

ROOT-INDUCED CHANGES IN SOME BIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF SOIL SOWN TO WHEAT (*TRITICUM AESTIVUM* L.) AND CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

In a greenhouse experiment, wheat and chickpea were compared for root-induced changes in bacterial/fungal population of rhizospheric soil, microbial biomass, immobilization/loss of N applied as NH_4^+ and NO_3^- , dehydrogenase and nitrate reductase (NRA) and potential nitrification activity (PNA). Unplanted soil was used as a control for changes due to plant growth. Four varieties each of wheat and chickpea were grown in a greenhouse. Dry weight and % dry matter content of root and shoot portions were significantly different for the two crop types. On an average, varieties of chickpea gathered *ca* 2 times greater biomass pot^{-1} as compared to wheat. Both wheat and chickpea varieties had a significantly positive effect on dehydrogenase activity (DA) of the soil, the effect being significantly greater for the latter. Microbial biomass N content of planted soil that accounted for 2.7% - 3.5% of total N was significantly ($P = 0.05$) higher as compared to that of unplanted soil, was higher in chickpea than wheat rhizosphere, and was significantly correlated ($P = 0.05$; $r = 0.092$) with DA. On an average, the wheat varieties had an inhibitory effect on potential nitrification activity (PNA), while the chickpea varieties caused a significant ($P = 0.05$) increase over unplanted soil. With reference to unplanted soil, a significant ($P = 0.05$) inhibition of nitrate reductase activity (NRA) in wheat rhizosphere (>63%) and significant enhancement (16 folds) in chickpea rhizosphere was observed. Microbial biomass N content of unplanted soil was $13.9 \mu\text{g g}^{-1}$ soil compared to 18.5 and $22.1 \mu\text{g g}^{-1}$ soil planted to wheat and chickpea, respectively. It accounted for 2.7 - 3.5% of the total N under different crop varieties and was significantly correlated with DA ($r = 0.92$; $n = 9$; $P = 0.05$). Immobilization of labeled NH_4^+ was significantly higher in the rhizosphere soil of wheat than chickpea. A higher percentage of NO_3^- -N than NH_4^+ -N was immobilized in the rhizosphere soil of wheat compared to chickpea (30.2 vs 22.7 % of the applied), while more NO_3^- -N was unaccounted in the rhizosphere soil of chickpea than wheat (37.3 vs 16.8%).

Introduction

Fertility and productivity of soil depends to a significant extent on the rhizospheric microbial activities that influence plant growth and health in multiple ways including nutrient release/mobilization, N_2 fixation, production of hormones, and by affecting root growth and proliferation (Höflich *et al.*, 1994). As a source of carbonaceous materials deposited in soil through roots (rhizodeposition), plants are the major determinant of rhizospheric microbial dynamics, diversity and functions (Germida *et al.*, 1998; Jaeger *et al.*, 1999; Gomes *et al.*, 2001; Alvey *et al.*, 2003). In fact, the root exudates selectively influence the growth of bacterial and fungal populations by altering the presence of substrates in the vicinity of roots (Jaeger *et al.*, 1999; Yang & Crowley, 2000). The structural and functional diversity of rhizosphere population is therefore affected by differences in rhizodeposition, soil type, plant species, growth stage, cultural practices such as tillage and crop rotation and other environmental factors (De Leij *et al.*, 1994; Gomes *et al.*, 2001). These mutualistic effects play a significant role in ecosystem functioning.

