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Ammonium *versus* nitrate nutrition of plants stimulates microbial activity in the rhizosphere

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Abstract

Using an alkaline calcareous soil, pot experiments were conducted to elucidate the effects of NH_4^+ vs. NO_3^- nutrition (50 or 100 mg kg^{-1} soil) of wheat and maize on microbial activity in the rhizosphere and bulk soils. Dicyandiamide was used as nitrification inhibitor to maintain NH_4^+ as the predominant N source for plants grown in NH_4^+-treated soil. While maize grew equally well on both N sources, root and shoot growth of wheat was higher under NH_4^+ than under NO_3^- nutrition. Bacterial population density on roots, but not in the rhizosphere soil, was higher under NH_4^+ than under NO_3^- supplied at 150 mg N kg^{-1} soil; whereas at both N levels applied, NH_4^+ compared to NO_3^- nutrition of wheat and maize significantly increased microbial biomass in the rhizosphere soil. Under both plant species, NH_4^+ vs. NO_3^- nutrition also increased aerobic and anaerobic respiration, and dehydrogenase activity in the rhizosphere. As microbial activity in the planted bulk and unplanted soils was hardly affected by the N-source, we hypothesize that the stimulation by NH_4^+ of the rhizosphere microbial activity was probably due to higher availability of root exudates under NH_4^+ than under NO_3^- nutrition.

Introduction

Increased microbial activity in the rhizosphere compared to bulk soil is a well established phenomenon and attributed to root-derived organic compounds known as rhizodeposits (Cheng et al., 1996; Kuzyakov and Domanski, 2000; Kuzyakov et al., 2002; Nobili et al., 2001; Qian et al., 1997). The amount of the root-born C compounds released into the rhizosphere may be as much as 40% of the net CO_2 assimilation in annual species, whereas values as high as 70% have also been reported for forest species like Douglas fir (Lynch and Whipps, 1990). Sugars, organic acids and amino acids are considered as the major constituents of the low-molecular weight root exudates, with sugars being in highest (65%) and amino acids in lowest (2%) proportion (Kraffczyk et al., 1984). Factors controlling the qualitative and quantitative release of root exudates include: plant species and developmental stage, soil physical stress factors, oxygen supply, mechanical or disease injury, herbivory, foliar-applied chemicals, presence of microbes, plant nutritional status, and nitrogen source (Grayston et al., 1998; Holland et al., 1996; Mahmood et al., 2002; Marschner, 1995; Smucker and Erickson, 1987; Warembourg et al., 2003). The stimulated microbial activity around roots increases the mineralization of native soil organic matter, thus leading to substantial nutrient fluxes (Kuzyakov, 2002). On the other hand, since microbes may also contribute to as high as

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27–66% of the CO_2 released in the rhizosphere (Gregory and Atwel, 1991; Keith et al., 1984; Swinnen et al., 1995; Trolldenier, 1972), high oxygen consumption by the rhizosphere microflora may impair root and shoot growth when soil oxygen is in short supply (Geisler, 1969).

Considerable information has been documented regarding the effect of N source on various aspects of plant growth and metabolism (Chaillou et al., 1991; Cramer and Lewis, 1993; Lang and Kaiser, 1994; Mahmood and Kaiser, 2003; Martins-Loucao et al., 2000; Walch-Liu et al., 2000). However, the impact of the N source on processes in the rhizosphere is relatively less well understood in soil (Söderberg and Bååth, 2004), and most of the studies dealing with this aspect have been carried out in hydroponics (Brown and Hornby, 1987; Marschner et al., 1999; Martins-Loucao et al., 2000; Trolldenier and Rheinbaban, 1981). Roots of NH_4^+- compared to NO_3^--grown plants often possess higher sugar levels (Martins-Loucao et al., 2000). Thus NH_4^+-grown plants may differ from NO_3^--grown plants with respect to passive sugar efflux from roots. Roots of NH_4^+- compared to NO_3^--fed plants are more highly branched (Martins-Loucao et al., 2000). Considering the major sites of exudation either root apices or the points of lateral root emergence (Egeraat, 1975; Frenzel, 1960; Schroth and Snyder, 1961), differences in root exudation driving rhizosperic microbial activity might also be expected in response to changes in the root architecture depending on the N source. Roots of kallar grass [*Leptochloa fusca* L. (Kunth)] indeed showed much higher sugar exudation under NH_4^+ than under NO_3^- nutrition, though the mechanism of increased exudation was unknown (Mahmood et al., 2002). Higher root and rhizosphere respiration with NH_4^+ than NO_3^- nutrition has been ascribed to increased root exudation and hence stimulation of bacterial growth (Trolldenier and Rheinbaban, 1981). The increased substrate availability in the rhizosphere of plants grown on NH_4^+ instead of NO_3^- may also have significant bearing on the root colonization and activity of the beneficial microorganisms as well as of root pathogens. Increased lesion severity of the take-all fungus *Gaeumannomyces graminis* under NH_4^+ compared to NO_3^- nutrition of wheat has been attributed to higher root sugar exudation (Brown and Hornby, 1987). Increased wheat root colonization by *Pseudomonas fluorescens* under NH_4^+ compared to NO_3^- nutrition was also related to impaired exudate retention due to high H^+ concentration in the rhizosphere or the apoplast (Marschner et al., 1999).

