Denitrification and total N losses from an irrigated sandy-clay loam under maize–wheat cropping system

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Abstract

Denitrification and total N losses were quantified from an irrigated field cropped to maize and wheat, each receiving urea at 100 kg N ha^{-1}. During the maize growing season (60 days), the denitrification loss measured directly by acetylene inhibition-soil cover method amounted 2.72 kg N ha^{-1} whereas total N loss measured by ^{15}N balance was 39 kg ha^{-1}. Most (87%) of the denitrification loss under maize occurred during the first two irrigation cycles. During the wheat growing season (150 days), the denitrification loss directly measured by acetylene inhibition-soil cover and acetylene inhibition-soil core methods was 1.14 and 3.39 kg N ha^{-1}, respectively in contrast to 33 kg N ha^{-1} loss measured by ^{15}N balance. Most (70–88%) of the denitrification loss under wheat occurred during the first three irrigation cycles. Soil moisture and NO_3^--N were the major factors limiting denitrification under both crops. Higher N losses measured by ^{15}N balance than C_2H_2 inhibition method were perhaps due to underestimation of denitrification by C_2H_2 inhibition method and losses other than denitrification, most probably NH_3 volatilization.

Abbreviations: AI – acetylene inhibition, HAI – hours after irrigation, WFPS – water-filled pore space

Introduction

Denitrification can be an important cause of low nitrogen use efficiency as well as a major source of atmospheric nitrous oxide, which, besides acting as a greenhouse gas (Watson et al., 1990), is implicated in destruction of the stratospheric ozone (Crutzen, 1981). Although extensive studies have been conducted since the development of methods for direct measurement of denitrification, the process still remains one of the least well-quantified sectors of the terrestrial nitrogen cycle. Quantitative estimates of denitrification vary tremendously. From heavily fertilized irrigated vegetable fields, denitrification loss as high as 200 kg N ha^{-1} a^{-1} has been reported (Ryden and Lund, 1980) whereas figures reported by others were quite low and ranged between <1 to 12 kg N ha^{-1} during the vegetation period (Benckiser et al., 1986; Bertelsen and Jensen, 1992; Myrold, 1988).

Methods used for direct measurement of denitrification gaseous flux are based on ^{15}N and acetylene inhibition (AI), the latter being used more commonly because of the lower cost and higher sensitivity (Ryden and Rolston, 1983). A close agreement between the two methods was achieved by Mosier et al. (1986b) and Aulakh et al. (1991) whereas Arah et al. (1993) reported higher figures with ^{15}N as compared to AI method. Soil cover (Ryden et al., 1979) and soil core (Ryden et al., 1987) versions of the AI technique have been widely used for quantification of denitrification loss under field conditions and higher figures are generally reported with soil core method (Arah et al., 1997; Aulakh et al., 1991). Inadequate supply of C_2H_2 in poorly drained soils, retarded N_2O diffusion, and

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downward lateral diffusion of N_2O from the site of production may lead to an underestimation of denitrification by soil cover method (Arah et al., 1991; Becker et al., 1990; Ryden et al., 1987). However, there have been few studies that compare the soil cover and soil core methods (Arah et al., 1991; Aulakh et al., 1991). An indirect approach to quantify denitrification loss is the ^{15}N balance (Broadbent, 1981). A close agreement has been found between AI and ^{15}N balance (Aulakh et al., 1983) or between ^{15}N gaseous flux and ^{15}N balance (Mosier et al., 1986a). However, higher figures by ^{15}N balance than AI method have also been reported (Bertelsen and Jensen, 1992). Overestimation of denitrification by ^{15}N balance is attributed to losses other than denitrification such as those during nitrification, those of NO and NH_3, and possible volatile losses from vegetative parts of plants (Farquhar et al., 1979; Nelson, 1982).

In Pakistan, crop husbandry largely depends on irrigation and other inputs including fertilizer N, annual consumption of which stands at 1.64 million tonnes on 21.93 million hectares of the cultivated land (Anonymous, 1994). The recovery of the applied fertilizer N is seldom more than 60% under upland conditions in Pakistan (Ahmed, 1985). Some laboratory studies conducted on the soils of Faisalabad region showed that, of the total N applied, upto 30% may be lost due to NH_3 volatilization (Hamid and Ahmad, 1987). However, the knowledge about denitrification loss under field conditions in Pakistan is lacking. This paper reports denitrification loss from an irrigated sandy-clay loam under maize-wheat cropping system, measured directly by AI technique and its comparison with the total N loss measured by ^{15}N balance. Moreover, soil cover and soil core versions of the AI technique were compared for measurement of denitrification in the wheat field.

Materials and methods

Study site

The soil at the study site (Nuclear Institute for Agriculture and Biology, Faisalabad) belongs to Hafizabad series (Haplic Yermosol; FAO, 1966) and is a deep, well-drained sandy-clay loam developed in a mixed calcareous medium-textured alluvium derived from Himalaya. The area has a semiarid subtropical climate with mean annual rainfall of 340 mm, most of which falls in the months of July and August in the form of high intensity downpours. The site has been under maize-wheat cropping system, receiving different fertilizer treatments, since 1980. Some physico-chemical characteristics of the soil are given in Table 1. The plot (128 m^2) selected for the present study has been receiving urea at 200 kg N ha^{-1} a^{-1}, 100 kg N ha^{-1} applied each to maize (August–October) and wheat (November–May). Maize (*Zea mays* L. cv. Akbar) was sown on 1 September 1992 and the fodder harvested on 31 October 1992. A total of four canal irrigations were applied during the maize season; all were equivalent to 75 mm, except the first (pre-plant irrigation) which was 100 mm. Wheat (*Triticum aestivum* L. cv. Pak 81) was sown on 9 December 1992 and harvested on 6 May 1993. During the wheat season, six irrigations were applied; all being 75 mm, except the first (pre-plant irrigation) which was 100 mm. Each crop received P_2O_5 at 75 kg ha^{-1} (as single superphosphate) and urea at 50 kg N ha^{-1} at sowing and another 50 kg ha^{-1} urea-N was applied with second irrigation (22 September, to maize; 10 January, to wheat).

