1}, and (T_3) 150 mg DOC-C kg^{-1}. Nitrate was supplied to all treatments to ensure an adequate source of substrate for denitrification, and we considered T_1 as the control against which the effect of the added carbon sources would be measured. Large leaching losses of nitrate-nitrogen (50-200 Kg N ha^{-1}) can occur from intensively grazed and/or fertilized pasture (Cameron and Haynes, 1986; Jarvis, 2000; Scholefield, 1993; Ledgard et al., 1996). Jarvis (1999) reported the mean surpluses of N in UK grassland of 257 Kg N ha^{-1} equivalent to 183 kg N Kg^{-1} soil. Richards and Webster (1999) measured denitrification potential in subsoils (0-2 m) collected from arable land being treated with 1000 ug C g^{-1} soil and 100 ug N g^{-1} soil. The WSOC was 80, 50 and 24 mg L^{-1}, respectively in A, B and C horizons. During MWHC and FC determination, water saturation and drain out can cause losses of indigenous WSOC and NO_3^-. Therefore, considerable amount of C and N were added (amendments) to compensate the losses and thereafter to ensure the availability of C and N as per the concept of denitrification potential.

Each of the three treatment sets of cores was incubated consecutively whilst maintaining exactly the same conditions. During each incubation, 12 soil cores were weighed and placed in a plastic tray of approximately 0.6m length x 0.5m width x 0.25m height and water was added slowly to bring water level until 3 cm below the top of soil. After 24 h, the fine mess placed over either end of the core to contain the soil was removed before placing soil cores on a fine screen metal sieve with sufficient space below the screen to drain out excess water for 30 minutes to achieve the maximum

water holding capacity (MWHC) (Scharenbroach, 2010). The saturated soil cores were kept covered to limit evaporation and were allowed to drain gravitational water for 48 h and weighed to estimate the field capacity (FC) (Scharenbroach, 2010). The amendment solutions were prepared with an amount of water required to maintain the soil WFPS (water-filled pore space) levels at a moisture content of 3% above the moisture content at FC: ca. 80, 85 and 88% for A, B and C horizons, respectively. Potential denitrification rates requires approximately anaerobic conditions (~90% WFPS). Considering the required anaerobic conditions and natural field conditions having higher O_2 concentrations in top soil than subsoils the present WFPS was satisfactory for top soil and subsoils.

2.4 Preparation of dissolved organic C (DOC) solution used

Surface soils (1 kg) from grazed grassland were collected; herbage, roots, stones and other extraneous materials were removed. Subsequently, 100 g soil was placed into a 500 ml plastic bottle and 150 ml deionised water was added (1:1.5 v/v ratio). The bottle was shaken mechanically for 1 h. The supernatant was removed following sedimentation, and was centrifuged for 30 minutes at 2500 rpm; filtered using filter paper (Whatman No. 41) and DOC was measured using a TOC analyser (TOC-Vcph/cpn; Shimadzu Corporation, Kyoto, Japan). The NO_3^- and NH_4^+ concentrations being * and * , respectively were negligible in compare to the added amendment concentration (I don't have these data).

2.5 Soil core pre-incubation, incubation and data recording

The denitrification study was carried out by incubating the soil cores at 15^{o}C, for 17 days, in an automated laboratory incubation system installed at the research centre at

North Wyke (Cardenas et al., 2003; Scholefield et al., 1997). The incubation system comprised of a 1.3 m^3 temperature controlled cabinet containing 12 incubation vessels (each fitted to an amendment vessel) and gas lines. Headspace temperatures inside the vessels were logged hourly. Each of 12 soil cores was then placed inside a cylindrical incubation vessel to an exact fit. A mixture of *He* + O_2 was passed through the soil core (via the bottom of the vessel) in order to purge (flow-through mode) the soil atmosphere, headspace and all gas lines of N_2 for 24 h. Flow rates of *He*+O_2 mixture were (20ml min^{-1}) were regulated using mass flow controllers to provide an O_2 concentration of ca. 20% (Scholefield et al., 1997a; Cardenas et al., 2003). The *He*+O_2 mixture was then directed to the vessel via the lid (flow-over mode) after reducing the flow rate to 10 ml min^{-1} and O_2 level to 20% for 72 h. The effluent gases from each vessel were passed through an outlet in the lid of the incubation vessels to an actuated 16-port selection valve to split and direct the gas stream from each outlet column to GC (automatic sample feeding). Flow-over continued for 72 h because measured N_2 levels reached the baseline by this time. After replacement of the atmosphere within the soil cores, amendments were added via a secondary vessel fitted to the centre of each lid after being flushed with *He* (to avoid any atmospheric N_2 contamination). Amendment distribution in soil core was found similar from subsequent analysis of 9 subsamples in each core after 3 vertical and 3 horizontal sections). The technique allowed the direct and independent measurement of N_2O and N_2 fluxes from each incubation vessel, which permitted an exact measurement of denitrified gas concentrations. Continuous recording of N_2O and N_2 concentrations were automated at a frequency of approximately 12 measurements per day using Shimadzu GC (Gas Chromatography) throughout the experiment. N_2O was detected by Electron Capture Detector (ECD) with separation achieved by a stainless steel packed column (2m long, 4m bore) filled with

'Porapak Q' (80-100 mesh) and using N_2 as a career gas. N_2 was detected by *He* Ionization Detector (HID) with separation achieved by a PLOT column (30m long, 0.53mm i.d.), with He as the carrier gas. The software 'Kontron' (Kontron Electronic, Munich, Germany) was used to measure the concentration of effluent gases.

Scholefield et al (1997) found that this technique is particularly suited to an investigation into the effects of O_2 concentration *per se*, because variation of the O_2 concentrations of the headspace gas in flow over mode would not be relevant to field conditions. They observed O_2 concentrations negatively correlated with WFPS in the automated technique of denitrification study. Therefore, higher WFPS in subsoil horizons (85-88%) than in A horizon (80%) indicated lower O_2 content and prevent further O_2 diffusion from headspace into soil cores. Because no changes in the estimated water contents, being measured at initial, highest peak and end of the experiment, was observed which indicated that there was no evidence air exchange into the soil cores during the incubation period. Therefore, the microbiological compositions were considered intact throughout the experimental procedures.

