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Pakistan Journal of Biological Sciences, 2 (4): 1451-1454, 1999 **Research Article**

Seasonal Changes In Denitrification Potential of an Irrigated Sandy-clay Loam Under A Wheat-maize Cropping System Receiving Different Fertilizer Treatments

T. Mahmood, A.S. Bhatti and K.A. Malik
Nuclear Institute for Agriculture & Biology, P.O. Box 128, Faisalabad 38000, Pakistan
*Pakistan Agricultural Research Council, P.O.Box 1031, Islamabad 44000, Pakistan

Abstract

tudy was conducted to follow seasonal changes in the denitrification potential (DNP) of an irrigated sandy-clay loam under wheat-maize cropping system receiving different fertilizer treatments for the past ten years. Fertilizer treatments included: -100 (urea at 100 kg N ha^{-1} a^{-1}), N-200 (urea at 200 kg N ha^{-1} a^{-1}), FYM-16 (farmyard manure at 16 t ha^{-1} a^{-1}), FYM-32 armyard manure at 32 t ha^{-1} a^{-1}) and the control (unfertilized). Urea-N was applied in four equal splits, two to each crop. hereas all farmyard manure was applied at the time of sowing wheat. Averaged across treatments during the growing eriod of wheat, DNP increased with crop growth, reached maximum at flowering, and declined at grain formation. Under aize, however, the DNP increased till flowering and remained unchanged at dough stage when the fodder was harvested. verage DNP during the wheat season was almost 1.6 times higher than that under maize. Averaged across sampling dates uring the wheat season, N-200 showed 13 per cent higher DNP than the control, whereas N-100 had no effect. Under aize, DNP was 37 per cent higher with N-100 treatment than the control while N-200 had no effect. During the wheat eason, DNP showed an increase of 66 and 114 per cent due to FYM-16 and FYM-32 treatments, respectively. However, he residual effect of FYM on DNP during the maize growing season was observed only with FYM-32 treatment which howed 34 per cent higher values relative to control. Denitrification potential was significantly correlated with aerobically ineralizable carbon and microbial biomass carrying capacity but not with microbial biomass carbon, total organic carbon nd actual denitrification rates in the field.

ntroduction

eing heterotrophic in nature, denitrifiers require arbonaceous substrate for growth and reduction of nitrate. herefore, denitrification in soil is strongly influenced by the vailability of organic C such as native soil organic matter, oot exudates, crop residues and manures (Bijay-Singh *et* ., 1988; Van Cleemput *et al.*, 1990). Seasonal changes in inputs from crop roots, rhizosphere products and crop esidues significantly influence the C availability to soil eterotrophs (Wheatley *et al.*, 1990; Franzluebbers *et al.*, 994). Besides seasonal changes, type of plant cover and rtilizer application may also have significant bearing on C vailability in soil and thus on dynamics of microbial ommunities (Shen *et al.*, 1989; Insam *et al.*, 1991; ahmood *et al.*, 1997).

hanges in the C availability in soil should also be reflected y changes in denitrification potential which is a measure f C availability to denitrifiers under nitrate non-limiting and ater saturated conditions. However, limited data are vailable on seasonal changes in denitrification potential nder field conditions (Wheatley and Williams, 1989). Previously, we reported carbon availability (Mahmood *et* ., 1997) and denitrification losses (Mahmood *et al.*, 1998) rom an irrigated sandy-clay loam under a wheat-maize ropping system. We now report seasonal changes in DNP f an irrigated sandy-clay loam under wheat-maize cropping ystem receiving different fertilizer treatments. elationships between DNP and some C availability indices uch as aerobically mineralizable C (AMC), microbial omass C (MBC), microbial biomass carrying capacity BCC) and total organic C (TOC) are also described.