Direct measurement of denitrification by AI-soil cover method

Quantification of denitrification loss from maize and wheat fields was carried out by AI-soil cover method as described by Ryden et al. (1979). However, at the time of irrigation, C_2H_2 was injected into the soil through dispersion probes followed by irrigation with C_2H_2-treated water (Hallmark and Terry, 1985). A randomly selected C_2H_2 treatment site was confined by inserting a steel frame (0.4 × 0.8 m) into the soil to a depth of 10 cm to prevent the mixing of C_2H_2-treated water with the irrigation water outside the C_2H_2 treatment site. Acetylene supply probes were made from PVC tubes (0.4 × 70 cm; inner diameter × length) which were closed at the lower end by PVC plugs while the side facing the soil cover site, was provided with nine 1.5 mm holes drilled at 5 cm intervals starting from the base. Inside the steel frame, eight C_2H_2 supply probes were inserted in holes (1 cm × 50 cm; diameter × depth), four along each side of the soil cover. Acid-washed commercial grade C_2H_2 was injected into the soil at 6 L h^{-1} $probe^{-1}$ for 2 h. For C_2H_2 treatment of the irrigation water, acid-washed C_2H_2 was bubbled (165 L h^{-1}) for 2 h through 120 L of canal water contained in a plastic can. After injecting C_2H_2 into the soil, the C_2H_2 supply probes were pulled out and the probe holes firmly packed with soil to check the entry of irrigation water. Acetylene-

Table 1. Some physico-chemical characteristics of the field soil

Soil depth (cm)	Organic C (%)	Total N (%)	WHC (%)	pH[a]	EC[b] (dS m^{-1})	Bulk density (g cm^{-3})	Porosity (%)	Sand (%)	Silt (%)	Clay (%)
0–20	1.05	0.09	36	7.9	0.6	1.5	44	58	23	20
20–40	0.98	0.08	35	7.9	0.8	1.5	45	61	19	19
40–60	0.65	0.05	35	7.8	1.3	1.5	47	63	19	18

[a] Saturation paste.
[b] Saturation extract.

treated water was then poured on the C_2H_2-treated site inside the steel frame. On events other than irrigation, C_2H_2 was supplied into the soil only through probes and the C_2H_2 flow rate/time was adjusted according to moisture status of the field. At selected time intervals, soil atmosphere was sampled through gas sampling probes (Benckiser et al., 1986) installed at various depths and analysed for C_2H_2 on a Carlo Erba Fractovap 2150 gas chromatograph equipped with a flame-ionization detector.

During each irrigation cycle, sampling for denitrification gaseous N flux started as soon as the irrigation water percolated (6–12 h). Sampling was done at 12 h intervals during the first 3 days and then at daily intervals till the water-filled pore space (WFPS) in the upper 0–20 cm soil layer fell to ~40–50%. Soil covers (10 × 50 × 15 cm; width × length × height) for sampling of N_2O flux were fabricated from 6 mm thick PVC sheet with upper side provided with 0.8 cm ports, one on each end. Base was made of stainless steel sheet (20 gauge) by which the covers were inserted in the soil to a depth of 5 cm. The enclosed air space was continuously swept with a diaphragm vacuum pump at a suction rate of 20 L h^{-1}. After a 2 h equilibration, the out coming air was passed for 4 h through columns (2.2 × 22 cm; inner diameter × length, Pyrex glass) of $CaCl_2$ (20 g), soda lime (30 g) and molecular sieves 5 Å (45 g) to trap H_2O, CO_2 and N_2O, respectively. The amount of N_2O swept from enclosed air space was corrected for that drawn into the soil cover by concurrent determination of the amount of N_2O adsorbed from an equivalent flow of external air. The N_2O adsorbed on molecular sieves was released by displacing with water (Ryden et al., 1978) and analysed on a Hitachi 263-30 gas chromatograph equipped with a ^{63}Ni electron-capture detector. Amount of N_2O released from molecular sieves was corrected for that dissolved in water using Bunsen absorption coefficients (Moraghan and Buresh, 1977). Each part of the apparatus was connected with 5 mm

(inner diameter) nylon pressure tubing and flow rate regulated by needle valves. Measurements of denitrification by soil cover method were replicated four times and C_2H_2 treatment sites rotated weekly.

Direct measurement of denitrification by AI-soil core method

In addition to AI-soil cover method, AI-soil core method (Ryden et al., 1987) was also employed for direct measurement of denitrification during wheat season. Design of the soil core sampler was similar to that described by Rice and Smith (1982) with slight modifications to facilitate sampling to a working depth of 50 cm. Measurements by soil core method were replicated 15 times. For each replicate, intact soil cores (3 cm diameter, contained in perforated PVC sleeves) were extracted in 12.5 cm increments upto a depth of 50 cm. For each replicate, soil cores (four) from all depths were placed together in the field 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 for gas sampling. After replacing the head space by 5% acid-washed C_2H_2, the jars were incubated in the holes made within the experimental field. After 2 and 12 h of incubation, the atmosphere in the jars was repeatedly mixed with a 50-mL syringe and gas sample removed for analysis of N_2O by gas chromatography. Amount of N_2O released from cores was corrected for that dissolved in H_2O. After gas sampling and other analyses, the soil from each core was back-filled in the respective hole/depth. This back-filling was necessary before subsequent irrigations to prevent the entry of irrigation water into the soil through these holes.