2.6 Physical and chemical analyses

In addition to the three treatment sets of cores (36 in total), an additional three cores from each horizon (9 cores) were sampled before pre-incubation. Another three cores were removed from incubation on the day following the highest recorded N_2O peak and before the N_2 peak was attained (this left three replicates out of the four original treatment sets to continue until the end of the incubation). At the end of each experiment, all soil cores were prepared for physical and chemical analyses. Pre-incubation, at peak N_2O and N_2 emission points and at the end of incubations, soil sub-samples were taken for microbial analysis, as described by Barrett et al. (2010). Soil

moisture content was measured gravimetrically after drying for 24 h at 105^{o}C. Dry bulk density (BD) was determined by a soil core method, using the oven dry weight of soil and the known volume of the soil corer. Soil mineral N as ammonium (NH_4^+) and nitrate (NO_3^-) were analysed using an Aquakem 600 Discrete Analyser (Askew and Smith, 2005; Standing Committee of Analysts, 1981) after extraction with 2 M KCl in 1:2.5 (w:w) of soil and KCl solution. Water soluble organic C (WSOC) was analysed on a TOC Analyser (TOC-Vcph/cpn; Shimadzu Corporation, Kyoto, Japan) after extraction with deionised water (soil water ratio 1:2.5). The WSOC extracts were first used to measure pH and then centrifuged at 1500 rpm for 30 min and then filtered through a 0.45 µm filter. Soil total organic C and N determination were determined by dry combustion analysis (Leco CNS 2000 analyzer; Leco Corporation, USA)

2.7 Calculation of potential denitrification

Denitrification potential is defined as the denitrification rate under anaerobic condition with abundant NO_3^- (Aulakh et al., 1992) and available organic C as an energy source for denitrifying organisms (Well and Myrold, 2002). N_2O and N_2 fluxes (mg N kg^{-1} dry soil d^{-1}) were calculated from the concentrations continuously measured by the GC during the entire incubation period. Approximately 12 measurements were recorded per sample per day and averaged to express flux as mg kg^{-1} d^{-1}. Denitrification rates and total denitrification (TDN) losses of added N were calculated from the N_2O and N_2 fluxes. The N_2O mole fractions were calculated using N_2O fluxes and the total fluxes of N_2O and N_2 [$N_2O/(N_2O+N_2)$]. All the calculated results were then compared for three soil depths and treatments.

2.8 Statistical methods

All statistical analyses were performed using SPSS 16 (SPSS Inc. USA). As the variables showed an approximately lognormal distribution, log transformations were used and residual checks indicated that the assumptions of the analyses were not violated and there was no evidence of heterogeneity of variances within each treatment. A factorial analysis was carried out to detect treatment and depths effects on the data at maximum fluxes, mean and cumulative emissions of N_2O, N_2, N_2O+N_2 and on the $N_2O/(N_2O+N_2)$ ratios over the incubation period with treatment and soil depths as fixed factors following univariate analysis under a General Linear Model. Multiple comparisons test between individual treatment and depth effects were carried out using the Bonferroni Post Hoc test. Simple and multiple linear regressions (stepwise) analyses using the data points at initial and highest flux stages were carried out to test relationships between potential denitrification rates and soil properties (soil pH, NH_4^+, NO_3^-, total N, organic N, inorganic N, WSOC, total C and organic C) after converting all non-normal data to log-transformed data. For correlation and regression study we used all the cores because our interest was to see what happens with soil physico-chemical properties at the very moment of maximum denitrification. For this, we removed additional soil cores during maximum denitrification from the incubation chamber for each depth in each experiment. A statistical probability of $p<0.05$ was considered significant for both significance test and regression analyses.

3. Results

3.1 N_2O and N_2 fluxes

Cumulative emissions of N_2O varied significantly between treatments ($p<0.01$), soil horizons ($p<0.001$) and the interaction of treatments and soil horizons ($p<0.05$), showing an episodic form of emissions in the A horizon that received nitrate + glucose-

C (T_2), (Figure 1). The maximum fluxes of N_2O varied significantly between treatments (p<0.001) and depths (p<0.001). The nitrate + glucose-C (T_2) and nitrate + DOC (T_3) treatments showed the highest peaks for N_2O fluxes on day 1 after the amendment in the A horizon (9.91 and 7.22 mg N kg^{-1} dry soil for T_2 and T_3, respectively). Though smaller (1.28 mg N kg^{-1} dry soil), the maximum emissions in the nitrate only (T_1) treatment was delayed for 2 days. The maximum peaks were several-fold lower in the subsoils (B and C horizons), ranging from 0.07-0.22, 0.20-0.44 and 0.47-1.04 (mg N kg^{-1} dry soil) for T_1, T_2 and T_3 treatments respectively, compared with the A horizon and observed between day 4 and 8 of incubation. Similarly, mean N_2O fluxes over the incubation period were significantly (p<0.001) greater in the A horizon (0.77 to 2.38 mg N kg^{-1} d^{-1}) than in the subsoil horizons (0.07 to 0.54 mg N kg^{-1} d^{-1}); the lowest being in the C horizon (Table 2). Overall, the soil cores amended with nitrate only (T_1) displayed significantly (p<0.01) lower cumulative N_2O emission than the T_2 and T_3 treatments whereas it was consistently (p>0.01) higher in the treatment with glucose-C (40.52 mg N kg^{-1}) than with DOC (23.82 mg N kg^{-1}). Despite lower emissions, subsoils that received DOC enhanced N_2O emissions (but not significantly) compared with those that received glucose-C (Table 2).