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Extensive studies have been reported on microbial diversity and functions in the rhizosphere of individual plant types (Germida *et al.*, 1998; Gomes *et al.*, 2001). Of the different microbial parameters related to soil fertility and nutrient availability, microbial biomass occupies a key position as a small but labile component of the soil organic matter (Jenkinson & Ladd, 1981; Dick, 1992), while dehydrogenase is an overall indicator of soil microbiological activity (Skujins, 1976; Bolton *et al.*, 1985; Nannipieri *et al.*, 1990). With regards to dynamics and plant-availability of N, mineralization-immobilization turnover (MIT) and nitrification/denitrification occupy the key position. The process is influenced mainly by the form of available N and relative complexity of the C source available, the process being faster in the presence of easily oxidizable C sources and slowing down with the complexity of the latter (Azam *et al.*, 1988). Microbial immobilization of NH_4^+ -N is reported to be faster and preferred over NO_3^- -N (Jansson *et al.*, 1955, Rice & Tiedje, 1989) while remineralization of immobilized N is slower in NH_4^+ - than NO_3^- -treated soil (Herrmann *et al.*, 2005). Under conditions of sufficient C supply, however, immobilization of both NH_4^+ - and NO_3^- -N may not be very different (Azam *et al.*, 1993). Plant roots will affect immobilization/remineralization turnover of N through C and N rhizodeposition that differ in both quantitative and qualitative terms. Besides MIT, both nitrification and denitrification are important components of the N cycle in soil (Granli & Bockman 1994). The former being mainly autotrophic (Wrage *et al.*, 2001) is strongly influenced by CO_2 (Azam *et al.*, 2005), while latter is driven by easily oxidizable C sources (Beauchamp *et al.*, 1989). Since plants are the predominant source of both organic C (released in soil as rhizodeposits; Gregory & Atwell, 1991; Kuzyakov & Domanski, 2000) as well as CO_2 (resulting from rhizorespiration; Kuzyakov & Domanski, 2002; Azam & Farooq, 2005; Kuzyakov, 2006), they will exert a significant influence on nitrification and denitrification processes. This influence will differ with the plant type as rhizodeposition and rhizorespiration vary between species both qualitatively and quantitatively (Grayston *et al.*, 1996). While denitrification may serve as a conduit for excessive amounts of NO_3^- in legumes, inhibition of nitrification could be advantageous for the non-legumes, particularly the cereals that are known to perform better when both NH_4^+ and NO_3^- are available in the soil (Gentry & Below, 1993; Gill & Reisenbauer, 1993).

While literature is available on root-induced changes in rhizospheric microbial/biochemical processes, a comparison of leguminous and non-leguminous plants is generally lacking. Studies are particularly needed to compare chickpea and wheat that are the major and traditional winter crops of arid and semi-arid regions. In recent years, increasing concerns about soil and environmental quality and economic innovations has stimulated changes in cropping systems with emphasis on crop diversification through rotation as well as mixing. In this context, chickpea has become an important option for either of the practices. Whether grown in rotation or mixed, wheat and chickpea will have diverse influences on rhizospheric microbial activities. Previously, we have reported root modifications in wheat in response to hormone treatment (Azam *et al.*, 2005) and root-induced changes in potential nitrification and nitrate reductase in the rhizosphere of wheat and chickpea (Gill *et al.*, 2006; 2008). Objectives of the present studies were to compare the two crops for growth and root-induced changes in some biochemical parameters as an indirect measure of plant influences. The parameters studied were i) microbial biomass N, ii) activity of dehydrogenase, potential nitrification, and nitrate reductase, and iii) immobilization and/or loss of NH_4^+ and NO_3^- .

Materials and Methods

Soil: Soil was collected from the top 0-15 cm of an experimental field at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Air-dried and sieved (<2mm) soil had the following characteristics: pH (1:1, soil: water suspension), 7.6; electrical conductivity (EC), 0.78 d Sm⁻¹; organic C, 0.58%; total N, 0.065%; NH₄⁺-N, 3.1 mg kg⁻¹ soil; NO₃⁻-N, 8.9 mg kg⁻¹ soil; sand, 30%; silt, 31%; clay, 39%; and water-holding capacity, 30%.

Plants: Four varieties of wheat (*Triticum aestivum* L.) i.e., U-2000, Inqlab, Chenab, and WL-1076 and four of chickpea (*Cicer arietinum* L.) i.e., 98004, P-2000, 90261, and 97086 were used in the study. Except for the wheat variety WL-1076 that has been produced through wide hybridization (Farooq *et al.*, 1995), the remaining varieties are the outcome of conventional breeding.