Measurement of the total N loss by ^{15}N balance method

The ^{15}N microplots consisted of PVC irrigation pipes (28.8 × 120 cm; inner diameter × length) pushed to

a depth of 1 m within the main plot. For each crop, microplots (in triplicate) were established one season before the start of actual ^{15}N balance experiment and maintained under same cropping/fertilizer regimes as the surrounding main plot. For each ^{15}N balance study, microplots received 75 kg ha^{-1} P$_2$O$_5$ (as single super-phosphate) and 50 kg N ha^{-1} [as (^{15}NH$_4$)$_2$SO$_4$; 31.72 atom% ^{15}N] at the time of sowing. Ten seeds were sown in each microplot and the population reduced to three plants per microplot. Another 50 kg N ha^{-1} of (^{15}NH$_4$)$_2$SO$_4$ (31.72 atom% ^{15}N) was applied with 2nd irrigation. After harvesting, shoots were dried at 60 °C to a constant weight and ground (<0.5 mm) before analysis for total N. The soil of the microplots was removed in 10 cm segments upto 1 m depth, air-dried, sieved (<0.5 mm) and analysed for total and mineral N. For each microplot, root material from all depths was pooled, washed, dried at 60 °C and ground (<0.5 mm) for analysis of total N.

Soil and plant analyses

While sampling for denitrification, soil from different depths was also collected for analyses of mineral N and WFPS. Mineral N was determined by micro-Kjeldahl method after extracting the soil with 2 N KCl (Keeney and Nelson, 1982) whereas WFPS was calculated by dividing the volumetric moisture content by total porosity (Anonymous, 1980). Total N of plant and soil was determined by micro-Kjeldahl method (Bremner and Mulvaney, 1982) and samples prepared for ^{15}N analysis (Hauck, 1982). Rittenberg method was used to convert ammonium to N$_2$ and ^{15}N content measured on a VG Isogas mass spectrometer fitted with a double inlet system. Soil temperature was measured by glass thermometers inserted at 5 cm depth whereas rainfall data were obtained from the meteorological station located at a distance of 2 km from the study site.

Results

Direct measurement of denitrification loss from the maize field

At irrigation events, C$_2$H$_2$ concentrations between 0.2–6.1% (v/v) were maintained for at least 24 h after irrigation (HAI). After 48 h of irrigation as the WFPS fell to <71%, the C$_2$H$_2$ concentrations also dropped
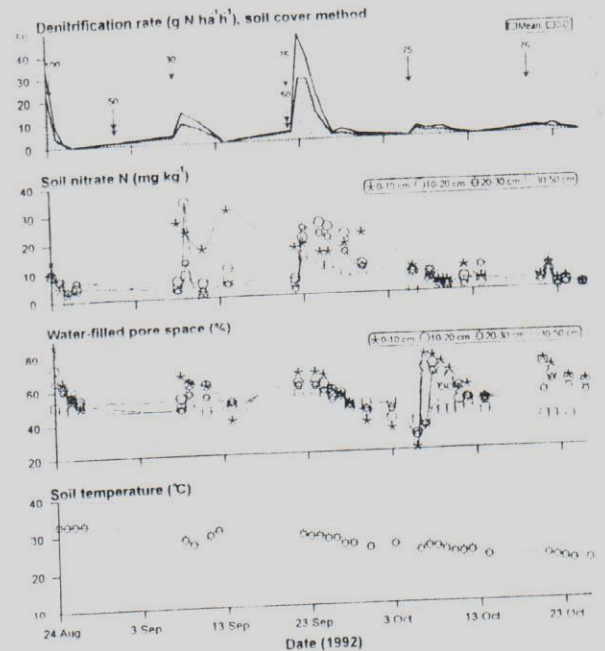

Figure 1. Temporal changes in denitrification rate and environmental conditions in the maize field. Long arrows indicate the depth of irrigation (mm); short arrows, rainfall (mm); double-headed arrows, application of urea (kg N ha^{-1}).

to <0.1%. After 24 h of irrigation, the desired level of C$_2$H$_2$ (≥0.1%) was maintained for the next 24 h with a 6 h flow of C$_2$H$_2$ at 6 L h^{-1} probe^{-1}. However, for WFPS 62% or less, a C$_2$H$_2$ supply of 6 L h^{-1} probe^{-1} for 4 h was sufficient to maintain a C$_2$H$_2$ level >0.2% for 24 h.

Figure 1 presents the temporal changes in denitrification rate in relation to environmental conditions in the maize field. Spatial variability in denitrification rate was highest (average CV = 51%; range = 4–115%), followed by soil NO$_3^-$–N (average CV = 41%; range = 9–100%) while WFPS was spatially uniform (average CV = 4; range = 1–8%). During each irrigation cycle under maize, WFPS remained higher in the upper 10 cm soil profile. Downward movement of irrigation water also increased the WFPS of the deeper soil profile but the effect was more pronounced in the upper 30 cm. Maximum WFPS (87%) was recorded 24 h after pre-plant irrigation whereas the two rainfalls and all later irrigations resulted in a WFPS in the range of 67–76% during the initial 24 h. Following each irrigation, the WFPS declined to < 50% within 4–5 days.