aterials and Methods

The study site at the Nuclear Institute for Agriculture and Biology, Faisalabad, has a semiarid subtropical climate with a mean annual rainfall of 340 mm. The hottest months are May and June, with mean maximum air temperatures of 39.3 and 41.1°C, respectively, whereas January is the coldest month with a mean minimum temperature of 5°C. The soil is a deep, well-drained Hafizabad sandy-clay loam (Typic Ustochrept), developed in a mixed calcareous medium-textured alluvium derived from Himalayas (Anonymous, 1972). The experiment comprised 20 field plots (7.5 × 8.5 m) that received five fertilizer treatments in a completely randomized design, each with four replicates. Details of the fertilizer treatments (which have been in effect since 1980) and some physicochemical properties of the plough layer are given in Table 1. Wheat (*Triticum aestivum* cv. Pak-81) was sown on 6 December, 1990 and harvested on 5 May, 1991 while maize (*Zea mays* cv. Akbar) was seeded on 29 August, 1991 and the fodder harvested on 30 October, 1991. Wheat received six canal irrigations (14 November, 26 December, 13 February, 28 February, 18 March and 2 April); all irrigations were equivalent to 7.5 cm of water except the first (presowing) and the fourth, which were 10 and 5 cm, respectively. During the maize season, five irrigations were applied (24 August, 12 September, 26 September, 9 October, 21 October); all being equivalent to 7.5 cm of water except the first (presowing) and the last, which were 10 and 3 cm, respectively. Soil was sampled five times during the wheat growing season and three times under maize. Four soil cores (3 × 15 cm, diameter × depth) were randomly collected from each replicate plot, pooled, mixed and

Table 1: Details of fertilizer treatments and some physico-chemical properties of the (0-15 cm) soil

Treatment[1]	Wheat	Maize	pH[2]	TOC (%)	Total N (%)	WHC (%)	Pore space (%)	Bulk density (g cm^{-3})
N[3]-100	50 kg N ha^{-1}	50 kg N ha^{-1}	7.3	1.14	0.07	37	46.9	1.44
N-200	100 kg N ha^{-1}	100 kg N ha^{-1}	7.3	1.05	0.09	36	47.4	1.42
FYM[4]-16	16 t ha^{-1}	None	7.4	1.17	0.08	36	47.5	1.42
FYM-32	32 t ha^{-1}	None	7.4	1.18	0.09	37	47.6	1.41
Control	None	None	7.4	0.78	0.07	35	43.8	1.52

[1] Treatments are in effect since 1980. [2] Saturation paste. [3] Urea-N: to each crop, the stated dose of N-fertilizer was appli in two equal splits, one at sowing and the other with second (in case of wheat) or third (in case of maize) irrigatio [4] Farmyard manure: stabilized for about six months in a pit, all applied in November at land preparation for wheat. The to N applied as FYM-16 and FYM-32 treatments was equivalent to 96 and 192 kg ha ^{1}, respectively; the amount of P applied as FYM-16 and FYM-32 treatments was equivalent to 96 and 192 kg ha^{-1}, respectively, which was balanced in 100 and N-200 treatments by application of single superphosphate.

Table 2: Denitrification potential (ng N g ^{1} h^{-1}) of the field soil under wheat receiving different fertilizer treatments

Treatment	Sampling date (1990-91)/Growth stage								
	13 Dec Tillering	14 Jan Tillering	24 Feb Flowering	27 Mar Grain	11 Apr Dough	Treatment mean	LSD	$P<0.05$	$P<0.01$
N-100	104	293	707	373	302	372	Treatment	40.5	54.1
N-200	166	334	525	461	392	375	Date	40.5	54.1
FYM-16	289	515	831	934	196	553	Overall	90.7	120.9
FYM-32	428	675	1090	846	529	713			
Control	112	301	513	467	271	333			
Data mean	220	424	733	632	338				

Table 3: Denitrification potential (ng N g ^{1} h ^{1}) of the field soil under maize receiving different fertilizer treatments

Treatment	Sampling date (1991)/Growth stage						
	17 Sep Active Growth	7 Oct Flowering	29 Oct Dough	Treatment	LSD	$P<0.05$	$P<0.01$
N-100	207	465	334	335	Treatment	44.6	60.0
N-200	147	299	304	250	Data	34.5	46.5
FYM-16	204	300	348	284	Overall	77.1	103.9
FYM-32	260	373	351	328			
Control	217	218	301	245			
Data nean	207	331	328				

sieved (< 2 mm). The soil moisture at the time of sampling was equivalent to ca. 50 per cent of the water-holding capacity.