The treatment and soil depth had pronounced effects on the time course of N_2 fluxes (Figure 1). In the A horizon, the highest peak was observed on day 6 after amendment with nitrate + glucose-C and nitrate + DOC (1.03 and 1.29 mg N kg^{-1} dry soil in T_2 and T_3, respectively) and on day 5 of incubation when treated with nitrate only (0.96 mg N kg^{-1} dry soil). In subsurface horizons, the highest peaks were observed on day 1 after amendment with nitrate only (0.66, and 0.38 mg N kg^{-1} dry soil at B and C horizons), but it was delayed by 4-7 days in the treatment that had C. The mean N_2 fluxes only differed significantly (p<0.05) between the A and C horizons. In the A horizon, it

ranged from 0.55 mg N kg^{-1} d^{-1} in T_1 to 0.98 mg N kg^{-1} d^{-1} in T_3 (Table 2). In the C horizon, it varied from 0.13 mg N kg^{-1} d^{-1} in T_1 to 0.92 mg N kg^{-1} d^{-1} in T_2. Added C did not affect the mean N_2 flux significantly ($p>0.05$). The T_2 treatment showed consistently higher emissions than T_3 though the difference was not significant. In contrast to the subsoil horizons, cumulative N_2 emissions in the A horizon were higher with added DOC than with added glucose-C (Table 2).

3.2 Total denitrification rates and the losses of added nitrogen

The TDN (N_2O+N_2) rate significantly ($p<0.05$) differed with regards to soil depth and treatments (Figure 2a). Cumulative TDN emissions were significantly higher in the A horizon than in the B ($p<0.05$) and C horizons ($p<0.01$), but the later two were not statistically different from each other. Considering multiple comparisons (pair-wise) between the treatments, the soil cores amended with nitrate alone (T_1) showed significantly ($p<0.01$ for T_2 and $p<0.05$ for T_3) lower TDN rates (ca. 22.4, 10.3 and 2.82 mg N kg^{-1} from A, B and C horizons, respectively) than the same horizons amended with either glucose-C (ca. 54.1, 26.2 and 16.69 mg N kg^{-1} for A, B and C horizons, respectively) or DOC (ca. 40.5, 25.5 and 15.49 mg N kg^{-1} from A, B and C horizons, respectively). The treatment and soil depth significantly affected ($p<0.05$-<0.01) the percentage losses of added N (Figure 2b). The loss of added N from T_1, T_2 and T_3 treatments, respectively were significantly ($p<0.05$-0.01) greater in the A horizon (ca. 25, 60 and 45%) compared with B (ca. 12, 29 and 29%) and C (ca. 3, 20 and 18%) horizons and the B and C horizons also differed significantly ($p<0.05$). Addition of C significantly increased N losses in T_2, nitrate + glucose-C ($p<0.05$) and T_3, nitrate + DOC ($p<0.01$) compared with the T_1, nitrate only treatment. There were no significant differences between the two C sources.

3.3 Nitrous oxide mole fractions at various soil depths

The mole fractions of N_2O varied significantly ($p<0.05$) with soil depth and did not response satisfactorily to either added N with or without C sources (Figure 2c). The A horizon had significantly ($p<0.05$ for B and $p<0.01$ for C) greater N_2O mole fractions (0.58-0.75) than the subsoil horizons (0.06-0.36). There was no significant effect of treatments on $N_2O/(N_2O+N_2)$ ratios, but glucose-C amended soils showed consistently higher ratios than the soils which received either nitrate alone or coupled with DOC ($p>0.05$).

3.4 Relationship between denitrification and soil properties

Pearson correlation coefficients between denitrification products and all the soil-related controlling factors with their levels of significance are shown in Table 3. There was a significant ($p<0.001$) positive correlation between N_2O flux and TDN rates with R^2 = 0.95. The N_2O mole fractions was also positively and significantly correlated with TDN rates and N_2O flux giving R^2 values of 0.50 and 0.55, respectively. The estimated coefficients of soil physico-chemical properties selected as significant explanatory variables for the models that best fitted to predict the observed flux following stepwise multiple linear regressions of potential denitrification rates and N_2O mole fractions during the incubation were summarized in Table 4.

Considering the three soil horizons, a significant positive correlation was observed between N_2O flux and total organic carbon ($p<0.001$) and soil total N ($p<0.05$) but a significant negative correlation was observed with NO_3^--N ($p<0.001$). The N_2 flux was significantly positively correlated with total organic N ($p<0.01$) and negatively with

NO_3^--N (p<0.05). The regression model developed could explain only 45% of the variances of N_2 emissions (Table 4). The TDN (N_2O+N_2) showed a significant positive linear relationship with total C (p<0.001), but a significant negative relationship with NO_3^--N (p<0.01). The empirical model which stepwise included the variables based on the changes in F value explained 76% (adjusted R^2=0.76) of variances (Table 4). A very strong positive relationship was observed between N_2O mole fraction ($N_2O/(N_2O+N_2)$) and total C (p<0.01) and pH (p<0.01).

4. Discussion

4.1 N_2O and N_2 fluxes

The maximum peaks for N_2O fluxes in the A horizon appeared on 1 day after the amendment was applied, in all treatments except the cores that received nitrate alone. In the other two subsoil horizons (B and C), the maximum peaks appeared between 4 and 8 days, regardless of the treatments applied. The A horizon time course for the peaks was slightly different from those observed by Scholefield et al. (1997), who reported the highest peak for N_2O in surface soil on day 2, i.e. 1 day later than we observed. This might be due to the different nutrient rates (nitrate 50-100 kg ha^{-1}, glucose 394 kg ha^{-1}) and soil conditions they used e.g. pH 5.1. However, in the A horizon cores, the highest peaks of N_2 appeared 3- 4 days later than (5-6 days after the amendment) the highest peaks of N_2O regardless of the treatments. The time course for A horizon N_2 peaks were quite similar to the finding of Scholefield et al. (1997) for the appearance of the N_2 peaks. In the A horizon, the N_2O and N_2 emissions for the consecutive days of their peaks were also in agreement with the findings of Cardenas et al. (2003) and Miller et al. (2009), where the highest N_2O and N_2 peaks appeared by 1 and 3 days after incubation, respectively.