During the 3-month fallow preceding the maize crop, considerable NO$_3^-$–N had accumulated but fol-



lowing pre-plant irrigation, the initial NO_3^-–N in the upper 10 cm soil layer (14 mg kg^{-1}) decreased to <4 mg kg^{-1}. Most of the urea applied at the time of sowing maize was nitrified within one week and due to low crop uptake, soil NO_3^-–N in the upper (10 cm) layer remained high (17–31 mg kg^{-1}) till mid-September. Following the second urea application (with the second irrigation on 22 September), NO_3^-–N in the upper (10 cm) soil layer ranged between 16–18 mg kg^{-1} but due to leaching its content increased in the deeper layers where it ranged between 14–25 and 5–18 mg kg^{-1} for 10–30 and 30–50 cm soil layers, respectively. Due to uptake by an actively growing crop, NO_3^-–N in the upper 30 cm soil layer was reduced to 2–9 mg kg^{-1} during the first week of October. During mid-October when the crop was at flowering, accumulation of NO_3^-–N was observed. This accumulation was, perhaps, due to decomposition of the root material. Following the fourth irrigation (21 October), the initial NO_3^-–N (5–8 mg kg^{-1}) in the 0–30 cm soil profile declined to <1–3 mg kg^{-1} within 5 days. A deficit in NO_3^-–N balance was recorded on two occasions during the maize growing season. Following the pre-plant irrigation (24 August), the initial NO_3^-–N (14 mg kg^{-1}) of the upper 0–10 cm decreased by one third within 48 h; the decrease was not compensated for by increased NO_3^-–N of the deeper layers. Similarly, after 30 mm rainfall (9 September), the initial NO_3^-–N (77 mg kg^{-1}) in the 0–50 cm soil profile decreased by one third within 24 h.

Denitrification rate was strongly influenced by irrigation. During all irrigation cycles except the second, peaks appeared 12 HAI followed by a gradual decline to background level within 5–7 days. During the second irrigation cycle, however, the peak denitrification rate was recorded 24–36 HAI. Peaks during the first two irrigation cycles (23–27 g N ha^{-1} h^{-1}) coincided with those of NO_3^-–N and were 7–14 times higher than those recorded during the last two irrigations. Of the total denitrification loss during the maize season (2.7 kg N ha^{-1}), major (87%) loss took place during the first and second irrigation cycles (Table 2). Contribution of the third and fourth irrigation cycles to the total denitrification was only 8 and 5%, respectively.

Direct measurement of denitrification loss from the wheat field

At irrigation events when C_2H_2 was supplied through probes followed by irrigation with C_2H_2-treated water, one such treatment was sufficient to maintain C_2H_2 concentration in excess of 0.1% (v/v) for 24 h (75 mm irrigation) to 72 h (100 mm irrigation). For samplings other than irrigation, C_2H_2 was supplied through probes only and the application rate/time depended on the moisture status of the soil. At 70% WFPS, one C_2H_2 treatment was required at 8 L h^{-1} $probe^{-1}$ for 6 h to maintain the desired C_2H_2 level (≥0.1%) for at least 24 h. At lower WFPS (60%), the C_2H_2 flow was reduced to 6 L h^{-1} $probe^{-1}$ for 4 h.

Figure 2 presents the temporal changes in denitrification rate in relation to environmental conditions in the wheat field. Spatial variability in denitrification rate by soil cover (average CV = 77%; range = 5–121%) was comparable to that observed with soil core method (average CV = 69%; range = 20–154%). Soil NO_3^-–N showed a lesser degree of spatial variation (average CV = 34%; range = 4–172%) whereas WFPS was spatially uniform (average CV = 4%; range = 0–10%). The major changes in WFPS following irrigation were observed in the 0–30 cm soil profile while WFPS of the deeper (30–50 cm) layers generally remained <50%. Maximum WFPS during each irrigation cycle was recorded in the upper 0–10 cm soil layer within 12 h of irrigation when it ranged between 68–98%, the lower values being for the last two irrigation cycles. The soil gradually dried to <60% WFPS within 4–7 days except during the fifth irrigation cycle when it dried quickly (2 days) due to higher evapotranspiration. During the sixth irrigation cycle however, rainfall maintained relatively higher moisture in the upper (0–30 cm) soil profile when the WFPS ranged from 67–76% during initial 3 days.