Studies with hydroponics suggested that NH_4^+ compared to NO_3^- nutrition of plants increases the microbial activity in the rhizosphere, and that this stimulatory effect is attributable mainly to the increased C availability in the rhizosphere as the result of enhanced root exudation. However, it is not known whether the observed stimulatory effect of NH_4^+ also prevails in soil-grown plants. In arable soils, though the added NH_4^+ is nitrified rapidly, it may become the predominant N source, at least for a certain period of time, when nitrification inhibitors are employed to control NO_3^- loss via leaching or denitrification. Besides, due to inhibition of nitrification, NH_4^+ may be the predominant N form in forest ecosystems (Lodhi and Killingbeck, 1980) or in saline habitats (Cartaxana et al., 1999). The present study was carried out to elucidate the comparative effects of NH_4^+ vs. NO_3^- nutrition on different indices of microbial activity in the rhizosphere of wheat and maize plants grown in soil. We selected wheat and maize since the two species respond differently to NH_4^+ compared to NO_3^- nutrition when grown in hydroponics (Cramer and Lewis, 1993).

Materials and methods

Soil

The soil (Hafizabad series; Typic Ustocrept) was a sandy clay loam collected from an experimental field at the Nuclear Institute for Agriculture & Biology Faisalabad and had been under a wheat–maize rotation for the past 20 years. The (0–20 cm) soil had the following physicochemical characteristics: bulk density, 1.52 g cm^{-3}; water-holding capacity (WHC), 35%; pH (saturation paste), 7.8; electrical conductivity, 0.66 dS m^{-1}; $CaCO_3$, 2.02%; total organic carbon, 0.78%; total N, 0.07%; NH_4^+-N, 10.53 mg kg^{-1} and NO_3^--N, 23.0 mg kg^{-1}. The soil was air-dried, sieved (< 2 mm) and stored at room temperature until used.

Experiments

In a preliminary experiment with different nitrification inhibitors, only dicyandiamide proved to be effective in this soil and inhibited nitrification when applied at 20 mg kg^{-1} (at 16 °C, the maximum soil temperature in the wheat field) to 30 mg kg^{-1} (at 33 °C, the maximum soil temperature in the maize field).

Before each experiment, the NO_3^--N was reduced by incubating the soil at 100% WHC with 2 g glucose kg^{-1}; after 1-week of incubation at 30 °C, soil NO_3^- and the glucose had been completely consumed. The soil was again air dried and sieved (< 2 mm), and filled in pots (11.5 × 11 cm, diameter × depth, accommodating 1 kg soil). Soil was mixed with 50 mg P kg^{-1} (as single super phosphate) and of 50 mg K kg^{-1} (as potassium sulphate). Pots (four replicates) were treated either with a solution (equivalent to 60% WHC) of $(NH_4)_2SO_4$ (NH_4^+ treatments), or of KNO_3 (NO_3^- treatments), each with two N levels, either 50 mg N kg^{-1} or 150 mg N kg^{-1} soil. Dicyandiamide at 20 mg kg^{-1} soil was applied (along with N solutions) to both NH_4^+ and NO_3^- treatments in order to balance the side effects of the nitrification inhibitor. Fifteen seeds of wheat (*Triticum aestivum* L. cv. Inqalab-91) were sown in each pot and upon germination, plants were reduced to 10 pot^{-1}. After germination, the wheat plants were grown for 4 weeks. Unplanted pots (four replicates) were also kept for each N treatment. The experiment was conducted under greenhouse conditions in November–December with soil temperature ranging between 12.5 and 19.0 °C. The soil moisture content was maintained at 60% WHC throughout the experiment period.

Conditions for the experiment with maize were the same as for wheat, except that dicyandiamide was applied at 30 mg kg^{-1} soil. Fifteen seeds of maize (*Zea mays* L. cv. Composit-20) were sown in each pot leaving 10 plants pot^{-1}. After germination, the maize plants were grown for 2 weeks. The experiment with maize was conducted in October with soil temperature ranging from 21.5–25.5 °C.