In the two subsoil horizons (B and C) the N_2 peaks appeared only 1 day later than the N_2O peaks and, interestingly, the addition of C sources delayed the appearance of peaks 2- 3 days.

N_2O emissions were observed at lower concentrations in the C horizon, compared to the shallower A and B horizons. Li et al. (2002) also reported N_2O production in the B and C horizons (0.016-0.233 μg l^{-1}). The decrease of denitrification rates with increasing soil depth has also been observed in previous studies (e. g. Dambreville et al., 2006; Dixon et al., 2010). The underlying causes of higher N_2O fluxes in the A horizon is probably due to the higher total organic C sources and greater denitrifier abundances compared with subsoil horizons. The N_2O emissions from the treatment, without the addition of C, were very similar to those reported by Castle et al. (1998), of 0.103-0.672 mg N kg^{-1} d^{-1}, and by Richards and Webster (1999) of 0.029-0.185 mg N kg^{-1} d^{-1} in subsoils (0.6 to 1.4 m depths). The addition of C as either glucose and DOC increased N_2O emissions by 45 and 67% in the A horizon; by 50 and 150% in the B horizon and by 25 and 55% in the C horizon, respectively. Our results also agree with other laboratory experiments, which reported between 30 and 50% of applied N lost as N_2O (Cardenas et al., 2003; Miller et al., 2009; Pfenning and McMahon, 1996) stimulated by C addition. In the A horizon, added glucose-C increased N_2O emissions more than added DOC, although not in the subsoil horizons. This is probably because of the labile C characteristics of DOC, irrespective of the solubility and availability to soil microbes. McCarty and Bremner (1992) found that DOC is rapidly metabolized by the microbial community. Contrasting effects of the added C sources on N_2O emissions in the top soil and subsoils might be attributed to the differences in the native organic C pools, water-

holding capacity, pH, bulk density, and mainly fungal and bacterial community structure dynamics (Anderson and Peterson, 2009; Laughlin and Stevenson, 2002).

Higher N_2 flux from the C horizon than the A horizon could possibly be due to the higher bulk density and WFPS in C horizon. A higher bulk density will alter pore geometry and connectivity resulting in higher N_2O generation and a longer residence which may allow a more complete reduction of N_2O to N_2 (Jacinthe and Dick, 1997; Elmi et al., 2003). The absence of treatment effects with the application of a high levels of NO_3^--N may be explained by the finding that high NO_3^- concentrations can inhibit the reduction of N_2O to N_2 (Blackmer and Bremner, 1978), which might mask the influence of added N and C on N_2 fluxes. By contrast, Miller et al., (2009) observed that C availability in soil could promote the reduction of N_2O to N_2. Scholefield et al. (1997) postulated that with an increasing concentration of NO_3^-, denitrification changes are dependent on NO_3^-, with first order to zero order kinetics. Interestingly, glucose-C showed consistently more potential to enhance further reduction of N_2O to N_2 in the top soil, as it provided lower N_2O but higher N_2 than measured following DOC application; a situation which was reversed in the subsoils. This may be due to the variability in effects of glucose-C and DOC on microbial functions, as fungi were reported to retard further reduction of N_2O to N_2 (Laughlin and Stevens, 2002).

4.2 Total denitrification (TDN) rates

The TDN rates decreased with increasing soil depth indicating that topsoil bio-, physico-chemical conditions were more favourable than subsoils for potential denitrification to occur. This suggestion was supported by analysis of the diversity and abundance of microbes (*Bacteria* and *Archaea*) harboring denitrifying functional genes

(*nirK*-nitrite reductase that contains copper; Cu-Nir, *nirS*-nitrite reductase that contains heme c and heme $d_{1;}$ cd_1-Nir, *nosZ*-nitrous oxide reductase), within each of the three soil horizons and the three separate sampling stages e. g. before incubation, following highest peak of N_2O and at the end of incubation, which was carried out by Barrett *et al.* (2010). Briefly, the authors reported a significantly higher abundance of denitrifying functional genes and bacteria in the A horizon, compared to the B (p<0.01) and C (p<0.01) horizons, but higher *nosZ* gene abundances in subsoil horizons than A horizon (p<0.001), irrespective of the treatments added. Among the two subsoil horizons, C horizon had significantly lower denitrifying functional and bacterial genes than B horizon (p<0.01). The concentration of archaeal gene copy numbers was similar across all horizons. In the A horizon, the analyzed gene copy numbers were 10^5-10^6 genes g-^{1} soil for *nirK*, 10^5-10^7 genes g^{-1} soil for *nirS* and 10^4-10^5 for *nosZ*. In the subsoil horizons the analysed copy number were 10^4-10^6 genes g^{-1} soil for *nirK*, 10^4-10^7 genes g^{-1} soil for *nirS* and 10^5-10^6 genes g^{-1} soil for *nosZ* (Barrett *et al.*, 2010). Frey et al. (1999) also reported a significantly higher total microbial biomass (bacterial and fungal) in top soil layer than in the lower layer. The treatment, which received NO_3^- only, registered lower losses of the applied N than the treatments receiving NO_3^- coupled with either glucose-C or DOC, with consistently lower losses found with DOC addition. Analysis of soil parameters at the end of incubation showed that a minimum of 20% of the added nitrate was remained in soil cores (e.g. in A horizon with T_2 where 61% nitrate was denitrified) which might have been denitrified if the incubation time was extended but another 20% of added nitrate might be immobilized due to C addition. The NH_4^+ concentrations at the end of incubation in all soil cores were approximately similar to the initial concentrations indicating that there was no evidence of dissimilatory nitrate reduction to ammonium. Stimulus of subsoil denitrification by

added C was reported from laboratory (Khalil and Richards, 2010) and field studies (Weier et al., 1993). Our results of TDN (15.49-26.15 mg N kg^{-1} dry soil) in the subsoil horizons (clay loam) under adequate C sources were higher than other studies. Jarvis and Hatch (1994) reported potential denitrification rates of 1.0 mg N kg^{-1} dry soil d^{-1} in grassland subsoils (loam) while Yeomans et al. (1992) found 1.4-5.1mg N kg^{-1} dry soil d^{-1} in subsoil with a non-limiting C source. Khalil and Richards (2010) reported a small denitrification capacity in subsoils (C horizon; sandy clay loam to clay loam) of grazed pasture (0.03-0.05 mg N kg^{-1} soil d^{-1}) and its potential was found to be significantly higher in subsoils of grazed ryegrass than clover-grass (1.15 vs. 0.50 mg N kg^{-1} soil d^{-1}).

