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SEASONAL CHANGES IN SOIL MICROBIAL BIOMASS NITROGEN UNDER AN IRRIGATED WHEAT-MAIZE CROPPING SYSTEM

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Abstract

Seasonal changes in soil microbial biomass nitrogen (N_{mic}) were followed under an irrigated wheat-maize cropping system receiving urea and/or farmyard manure (FYM) at 0–200 kg N ha^{-1} year^{-1} for the past ten years. The N_{mic} was maximum (109–218 kg N ha^{-1}) at the wheat stem elongation stage and minimum (80–148 kg N ha^{-1}) at the wheat tillering stage. Fertilizer application significantly increased the N_{mic}, indicating that the belowground processes were limited by N availability. Averaged across sampling dates, the N_{mic} ranged from 93 kg N ha^{-1} (in unfertilized) to 176 kg N ha^{-1} (in FYM applied at 32 t ha^{-1} year^{-1}), whereas the annual N_{mic} flux ranged from 57 kg N ha^{-1} (in unfertilized) to 118 kg N ha^{-1} (in FYM applied at 16 t ha^{-1} year^{-1}). Crop dry matter and N yields increased due to fertilizer application. At an equivalent N application rate, urea applied alone produced maximum yields, followed by urea combined with FYM, whereas FYM applied alone yielded minimum. The size and flux of N_{mic} were poor indicators of the crop N availability, whereas the soil mineralizable N determined by alkaline permanganate method at the crop sowing stage was significantly correlated with the soil mineral N, and with the crop dry-matter and N yields.

Introduction

Soil microbial biomass (SMB) though constitutes a minor fraction of the soil organic matter, it plays a pivotal role in nutrient cycling in terrestrial ecosystems by acting as a highly labile source of plant nutrients (Jenkinson & Lad, 1981). Primary productivity of an ecosystem is closely linked with the dynamics of SMB (Jenkinson *et al*., 1992), suggesting SMB as a more sensitive indicator of changes in the soil quality as compared to the total organic carbon (C) (Sparling, 1992). Besides, a close relationship has been reported between plant nitrogen availability and SMB (Jenkinson & Lad, 1981; Houot & Chaussod, 1995), signifying SMB as a reliable index of soil productivity that considerably depends on nutrient fluxes (Hassink, 1994).

The crucial factor governing the size and activity of SMB pool is the quality and quantity of C input. Organic amendments like post-harvest crop residues, manures, sewage sludge and municipal wastes are well known to increase the content of soil organic C and that of SMB (He *et al*., 1997; Thomsen *et al*., 2003). Carbon inputs in the form of rhizodeposits by the actively growing crop often lead to marked temporal fluctuations in SMB (Franzluebbers *et al*., 1994), whereas the type of plant cover and cropping history have also significant bearing on the dynamics of SMB (Mahmood *et al*., 1997, 2005; Moore *et al*., 2000). The size and activity of SMB are also strongly influenced by soil temperature (Nicolardot *et al*., 1994), soil type (Groffman *et al*., 1996), and tillage regimes (Franzluebbers *et al*., 1994). There have been conflicting reports regarding the effect of mineral N fertilizers on dynamics of soil microbial biomass nitrogen (N_{mic}). Though N_{mic} under different crops was not affected by the level of mineral N fertilizers (Nieder *et al*., 1996; Moore *et al*., 2000), the fertilizer-derived N_{mic} and its turnover increased by about two-folds with increasing the fertilizer N application rate from 100 to 200 kg ha^{-1} (Jensen *et al*., 1997a).