Changes in the NO_3^-–N were more pronounced in the upper 0–10 cm soil layer where peaks were recorded during the first two irrigation cycles. The first peak (26 mg kg^{-1}) was recorded at pre-plant irrigation and resulted due to mineralization during the 20 d fallow after the maize harvest. Other peaks, each corresponding to 50 kg N ha^{-1}, appeared 32 and 7 days after the first (9 December) and second (10 January) urea application. Due to uptake by the actively growing crop, NO_3^-–N in the upper 10 cm soil layer decreased to 9 mg kg^{-1} by mid-February. During the last four irrigation cycles (9 February–20 April), small peaks were also recorded which ranged between 5–16 mg N kg^{-1} due to mineralization. During different irrigation cycles, the NO_3^-–N in the 10–20 and 20–30 cm depths ranged between 0–35 and 0–18 mg kg^{-1}, respectively. The higher values were recorded on occasions of higher NO_3^-–N concentration in the 0–10 cm layer (second irrigation cycle). Though leaching
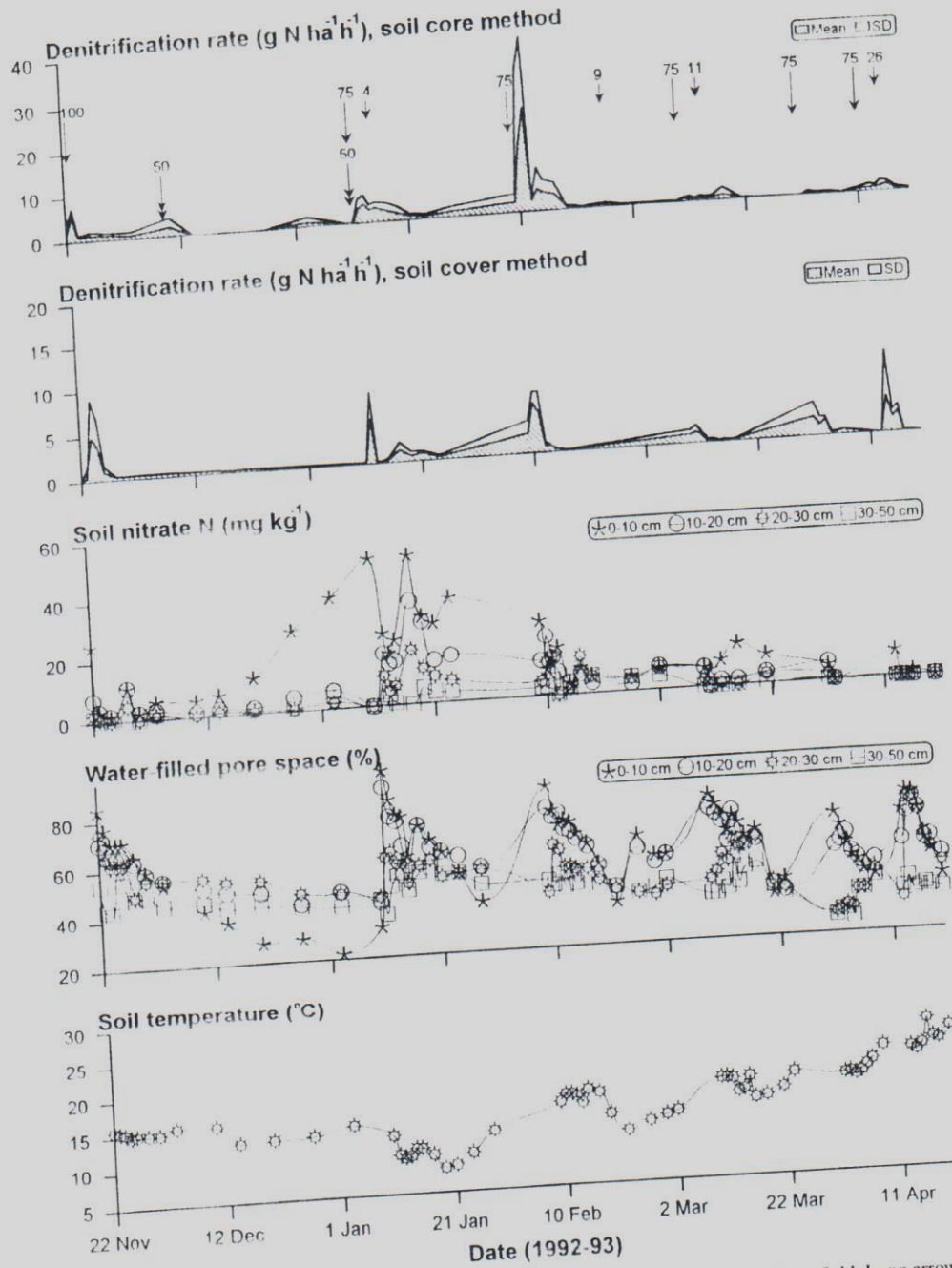
Figure 2. Temporal changes in denitrification rate and environmental conditions in the wheat field. Long arrows indicate the depth of irrigation (mm); short arrows, rainfall (mm); double-headed arrows, application of urea (kg N ha^{-1}).

Table 2. Denitrification loss integrated over different irrigation cycles during maize and wheat growing seasons

Crop	Irrigation applied (mm)	Measurement period	Denitrification loss (kg N ha^{-1})	
			C_2H_2 inhibition-soil cover method[a]	C_2H_2 inhibition-soil core method[b]
Maize	100	24 Aug–14 Sep	1.15±0.35	not determined
	75	23 Sep–6 Oct	1.21±0.60	
	75	7 Oct–14 Oct	0.22±0.08	
	75	21 Oct–26 Oct	0.14±0.05	
		Maize season total	2.72±1.09	
Wheat	100	22 Nov–10 Jan	0.29±0.18	0.88±0.37
	75	11 Jan–28 Jan	0.25±0.10	0.70±0.19
	75	9 Feb–2 Mar	0.26±0.04	1.41±0.36
	75	10 Mar–23 Mar	0.08±0.02	0.20±0.10
	75	1 Apr–8 Apr	0.11±0.02	0.05±0.04
	75	13 Apr–20 Apr	0.16±0.12	0.16±0.05
		Wheat season total	1.14±0.47	3.39±1.11

[a]Mean of four replicates with standard deviation.
[b]Mean of fifteen replicates with standard deviation.

of NO_3^-–N into deeper (30–50 cm) soil layer was observed during the first three irrigation cycles, the contents remained <5 mg kg^{-1}. As observed during the maize growing season, a deficit in NO_3^-–N balance was also recorded under wheat. During the pre-plant irrigation cycle (22 November), the initial NO_3^-–N of the 0–10 cm layer decreased from 26 to 3 mg kg^{-1} within 24 h. The decrease in NO_3^-–N of the upper (0–10 cm) soil layer was not accounted for by increased NO_3^-–N concentrations at greater depths. Similarly, following the third irrigation (2 February), the initial NO_3^-–N (46 mg kg^{-1}) in the 0–50 cm layer decreased by one half within 72 HAI. A deficit in NO_3^-–N was also recorded during the fourth irrigation cycle (10 March) when the NO_3^-–N in the 0–50 cm soil profile decreased from 15 to 5 mg kg^{-1} within 24 h.