Pot experiment: Portions of soil (500 g) contained in plastic pots (9 x 20 cm) were fertilized with a solution of ammonium sulphate and potassium dihydrogen phosphate to obtain N, P and K concentration of 25, 25 and 32 mg kg⁻¹ soil, respectively. Fifteen seeds of wheat and 8 of chickpea were planted per pot using triplicate pots for each variety. Triplicate pots were left unplanted to serve as control against root-induced changes. After germination, the crop stand was thinned to 10 and 5 seedlings pot⁻¹ in case of wheat and chickpea, respectively. Our experience shows that this plant number was sufficient to achieve effective root distribution in the entire soil mass and to get good rhizospheric effect. Both planted and unplanted pots were weighed twice daily and the loss in weight made up by adding water to maintain the moisture content of the soil to 15% (w/w) throughout the experimental period. The pots were placed in a netted house with day/night temperature of 20/14 °C, relative humidity 40-52%, and photosynthetic photon flux density of 750-975 μmol m⁻² s⁻¹.

After 4 weeks of growth, the entire soil-plant system was removed from the pots by gently tapping the pots. Before this, however, it was determined that soil moisture content was about 13-14%, a moisture level suitable for removing root-adhering soil with minimum root breakage according to our experience. The soil adhering to the roots was removed by gentle tapping of the root system with hand. The potted soil was considered as rhizosphere, while that from the unplanted pots as non-rhizosphere or bulk soil. In either case, the wet soil was passed through 1 mm sieve and visible pieces of roots removed to the maximum extent. The roots were washed free of remnants of soil using running tap water and blotted. Root and shoot portions were weighed fresh and then dried at 65°C.

Nitrogen transformations: The potential of rhizosphere and non-rhizosphere soil to immobilize and/or loose inorganic N was determined after moistening 20 g portions of relatively dried soil (10% moisture) by adding appropriate volumes of (¹⁵NH₄)₂SO₄ and K¹⁵NO₃ (atom % ¹⁵N excess of 2.0211 and 2.0234, respectively) solutions so as to have a N concentration of 100 mg kg⁻¹ and soil moisture content of 15% (w/w). The treated soil samples in triplicate were incubated at 30°C for one week and extracted with 100 ml of 2N KCl (60 min shaking on a reciprocating shaker followed by centrifugation at 5000 g). Supernatant was recovered and the residue washed twice with 25 ml of 1N KCl. All the KCl fractions were pooled and analyzed for NH₄⁺ + NO₃⁻-N and ¹⁵N. The residual soil was freeze-dried and portions analyzed for total N and ¹⁵N. The proportion of applied N

(expressed as percent) in KCl-extracted residual soil (organic N) was attributed to N immobilization. Applied N not accounted for in organic and inorganic forms was assumed to be lost from the soil.

Analyses: Organic C in soil was determined using a modified wet oxidation method (Azam & Sajjad 2005). Steam distillation method was used for the quantification of NH_4^+ or NO_3^- -N in the KCl extracts (Keeney & Nelson, 1982) and total N in the Kjeldahl digests (Bremner, 1996). The distillates obtained were titrated and processed for ^{15}N isotope ratio analysis using a mass spectrometer (Mulvaney *et al.*, 1997). Nitrogen derived from the applied NH_4^+ or NO_3^- was calculated by using isotope dilution equations as described by Rennie *et al.*, (1978). Analyses of EC, pH and texture were performed using methods described in USDA Handbook 60 (Richards, 1954). Microbial biomass N was determined by the chloroform-fumigation extraction method of Wits *et al.*, (2000). Difference in the 0.5M K_2SO_4 -extractable Kjeldahl-N (Bremner 1996) of fumigated and unfumigated soil samples was divided by 0.54 (Brookes *et al.*, 1985) for calculating microbial biomass N. Dehydrogenase activity, potential nitrification and nitrate reductase activity were determined as described by Schinner *et al.*, (1996).

Statistical analyses: Significance of differences between treatment means was determined using the SAS statistical package (SAS Institute, 1998). Microsoft Excel software was employed for calculating means and coefficient of correlations.

Results and discussion

Plant growth: During 4 weeks of growth, average root dry matter of wheat varieties was similar to that of chickpea varieties with no root accumulation at the bottom of the pots suggesting that root length was not restricted by the depth of soil column. Shoot dry matter and total plant biomass was 80% and 44% higher, respectively, in chickpea as compared to wheat in spite of the fact that the number of plants pot^{-1} was twice in the latter; inter-variety differences within a crop type were significant in most cases. The difference in biomass of the two crop types could partly be attributed to the seed reserves, particularly the protein content and concentration. Chickpea grain weighed 5 times more than wheat grain (268 mg vs 55 mg) and contained 9 times more proteins (62 mg vs 7 mg seed^{-1}) and hence would have helped plants sustain higher photosynthesis and accumulation of dry matter over the short period of 4 weeks as both the crops had similar amounts of available N (added + native) in the soil and no nodules were formed on chickpea roots.