Although substantial information is available on the dynamics of SMB under different agroclimatic conditions (McGill *et al*., 1986; Franzluebbers *et al*., 1994; Groffman *et al*., 1996; Witt *et al*., 1998; Moore *et al*., 2000; Thomsen *et al*., 2003), relatively few studies pertain to irrigated systems, particularly under semiarid subtropical conditions (Azam *et al*., 1986; Mahmood *et al*., 1997, 2005; Raiesi, 2004). In such systems, drying-rewetting cycles coupled with relatively high soil temperatures might be conducive to a rapid turnover of soil microbial biomass, thus leading to substantial nutrient fluxes (Piao *et al*., 2000; Fierer & Schimel, 2002). However, under semiarid subtropical conditions, such as those prevailing in Pakistan, field studies have been lacking to ascertain the practical significance of N_{mic} turnover and its relationships with the crop dry matter yield and N uptake. We previously reported seasonal fluctuations in soil microbial biomass C in an irrigated wheat-maize cropping system under different fertilizer management practices (Mahmood *et al*., 1997, 2005). Objectives of the present study were (i) to follow seasonal fluctuations in N_{mic} under an irrigated wheat-maize cropping system receiving for the past ten years urea and/or FYM at 0–200 kg N ha^{-1} $year^{-1}$ and (ii) to elucidate the relationships of crop dry matter and N yields with N_{mic} parameters and with other indices of N availability.

Materials and Methods

The study site at the Nuclear Institute for Agriculture & Biology, Faisalabad is located at 73.2°longitude and 31.4°latitude. The area has a semiarid subtropical climate with a mean annual rainfall of 340 mm, most of which is received as high intensity monsoon downpours during July and August. The annual excess of pan evaporation over rainfall is 1600 mm, with greatest deficit occurring during May (203 mm) and June (314 mm). The air temperature varies from 5°C (mean minimum during January) to 41.1°C (mean maximum during June). The soil belongs to Hafizabad Series (Haplic Yermosol; Anon., 1966) and is a deep, well-drained sandy-clay loam developed in a mixed calcareous medium-textured alluvium derived from Himalayas (Anon., 1967). The site has been under a long-term fertilizer trial with irrigated wheat-maize cropping system receiving different urea and/or FYM treatments for the past 10 years.

Thirty six experimental plots (7.5×8.5 m) were established for nine treatments in a randomized complete block design, each with four replicates. Treatments included: urea at 100 and 200 kg ha^{-1} $year^{-1}$ (U_{50} and U_{100} treatments, respectively), FYM at 16 and 32 t ha^{-1} $year^{-1}$ (F_{16} and F_{32} treatments, respectively), urea at 50 kg ha^{-1} $year^{-1}$ combined with FYM at 8 and 16 t ha^{-1} $year^{-1}$ (U_{50}+ F_8 and U_{50}+ F_{16} treatments, respectively), urea at 100 kg ha^{-1} $year^{-1}$ combined with FYM at 8 and 16 t ha^{-1} $year^{-1}$ (U_{100}+ F_8 and U_{100}+ F_{16} treatments, respectively) and an unfertilized control. The FYM was stabilized for about 6 months in a pit and the entire stated dose was broadcast in the field plots before land preparation for wheat in November. The FYM contained 0.6% total N (NH_4^+ and NO_3^-, each 149 mg N kg^{-1}) and 0.6% P_2O_5; the amount of P_2O_5 was balanced through the application of single superphosphate. One half of the stated dose of urea was applied to wheat and the other half to maize. For each crop, urea-N was again split into two; one half applied at sowing and the other half with the first irrigation (wheat, 22 December; maize, 6 September). Some physicochemical characteristics of the plough layer after 10 years under different fertilizer regimes are given in Table 1. Wheat (*Triticum aestivum* L. cv. Pak-81) was seeded on 1 December and harvested on 30 April, whereas maize (*Zea mays* L. cv. Akbar) was sown on 2 August and the fodder harvested on 27 October. Crops were irrigated with canal water; in addition to the pre-sowing irrigations (one to each crop), wheat and maize crops received five and four irrigations, respectively. Irrigations during the wheat season were equivalent to 75 mm, except the first (pre-sowing) and the fourth, which were 100 mm and 50 mm, respectively; whereas those during the maize season were also equivalent to 75 mm, except the first (pre-sowing) and the last, which were 100 mm and 50 mm, respectively.

