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NITROGEN LEACHING OF SPRING WHEAT GENOTYPES (*TRITICUM AESTIVUM* L.) VARYING IN NITROGEN-RELATED TRAITS

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□ *Efficient use of nitrogen (N) by wheat crop and hence prevention of possible contamination of ground and surface waters by nitrates has aroused environmental concerns. The present study was conducted in drainage lysimeters for three years (1998–2000) to identify whether spring wheat genotypes (*Triticum aestivum *L.) that differ in N-related traits differ in N leaching and to relate parameters of N use efficiency (NUE) with parameters of N leaching. For this reason two spring wheat cultivars ('Albis' and 'Toronit') and an experimental line ('L94491') were grown under low (20 kg N ha*$^{-1}$*) and ample N supply (270 kg N ha*$^{-1}$*). The genotypes varied in parameters of NUE but not in N leaching. Grain yield of the high-protein line ('L94491') was, on average, 11% lower than that of 'Toronit' but among genotypes had significantly higher N in the grain (%), grain N yield, and N harvest index. Nitrogen lost through leaching was considerably low (0.42–0.52 g m*$^{-2}$*) mainly due to low volume of percolating water or the ability of the genotypes to efficiently exploit soil mineral N. There were no clear relationships between N-related genotype traits and N leaching, but across all treatments significantly negative correlations between volume of leachate and the amount of N in the total biomass and grain N yield existed.*

Keywords: lysimeters, N leaching, nitrogen use efficiency parameters, spring wheat

INTRODUCTION

Wheat is the oldest, most widespread and the most important agricultural product since worldwide occupies 225,437,694 ha, with production of 681,915,838 Mt and yield, 3.02 Mt ha^{-1} (FAO, 2009). Approximately 90%

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of its production is consumed directly by humans (bread, pasta, cookies, cakes breakfast cereals, etc), whereas it grows among diverse environments (it grows from the arid plains to Africa to the humid valleys of Vietnam and from the cold of Nepal to the heat of India) (Gooding and Davies, 1997). Nitrogen (N) is the most limiting yield factor in many growing areas whereas worldwide, nitrogen use efficiency (NUE) for cereal production is somehow low ($\sim$33%) and the unaccounted 67% represents approximately $16 billion annual loss of N fertilizer (Raun and Johnson, 1999).

Economic and environmental concerns arises the need to find ways of more efficient N use or efficient fertilizer use. The use of N throughout crop production needs to be optimized since agricultural N losses negatively impact groundwater quality and environment (Prakasa Rao and Puttanna, 2000; Galloway et al., 2008). Better use of N could provide more food and reduce the environmental impact since it is considered from three interrelated points of view: agronomy (in terms of grain yield produced per unit of N supply), environment [possible contamination of ground water, eutrophication of surface waters, or ozone depletion by release of nitrous oxide (N_2O)], economics (maximization of farmers' income) (Bock, 1984; Huggins and Pan, 1993; Raun and Johnson, 1999; Fageria and Baligar, 2005). The importance of N in wheat grain yield and quality is well documented (Gauer et al., 1992; Ehdaie and Waines, 2001; Ma et al., 2006). The protein concentration in the grain is an important quality criterion for baking wheat and is positively related to the amount of N supply by the soil (Ayoub et al., 1994; Mason and Brennan, 1998). Since there is a negative correlation between grain protein concentration and grain yield (Feil, 1997), either more N fertilizer has to be applied or high-protein genotypes must be grown to meet the quality standards. Both strategies are expected to influence soil N dynamics and thus the leaching of N, which is a highly relevant environmental and economic issue (Vitousek et al., 1997).

Nitrate leaching is a global phenomenon and occurs when the soil nitrate-N (NO_3-N) concentrations are high and water moves away from the root zone. In order to reduce nitrate leaching practices that should be based on avoiding excess N by applying N rate to meet expected yields, and applying N in phase with crop demand should be addressed. Leaching of nitrates can contribute to nitrate enrichment of groundwater, and to eutrophication of surface waters (David et al., 1997; Mosier et al., 2004; Meisinger et al., 2006).

Assessing nitrate leaching from agricultural sources is complicated and requires intensive field and laboratory measurements. Lysimeters is one of the five potentially suitable methods (porous ceramic cups, pan/trench samplers, large-scale drainage collection and soil coring) for measuring nitrate leaching from agricultural sources. All of them have typical advantages and limitations with regard to implementation, costs, reproducibility, relevance, and data interpretation (Addiscott, 1990). Lysimeters are valuable tools for studying the fate and transport of chemicals in soil and were used to study the mass balance analysis of water, pesticides, and nutrients leaching (Caspari et al., 1993; Winton and Weber, 1996; Marcinkonis 2006; Wegehenkel et al., 2008). Other studies investigated the leaching behavior of N and other elements in lysimeters (Uhlen, 1994), the influence of different agricultural management systems on N leaching (Knappe at al., 2002), or used simulation models to assess N leaching out of lysimeter tanks (Bohne et al., 2007). Webster et al., (1993), stated that lysimeters and ceramic suction cups can be used to quantify leaching losses, but in contrast to suction cups, lysimeters enable the measurement of the amount of percolated water and the concentration of different N forms in the drained water. Several other methods were used to measure nitrate leaching such as extracting the soil solution into a porous ceramic cup, collecting water leached in a lysimeter, collecting water from field drainage systems, computer modeling, collecting nitrate in an ion exchange resin, using bromide as a tracer for nitrate, using ^{15}N enriched (or depleted) fertilizer (Stephano et al., 1986; Addiscot et al., 1991; Ronalghi et al., 1993; Patra and Rego, 1997). However, information on possible relationship between NUE related parameters and N leaching parameters is still meager. The genotypes used in the present experiments are known to vary in NUE and fertilizer N recovery efficiency whereas the suitability of the lysimeter facility for NUE studies and small-plot trials with crop plants was confirmed (Keller et al., 1987; Noulas et al., 2004). The basic advantage of the lysimeter facility used in the present experiments is that allows the study of various treatments using a sound experimental design and statistical analysis (Liedgens at al., 2000). The present study was undertaken to determine (i) whether spring wheat genotypes that differ in N-related traits differ in nitrate leaching and (ii) to relate N accumulation and parameters of NUE with parameters of N leaching.