With the soil cover method, peaks of denitrification during different irrigation cycles under wheat ranged between 1.3–5.6 g N ha^{-1} h^{-1} compared to 0.7–23.9 g N ha^{-1} h^{-1} measured by the soil core method. With both methods, higher peaks corresponded to higher soil NO_3^-–N during the first three irrigation cycles. During the periods of peak denitrification, the WFPS and NO_3^-–N in the upper (0–10 cm) soil profile were in the range of 68–83% and 5–50 mg kg^{-1}, respectively.

On 26 of the 45 sampling occasions, the soil core method revealed 2–28 times higher rates than the soil cover method. Most of the events when the soil core method showed higher denitrification, were recorded during the first three irrigation cycles and were characterized with denitrification rates in the range of 2–24 g N ha^{-1} h^{-1}. Duration of the denitrification cycles was also different with two methods. This was most evident during the first three irrigation cycles when most of the denitrification occurred. Twelve h after the first irrigation, the denitrification rate was 13 times higher with soil core than soil cover method. Comparable peak rates were recorded 24 HAI with the soil core method and a lag of 48 h was observed with soil cover method. Moreover, the rate declined to 1 g N ha^{-1} h^{-1} within 4 days with soil cover but took much longer to decline to this rate with soil core method. During initial period of the second irrigation, the rate was low with both methods. The peak, 4.9 g N ha^{-1} h^{-1}, appeared 24 HAI with soil cover and declined to less than 1 g N ha^{-1} h^{-1} during the next 12 h. On the other hand with soil core method, the rate continued to increase till 48 h and took a further 10 days to decline below 1 g N ha^{-1} h^{-1}. During the third irrigation cycle, the peak rate by soil cover, 5.6 g N ha^{-1} h^{-1}, recorded after 24 h following irrigation, declined to <1 g N ha^{-1} h^{-1} within next 2 days. In contrast, the soil core method showed 3–5 times higher rates during this period and the decline was attenuated for 7 days. Total denitrification loss during the wheat growing season was small and amounted only 1.1 and 3.4 kg N ha^{-1} by soil cover and soil core methods, respectively (Table 2). Major loss occurred during the first three

Table 3. Denitrification rate during a 96 h irrigation cycle as measured by soil core and soil cover methods[a]

Method	Denitrification rate (g N ha^{-1} d^{-1})			
	Hours after irrigation			
	24	48	72	96
Soil core (+C$_2$H$_2$)				
Depth (cm)				
0–10	128±63[b]	71±24	24±17	6±2
10–20	121±131	77±69	33±20	12±10
20–30	12±9	11±14	11±9	1±0
30–40	10±5	3±1	19±27	15±11
40–50	12±6	3±2	7±8	4±4
0–50	283±211	165±110	94±81	38±27
Soil cover				
(+C$_2$H$_2$)	125±25[c]	11±3	3±4	8±5
(–C$_2$H$_2$)	8±10	2±1	–3±4	0±3

[a] For both methods, working soil depth was up to 50 cm.
[b] Mean of fifteen replicates with standard deviation.
[c] Mean of four replicates with standard deviation.

Table 4. Nitrous oxide concentration in soil during a 96 h irrigation cycle with and without C$_2$H$_2$ treatment

Soil depth (cm)	C$_2$H$_2$ treatment	N$_2$O concentration (μg N L^{-1})				
		Hours after irrigation				
		BI[a]	24	48	72	96
5	+	0.38[b]	54.02	6.52	1.25	2.56
15	+	0.45	38.12	7.47	5.15	6.15
25	+	0.58	14.40	7.41	7.22	8.70
50	+	0.52	5.69	6.39	6.04	5.37
75	+	0.81	1.82	4.56	4.17	2.58
100	+	0.44	0.36	2.89	3.43	2.29
5	–	0.56	0.48	0.29	0.22	0.22
15	–	0.50	0.35	0.31	0.24	0.28
25	–	0.50	0.36	0.35	0.29	0.33
50	–	0.67	0.30	0.33	0.29	0.35
75	–	0.43	0.28	0.31	0.29	0.34
100	–	0.59	0.27	0.30	0.30	0.37

[a] Before irrigation.
[b] Mean of three replicates

irrigation cycles which collectively contributed to 70% (soil cover method) and 88% (soil core method) of the total denitrification.

Surface flux versus N$_2$O concentration in the soil

In a short-term field experiment in which denitrification rates were measured by soil cover and soil core methods, N$_2$O concentration at different soil depths was also monitored. Moreover, in this experiment, soil cores from different depths were incubated separately to study the contribution of different soil layers. The soil core method exhibited 2–31 times higher denitrification rate compared to soil cover (Table 3). The major zone of denitrification was restricted to upper 20 cm where highest denitrification was recorded 24 HAI followed by a gradual decline during the next 72 h. The concentration of N$_2$O (Table 4) in the C$_2$H$_2$-treated soil was highest in the upper 5 and 15 cm depth and was recorded 24 HAI followed by an abrupt decrease during the next 24 h. This temporal pattern is similar to that observed for the surface flux by soil cover method (Table 3). An increase in the N$_2$O concentration was observed in deeper (25–100 cm) soil layers during 24–72 HAI. This increase may not be attributable to denitrification since little denitrification occurred in soil cores below the 20 cm depth during 24–72 HAI. The increase in N$_2$O concentration in the deeper soil layers probably resulted from downward movement of N$_2$O produced in the upper (0–20 cm) soil layers during initial 24 HAI. In the absence of C$_2$H$_2$, most of the denitrification occurred as N$_2$ as revealed by the low N$_2$O flux by soil cover method as well as by the N$_2$O concentration in the soil. Following irrigation, the N$_2$O concentration decreased to below ambient and was maintained at this low level throughout the 96 h monitoring period.