Plant harvest and isolation of the rhizosphere soil

Soil was knocked out of the pots and the plants were shaken (60 s) on a vibrating arm shaker to remove the loosely adhering soil. The soil tightly adhering to the roots was defined as the rhizosphere soil, whereas the rest was considered as planted bulk soil. For isolation of the rhizosphere soil (Priha et al., 1999), intact roots systems were transferred to 120-ml glass bottles containing 25 ml distilled H_2O. The bottles were vortexed (5 s), sonicated in an ultrasonic water bath (60 s) and vortexed again (10 s). The planted bulk and unplanted soils were moist-sieved to pass a 2-mm screen. Soils were not stored and all measurements commenced within 2 h of the soil processing. For dry weight determination, portions of the rhizosphere soil suspension and those of planted bulk and unplanted soils were dried at 105 °C for 48 h. The rhizosphere soil suspension contained ca. 10% soil on dry weight basis. Plants were fractioned into roots and shoots, dried at 70 °C to a constant weight, and ground (< 0.5 mm) before analysis of total N (Bremner and Mulvaney, 1982).

Bacterial population density and soil microbial biomass

The bacterial population density on roots (fresh root biomass obtained after isolation of the rhizosphere soil) and in soil was determined by plate count method using tryptic soy agar medium as described by Wollum (1982). For the measurement of soil microbial biomass, $CHCl_3$ fumigation–incubation method of Jenkinson and Powlson (1976) was followed using ethanol-free $CHCl_3$ and 10 ml of the rhizosphere soil suspension or 10-g portions of the moist planted bulk and unplanted soils. Before microbial biomass measurements, the soils were conditioned for 72 h at 30 °C. In a preliminary test, fumigation of the rhizosphere soil suspension as described by Priha et al. (1999) did not work for $CHCl_3$ fumigation–incubation procedure, whereas mixing the soil suspension with appropriate amounts of inert sand made it possible to fumigate the soil and to remove $CHCl_3$ vapors. Moreover, in a preliminary test, aerobic incubation of the soil suspension evolved much lower CO_2 as compared to the soil suspension mixed with inert sand to facilitate gaseous diffusion (data not shown). Consequently, to overcome diffusional constraints, we added 20 g of dry inert sand to the rhizosphere soil suspension. The sand was also added to the planted bulk and unplanted soils and the moisture

content adjusted. Prior to use, the sand was heated at 550 °C for 6 h, washed with HCl and then with distilled H_2O, and dried. No CO_2 was evolved from the moist sand control during a 10-d incubation. The fumigated-reinoculated (with 1% respective fresh soil) and unfumigated (control) soils were incubated at 30 °C for 10 days in air-tight 100-ml serum vials, and the headspace analyzed for CO_2 by gas chromatography using a thermal-conductivity detector. Microbial biomass C was calculated as: $SMBC = F/k_C$; where F is the flush of CO_2-C (CO_2-C evolved from fumigated minus that from the unfumigated) and k_C is the recovery factor, the value of which was taken as 0.41 (Voroney and Paul, 1984).

Soil respiration, denitrification capacity, dehydrogenase activity and mineral nitrogen

For aerobic soil respiration, 10 ml of the rhizosphere soil suspension or 10-g portions of the planted bulk and unplanted soils with 10 g sand contained in 100-ml gas tight serum vials were incubated at 30 °C for 24 h. The headspace was analyzed for CO_2 by gas chromatography. For the measurement of denitrification capacity (an index of the C availability to denitrifiers; Smith and Tiedje, 1979) and anaerobic soil respiration, 10 ml of the rhizosphere soil suspension or 10-g portions of the planted bulk and unplanted soils in 100-ml serum vials were treated with of KNO_3 solution to provide the NO_3^--N concentration equivalent to 200 mg kg^{-1} soil and the final H_2O level equivalent to 12 ml $bottle^{-1}$. Vials were sealed, made anaerobic by evacuation and flushing with O_2-free N_2 (three times), the head space was replaced by 5% acid-washed C_2H_2 and the vials incubated at 30 °C. After 24 h, the vials were manually shaken and the head space analyzed for N_2O and CO_2 by gas chromatography. The data were corrected for N_2O dissolved in the aqueous phase (Moraghan and Buresh, 1977). Soil dehydrogenase activity was determined by the method of Casida et al. (1964). Soil mineral N was measured by a micro-Kjeldahl method after extracting the soil with 2*N* KCl (Keeney and Nelson, 1982).

Statistics

Data were subjected to an analysis of variance, followed by Duncan's multiple range test (Steel and Torrie, 1980). Results are reported as means of 4 replicate pots and expressed on dry weight basis.