4.3 N_2O mole fractions ($N_2O/(N_2O+N_2)$ at various soil depths

In the A horizon, N_2O was the dominant denitrification end product (58-75%) that increased by 2 to 30% with the addition of C sources. The N_2O mole fractions were significantly lower (6-36%) in the two deeper soil horizons, compared with the A horizon, suggesting more complete reduction of N_2O to N_2. As N_2O mole fraction did not differ significantly between the treatments but differed significantly between the soil horizons, it can be postulated that N_2O mole fraction was a function of soil depths which had different WFPS and thus different O_2 concentrations. The N_2O-to-N_2 ratios do generally decrease with increasing WFPS and from an experiment in grassland soil Scholefield (1997) reported that with increasing WFPS from approximately 70-90%, there was a greater than 50-fold increase in denitrification (Scholefield, et al., 1997). It is well known that denitrification is inhibited progressively by increasing O_2 concentrations in the soil, with the nitrate reductase enzyme system perhaps being the most sensitive, and leading to a decreasing N_2O-to-N_2 ratio with increasing soil water

content (Knowles, 1981). Even trace amounts of O_2 can inhibit nitrous oxide reductase activity (Zumft, 1997; Knowles, 1982). Therefore, decrease in $N_2O/(N_2O+N_2)$ with increasing depths may be due to the reduction of N_2O to N_2 at increased moisture levels. Ciarlo et al. (2007) found highest N_2O emission in 80% WFPS compared to 40, 100 (saturated) and 120% (oversaturated with about 2 cm overlying surface water layer) and $N_2O/(N_2O+N_2)$ was lowest at 120% WFPS and postulated that $N_2O/(N_2O+N_2)$ decreased with increasing moisture contents. This finding is in agreement with Granli and Bockman (1994) who reported that within the range 60-90% WFPS aeration could increase the proportion of N_2O produced by denitrification.

Lower bulk density with correspondingly lower permeability in subsoils than A horizon (see Table 1) can increase the residence time of N_2O by slowing down of the diffusion rate. When denitrification occurs in subsoil, denitrified gas has to diffuse back up the soil profile before detection at the soil surface and during this slow diffusion process there is an increased likelihood of N_2O undergoing further microbial reduction to N_2 (Castle et al., 1998; Ciarlo et al., 2007). Farquharson and Baldock (2008) suggest that the amount of N_2O that moves through the entire denitrification pathway to N_2 depends on the ability of N_2O to diffuse out of the soil before it can be further reduced. The slow diffusion rate through the subsoil also results in longer periods of time before denitrified gas is measurable at the soil surface. Another reason of higher $N_2O/(N_2O+N_2)$ ratios in the A horizon is that the nitrification process might have contributed to the N_2O emitted from the A horizon where WFPS was comparatively lower (80%) than that of the two other horizons (85-88%). Aulakh et al. (1996) in a laboratory experiment showed 100% nitrification of applied ammonium at 80% WFPS within 10 days which declined to 82-90% at 120% WFPS (flooded soil) within 30 days of ammonium application indicating that very trace level of O2 is sensitive to both

nitrification and denitrification. Total organic N, being higher in the A horizon than the two subsoil horizons, can be transformed to nitrate and thus contributed to higher N_2O production by nitrification because A horizon had comparatively higher (WFPS 80%) aeration than B and C horizons (WFPS 85-88%). High $N_2O/(N_2O+N_2)$ ratios are the characteristic of fairly well-aerated soil, in which N_2O can easily diffuse away, and thus is not further reduced to N_2 by denitrifying organisms (Webster and Hopkins, 1996) and also the presence of high NO_3^- in top soil can decrease further reduction of N_2O to N_2 (Bandibas, et al., 1994). Schlegel (1992) explained this phenomenon by stating that NO_3^- is preferred as an electron acceptor with respect to N_2O. The N_2O can also be produced simultaneously by nitrification and denitrification (Khalil and Baggs, 2005), so the production of N_2O from nitrification could affect calculated N_2O to (N_2O+N_2) ratios (Elmi et al., 2005). These factors result in subsoil conditions favoring N_2 as the dominating end product of denitrification. N_2O produced by nitrification is prone to be consumed by denitrification via N_2O uptake and reduction by N_2O reductase activity (Dannenmann et al., 2008). Thus, N_2O and N_2 can be produced simultaneously under adequate supplies of nitrate and C sources in the A horizon. On the other hand, subsoil denitrification could be an important NO_3^- removal pathway to limit nitrate contamination to surface water and groundwater as well as atmospheric build-up of N_2O, provided that there is an available C source to drive the denitrification sequence to completion.

4.4 Relationships between potential denitrification rates and their controlling factors

The strong positive relationships of potential denitrification rates with total soil organic C content and not with water-soluble organic C (WSOC) suggests that this fraction is not the only candidate for an electron donor and that the total organic C contains other

C sources, which might also influence denitrification. Similarly, Hill and Cardaci (2004) reported a weak and insignificant correlation between WSOC and denitrification potential in mixed and conifer forest soils. Well et al. (2001) found a positive linear relationship between denitrification and total organic C in a shallow groundwater zone. Richards and Webster (1999) and Brettar et al. (2002) also observed a similar relationship in a soil that contained labile C, which was assumed to have been relatively bioavailable. It is likely that the organic C in grassland produced more mineralisable C fractions which are more important than the WSOC (assumed to be equal to DOC) for denitrification to occur. Siemens et al. (2003) revealed that the DOC leached from some agricultural soils contributed negligibly to the denitrification process because the DOC appeared not to be bioavailable. Khalil and Richards (2010), however, postulated that dissolved organic C, oxidation-reduction potential and the substrates (C and N) load differences between the land uses could regulate the degree of denitrification capacity/potential in soils.