Percent dry matter content of roots was significantly lower and that of shoot significantly higher in chickpea as compared to wheat. Thus chickpea roots appeared to maintain higher water content in the roots as compared to wheat when both were grown under similar conditions of soil moisture. The difference in root water content could be an important factor in determining plant growth under water limiting conditions. Of the 4 wheat varieties, WL-1076 had lowest root dry matter content (and thus highest water content). This wheat has been produced by crossing bread wheat with a wild grass *Aegilops cylindrica* (Farooq *et al.*, 1995) and is reported as tolerant to low water availability and soil salinity (Farooq & Azam, 2001). The observations on water content of roots though not very relevant to this study *per se* do suggest that this parameter could be important in influencing plant growth and root-induced rhizospheric microbial functions. Some of our unpublished studies suggest that root water content could serve as a selection criterion against salinity and drought stress, while plant types that withstand water stress maintain higher microbial activity in the rhizosphere.

Table 1. Fresh biomass and water concentration of root and shoot portions of different varieties of wheat and chickpea.

Crop/variety	Dry matter, g pot ⁻¹			% Dry matter	
	Root	Shoot	Total	Root	Shoot
Wheat					
U-2000	0.54b	1.13c	1.67de	16.22a	18.26bc
Inqlab	0.57b	0.79e	1.37f	13.74b	14.58e
Chenab	0.65a	0.92d	1.58e	16.33a	17.78c
WL-1076	0.54b	0.86de	1.40f	12.28b	13.23f
Average	0.58	0.92	1.50	14.64	15.96d
Chickpea					
98004	0.54b	1.66b	2.19b	7.85e	18.64b
P-2000	0.44c	1.30b	1.73d	8.89d	16.60d
90261	0.52b	1.45b	1.97c	9.28c	16.48d
97086	0.64a	2.12a	2.76a	8.93d	20.01a
Average	0.53b	1.63b	2.16b	8.74d	17.93c

*Values sharing a similar letter for a parameter in a column are not significantly different from each other at 5% level of probability

Dehydrogenase enzyme activity and microbial biomass: Dehydrogenase is considered an indicator of microbial activity in soil (Skujins, 1976; Nannipieri *et al.*, 1990) as it is linked with respiratory processes (Bolton *et al.*, 1985). Hence, changes in the activity of this enzyme could provide a useful index of soil quality vis-à-vis availability of carbonaceous materials to rhizospheric microflora (Dick, 1992; Visser & Parkinson, 1992). The differences in DA could also be used as an indirect measure of rhizodeposition. In the present study, both wheat and chickpea varieties had a significantly positive effect on DA of the soil, the effect being significantly greater for the later although the root mass was statistically similar in both crop types (Table 2). This suggests that chickpea supports higher microbial activity that differed significantly with the varieties. Higher plant biomass of chickpea varieties (Table 1) and consequently higher rhizodeposition would have led to enhanced rhizospheric microbial activities including DA. The inter-varietal difference in DA was observed for both the crops but chickpea varieties were more diverse compared to wheat varieties (deviation for wheat and chickpea varieties was 20 and 80%, respectively). A significant correlation between DA and total plant biomass ($r = 0.91$, $n = 8$) and DA and shoot biomass ($r = 0.92$, $n = 8$) supports this contention. A close relationship between soil microbial biomass and rhizodeposition has also been reported (Kuzyakov & Domanski, 2002), while 13-16% of the total plant N and 80% of the below-ground plant N could be attributed to rhizodeposits in legumes (Mayer *et al.*, 2003). In addition, rhizodeposits from leguminous plants are reported not only to be greater in quantity compared to non-legumes but more labile as well (Jensen, 1996).