Factors controlling denitrification

Denitrification was strongly influenced by soil moisture, as revealed by highly significant correlation between denitrification rate and WFPS during maize (r = 0.519; p < 0.01) and wheat (r = 0.578; p < 0.001). Soil NO$_3^-$–N was another important factor governing denitrification as indicated by its significant correlation with denitrification rate under maize (r = 0.414; p < 0.05) and wheat (r = 0.274; p < 0.05). Since denitrification potential (results not presented) was several-fold higher than the actual denitrification rate in the field, the process does not seem to be limited by C availability. Denitrification was also influenced by soil temperature during the maize season (r = 0.408; p < 0.05) though the effect was masked during the wheat season. However, a highly significant correlation between denitrification and soil temperature was recorded when the data for both crops were combined (r = 0.466; p < 0.001), indicating that higher denitrification rates during the maize than the wheat season

Table 5 Fertilizer N recovery during maize and wheat growing seasons[a]

Source		Fertilizer N recovery (kg N ha^{-1})[b]	
		Maize fodder	Wheat
Plant			
Grain		–	17.47±2.89
Shoot		35.97±2.50	20.08±1.61
Root		1.35±0.11	1.66±0.42
Soil			
Depth (cm)	0–10	13.50±1.75 (0.61±0.24)[c]	16.32±1.84 (0.54±0.02)
	10–20	5.38±2.02 (0.18±0.08)	3.07±0.47 (0.18±0.08)
	20–30	2.10±1.26 (0.56±0.64)	2.76±1.16 (0.16±0.08)
	30–40	0.87±0.07 (0.22±0.26)	2.02±0.83 (0.25±0.28)
	40–50	0.39±0.12 (0.12±0.14)	0.97±0.19 (0.14±0.20)
	50–100	1.27±0.68 (0.27±0.22)	2.59±0.47 (0.44±0.21)
Total (plant+soil)		60.82±4.10	66.94±1.97

[a] Each crop received $(NH_4)_2SO_4$ at 100 kg N ha^{-1} with 31.72 atom% ^{15}N

[b] Mean of three replicates with standard deviation.

[c] Figures in parentheses are the fertilizer-N present in mineral form

were due in part to the higher soil temperature under maize.

Measurement of the total N loss (^{15}N balance)

Results of the fertilizer N balance studies on maize fodder and wheat are presented in Table 5. During the maize growing season (1 September–31 October), 37.3% of the applied fertilizer N was utilized by crop and 23.5% remained in the soil at harvest. Most (92%) of the residual fertilizer N was present in the organic form. At maize harvest, 89% of the residual fertilizer N in soil was found in the 0–30 cm depth. During the wheat growing season (9 December–6 May), 39.2% of the fertilizer N was used by the crop; the recovery in grain (17.5%) being slightly less than that in shoot and root (21.7%). At wheat harvest, 27.7% of the applied fertilizer N remained in soil, most (80%) of which was present in the 0–30 cm depth. Of the residual fertilizer N, 94% was found in the organic form at wheat harvest. Total fertilizer N losses as measured by ^{15}N balance method, were 39.2 and 33.1 kg N ha^{-1} during maize and wheat growing seasons, respectively. These values are 10–14 times higher than the denitrification loss measured directly by AI technique.

Discussion

With the AI-soil cover method, peaks of denitrification are generally reported after one day following irrigation (Mosier et al., 1986a,b; Ryden et al., 1979; Terry et al., 1986). A delay of 4 days between the heavy rain and peak denitrification rate was observed by Goulding et al. (1993). This lag in the appearance of denitrification peaks is partly attributed to the time necessary to establish conditions for denitrification following soil wetting (Smith and Tiedje, 1979) and to the time required for N_2O to diffuse through soil matrix to the soil surface (Jury et al., 1982). In the present study with maize, a lag of 12 h was observed during the first, third and fourth irrigation cycles in contrast to the second irrigation where it required 24 h for the denitrification peak to appear. It seems that conditions were established for denitrification within 12 h of irrigation. However, the diffusion of N_2O was relatively slower following the second irrigation to maize because of disturbed soil structure due to ploughing. During the wheat season, although highest WFPS during all irrigation cycles were recorded after 12 h of irrigation, the denitrification peaks generally appeared 24 HAI with both soil cover and soil core methods. The slightly higher lag recorded during the wheat than maize season may be attributed to lower soil temperature which caused slower consumption of O_2 and thus delayed the establishment of denitrifying conditions.

Nitrous oxide diffusion should also be slower at lower soil temperatures. During pre-plant irrigation under wheat, a longer lag was observed with the soil cover (48 h) than the soil core method (24 h), probably due to physical constraints in diffusion of N_2O from the site of its production to the soil surface.

The low denitrification rates measured during this study (0.1–26.9 g N ha^{-1} h^{-1}) compare favourably with some earlier studies using AI-soil cover method on irrigated (Mosier et al., 1986a; Rolston et al., 1982) and non-irrigated systems (Benckiser et al., 1986; Goulding et al., 1993). However, the values for maximum denitrification rate recorded in our study with wheat (5.6 g N ha^{-1} h^{-1} with soil cover and 23.9 g N ha^{-1} h^{-1} with soil core) and maize (26.9 g N ha^{-1} h^{-1} with soil cover) are much lower than the maxima (80–183 g N ha^{-1} h^{-1}) reported in several other irrigated systems employing the AI-soil cover technique (Hallmark and Terry, 1985; Rolston et al., 1976; Ryden and Lund, 1980; Ryden et al., 1979; Terry et al., 1986).