Both positive and negative correlations have been reported between soil pH and potential denitrification rates (N_2O, N_2) (Scholefield et al., 1997; Brady and Weil, 2002). The activity of N_2O reductase enzyme is generally thought to increase with increasing pH values (Chapuis-Lardy et al., 2007). Denitrification itself can increase pH by releasing CO_2 and hydroxide (OH^-). However, strongly acidic environments (pH < 5) inhibit denitrification and tend to arrest the denitrification chain with the formation of nitrite or N_2O (Brady and Weil, 2002). In our case, the soil was a gleysol with pH values close to 5 in the 1.20-1.30 m soil depth which had lower denitrifier populations than A horizon affecting overall relationships.

The negative correlation between potential denitrification rates and the soil NO_3^- content might be attributed to the reduction of NO_3^- to N_2O and N_2 and/or it might also be immobilized (Scholefield et al., 1997), as the NH_4^+ concentrations at the end were similar to the initial level. Figure 2 showed that 3-61% of applied nitrate converted to N_2O+N_2 (TDN) by denitrification, regardless of treatments used and depths. The NH_4^+-N was positively correlated with denitrification rates, whereas total inorganic N showed a rather weaker and negative correlation. This indicates that NH_4^+ was assimilated into the cells of denitrifiers and enhanced both the denitrifying population and activity (Buss et al., 2005).

The potential denitrification rates (N_2O, N_2 and N_2O+N_2 fluxes) were positively correlated with total N and total organic N content, the former is in line with the findings of Ciarlo et al. (2007). This indicates that soil total N might have provided adequate amounts of NO_3^- and NH_4^+ to the substrate pool after mineralization. Bandibas et al. (1994) proposed that N_2O emissions were affected by the $N_2O/(N_2O+N_2)$ ratio. Thus, denitrification is a complex process and the soil and environmental factors that influence the process are interrelated. Any variable controlling the N_2O emissions can be a rate-limiting one at different times, depending on particular conditions (Dobbie and Smith, 2003).

There is potential for subsoil denitrification to be enhanced by the introduction of available C sources into subsoils which can be directly or indirectly managed. Fenton et al. (2008) recommended the use of C substrates directly in constructed permeable reactive barriers in subsoils to treat NO_3^- contaminated groundwater, but this is not likely to be cost effective. Manipulation of plant composition and abundance to

increase C leaching might indirectly enhance subsoil denitrification. For example, in arable systems the use of cover crops during the winter recharge has been shown to significantly increase groundwater DOC concentrations (Premrov et al., 2010) and this could also enhance denitrification. In groundwater under dirty water irrigated grassland Jahangir et al. (2010) observed substantial amount of DOC (25 mg L^{-1}) with nitrate concentration nearly 0 mg L^{-1} and $N_2O/(N_2O+N_2)$ ratio of 0.01 which is indicating that land use and management could play significant role in groundwater nitrate and N_2O reduction by supplying necessary energy sources. The potential implication of denitrification in subsoil implies that NO_3^- will be reduced to N_2O, so leaching would be reduced due to nitrate reduction and N_2O emissions would be further reduced due to conversion to N_2.

5. Conclusions

The rates of N_2O emission and TDN (N_2O+N_2) were generally greater in the surface soil than in the subsoils, irrespective of the supply of NO_3^- and two added C sources in the form of glucose and DOC treatments. Addition of C markedly increased soil denitrification rates, giving higher $N_2O/(N_2O+N_2)$ ratios in the surface soil than in the subsoils. This clearly indicates the potential of subsoils for more complete reduction of N_2O to N_2 while the energy sources for denitrifiers are available. Denitrification potentials were mainly regulated by substrates including total organic C, total N and total organic N. The findings suggest that both glucose-C and DOC were highly effective for the complete reduction of NO_3^- to occur in subsoil environments and subsoils could have a large potential to attenuate NO_3^- that has leached below the root zone, with the production of more N_2 than N_2O, if available C is not limiting.

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Table 1 Soil physical and chemical properties of the experimental site

Soil Horizon	Sampling Depth	Drainage*	Texture	Dry bulk density	pH (H_2O)	NO_3^--N	NH_4^+-N	Total N	Organic C	Moisture at Field Capacity	Moisture at MWHC*
	(m)			(g cm^{-3})		(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(%)	%	%
A	0-0.10	MWD	Loam	1.21 - 1.32	5.71 - 6.97	5.5 - 6.0	3.5-5.5	2950-3200	2.80 - 2.90	41	44
B	0.45-0.55	MWD-PD	Loam-Clay loam	1.45 - 1.55	5.47 - 6.64	3.0 - 3.5	2.5-3.5	1000-1050	0.78 - 0.82	28	30
C	1.20-1.30	PD-ID	Clay loam-Clay	1.50 - 1.64	4.58 - 5.64	2.0 - 2.5	1.5-3.0	390-395	0.20 - 0.25	24	25

*MWD-moderately well drained, PD-poorly drained, ID-imperfectly drained; MWHC-maximum water holding capacity

Table 2 Mean and cumulative N_2O and N_2 fluxes/emissions at various soil horizons as affected by N and C sources during the 17-day incubation period (n=3).