Results and discussion

Plant growth

Application of dicyandiamide effectively inhibited the nitrification process during the 40 and 18-day experiment period under wheat and maize, respectively. However, analyzing the unplanted soils at the end of experiments showed that nitrification was not completely inhibited by dicyandiamide and a part of NH_4^+ was converted into NO_3^- in NH_4^+-treated soils, whereas some NH_4^+ also had accumulated in NO_3^- treatments due to mineralization of the soil organic N or that of dicyandiamide (Table 1). Consequently, by the end of experiments, the initial NH_4^+-N: NO_3^--N ratio had decreased in NH_4^+ treatments and increased in NO_3^- treatments. Under these experimental conditions, therefore, NH_4^+ or NO_3^- treatments did not represent as the sole N sources throughout the study period. Instead, NH_4^+ and NO_3^- remained as the predominant N sources in NH_4^+ and NO_3^- treatments, respectively. The much lower NO_3^--N content in the unplanted soil during maize compared to wheat experiment was probably due to higher gaseous N loss because of the higher soil temperature during the maize experiment.

At the lower N level applied, the N form had no effect on the wheat root growth, whereas with higher N level, NH_4^+ compared to NO_3^- nutrition produced 32% higher root yield (Table 2). Wheat shoot yield was 31% (at 50 mg N kg^{-1}) to 39% (at 150 mg N kg^{-1}) higher under NH_4^+ than under NO_3^- nutrition ($P < 0.05$). Root and shoot growth of maize was not affected by the N form, whereas increasing the concentration of both NH_4^+ and NO_3^- reduced the root biomass ($P < 0.05$). At the higher N application, NH_4^+-compared to NO_3^--grown wheat and maize plants maintained higher total N concentration both in roots and in shoots, whereas at lower N application , the N form had no effect on the tissue N concentration.

In our conditions, the pH of the rhizosphere soil was only slightly lower under NH_4^+ than that

Table 1. Ammonium- and nitrate-N concentrations of the unplanted soil treated with ammonium (Am) or nitrate (Nit) in the presence of dicyandiamide[a]

Experiment	Treatment	NH_4^+-N (mg kg^{-1} DW)	NO_3^--N (mg kg^{-1} DW)	NH_4^+-N: NO_3^--N ratio
Wheat	Am-50 mg N kg^{-1}	33.9 b[b]	11.9 c	3.04 b
	Nit-50 mg N kg^{-1}	10.9 c	62.7 b	0.17 c
	Am-150 mg N kg^{-1}	127.5 a	16.6 c	8.07 a
	Nit-150 mg N kg^{-1}	17.0 c	143.3 a	0.12 c
Maize	Am-50 mg N kg^{-1}	48.4 b	4.3 c	12.65 b
	Nit-50 mg N kg^{-1}	22.0 c	35.0 b	0.64 c
	Am-150 mg N kg^{-1}	99.8 a	4.3 c	23.95 a
	Nit-150 mg N kg^{-1}	24.1 c	94.6 a	0.26 c

[a]Measured at the end of experiments (wheat, 40 days after N treatments; maize, 18 days after N treatments).
[b]For each experiment, values within a column followed by different letter are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 2. Dry matter yield and nitrogen content of wheat and maize grown under ammonium (Am) or nitrate (Nit) as nitrogen source

Treatment	Wheat		Maize	
	Root	Shoot	Root	Shoot
	Dry weight (mg $plant^{-1}$)			
Am-50 mg N kg^{-1}	38.8 a[a]	79.4 b	83.8 a	138.5 a
Nit-50 mg N kg^{-1}	31.3 ab	60.7 c	88.3 a	141.5 a
Am-150 mg N kg^{-1}	38.9 a	95.2 a	64.3 b	131.8 a
Nit-150 mg N kg^{-1}	29.5 b	68.7 bc	65.5 b	139.0 a
	Total nitrogen (% of dry matter)			
Am-50 mg N kg^{-1}	1.97 b	4.97 b	2.27 c	4.41 c
Nit-50 mg N kg^{-1}	1.79 b	4.74 b	2.02 c	4.33 c
Am-150 mg N kg^{-1}	3.08 a	5.51 a	3.02 a	6.07 a
Nit-150 mg N kg^{-1}	2.03 b	4.92 b	2.70 b	4.98 b

[a]For each parameter, values within a column followed by different letter are significantly different by Duncan's multiple range test ($P < 0.05$).

under NO_3^- nutrition (Table 3). The calcareous soil used in the present study had enough buffering capacity to maintain the pH, thus avoiding the pH-related growth disorders. By and large, at the concentrations applied in the present study, NO_3^- as the dominating N source did not show an advantage over NH_4^+ nutrition, as generally reported in poorly buffered hydroponics (Cramer and Lewis, 1993; Gill and Reisenauer, 1993).