Microbial biomass N content of planted soil was significantly higher as compared to that of unplanted soil i.e., 18.5 and 22.1 $\mu\text{g g}^{-1}$ soil in wheat and chickpea, respectively, as compared to 13.9 $\mu\text{g g}^{-1}$ in unplanted soil (Table 2); an observation in line with other reports (Wheatley *et al.*, 1990). Microbial biomass N accounted for 2.7–3.5% of the total N under different crop varieties and was significantly correlated with DA ($r = 0.92$; $n=9$; $P=0.05$). The differences within chickpea varieties were also generally significant, while wheat varieties were similar in this respect. The two crop types not only differed in

Table 2. Deyhydrogenase activity (DA) and microbial biomass N in unplanted soil and soil planted to different varieties of wheat and chickpea.

Crop/variety	DA, $\mu\text{g TPF g}^{-1} \text{soil h}^{-1}$	0.5M K ₂ SO ₄ -extractable N, $\mu\text{g g}^{-1} \text{soil}$		Biom-N, $\mu\text{g g}^{-1} \text{soil}$	Biomass-N, % of soil N	DA: Biom-N
		Unfumigated	Fumigated			
Wheat						
U-2000	167.9d*	5.9bc	15.3c	17.3d	2.7d	9.71c
Inq-91	192.5c	5.6bc	15.2c	17.8d	2.7d	10.81b
Chenab	174.2d	6.3b	16.5c	18.9d	2.9d	9.22c
1076	169.2d	5.4c	16.3c	20.1cd	3.1cd	8.42d
Average	176.0	5.8	15.8	18.5	2.9	9.51
Chickpea						
98004	249.5b	6.9ab	20.8a	25.8a	4.0a	9.67c
P-2000	180.2cd	6.3b	17.5bc	20.8c	3.2c	8.66d
90261	171.8cd	5.4c	16.0c	19.5cd	3.0cd	8.81d
97086	277.7a	7.4a	19.6a	22.5b	3.5b	12.34a
Average	221.3	6.5	18.5	22.1	3.4	10.01
Unplanted	123.0e	4.8d	12.2d	13.9e	2.1e	8.85d

*Values sharing a similar letter in a column are not significantly different from each other at 5% level of probability

microbial biomass N, but significantly ($P=0.05$) in the amount of extractable N from unfumigated soil as well (average for different varieties of wheat and chickpea being 5.8 and 6.5 $\mu\text{g g}^{-1}$ soil, respectively) suggesting that chickpea maintains a higher proportion of N in relatively labile fractions. In a pot experiment, Breland and Bakken (1991) found that microbial biomass N was higher in soil cropped to clover than that cropped to barley and ryegrass. Such differences have also been reported in studies on crop rotations. For example, Balota *et al.* (2003) found higher microbial biomass N in soil under soybean-wheat rotation than that under maize-wheat or under cotton-wheat rotations.

Potential nitrification and nitrate reductase activity: The two crop types differed significantly (Table 3) in root-induced effects on potential nitrification activity (PNA) and nitrate reductase activity (NRA) in the rhizosphere soil. Unplanted soil was used as a reference for both the assays. On an average, the wheat varieties had an inhibitory effect on PNA with Chenab and WL-1076 being significantly different from others as well as amongst themselves. In comparison to wheat, all the chickpea varieties caused a significant increase in PNA over unplanted soil; average increase for different varieties was 98%. A significant inhibition of NRA in wheat rhizosphere ($> 63\%$) and significant enhancement (16 folds) in chickpea rhizosphere was observed with reference to unplanted soil. Wheat varieties were not significantly different from each other. The rhizosphere is generally thought to be inhibitory to nitrification (Schmidt, 1982), but stimulation has been reported in some crops (Berg & Rosswall, 1987). Gramineous plants including wheat are known to release allelochemicals in the soil that are inhibitory to nitrification (Sanchez-Moreias *et al.*, 2003).