Denitrification potential was measured on the field-moist soil within 6 h of collection. Ten gram portions of the soil were taken in 100-mL serum vials and treated with 10 mL of KNO_3 solution to establish NO_3^--N level of approximately 200 mg kg ^{1}. After sealing with silicone rubber septum, the vials were evacuated and flushed thrice with O_2-free N_2. The headspace was then replaced by 5 per cent acid-washed C_2H_2 and vials incubated at 30 °C. After 48 h incubation, the contents were vigorously shaken by hand and the headspace sample withdrawn for N_2O analysis. Nitrous oxide was analysed on a Gasukuro Kogyo 370 gas chromatograph equipped with a thermal conductivity detector. The amount of N_2O was corrected for that dissolved in water using Bunsen absorption coefficients (Moraghan and Buresh, 1977). Results and procedures for the concurrently measured C availability indices viz. TOC, AMC, MBCC and MBC have been reported earlier (Mahmood *et al*., 1997). Briefly, AMC was taken as the CO_2-C evolved after 10 d aerobic incubation of the unfumigated soil at 30 C. Microbial biomass car capacity represents the CO_2-C evolved after 10 d aer incubation of the $CHCl_3$-fumigated soil at 30 C, divide a k_c factor of 0.45. Microbial biomass C was calculate the flush of CO_2-C from the $CHCl_3$-fumigated soil m CO_2-C evolved from the unfumigated soil, using a k_c f of 0.45. The data were subjected to analysis of vari followed by the Least Significant Difference (LSD) (Steel and Torrie, 1980). Relationships between DNP different C availability indices were evaluated by si linear regression analyses.

Results

Results of DNP of the field soil under wheat and maiz presented in Tables 2 and 3, respectively. Denitrific potential exhibited a marked seasonal pattern. Aver across treatments during the wheat growing seas increased with the crop growth, reached maximu flowering and then declined at grain formation ($P < 0$ Under maize, however, the DNP increased till floweri < 0.01) and remained unchanged at dough stage whe fodder was harvested. Average DNP during the ason was almost 1.6 times higher than that during the aize season. Fertilizer treatments had pronounced effects DNP under both crops. Averaged across sampling dates ring the wheat season, N-200 showed slightly (13%) gher DNP than the control ($P<0.05$), whereas N-100 d no effect. Under maize, DNP with N-100 treatment as 37 per cent higher than the control ($P<0.01$) while 200 had no effect. Application of FYM to wheat, sulted in a 66 and 114 per cent increase in DNP in M-16 and FYM-32 treatments, respectively ($P<0.01$). owever, the residual effect of FYM on DNP during the aize season was observed only with FYM-32 treatment hich showed 34 per cent higher values relative to control <0.01). Comparing the two urea treatments, DNP was milar under wheat but 34 per cent higher in N-100 than N-0 treatment in maize ($P<0.01$). Although 29 per cent gher DNP was recorded with higher rate of FYM during e wheat season ($P<0.01$), the two FYM treatments had milar effect under maize. Regarding the treatment effects various sampling dates, the N-100 treatment exhibited NP similar to the control except at the active growth stage wheat (24 February) and maize (7 October) when it was gher at N-100 ($P<0.01$). A stimulatory effect on DNP as also observed with N-200 at flowering in maize <0.05) and at maturity in wheat ($P<0.01$). Comparing e control with FYM treatments, the latter generally owed higher DNP during the wheat season ($P<0.01$). uring the maize growth, however, the stimulatory effect FYM was observed only at the active growth stage when e FYM treatments showed 38-71 per cent higher DNP an the control ($P<0.05$).

enitrification potential showed a strong correlation with MC when data from both crops were pooled ($n = 40$; $r =$ .732; $P<0.001$). The correlation between DNP and AMC as still high for wheat data ($n = 25$; $r = 0.720$; <0.001) but relatively low under maize ($n = 15$; $r =$ .681; $P<0.01$). The relationship between DNP and TOC ased on point data ($n = 40$) or date means ($n = 8$) was on-significant. However, when averaged across sampling ates (i.e. treatment means, $n = 5$), the two showed highly ignificant correlation ($r = 0.979$, $P < 0.001$). enitrification potential was also significantly correlated with MBCC ($n = 40$; $r = 0.514$; $P<0.001$). However, the elationship between DNP and MBC was non-significant whether combined or individual crop data were used for egression analyses. Averaged across sampling dates during he wheat or maize growing season (i.e. treatment means; $= 5$ for single crop, $n = 10$ for both crops), the lationship between DNP (data from the present study) and e actual denitrification rate (data from Mahmood *et al.*, 998) was non-significant.

iscussion

gher DNP observed in the FYM treatments may be ributed to two different effects of FYM. The readily ailable carbon contained in FYM could be related to her DNP. However, this carbon substrate might have n exhausted near the wheat maturity in FYM-16 tment and also in FYM-32 treatment during the fallow od preceding maize. An indirect effect of FYM on DNP ld be through increased supply of root-derived carbon. above-ground dry matter yields of wheat for FYM-16, FYM-32 and control were 4.3, 5.0 and 3.8 t ha^{-1}, respectively, whereas maize fodder yields were 10.6, 13.3 and 8.3 t ha^{-1}, respectively. As crop yields are also linked with carbon input into the soil (Russel, 1977), the higher biomass in FYM treatments probably resulted in a greater below-ground flow of the fixed carbon as compared to the unfertilized crops. A substantial increase in DNP due to FYM at active growth stages of wheat and maize also indicates the higher availability of plant-derived carbon in FYM treatments compared to the control. Stimulatory effect of organic amendments on denitrification activity is well documented (Van Cleemput *et al.*, 1990; Artiola and Pepper, 1992) and an increase in the denitrifying capacity during the active plant growth has also been reported (Haider *et al.*, 1985). However, the stimulatory effect of organic amendments on DNP through controlling the availability of plant-derived C is less well understood.

