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SHORT COMMUNICATION

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# Comparison of two versions of the acetylene inhibition/soil core method for measuring denitrification loss from an irrigated wheat field

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**Abstract** Two versions of the acetylene inhibition (AI)/soil core method were compared for the measurement of denitrification loss from an irrigated wheat field receiving urea-N at a rate of 100 kg $ha^{-1}$. With AI/soil core method A, the denitrification rate was measured by analysing the headspace $N_2O$, followed by estimation of $N_2O$ dissolved in the solution phase using Bunsen absorption coefficients. With AI/soil core method B, $N_2O$ entrapped in the soil was measured in addition to that released from soil cores into the headspace of incubation vessels. In addition, the two methods were also compared for measurement of the soil respiration rate. Of the total $N_2O$ produced, 6–77% (average 40%) remained entrapped in the soil, whereas for $CO_2$, the corresponding figures ranged from 12–65% (average 44%). The amount of the entrapped $N_2O$ was significantly correlated with the water-filled pore space (WFPS) and with the $N_2O$ concentration in the headspace, whereas $CO_2$ entrapment was dependent on the headspace $CO_2$ concentration but not on the WFPS. Due to the entrapment of $N_2O$ and $CO_2$ in soil, the denitrification rate on several (18 of the 41) sampling dates, and soil respiration rate on almost all (27 of the 30) sampling dates were significantly higher with method B compared to method A. Averaged across sampling dates, the denitrification rate measured with method B (0.30 kg N $ha^{-1}$ $day^{-1}$) was twice the rate measured with method A, whereas the soil respiration rate measured with method B (34.9 kg C $ha^{-1}$ $day^{-1}$) was 1.6 times the rate measured with method A. Results of this study suggest that the $N_2O$ and $CO_2$ entrapped in soil should also be measured to ensure the recovery of the gaseous products of denitrification by the soil core method.

**Key words** Acetylene inhibition · Soil core technique · Denitrification · Irrigation · Nitrous oxide entrapment

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## Introduction

Methods for the direct measurement of denitrification gaseous N flux are based on $^{15}N$ and acetylene inhibition (AI), the latter being used more commonly because of its lower cost and higher sensitivity (Ryden and Rolston 1983). Soil cover (Ryden et al. 1979) and soil core (Ryden et al. 1987) versions of the AI method have been widely used for the direct measurement of denitrification loss under field conditions, and the soil core version has generally been reported to yield higher figures (Arah et al. 1991; Mahmood et al. 1998). However, the conventional AI/soil core method may still underestimate denitrification because substantial amounts of $N_2O$ may remain entrapped in soil (Aulakh and Doran 1990). Different procedures adopted to release the $N_2O$ entrapped in soil cores include: vigorous shaking of soil cores (Aulakh and Doran 1990), breaking up of soil cores followed by shaking (Ambus and Christensen 1993), and shaking of the broken up soil cores with excess water (Rice and Smith 1982). However, in most of the denitrification studies employing the AI/soil core method, $N_2O$ entrapment in soil was not considered.

In a previous study with wheat (Mahmood et al. 1998), the denitrification loss measured by the AI/soil core method was 3.4 kg N $ha^{-1}$, in contrast to 33.1 kg N $ha^{-1}$ of the total fertilizer-N loss recorded with the $^{15}N$-balance method. A higher N loss with the $^{15}N$-balance compared to the AI/soil core method was partly attributed to losses other than denitrification, and to the underestimation of denitrification, most probably due to the entrapment of $N_2O$ in soil cores. The present

study was carried out to investigate the significance of $N_2O$ (and $CO_2$) entrapment in soil while comparing two versions of the AI/soil core method for the measurement of denitrification (and soil respiration) from an irrigated wheat (*Triticum aestivum* L. cv Pak-81) field.

## Materials and methods

The experimental site was located at the Nuclear Institute for Agriculture and Biology, Faisalabad. The area has a semiarid subtropical climate and the soil is a deep, well-drained sandy-clay loam (Hafizabad series, Typic Ustochrept) developed in a medium-textured mixed calcareous alluvium originating from the Himalayas. The field plot (450 $m^2$) selected for the present study has been under cotton since 1975 and receives urea-N at a rate of 150–170 kg $ha^{-1}$ $year^{-1}$, in addition to a regular input of cotton crop residues. Some physicochemical properties of the soil (0–20 cm) were: total organic C 2.3%, total N 0.06%, pH (saturation paste) 7.9, electrical cinductivity (saturation extract) 0.06 S $m^{-1}$, water-holding capacity 35.5%, bulk density 1.5 g $cm^{-3}$, porosity 44.5%, sand 57.8%, silt 22.7% and clay 19.5%.