Bacterial population density and soil microbial biomass

Averaged across treatments, the bacterial population density under both plant species was highest on the roots (Table 4). Under wheat, the population density was similar in the rhizosphere and in the planted bulk and unplanted soils. In contrast, the maize rhizosphere had significantly higher population density as compared to the planted bulk and unplanted soils. Comparing the overall treatment effects, the population density was generally higher under NH_4^+ nutrition than under NO_3^- ($P < 0.05$). Only at higher N concentrations applied, NH_4^+ compared to NO_3^- nutrition caused higher bacterial population density on roots of both plant species. In the rhizosphere soil, however, the stimulatory effect of NH_4^+ was observed only in wheat supplied with the lower N level. In the unplanted soil receiving higher N level the increased bacterial population density under NH_4^+ than under NO_3^- indicates the preferential utilization of NH_4^+ by microbes (Recous et al., 1990).

Table 3. pH of the rhizosphere, planted bulk and unplanted soils under wheat and maize fertilized with ammonium (Am) or nitrate (Nit)

Plant species	Treatment	Soil type			Treatment mean
		Rhizosphere	Planted bulk	Unplanted	
Wheat	Am-50 mg N kg^{-1}	7.28 g[a]	7.80 b	7.73 cd	7.60 B
	Nit-50 mg N kg^{-1}	7.60 f	8.00 a	7.65 ef	7.75 A
	Am-150 mg N kg^{-1}	7.20 h	7.70 de	7.64 ef	7.51 C
	Nit-150 mg N kg^{-1}	7.78 bc	7.80 b	7.60 f	7.73 A
	Soil type mean	7.47 C	7.83 A	7.66 B	
Maize	Am-50 mg N kg^{-1}	7.14 f	7.81 c	7.98 ab	7.64 B
	Nit-50 mg N kg^{-1}	7.34 e	8.08 a	8.05 a	7.82 A
	Am-150 mg N kg^{-1}	7.24 ef	7.59 d	7.85 bc	7.56 C
	Nit-150 mg N kg^{-1}	7.64 d	8.03 a	7.93 abc	7.86 A
	Soil type mean	7.34 C	7.88 B	7.95 A	

[a]For each plant species, means followed by different letter (lower case) are significantly different by Duncan's multiple range test ($P < 0.05$); treatment means or soil type means followed by different letter (upper case) are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 4. Bacterial population density and soil microbial biomass under wheat and maize fertilized with ammonium (Am) or nitrate (Nit)

Plant species	Treatment	Roots	Rhizosphere	Planted bulk	Unplanted	Treatment mean
		Bacterial population density (^{10}log g^{-1} DW)				
Wheat	Am-50 mg N kg^{-1}	7.94 a[a]	7.38 bcd	6.88 ef	7.08 de	7.32 A
	Nit-50 mg N kg^{-1}	7.62 ab	6.92 ef	7.04 de	6.66 f	7.06 B
	Am-150 mg N kg^{-1}	7.62 a	6.65 f	7.18 e	7.08 de	7.13 B
	Nit-150 mg N kg^{-1}	7.48 bc	6.65 f	6.94 ef	6.86 ef	6.98 B
	Soil type mean	7.66 A	6.90 B	7.01 B	6.92 B	
Maize	Am-50 mg N kg^{-1}	9.15 b	8.24 e	7.78 fg	8.75 cd	8.48 B
	Nit-50 mg N kg^{-1}	8.99 bc	8.02 ef	7.22 h	8.69 d	8.23 C
	Am-150 mg N kg^{-1}	9.97 a	9.15 b	7.68 g	8.95 bcd	8.94 A
	Nit-150 mg N kg^{-1}	9.15 b	9.06 b	7.13 h	7.57 g	8.23 C
	Soil type mean	9.31 A	8.62 B	7.45 D	8.49 C	
		Soil microbial biomass (μg C g^{-1} DW)				
Wheat	Am-50 mg N kg^{-1}	nd	192 a	54 g	76 ef	107 A
	Nit-50 mg N kg^{-1}	nd	131 c	67 f	86 de	95 B
	Am-150 mg N kg^{-1}	nd	171 b	65 fg	94 d	110 A
	Nit-150 mg N kg^{-1}	nd	96 d	67 f	90 d	84 C
	Soil type mean	nd	148 A	63 C	87 B	
Maize	Am-50 mg N kg^{-1}	nd	309 a	66 c	80 c	152 AB
	Nit-50 mg N kg^{-1}	nd	185 b	66 c	66 c	106 BC
	Am-150 mg N kg^{-1}	nd	367 a	69 c	68 c	168 A
	Nit-150 mg N kg^{-1}	nd	109 bc	67 c	76 c	84 C
	Soil type mean	nd	242 A	67 B	73 B	

[a]For each parameter, means followed by different letter (lower case) are significantly different by Duncan's multiple range test ($P < 0.05$); treatment means or soil type means followed by different letter (upper case) are significantly different by Duncan's multiple range test ($P < 0.05$).
nd = not determined.