Higher stimulatory effect with lower urea application as recorded at flowering stage under both crops may be attributed to higher carbon allocation to roots under N-deficient conditions, since C allocation to roots may be curtailed in adequately fertilized soils (Russell, 1977; Roder *et al.*, 1988). However, the same may not hold true while comparing N-100 treatment with the control. The above-ground dry matter yields of wheat for N-100, N-200 and control were 7.8, 12.2 and 3.8 t ha^{-1}, respectively, whereas maize fodder yields were 14.3, 16.7 and 8.3 t ha^{-1}, respectively. As crop yields in the control were severely affected due to nutrient stress prevailing over the past 10 years, this might have lead to lower belowground C inputs. These results suggest that under a moderate degree of nutrient deficiency (as in N-100), the flow of carbon into soil may be greater than that under conditions of sever nutrient stress (as in control) or no stress at all (as in N-200 treatment). This is supported by the higher AMC in N-100 treatment as compared to the control and N-200 treatment observed at flowering stage under both crops (Mahmood *et al.*, 1997). A moderate degree of nutrient stress might also have prevailed under both FYM treatments as indicated by the lower dry matter yields in FYM than the corresponding urea treatments. Although the amount of N in FYM treatments was equivalent to that of the corresponding urea treatments, the plant availability of FYM-N was perhaps lower as compared to urea-N.

Temporal changes in DNP were concomitant to the changes in AMC, the two showing a strong correlation under both crops. This indicates the availability of root exudates from growing plants to the soil heterotrophs both under aerobic and anaerobic (denitrifying) conditions. A significant relationship observed between DNP and AMC is also in agreement with earlier reports (Beauchamp *et al.*, 1980; Bijay-Singh *et al.*, 1988). Griffiths *et al.* (1993) also reported DNP to be highly correlated with soil respiration rate. The highly significant relationship between DNP and TOC (treatment means) is also consistent with the findings of others (Beauchamp *et al.*, 1980; Bijay-Singh *et al.*, 1988). These authors studied soils of different organic matter content and found highly significant correlation between DNP and TOC, since bio-availability of carbon may depend on TOC. During the present study, however, the lack of relationship between DNP and TOC based on point data suggests that, TOC may not account for the temporal pattern of carbon availability. It is the amount of available carbon (e.g. AMC) which appears to be more important than TOC in determining such relationship. This is why DNP and TOC are not always significantly correlated as found in the present study and by others (McGarity, 1961; McGarity and Myers, 1968). A highly significant correlation between DNP and MBCC conforms with the results of Griffiths *et al.* (1993) who used chloroform-fumigation flush of carbon as a measure of microbial biomass. Similarly, Groffman and Tiedje (1989) used carbon released after chloroform-fumigation as an indicator of the microbial biomass 'carrying capacity' that was highly correlated with the annual loss of N through denitrification. Since the relationship between DNP and MBC was non-significant, a highly significant relationship between DNP and MBCC appears to be due to AMC which is included with MBCC. This is supported by a highly significant correlation between AMC and MBCC ($n = 40$; $r = 0.893$; $P < 0.001$).

The lack of relationship between DNP and actual denitrification rate indicates that, the latter was not limited by the supply of energy source in this particular system. In all treatments, DNP was always several-fold higher than the actual denitrification rates in the field. Nevertheless, higher denitrification rates recorded with FYM treatments during the maize growing season (Mahmood *et al.*, 1998) indicated that, the C contained in FYM may also influence the actual denitrification rates in the field. However, the stimulatory effect of FYM-C on denitrification appears to be indirect i.e. by promoting anoxic microsites rather than directly acting as energy source for denitrifiers.

Results of the present study confirm that marked seasonal fluctuations in DNP occur due to C inputs in cropped soils and that different fertilizer treatments have significant bearing on the C availability to denitrifiers through controlling the availability of plant-derived C or due to the C contained in natural fertilizers such as manures. Moreover, AMC can serve as a reliable indicator for the temporal changes in C availability to denitrifiers.

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