Due to low denitrification rates, the magnitude of denitrification during the present study was also low (2.7–3.4 kg N ha^{-1}) and compares reasonably with the figures reported for other irrigated (Hallmark and Terry, 1985; Mosier et al., 1986a) or non-irrigated croplands (Aulakh et al., 1982; Benckiser et al., 1986; Bertelsen and Jensen, 1992; Goulding et al., 1993; Myrold, 1988). However, several studies with irrigated or non-irrigated systems have reported much higher denitrification loss due to heavy inputs of fertilizer and crop residues or due to rainy and mild weather conditions (Estavillo et al., 1994; Ryden and Lund, 1980; Ryden et al., 1979). The primary reason for the low denitrification loss observed in the present study appears to be the lack of sufficiently reduced soil environment for extended period of time to support high denitrification rates. Moreover, denitrification during the later period of crop growth was also reduced due to limited supply of NO_3^-–N as a result of crop uptake.

Higher denitrification loss during the first two irrigation cycles in maize may be attributed to higher soil NO_3^-–N and temperature. This is supported by the significant correlation of denitrification loss during different irrigation cycles under maize ($n = 4$) with the average soil NO_3^-–N ($r = 0.999$; $p < 0.001$) and temperature ($r = 0.879$; $p < 0.05$). During the wheat season, the differences in denitrification loss among different irrigation cycles probably resulted from variation in the WFPS. This is supported by the significant and positive correlation of denitrification loss with the average WFPS during different irrigation cycles under wheat ($r = 0.733$; $p < 0.05$; $n = 6$). Higher denitrification loss during the maize than wheat season may be attributed to the higher soil temperature under maize since denitrification loss during different irrigation cycles was positively correlated with the average soil temperature ($r = 0.683$; $p < 0.05$; $n = 10$).

As observed in the present study, lower denitrification loss with soil cover as compared to soil core method has also been reported in few earlier studies. Ryden et al. (1987) and Arah et al. (1991) attributed this to an inadequate supply of C_2H_2 in poorly-drained soils. However, as indicated by Arah et al. (1991), the vertical holes around soil covers for installation of C_2H_2 supply probes may also act as preferred routes for the entrance of O_2 into and escape of N_2O from the soil beneath the soil cover. Retarded diffusion of N_2O through soil may be another reason for lower denitrification measured by soil cover method. Flood-irrigation of soil is known to cause surface crust and decrease in the aggregate stability (Terry et al., 1986). As a result of the disintegrated structure of surface layer of irrigated soils, gaseous diffusion is retarded. Besides retarded upward diffusion of N_2O, its lateral and downward movement is also envisaged to cause underestimation of denitrification by soil cover method (Becker et al., 1990; Benckiser et al., 1986; Minzoni et al., 1988). Results of the present study also indicate the downward movement of denitrification N_2O from the sites of its production.

On several occasions, the soil NO_3^-–N in the upper (0–10 cm) soil profile decreased following irrigation or rainfall whereas this decrease was not compensated for by increased NO_3^-–N content of the deeper layers. On such occasions the soil moisture conditions were favourable for denitrification, though the observed denitrification rates were too low to account for the deficit in NO_3^-–N balance. This deficit in the NO_3^-–N balance may partly be attributed to the crop uptake, microbial immobilization and the denitrification loss which was perhaps not detected by the methods employed. Crop uptake may not be responsible for the depletion of NO_3^-–N during the pre-plant irrigation cycles. However, microbial immobilization of NO_3^-–N in the presence of readily oxidizable C source is well documented (Azam and Malik, 1985; Azam et al., 1988). Ample amounts of easily oxidizable C should be available for the microbial immobilization of NO_3^-–N at the time of pre-plant irrigations (from decomposing residues of the previous crop) as well as during the period of active crop growth (in the form root exudates). As regards underestimation of denitrification

by the AI, possible reasons have already been discussed for soil cover method. With soil core method, incomplete recovery of the denitrification N_2O in the head space of incubation vessel has also been reported in some studies. Holt et al. (1988) reported a 6–12 fold increase in the recovery of denitrification gaseous N products when the soil in incubation vessel was shaken for one hour prior to the gas analysis. In a study by Aulakh and Doran (1990), 65% of the N_2O produced via denitrification remained entrapped in the soil cores incubated at 120% WFPS. Similarly, Ambus and Christensen (1993) working with soil cores at a WFPS of 103%, found 43% higher denitrification rate when the soil cores were disrupted and shaken to release the entrapped N_2O. The results emphasize that besides analysing the head space of incubation vessel, the N_2O entrapped in the soil cores should also be measured to obtain its quantitative recovery.

Recovery of the fertilizer N as measured by ^{15}N balance (61–67%) is comparable with the figure (60%) reported for different Pakistani soils under upland conditions (Ahmed, 1985). However, loss of the fertilizer N measured by ^{15}N balance (33–39%) was 10–14 times higher than the denitrification loss directly measured by AI method. A probable reason for the higher N loss measured by ^{15}N balance may be losses other than denitrification, most probably NH_3 volatilization. However, underestimation of denitrification by AI can not be entirely excluded. The most probable reason for this underestimation appears to be the entrapment of denitrification gaseous N products in the soil matrix. Field experiments are in progress to address the question of N_2O entrapment in the soil.

Acknowledgements

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