Averaged across treatments, the rhizosphere soil had 2–4-fold higher microbial biomass content than the planted bulk and unplanted soils. This was apparently associated with the microbial use of root-derived C, indicating higher substrate availability in the rhizosphere (Priha et al., 1999; Qian et al., 1997). Ammonium compared to NO_3^- nutrition substantially increased the microbial biomass in the rhizosphere of wheat (47–78% increase) and that of maize (67–278% increase), the stimulatory effect being more pronounced at higher N application rate ($P < 0.05$). Microbial biomass content of the planted bulk and unplanted soils was generally not affected by the N form. Although, NH_4^+ compared to NO_3^- supplied at 150 mg kg^{-1} increased the bacterial population density on roots of both wheat and maize (Table 4), the population density in soil was not correlated with the microbial biomass (Table 7). This might indicate a major contribution of fungi to the soil microbial biomass. Besides other factors, the soil microbial community structure is strongly influenced by the substrate loading rate, with fungi dominating over bacteria at high substrate loading rate (Griffiths et al., 1998), and this may also apply to microbial community structure in the rhizosphere. We may not rule out the possibility that bacterial population density in the rhizosphere and bulk soils might also have been affected by N treatments without being detected by the method used since the culture-dependent methods are well known to underestimate the bacterial density in soils. Nevertheless, results of soil microbial biomass indicate an overall increase in the rhizosphere microbial population under NH_4^+ than under NO_3^- nutrition of both wheat and maize.

Microbial activity

Aerobic soil respiration in the rhizosphere was 7–10 times and 17–20 times higher than that of the planted bulk and unplanted soils, respectively (Tables 5 and 6). Both under wheat and maize, the N form had no effect on soil respiration in the planted bulk and unplanted soils. However, the rhizosphere soil of NH_4^+ compared to NO_3^- grown plants showed 39–67% (under wheat) and 63–65% (under maize) higher soil respiration ($P < 0.05$). Aerobic respiration was generally higher under wheat than under maize, whereas anaerobic respiration was substantially higher under maize than under wheat. Anaerobic soil respiration in the rhizosphere, on the average, was 6 times (under wheat) to 34 times (under maize) higher than that of planted bulk and unplanted soils ($P < 0.05$) (Tables 5 and 6). These very large differences may indicate differences in the quality/quantity of root exudates of C_3 and C_4 plant species as hypothesized by Kuzykov (2002). We speculate that root exudates from maize were dominated by sugars thus supporting much higher anaerobic respiration as compared to organic acids which probably dominated in wheat root exudates, and were not efficient C sources under anaerobic conditions. As observed for the aerobic soil respiration, N form had no effect on the anaerobic respiration in planted bulk and unplanted soils. For the rhizosphere soil, however, NH_4^+ compared to NO_3^- nutrition caused 81–199% (under wheat) and 12–17% (under maize) increase in the anaerobic respiration ($P < 0.05$), the effect being higher with higher N application rate. The correlation of anaerobic soil respiration with the denitrification capacity was highly significant (Table 7), indicating that much of the CO_2 had originated from the activity of denitrifiers since the assay was carried out under denitrifying conditions. Denitrification capacity of the rhizosphere soil showed variable response to the N form, but was significantly higher than that of the planted bulk and unplanted soils ($P < 0.05$) (Tables 5 and 6). At lower N application rate, N form had no effect on the denitrification capacity of the rhizosphere soil, whereas at higher N application rate the capacity under NH_4^+ compared to NO_3^- nutrition was either higher (under wheat) or lower (under maize) indicating differential effects of plant species.

Rhizosphere and N form effects were also pronounced for dehydrogenase activity that was 5 times higher in the rhizosphere than the planted bulk and unplanted soils ($P < 0.05$) (Tables 5 and 6). The activity in the planted bulk and unplanted soils was generally not influenced by the N form. However, NH_4^+ compared to NO_3^- nutrition caused almost 2 times higher dehydrogenase activity in the rhizosphere soil, both under wheat and maize ($P < 0.05$). The increased dehydrogenase activity confirms higher substrate availability under NH_4^+ compared to NO_3^- nutrition. Under both plant species, the

Table 5. Dehydrogenase activity, aerobic and anaerobic respiration and denitrification capacity of the soil under wheat fertilized with ammonium (Am) or nitrate (Nit)