Treatment	Soil Horizon	N_2O		N_2	
		Cumulative emissions (mg N kg^{-1})	Flux rate (mg N kg^{-1} d^{-1})	Cumulative emissions (mg N kg^{-1})	Flux rate (mg N kg^{-1} d^{-1})
T_1: NO_3^- only	A	13.05	0.77	9.35	0.55
	B	1.27	0.07	9.01	0.53
	C	0.67	0.04	2.15	0.13
T_2: NO_3^-+ Glucose-C	A	40.52	2.38	13.56	0.80
	B	2.54	0.15	23.60	1.39
	C	0.99	0.06	15.70	0.92
T_3: NO_3^- + DOC	A	23.81	1.40	16.69	0.98
	B	9.21	0.54	16.30	0.96
	C	1.59	0.09	13.90	0.82

Table 3 Pearson correlation coefficients 'r' between N_2O, N_2, N_2O+N_2 and $N_2O/(N_2O+N_2)$ ratio and measured soil properties; soil properties were expressed as ‡mg kg^{-1} dry soil except pH; denitrification rates were expressed as ‡mg kg^{-1} dry soil d^{-1} except the N_2O/TDN

	pH	WSOC‡	TOC‡	TC	$NH4^+$-N‡	NO_3^-N‡	TIN‡	TORG-N‡	TN	N_2O‡	N_2‡	TDN‡	N_2O/TDN‡
pH	1												
WSOC‡	0.38ns	1											
TOC‡	0.92**	0.47*	1										
TC	0.89**	0.44*	0.94**	1									
NH_4^+-N‡	0.44*	0.34ns	0.54*	0.62**	1								
NO_3^--N‡	0.19ns	-0.06ns	-0.07ns	-0.02ns	-0.12ns	1							
TIN‡	0.45*	0.24ns	0.47*	0.39ns	0.26ns	0.16ns	1						
TORG-N‡	0.90**	0.43*	0.94**	0.99**	0.61**	-0.02ns	0.36ns						
TN	0.90**	0.43*	0.95**	0.99**	0.62**	-0.01ns	0.38ns	0.99**	1				
N_2O‡	0.47*	0.43*	0.64**	0.75**	0.56**	-0.59**	0.14ns	0.74**	0.74**				
N_2‡	0.43*	0.22ns	0.48*	0.51*	0.35ns	-0.43*	-0.08ns	0.52*	0.52*	0.53*	1		
TDN‡	0.57**	0.38ns	0.66**	0.75**	0.57**	-0.56*	0.03ns	0.75**	0.74**	0.85**	0.86**	1	
N_2O/TDN‡	0.58*	0.36ns	0.47*	0.58**	0.42ns	-0.40ns	0.20ns	0.56**	0.56**	0.90**	0.13ns	0.52*	1

WSOC, TOC, TC, TIN, TORG-N, TN, and TDN stand for water soluble organic C, total organic C, total C, total inorganic N, total organic N, total N, and total denitrification (N_2O+N_2), respectively; **p <0.01; *p<0.05; and ns, non significant; ‡ln=natural logarithm

Table 4 Estimated coefficients of physico-chemical properties selected as significant explanatory variables using a stepwise procedure for models of denitrification products and ratios (n=27)

Denitrification products and ratio‡	Equation element‡ (mg kg^{-1})	Estimate	s. e.	Significance	Partial R^2
lnN_2O	Intercept	11.769	2.208	***	
	lnTOC	0.002	0.001	***	0.57
	$lnNO_3^-$-N	-1.776	0.292	**	0.22
	TN	0.715	0.207	*	0.09
lnN_2	Intercept	2.036	1.040	**	
	TORG-N	0.001	0.001	**	0.27
	$lnNO_3^-$-N	-0.581	0.239	*	0.18
lnTDN	Intercept	3.040	0.892	***	
	TC	0.002	0.001	***	0.56
	$lnNO_3^-$-N	-0.800	0.205	**	0.22
$Ln(N_2O/(N_2O+N_2))$	Intercept	3.200	1.135	**	
	TC	0.001	0.001	**	0.34
	pH	0.900	0.232	**	0.29

‡ln = unit in natural logarithm; TN, TOC, TC and TORG-N represent respectively, total N, total organic C, total C and total organic N

Figure 1 N_2O and N_2 fluxes from three different soil horizons, A (a, d); B (b, e) and C (c, f) as influenced by nitrate only (T_1); nitrate+glucose C, (T_2) and nitrate+DOC, (T_3).

Figure 2 Cumulative denitrification (N_2O+N_2) (a), percentage losses of the applied N (b) and N_2O mole fractions (c) from three different treatments and soil horizons during the 17-day incubation period.