The soil was sampled five and three times during the wheat and maize growing season, respectively (Table 2). From each replicated plot, 10 soil cores (3×15 cm, diameter×depth) were randomly extracted, pooled, mixed, visible roots manually removed, and the soil sieved (<2 mm). The soil moisture content at the time of sampling was equivalent to field capacity. The soil was not stored or conditioned before the N_{mic} measurement for which the $CHCl_3$ fumigation-incubation method of Jenkinson & Powlson (1976) was followed using 10-g portions of the field moist soil. The fumigated-reinoculated and unfumigated (control) soils contained in air-tight 100-ml serum vials were incubated at 30°C for 10 days and then extracted with 2*N* KCl for the analysis of mineral N by a micro-Kjeldahl method (Keeney & Nelson, 1982). The N_{mic} was calculated as $N_{mic}=F/k_N$; where F is the flush of NH_4^+-N (NH_4^+-N released from the fumigated soil minus that from the unfumigated) and k_N is the recovery factor equivalent to 0.41 (Carter & Rennie, 1982). The N_{mic} turnover rate was calculated by dividing the sum of losses of N_{mic} at different sampling intervals by the average values of N_{mic} (McGill *et al*., 1986). Total organic C of soil was measured by an acid-dichromate method (Riehm & Ulrich, 1954), whereas soil mineralizable N was determined by an alkaline-permanganate method (Hussain & Malik, 1985). Data were subjected to an analysis of variance, followed by Duncan's multiple range test (Steel & Torrie, 1980). Results are reported as means of four replicate plots and expressed on soil dry weight basis.

Results

Seasonal changes in N_{mic}: Averaged across sampling dates, the N_{mic} constituted 6.5–9.1% of the total soil N; the lowest figure corresponding to the unfertilized, and the highest to the treatments receiving FYM. The N_{mic} values ranged from 80–109 kg ha^{-1} in the unfertilized, and from 99–218 kg ha^{-1} in the fertilized. Averaged across sampling dates, the N_{mic} was lowest (93 kg ha^{-1}) for the unfertilized, whereas the increase due to fertilizer application ranged from 25% (with U_{200}) to 87% (with F_{32}) (P<0.05) (Table 2). Comparing the fertilizer application rates, increasing the urea application from 100 to 200 kg N ha^{-1} year^{-1} caused a slight (6%) decrease in the N_{mic} (P<0.05), whereas doubling the FYM application rate from 16 to 32 t ha^{-1} year^{-1} produced a 21% increase (P<0.05). With urea and FYM applied in different combinations, increasing the FYM application rate from 8 to 16 t ha^{-1} year^{-1} increased the N_{mic} (8 and 22% increase with U_{50} and U_{100}, respectively; P<0.05); whereas increasing the urea application rate from 50 to 100 kg N ha^{-1} year^{-1} either had no effect (with F_{16}), or even decreased the N_{mic} (with F_8). Averaged across sampling dates, the N_{mic} was highly significantly correlated with the total organic C and with the total N (P<0.001) (Table 4). Considering the point data (n=72) also revealed a highly significant correlation between the N_{mic} and the total organic C (r=0.487, P<0.001).

Table 3. Annual flux and turnover rate of soil microbial biomass nitrogen (N_{mic}) under wheat-maize cropping system receiving different fertilizer treatments.

Treatment	$\sum N_{mic}$ flux[a] kg N ha^{-1}	N_{mic} turnover rate[a] Year^{-1}
U_{100}	65 de	0.53 de (694)[b]
U_{200}	71 cd	0.61 c (596)
F_{16}	118 a	0.82 a (445)
F_{32}	80 c	0.46 e (798)
$U_{50}+F_8$	44 f	0.33 f (1096)
$U_{50}+F_{16}$	76 cd	0.54 d (681)
$U_{100}+F_8$	56 e	0.45 e (803)
$U_{100}+F_{16}$	104 b	0.73 b (501)
Unfertilized	57 e	0.61 c (597)

[a]Figures within a column followed by different letter are significantly different by Duncan's multiple range test (P<0.05).
[b]Figures in parentheses indicate the N_{mic} turnover time (days).

The temporal variability in the N_{mic} was moderate (average CV=11%; range=8–14%), and was relatively higher during the wheat season (average CV=12%) than under maize (average CV=7%). The highest N_{mic} values were observed at the wheat stem elongation stage; at this stage, the gain in the N_{mic} compared to the preceding sampling date varied from 20 kg ha^{-1} (U_{200}) to 70 kg ha^{-1} (F_{32}). Moreover, the figures for the N_{mic} were higher under wheat (average, 155 kg ha^{-1}; range 109–218 kg ha^{-1}) than those under maize (average 128 kg ha^{-1}; range, 101–177 kg ha^{-1}).