Treatment	Soil type			Treatment mean
	Rhizosphere	Planted bulk	Unplanted	
	Dehydrogenase activity (μg triphenyl-formazan g^{-1} DW h^{-24})			
Am-50 mg N kg^{-1}	1224 a[a]	209 e	142 ef	523 A
Nit-50 mg N kg^{-1}	552 c	122 f	149 ef	276 C
Am-150 mg N kg^{-1}	878 b	103 f	158 ef	379 B
Nit-150 mg N kg^{-1}	478 d	137 ef	168 ef	259 C
Soil type mean[c]	782 A	144 B	154 B	
	Aerobic respiration (μg CO_2-C g^{-1} DW h^{-24})			
Am-50 mg N kg^{-1}	27.72 b	3.00 d	1.49 d	10.73 B
Nit-50 mg N kg^{-1}	19.94 c	2.42 d	1.58 d	7.99 C
Am-150 mg N kg^{-1}	38.18 a	2.66 d	1.27 d	14.04 A
Nit-150 mg N kg^{-1}	22.90 c	2.59 d	1.80 d	9.10 C
Soil type mean	27.46 A	2.66 B	1.54 B	
	Anaerobic respiration (μg CO_2-C g^{-1} DW h^{-24})[b]			
Am-50 mg N kg^{-1}	27.02 b	5.45 d	3.41 d	11.95 B
Nit-50 mg N kg^{-1}	14.88 c	4.66 d	2.71 d	7.82 C
Am-150 mg N kg^{-1}	46.85 a	6.31 d	4.46 d	19.20 A
Nit-150 mg N kg^{-1}	15.77 c	5.14 d	4.42 d	8.40 C
Soil type mean	26.11 A	5.38 B	4.06 B	
	Denitrification capacity (μg N g^{-1} DW h^{-24})			
Am-50 mg N kg^{-1}	64.56 bc	7.68 d	6.31 d	26.18 B
Nit-50 mg N kg^{-1}	53.21 c	8.28 d	2.88 d	21.46 B
Am-150 mg N kg^{-1}	105.91 a	17.47 d	5.23 d	43.10 A
Nit-150 mg N kg^{-1}	67.90 b	8.95 d	5.23 d	27.34 B
Soil type mean	72.89 A	10.58 B	4.90 B	

[a]For each parameter, means followed by different letter (lower case) are significantly different by Duncan's multiple range test ($P < 0.05$); treatment means or soil type means followed by different letter (upper case) are significantly different by Duncan's multiple range test ($P < 0.05$).
[b]Measured under denitrifying conditions in the presence of NO_3^-.

correlation of dehydrogenase activity with the aerobic and anaerobic soil respiration, denitrification capacity, and with microbial biomass was highly significant ($P < 0.001$) (Table 7).

The lack of treatment effects on the soil microbial biomass and activity in the unplanted soil indicates that increased microbial biomass and activity in the rhizosphere of NH_4^+ EN 1-1 compared to NO_3^- EN 1-1 grown plants was not attributable to the preferential utilization of NH_4^+ by soil microbes, but was most probably due to increased root exudation by NH_4^+-fed plants. We reported previously that root sugar exudation by hydroponically grown kallar grass [*Leptochloa fusca* (L.) Kunth] was up to 79-fold higher under NH_4^+ compared to NO_3^- nutrition (Mahmood et al., 2002). Therefore, we speculate here that NH_4^+ compared to NO_3^- nutrition might have stimulated root sugar exudation also in wheat and maize. However, possible effects of N source on the composition of root exudates and their availability to the rhizosphere microflora may not be ruled out; the higher N content of the NH_4^+-fed plants could have changed the quality of the exudates. Roots of NH_4^+ grown kallar grass exuded 3-fold higher amino acids than NO_3^--grown plants (Mahmood et al., 2002). Thus the higher sugar exudation in the rhizosphere of NH_4^+ plants (Mahmood et al., 2002), the abundance of NH_4^+ in NH_4^+-treated soil, and the preferential use of NH_4^+ by soil microbes (Rangel-Castro and Taylor, 2002; Recous et al., 1990) might lead to the increased rhizosphere microbial activity under NH_4^+ compared to NO_3^- nutrition as observed in the present study.

Table 6. Dehydrogenase activity, aerobic and anaerobic respiration and denitrification capacity of the soil under maize fertilized with ammonium (Am) or nitrate (Nit)