Figure 1

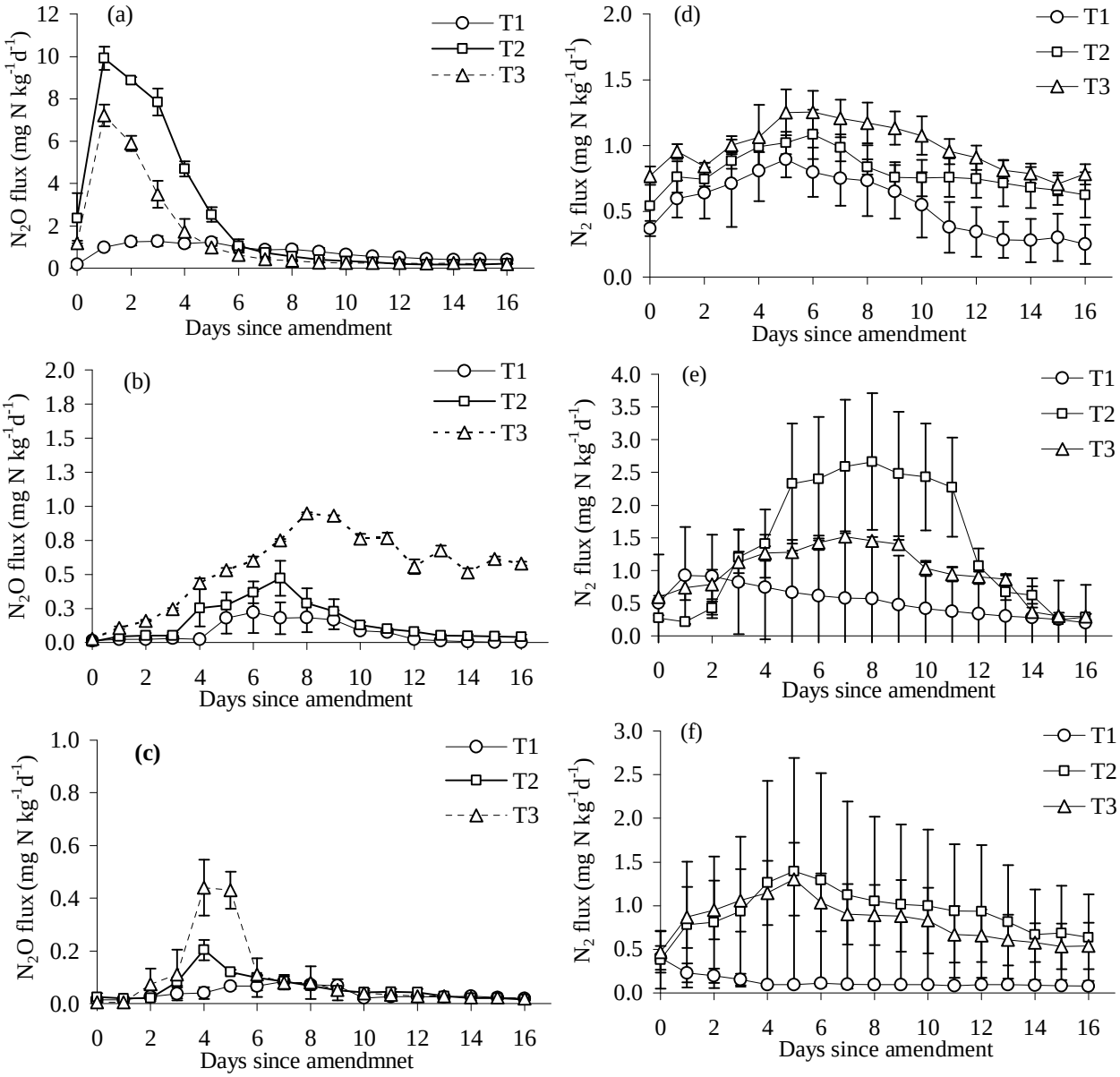
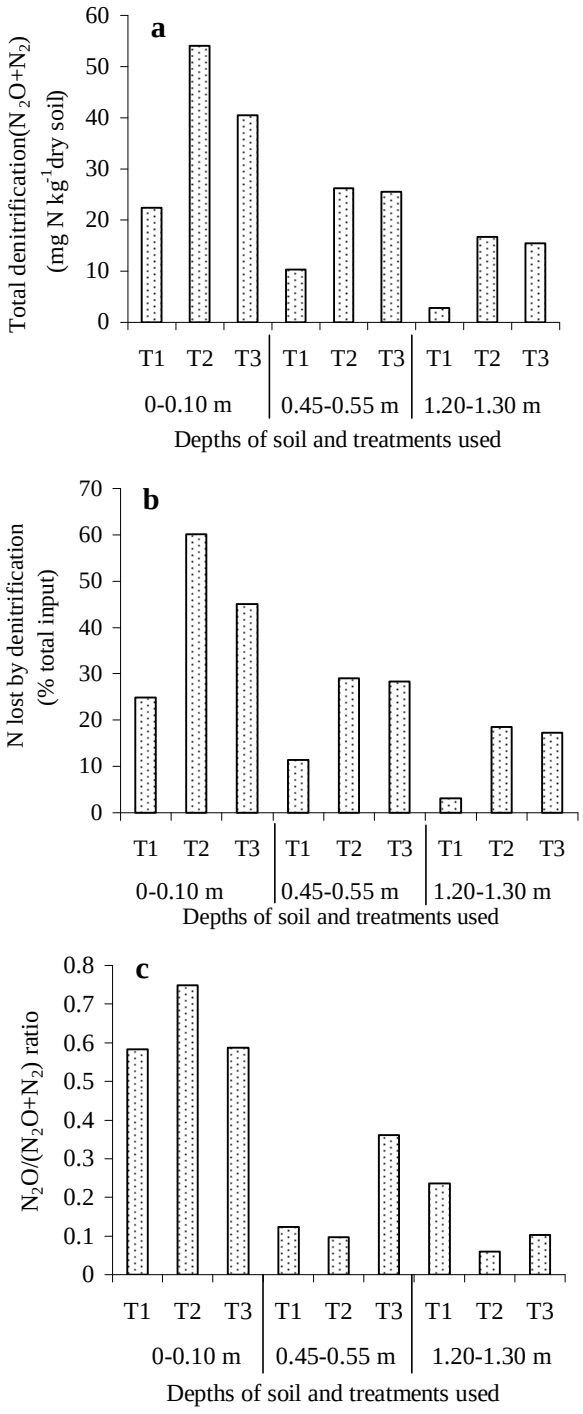

(a)
T1
T2
T3
N_2O flux (mg N kg^{-1}d^{-1})
Days since amendment
(b)
(c)
Days since amendmnet
(d)
N_2 flux (mg N kg^{-1}d^{-1})
(e)
(f)

Figure 2


a
Total denitrification(N_2O+N_2) (mg N kg^{-1}dry soil)
60
50
40
30
20
10
0
T1 T2 T3 T1 T2 T3 T1 T2 T3
0-0.10 m
0.45-0.55 m
1.20-1.30 m
Depths of soil and treatments used
b
N lost by denitrification (% total input)
70
60
50
40
30
20
10
0
T1 T2 T3 T1 T2 T3 T1 T2 T3
0-0.10 m
0.45-0.55 m
1.20-1.30 m
Depths of soil and treatments used
c
$N_2O/(N_2O+N_2)$ ratio
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
T1 T2 T3 T1 T2 T3 T1 T2 T3
0-0.10 m
0.45-0.55 m
1.20-1.30 m
Depths of soil and treatments used