Flux and turnover of N_{mic}: The annual N_{mic} flux ranged from 57 kg ha^{-1} (in unfertilized) to 44–118 kg ha^{-1} (in fertilized) (Table 3). Comparing fertilizer treatments, urea applied at 200 kg N ha^{-1} year^{-1} caused a 24% increase in the N_{mic} flux, whereas the lower urea application rate had no effect. The effect of FYM on the N_{mic} flux was much more pronounced than that of urea, causing 108% and 36% increase in F_{16} and F_{32} treatments, respectively. Combined application of urea and FYM also increased the N_{mic} flux relative to the unfertilized (P<0.05), but only when FYM was applied at higher rate ($U_{50}+F_{16}$ and $U_{100}+F_{16}$ treatments). Treatment effects on the N_{mic} turnover rate were almost similar to those observed for the N_{mic} flux and the two parameters were significantly correlated (r=0.842, P<0.01; Table 4). Urea applied alone either had no effect (at higher application rate) or even decreased the N_{mic} turnover rate when applied at lower rate. With FYM applied at lower rate, the turnover rate was highest among all treatments, whereas with higher FYM application the stimulatory effect decreased. When urea and FYM were applied in combinations, only $U_{100}+F_{16}$ treatment increased the N_{mic} turnover rate. The annual N_{mic} flux and N_{mic} turnover rate were not correlated with any of the soil parameters tested (Table 4).

Fertilizer treatments also strongly influenced the temporal pattern of N_{mic} flux (Table 2). During the wheat season, urea compared to FYM applied alone caused higher flux than gain in the N_{mic} and *vice versa* during the maize season. In the unfertilized, whereas the flux was recorded only at the flowering stage, in the fertilized plots the release also occurred at other growth stages. Of the total N_{mic} flux during the wheat season, the flux till flowering stage contributed 28–49% when urea and FYM were applied alone and 70–100% in urea+FYM treatments indicating that the combined application of urea and FYM accelerated the turnover of N_{mic} during the active growth period of wheat. During the maize season, the total N_{mic} flux and the flux during planting to flowering were much lower in magnitude as compared to the flux recorded during the wheat season, except in U_{100} +FYM_{16} treatment in which the flux was almost twice higher during the maize season than under wheat.

Crop dry matter and N yields and their relationship with N availability indices: Fertilizer N application significantly increased the crop dry matter and N yields, which further increased with increasing the N application rate (P<0.05) (Table 5). At equivalent N application rates, urea applied alone produced the highest yields, followed by urea combined with FYM; whereas applying FYM alone produced the lowest. Considering the individual crop data, the dry matter and N yields did not correlate with the total N_{mic} flux under either crop, with the N_{mic} flux at the active growth stage, or with the N_{mic} flux beyond flowering stage (Table 6). None of the other N_{mic} parameters showed significant relationship with the crop dry matter and N yields. Among the indices of plant N availability, the total organic C and the total N of soil showed no relationship with the yield parameters. However, N uptake by wheat and maize crops was highly significantly correlated with the soil mineral N (P<0.001) and with the soil mineralizable N (P<0.01) (Table 6). Combining the data of both crops, crop dry matter and N yields were significantly correlated only with the annual mean of the soil mineral N (P<0.001) and of the soil mineralizable N (P<0.01) (Table 6).