Treatment	Soil type			Treatment mean
	Rhizosphere	Planted bulk	Unplanted	
	Dehydrogenase activity (μg triphenyl-formazan g^{-1} DW h^{-24})			
Am-50 mg N kg^{-1}	1927 a[a]	276 c	269 c	823 A
Nit-50 mg N kg^{-1}	941 b	259 c	259 c	487 B
Am-150 mg N kg^{-1}	1747 a	283 c	238 c	756 A
Nit-150 mg N kg^{-1}	739 b	271 c	288 c	434 B
Soil type mean[c]	1339 A	271 B	264 B	
	Aerobic respiration (μg CO_2-C g^{-1} DW h^{-24})			
Am-50 mg N kg^{-1}	18.70 a	2.42 d	0.53 d	7.22 A
Nit-50 mg N kg^{-1}	11.33 b	2.42 d	0.53 d	4.75 B
Am-150 mg N kg^{-1}	13.03 b	0.94 d	0.77 d	4.92 B
Nit-150 mg N kg^{-1}	7.99 c	1.37 d	0.72 d	3.36 C
Soil type mean	12.77 A	1.80 B	0.65 C	
	Anaerobic respiration (μg CO_2-C g^{-1} DW h^{-24})[b]			
Am-50 mg N kg^{-1}	99.50 c	4.37 e	2.76 e	35.54 BC
Nit-50 mg N kg^{-1}	88.49 d	3.07 e	3.19 e	31.58 C
Am-150 mg N kg^{-1}	132.86 a	3.67 e	3.89 e	46.80 A
Nit-150 mg N kg^{-1}	113.95 b	3.10 e	2.28 e	39.77 B
Soil type mean	108.70 A	3.55 B	3.02 B	
	Denitrification capacity (μg N g^{-1} DW h^{-24})			
Am-50 mg N kg^{-1}	456.24 bc	20.38 d	14.90 d	163.85 A
Nit-50 mg N kg^{-1}	408.00 c	21.94 d	16.39 d	148.78 A
Am-150 mg N kg^{-1}	457.44 b	19.61 d	17.81 d	164.95 A
Nit-150 mg N kg^{-1}	516.96 a	21.98 d	19.63 d	186.19 A
Soil type mean	459.67 A	20.98 B	17.18 B	

[a]For each parameter, means followed by different letter (lower case) are significantly different by Duncan's multiple range test ($P < 0.05$); treatment means or soil type means followed by different letter (upper case) are significantly different by Duncan's multiple range test ($P < 0.05$).
[b]Measured under denitrifying conditions in the presence of NO_3^-.

Table 7. Relationships among different indices of microbial activity in soil (r-values)[a]

Plant species	Parameter	Aerobic respiration	Anaerobic respiration	Denitrification capacity	Bacterial density	Microbial biomass
Wheat	Dehydrogenase activity	0.902***	0.852***	0.844***	0.114	0.950***
	Aerobic respiration	–	0.951***	0.991***	−0.185	0.867***
	Anaerobic respiration	–	–	0.946***	−0.168	0.847***
	Denitrification capacity	–	–	–	−0.231	0.809***
	Bacterial density	–	–	–	–	0.108
Maize	Dehydrogenase activity	0.965***	0.885***	0.848***	0.180	0.976***
	Aerobic respiration	–	0.901***	0.901***	0.267	0.905***
	Anaerobic respiration	–	–	0.984***	0.388	0.857***
	Denitrification capacity	–	–	–	0.436	0.786**
	Bacterial density	–	–	–	–	0.141

[a]*** and ** indicate the correlation coefficients significant at $P < 0.001$ and $P < 0.01$, respectively ($n = 12$).

Table 8. Aerobic and anaerobic respiration, and denitrification capacity of the rhizosphere soil of the maize hybrid FHY-434 after 10 days growth under ammonium (Am) or nitrate (Nit)

Treatment	Aerobic respiration (μg C g^{-1} DW h^{-24})	Anaerobic respiration (μg C g^{-1} DW h^{-24})	Denitrification capacity (μg N g^{-1} DW h^{-24})
Am-50 mg N kg^{-1}	533 a[a]	143 b	758 a
Nit-50 mg N kg^{-1}	238 b	111 b	463 b
Am-250 mg N kg^{-1}	535 a	348 a	778 a
Nit-250 mg N kg^{-1}	439 a	102 b	797 a

[a]Values in a column followed by different letter are significantly different by Duncan's multiple range test ($P < 0.05$).

In another experiment carried out with a fast-growing maize hybrid FHY-434, NH_4^+ compared to NO_3^- even supplied at 250 mg N kg^{-1} soil was not detrimental to the maize plant growth, whereas it significantly increased the aerobic and anaerobic respiration and the denitrification capacity of the rhizosphere soil, confirming higher substrate availability in the rhizosphere of NH_4^+-grown plants (Table 8).

Conclusion

The overall results of the present study conform to those of earlier studies carried out in hydroponics (Brown and Hornby, 1987; Trolldenier and Rheinbaban, 1981) and suggest that NH_4^+ compared to NO_3^- nutrition also stimulates microbial activity in the rhizosphere of soil-grown plants. As a working hypothesis we suggest that the observed stimulation of the rhizosphere microbial activity is due to enhanced root exudation and utilization of root exudates under NH_4^+ compared to NO_3^- nutrition.

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