When data for different varieties within a crop type were averaged, PNA and NRA were *ca* 2 and 45 times higher, respectively, in chickpea as compared to wheat. As expected, the two parameters were significantly correlated ($r = 0.97$, $n = 9$), an observation in conformity with the reports that show dependence of nitrate reductase on nitrate (Beevers & Hagemann, 1980; Caba *et al.*, 1995). Thus the plant types that encourage nitrification may induce enhanced NO_3^- reduction as well due to the improved level of NO_3^- availability. However, ratio of NRA/PNA suggested that chickpea varieties are more efficient in inducing NO_3^- reduction than nitrification. The same was not true for wheat varieties where NRA was not induced to the extent observed in chickpea although NO_3^- was being formed at rates similar to that of the unplanted soil and only slightly (though significantly) less than in soil planted to two of the chickpea varieties i.e., P-2000 and 90261. Average ratio for 4 chickpea varieties was *ca* 21 times higher as compared to wheat varieties (Table 3) suggesting that in the chickpea rhizosphere, NO_3^- will be much more quickly removed through increased rhizospheric NRA; a higher uptake and transport of NO_3^- in leguminous than non-leguminous plants has been reported (Rao *et al.*, 1991). It was interesting to note that chickpea varieties P-2000 and 90261 were not different in affecting PNA, but the latter was >3 times more efficient in reducing NO_3^- as compared to the former. Such variations are useful in the sense that the varieties with a higher NRA could support higher nodulation and N_2 fixation.

The removal of NO_3^- through reduction from the chickpea rhizosphere can be considered advantageous for the process of N_2 fixation that is inhibited more by NO_3^- than NH_4^+ (Wasfi & Prioul, 1986; Marschner, 1995); the latter being reported as a better source of N than NO_3^- in legumes (Atwell, 1992). However, legume species differ in the degree to which soil NO_3^- impairs legume nodulation and N_2 fixation (Tang *et al.*, 1999).

Table 3. Potential nitrification (PNA) and nitrate reductase activity (NRA) in planted and unplanted soil.

Crop/variety	$\mu\text{g NO}_2^- \text{ g}^{-1} \text{ soil h}^{-1}$		NRA/PNA
	PNA	NRA	
Wheat			
U-2000	0.720d	0.022e	0.031d
Inqlab	0.704d	0.006e	0.007e
Chenab	0.656e	0.018e	0.027d
WL-1076	0.588f	0.014e	0.024d
Average	0.667	0.014	0.021
Chickpea			
98004	2.020a	0.962a	0.477b
P-2000	0.870c	0.158d	0.182c
90261	0.848c	0.542c	0.640a
97086	1.802b	0.836b	0.464b
Average	1.385	0.626	0.440
Unplanted	0.701d	0.030d	0.043d

*Values in a column sharing a similar letter are not significantly different from each other at 5% level of probability

Immobilization/loss of NH_4^+ and NO_3^- -N: Unplanted soil contained significantly lower amounts of NH_4^+ and higher amounts of NO_3^- as compared to cropped soil at the time of harvesting plants (Table 4). Wheat and chickpea had a significantly different effect on the relative amounts of the two N forms. Soil planted to chickpea had >2 times higher NO_3^- -N as compared to wheat soil suggesting thereby that the nitrification rate was higher in the former than in the latter. These observations confirm that potential nitrification activity was higher in the rhizosphere soil of chickpea than in the rhizosphere soil of wheat (Table 3). In addition, the chickpea soil may maintain higher inorganic N content due to the release of N-rich rhizodeposits a part of which is constantly being mineralized and nitrified. The same may not be true for wheat rhizodeposits that will have low N concentration and a wider C/N ratio. As stated earlier, N rhizodeposition is not only higher in legumes but the compounds are also more labile in terms of N release (Jensen, 1996).

In the present study, unplanted soil and the rhizosphere soil obtained after removing the plants were incubated with ^{15}N -labeled NH_4^+ or NO_3^- for 7 days. Of the applied NH_4^+ -N, ca 60% was determined as NH_4^+ + NO_3^- -N in both wheat and chickpea varieties with negligible inter-crop and inter-varietal differences. However, immobilization of NH_4^+ was significantly higher in the rhizosphere soil of wheat than chickpea; average for 4 varieties being 35.8 and 24.2% of the applied, respectively. This difference could be attributed to the C/N ratio of the rhizodeposits that is understandably wider in case of wheat as compared to chickpea. Jensen (1996) reported 79% and 48% of the pea and barley root N, respectively, to be deposited in soil, while a greater immobilization of N in the presence of plant residues with wider C/N ratio is a common observation (Azam *et al.*, 1988). Inter-varietal difference for wheat was non-significant, while that for chickpea it varied in significance. In unplanted soil also, immobilization of NH_4^+ -N was almost comparable to that observed in wheat soil and could be attributed to the release of easily decomposable carbonaceous materials during partial drying before incubation. The labile pool of organic C would have been exhausted (augmented by rhizodeposits at the same time) in soil planted to wheat but not in unplanted soil. Root-induced enhancement in the

Table 4. Mineral N status of planted (at harvest) and unplanted soil and recovery of N applied as $(^{15}\text{NH}_4)_2\text{SO}_4$ and K^{15}NO_3 in organic, inorganic and unaccounted (UA) forms after 7 days of incubation. Coefficient of variance for triplicate analyses ranged between 3 and 8%.