Discussion

The close relationship observed between the N_{mic} and the total N is in agreement with some earlier reports (MacCarty *et al*., 1995; Moore *et al*., 2000), whereas the significant correlation between the N_{mic} and the total organic C was probably an indirect effect resulting from a highly significant correlation between the total N and the total organic C. The N_{mic} values (average, 133kg ha^{-1}; range, 80–218 kg ha^{-1}) recorded under the wheat-maize cropping system are within the range (64–186 kg ha^{-1}) reported for different arable soils of the Punjab region (Azam *et al*., 1986), but are much higher than those (51–76 kg ha^{-1}) reported for some other systems (Jensen *et al*., 1997 b; Moore *et al*., 2000). The increased N_{mic} due to mineral N application is also not consistent with earlier reports (Niether *et al*., 1996; Moore *et al*., 2000), which indicates that the belowground processes are limited by N availability in semiarid environment (Qishui & Zak, 1998)). Higher levels of microbial biomass have been attributed to favourable conditions including nutrient availability and available organic C (He *et al*., 1997). Under our experimental conditions, relatively high soil temperatures and wetting-drying cycles increased the turnover of microbial biomass thus causing high nutrient fluxes, a part of which might have been available to soil microbes also. Moreover, while fertilizer N application increased the overall N availability, the enhanced plant growth increased the C availability thus sustaining relatively higher microbial N pool in the fertilized treatments. However, relatively higher N_{mic} in treatments receiving FYM compared to urea treatments also indicates the direct effect of readily available C contained in FYM; the effect persisted up to two months following the FYM application. Higher N_{mic} values under organic than mineral N treatments conform to some earlier reports (Gunapala & Scow, 1998; Fließbach & Mäder, 2000), whereas suppression of the N_{mic} pool with increasing mineral N application has also been reported (McCarty & Meisinger, 1997).

The moderate degree of temporal variability in the N_{mic} compares reasonably with the variability (CV=11–22%) reported in other cropping systems (Jensen *et al*., 1997a, b). However, contrary to Wardle (1998), temporal fluctuations in the N_{mic} were not related to variations in the soil temperature, but were more influenced by the crop growth stage. The highest N_{mic} values recorded during the active growth period of wheat agree with the results of Nieder *et al*., (1996), indicating the contribution of plant-derived C in microbial N immobilization. The lower N_{mic} values at the wheat tillering stage coincided with the 30-day dry spell following pre-sowing irrigation that favoured the microbial decay. The lower N_{mic} values after the wheat flowering stage may be attributed to decreased substrate availability; at this stage, the soil mineral N had almost been exhausted due to crop uptake (point data not shown) and the C availability in the form of root exudates was probably also lower than that during the preceding active crop growth period.

Despite of the substantial N_{mic} flux, the flux did not correlate with the crop dry matter or N yields; nor did the N_{mic} flux during the active crop growth periods (planting to flowering) correlate with the yield parameters. Our results are contrary to those of Deng *et al*., (2000) who reported N_{mic} as a sensitive indicator of N mineralization and availability. Under our experimental conditions, it was not possible to elucidate dynamics of different N pools since labelled N was not used. However, it appears that the released N_{mic} did not synchronize with the crop N uptake. It was either transformed into non-biomass organic N, or was probably reutilized by the soil microbes competing with plant roots. Fluxes of N_{mic} at the wheat tillering stage, followed by much higher gains in N_{mic} at the stem elongation stage indicate a substantial microbial immobilization during the active crop growth period when the C availability was probably high due to root exudation. High N_{mic} fluxes also occurred during the period between wheat flowering and dough stage. We may not rule out that a part of the N_{mic} released was also susceptible to denitrification and leaching losses following irrigation, particularly during the later period of crop growth when the crop N uptake from soil is relatively low. Although a significant correlation existed (r=0.794; P<0.01) between the N_{mic} flux at grain-filling+dough stage of wheat and the concurrently measured denitrification loss during this period, the magnitude of denitrification loss (64–670 g N ha^{-1}), however, was a minor fraction of the N_{mic} released during this period (5.6–23.5 kg N ha^{-1}).

The present study compares the dynamics of N_{mic} in the plough layer under different fertilizer regimes. Considering processes in the deeper layers, the magnitude of N_{mic} flux might be higher than that recorded in the present study. However, the observed lack of relationship between microbial N flux and crop yield parameters suggested N_{mic} parameters as poor indicators of N mineralization and crop N availability, which conforms to some earlier reports (Bending *et al*., 1998; Puri & Ashman, 1998; Witt *et al*., 1998). Instead, the soil mineralizable N determined by alkaline-permanganate method (Hussain & Malik, 1985) proved to be a reliable index of the N availability, and the crop dry matter and N yields.

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