The aboveground residues of cotton were removed before the land was prepared for wheat which was sown on 13 December 1995 with plant-plant and row-row distances of 10 cm and 20 cm, respectively. At land preparation, $P_2O_5$ at a rate of 60 kg $ha^{-1}$ (as single superphosphate) and urea-N at a rate of 50 kg $ha^{-1}$ were mixed in the upper 10-cm layer, whereas another 50 kg $ha^{-1}$ urea-N was broadcast just before the second irrigation (14 February). A total of five canal irrigations were applied; all were equivalent to 75 mm except the first (presowing) and the second, which were 100 mm and 50 mm, respectively.

The AI/soil core method (Ryden et al. 1987) was used for the direct measurement of denitrification (and soil respiration). The design of the soil corer was similar to that used by Rice and Smith (1982), with slight modifications to facilitate core sampling up to 50 cm depth. Measurements were replicated 15 times and for each replicate, intact soil cores (3 cm diameter, contained in perforated PVC sleeves) were extracted in 12.5-cm increments up to 50 cm depth. For each replicate, soil cores (four) from all depths were placed together in the incubation jar (8.5 × 20 cm, inner diameter × height; nominal volume 800 ml) and sealed with a silicone rubber stopper provided with a septum port. The headspace was replaced by 5% acid-washed acetylene and the jars were incubated in holes made in the field. After 18 h, the atmosphere in the jars was repeatedly mixed using a 50-ml syringe and the gas sample removed for analyses of $N_2O$ and $CO_2$. $N_2O$ was analysed on a Hitachi 263–30 gas chromatograph equipped with a $^{63}Ni$ electron-capture detector, whereas $CO_2$ analysis was carried out on a Gasukuro Kogyo 370 gas chromatograph using a thermal-conductivity detector. The amount of $N_2O$ released from the soil cores was corrected for that dissolved in $H_2O$ using Bunsen absorption coefficients (Moraghan and Buresh 1977), and for $N_2O$ in the headspace at the start of incubation. This method is termed as method A and measures the amount of $N_2O$ and $CO_2$ evolved into the headspace of incubation vessels (plus $N_2O$ dissolved in the solution phase, in the case of denitrification). In contrast, method B measures the $N_2O$ and $CO_2$ released from soil cores into the headspace plus that entrapped in the soil cores. The entrapped $N_2O$ and $CO_2$ were released according to Rice and Smith (1982). Briefly, after sampling the headspace (for method A), the soil cores were immediately transferred into another jar, broken up and the jar sealed after adding 200 ml distilled $H_2O$. The jars were vigorously shaken for 10 min on a reciprocal shaker and the headspace analysed for $N_2O$ and $CO_2$, considering the $N_2O$ dissolved in the solution phase.

While sampling for denitrification, soil samples (four replicates) were also collected to determine the water-filled pore space (WFPS, Anonymous 1980) and mineral N content (Keeney and Nelson 1982). The soil temperature was measured by glass thermometers inserted at a depth of 5 cm, whereas rainfall data was obtained from the meteorological station at a distance of 2 km from the study site. The holes in the field due to soil core sampling were backfilled with soil of respective depths obtained from an adjacent plot. This backfilling was necessary to check the entry of irrigation or rain water into the soil through these holes.

Data for denitrification and soil respiration rates were subjected to an analysis of variance, followed by the least significant difference test (Gomez and Gomez 1984). A simple linear regression analysis was performed to evaluate the effect of different factors on $N_2O$ and $CO_2$ entrapment in the soil. The data of $N_2O$ and $CO_2$ were log-transformed before statistical analyses to satisfay the assumption of variance homogeneity.

## Results

The spatial variability (variation among replicates) of denitrification rates was high and the mean coefficient of variation (CV) during the study period was 99% and 88% for methods A and B, respectively. For the soil respiration rate, the mean CV for method A and B was 38% and 28%, respectively. The soil $NO_3^-$-N content also showed a high degree of spatial variability (mean CV 41%), while the WFPS was spatially uniform (mean CV 8%).

Figure 1 shows the temporal fluctuations in denitrification and soil respiration rates in relation to environmental conditions in the wheat field. Total rainfall during the wheat season amounted to 104 mm, most of which was received during February (49 mm) and March (44 mm). The maximum WFPS following irrigation or rainfall was observed in the upper soil layer (0–10 cm) after 24 h when it ranged between 67–80%. The WFPS of the deeper layers (10–50 cm) generally remained $<60\%$, and fluctuations were less pronounced as compared to the upper layer. Changes in the soil $NO_3^-$-N content were also more pronounced in the upper 0–10 cm layer. The $NO_3^-$ content at the time of the presowing irrigation was low ($<6$ mg N $kg^{-1}$), but due to mineralization it had increased to 21 mg N $kg^{-1}$ by the time wheat was sown. Peaks of soil $NO_3^-$ were recorded 5–9 days after the first and second urea applications, i.e. on 22 January (18 mg N $kg^{-1}$) and 14 February (50 mg N $kg^{-1}$), followed by a decline to $>10$ mg N $kg^{-1}$. A rise in the $NO_3^-$-N level of the deeper soil layers (10–50 cm) was also observed, particularly after fertilizer applications and irrigation. The average soil temperature during the growing season was 16.9 °C, and ranged from 10.5–24.0 °C.