Crop/variety	Mineral N status of soil at plant harvest				% Recovery of applied N after 7 days incubation with					
	$\mu\text{g g}^{-1}$ soil				$(^{15}\text{NH}_4)_2\text{SO}_4$			K^{15}NO_3		
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	NH_4^+	$\text{NO}_3^-\text{-N}$	Inorganic	Organic	UA	Inorganic	Organic	UA
Wheat										
U-2000	5.3b	4.1h	9.4g	9.4g	60.9ab	34.9b	4.2e	53.7ab	29.1a	17.2de
Inq-91	7.1a	5.1f	12.2e	12.2e	59.1b	35.2b	5.7d	51.1b	30.9a	18.0d
Chenab	5.4b	5.2f	10.6f	10.6f	62.6ab	34.8b	2.6g	55.7a	29.0a	15.3g
1076	3.3c	3.1g	6.4h	6.4h	58.5ab	38.2a	3.3f	51.6b	31.8a	16.5ef
Average	5.3	4.4	9.7	9.7	60.3	35.8	4.0	53.0	30.2	16.8
Chickpea										
98004	4.9b	13.8b	18.7b	18.7b	61.7ab	20.8e	17.5a	42.1c	19.9c	38.0ab
P-2000	5.1b	11.4c	16.5c	16.5c	59.9b	26.8d	13.3b	36.9d	28.8a	34.3b
90261	3.0c	8.3d	11.3f	11.3f	59.6b	29.2c	11.2c	37.8d	25.4b	36.8ab
97086	4.0c	10.3e	14.3d	14.3d	62.0ab	20.1e	17.9a	43.2c	16.8d	40.1a
Average	4.2	10.9	15.2	15.2	60.8	24.2	15.0	40.0	22.7	37.3
Unplanted	1.4d	22.3a	23.7a	23.7a	65.1a	32.8b	2.1h	51.3b	26.5b	23.2c

*Values in a column sharing a similar letter are not significantly different from each other at 5% level of probability

decomposition of soil organic matter has been reported (Christensen, 1987; Personeni & Loiseau, 2004). The loss of $\text{NH}_4^+\text{-N}$ was significantly more from the rhizosphere soil of chickpea than wheat (15 vs 4% of the applied) suggesting a rapid nitrification followed by denitrification. This suggestion is supported by the results of nitrification potential and nitrate reductase activities (Table 3). A higher percentage of $\text{NO}_3^-\text{-N}$ than $\text{NH}_4^+\text{-N}$ was immobilized in the rhizosphere soil of wheat compared to chickpea (30.2 vs 22.7 % of the applied), while a reverse was true for $\text{NO}_3^-\text{-N}$ unaccounted (16.8 vs 37.3%). Again, the differences could be attributed to the C/N ratio of the rhizodeposits and a higher potential of nitrification/denitrification in the chickpea rhizosphere. Losses of $\text{NO}_3^-\text{-N}$ from unplanted soil were significant. These were higher than that from wheat but lower than that from chickpea rhizosphere soil. Release of easily decomposable carbon sources during disturbance and partial drying could have led to denitrification from unplanted soil. Myrold and Tiedje (1985) suggested that denitrifiers compete effectively with heterotrophs for available C.

Concluding remarks

Widely divergent root-induced changes in rhizospheric microbial functions of leguminous and non-leguminous crops could have significant implications. Besides the benefits arising from N_2 fixation, the leguminous crop could exert a positive effect on the associated/succeeding non-leguminous crop by affecting soil microbial functions beneficial to the latter. In the present study, chickpea had a more positive effect compared to wheat on different microbial functions. Of particular significance for the crop that follows chickpea will be the build up of higher microbial biomass (and thus transformation of nutrients into more labile forms) and enhanced microbial activity in terms of dehydrogenase.

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