Peaks of denitrification were recorded 1–4 days after irrigation or rainfall, and ranged from 0.02 to 0.50 kg N $ha^{-1}$ $day^{-1}$ with method A and from 0.05 to 1.21 kg N $ha^{-1}$ $day^{-1}$ with method B; the lower peaks corresponded to the presowing irrigation cycle. The rate of soil respiration during the growing season was 6.9–76.8 kg C $ha^{-1}$ $day^{-1}$ with method A, and 14.5–106.3 kg C $ha^{-1}$ $day^{-1}$ with method B; the peaks during the initial stages of crop growth (29 November–22 January) were lower as compared to those recorded later (11 February–7 April).

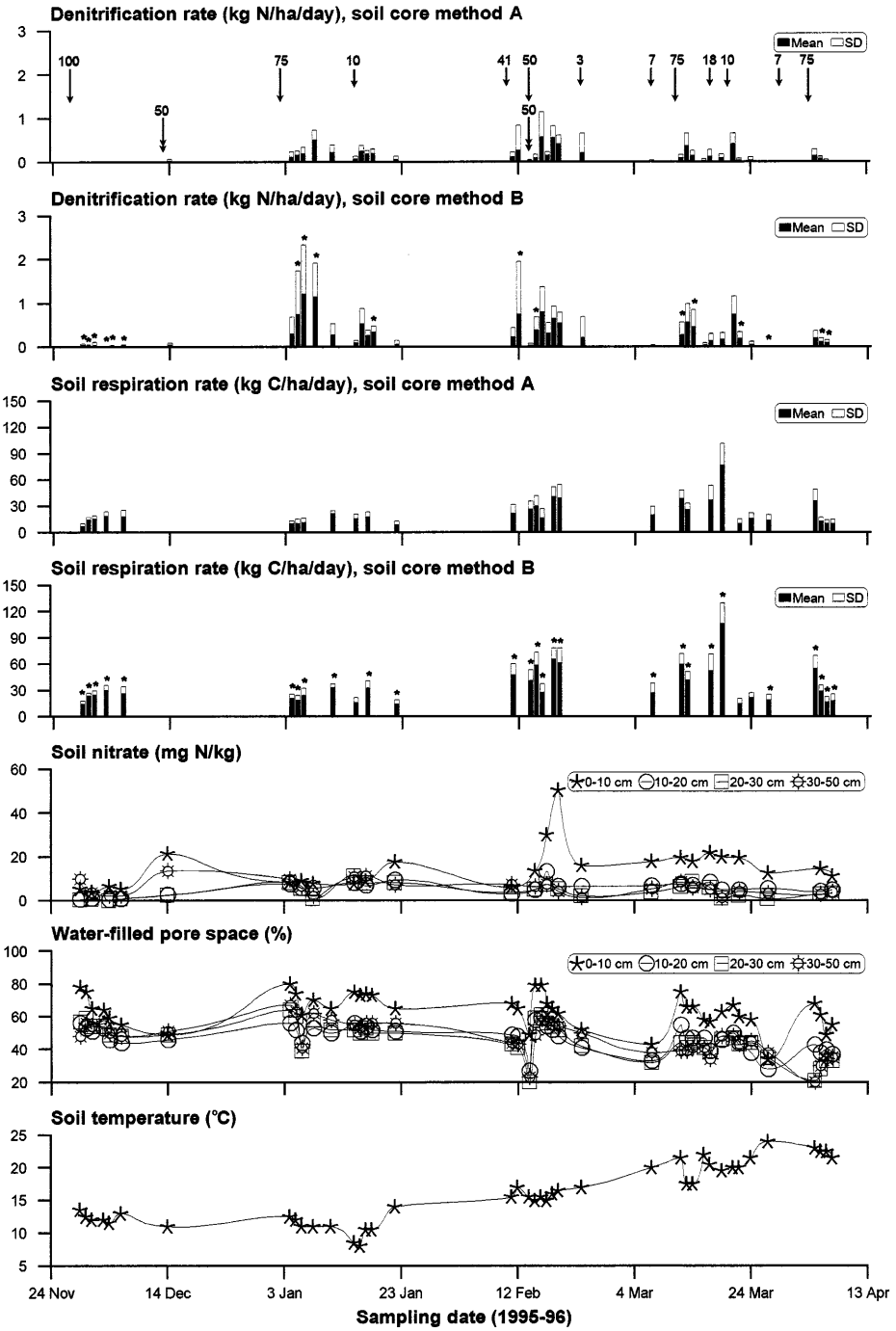


**Fig. 1** Temporal changes in denitrification and soil respiration rates in relation to environmental conditions in a wheat field. *Long arrows* represent irrigation (mm); *short arrows*, rainfall (mm); *double-headed arrows*, application of urea (kg N $ha^{-1}$). *Bars surmounted by asterisks* indicate the sampling dates when denitrification and soil respiration rates measured with soil core method B were significantly higher than the rates measured with soil core method A ($P<0.05$). *Nov* November, *Dec* December, *Jan* January, *Feb* February, *Mar* March, *Apr* April

On average, 40% (range 6–77%) of the $N_2O$ remained entrapped in the soil and showed a highly significant correlation both with the headspace $N_2O$ concentration ($r=0.707$, $P<0.001$, $n=41$) and WFPS ($r=0.568$, $P<0.001$, $n=41$). Of the total $CO_2$ produced, 12–65% (mean 44%) was not released from soil cores into the headspace. The entrapped $CO_2$ was significantly correlated with the headspace $CO_2$ concentration ($r=0.583$, $P<0.001$, $n=30$) but not with the WFPS. Due to the entrapment of $N_2O$ and $CO_2$ in soil, the denitrification rate on several (18 of the 41) sampling dates, and soil respiration rate on almost all (27 of the 30) sampling dates were significantly higher when determined with method B than with method A ($P<0.05$). Averaged across sampling dates, the denitrification rate measured by method B (0.30 kg N $ha^{-1}$ $day^{-1}$) was twice as high as that measured by method A ($P<0.01$), whereas the soil respiration rate measured by method B (34.9 kg C $ha^{-1}$ $day^{-1}$) was 1.6 times the rate measured by method A ($P<0.01$). The correlation between the two methods was highly significant for both denitrification ($r=0.946$, $P<0.001$, $n=41$) and soil respiration ($r=0.953$, $P<0.001$, $n=30$). Taking into account the $N_2O$ released from soil cores into the head-

space and that entrapped in the soil (method B), a total of 18.82 kg N $ha^{-1}$ was lost due to denitrification during the wheat growing season. In comparison, the total denitrification loss was 9.84 kg N $ha^{-1}$ when measured with method A.

## Discussion

A lag of 1–4 days recorded for denitrification peaks following irrigation or rainfall is consistent with earlier reports (Mosier et al. 1986; Goulding et al. 1993). This lag is partly attributed to the time required for the establishment of conditions favourable for denitrification (Smith and Tiedje 1979) and for the diffusion of $N_2O$ from the site of production to the soil surface (Jury et al. 1982). As soil core method B does not constrain the diffusion of gases, the observed lag could be attributed entirely to the time required for establishing denitrification conditions. The lower denitrification peak during the first irrigation cycle corresponded to the lower soil $NO_3^-$ level (3.7 mg N $kg^{-1}$) before the land was prepared and urea applied, whereas higher peaks during the later irrigation cycles could be related to a higher soil $NO_3^-$ content (6.1–20.0 mg N $kg^{-1}$).

A substantial amount of the denitrification $N_2O$ remained entrapped in the soil, which was in good agreement with some earlier reports (Rice and Smith 1982; Aulakh and Doran 1990; Ambus and Christensen 1993). These authors also reported incomplete recovery of denitrification $N_2O$ when measurements were based on headspace analysis and estimates of $N_2O$ dissolved in the solution phase made using Bunsen absorption coefficients. As noted by Aulakh and Doran (1990) and also in the present study, the highly significant correlation of the entrapped $N_2O$ and $CO_2$ with the headspace gas concentrations indicated that the gas diffusion from soil cores into the headspace was retarded due to a decreased concentration gradient. The observed positive relationship between the entrapped $N_2O$ and WFPS may have also been related to the reduced rate of gas diffusion, partly because of the decrease in air-filled pores and the increased $N_2O$ concentration in the headspace resulting from higher denitrification rates with increasing WFPS.

Results of the present study showed that the conventional AI/soil core method underestimates denitrification. In order to ensure the recovery of gaseous N products of denitrification, the $N_2O$ entrapped in soil should also be measured, besides analysing the $N_2O$ released from soil cores into the headspace of incubation vessels. The same may also hold true when measuring soil respiration by the soil core method.

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