MATERIALS AND METHODS

Experimental Conditions

The experiment was conducted for three years (1998–2000) in the Swiss midlands near Zurich (47° 26′ N, 8° 40′ E) in drainage-lysimeters (n = 24). The basic drainage lysimeter unit was a water-tight, double-walled fiberglass container (Figure 1). The inner surface area of the container was of 1.0 m^2 (1.0 × 1.0 m) and the depth of the soil column in the lysimeters was 1.1 m. The lysimeters contained Alporit® (Alporit AG, Boswil, Switzerland) (40 mm) sheets and a layer of batal® plates (16 mm) to provide temperature insulation from outside. At both ends of the two rows of the lysimeters, there were additional lysimeter-like containers to reduce border effects.

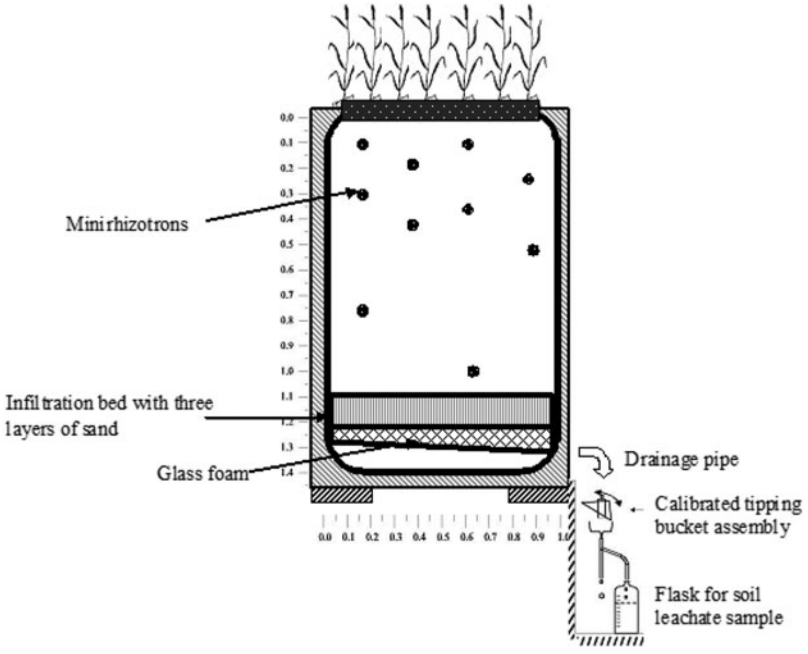

FIGURE 1 Simplified schematic representation of a drainage lysimeter used in the experiments. Figures of the rulers are in meters (m). For detailed instrumentation of the lysimeter facility please refer to the text.

Shading screens along the edges of the lysimeters simulated border rows of plants.

The experiment was conducted in such a facility because each lysimeter contained special instrumentation allowing N leaching studies. Specifically, each drainage lysimeter carried special instrumentation (Figure 1) allowing quantitative *in vivo* analysis of root dynamics [non-destructive–root observation devices: minirhizotrons (Herrera et al., 2007)], monitoring leaching control (leachate samplers, bucket assembly) and water content (soil water content–Time-Domain Reflectometer probes; TDR), measuring N dynamics in the soil profile (soil solution–suction cups), soil temperature (thermistors) and yield and NUE components. The facility used in the experiments was described and evaluated in detail in the study by Liedgens et al., (2000).

The soil of the lysimeter experiment was sandy loam (SL) [54% sand, 29% loam, 17% clay; Bouyoucos hydrometer method (Gee and Bauder, 1986)]. Below 1.1 m there were two layers of glass foam and three layers of quartz sand. The soil was sufficient in phosphorous [Olsen method (Olsen and Sommers 1982); 0.40–0.50 g phosphorus (P) kg^{-1}] and potassium content [assimilable dipotassium oxide (K_2O), ammonium (NH_4) acetate; 26.7–36.5 ppm] to a depth of 30 cm, poor in organic matter content

[2.8–3.0%, Walkley and Black method (Allison, 1965)], and a slightly alkaline [pH (H_2O) = 7.2 to 8.0].

In all years, 60 kg ha^{-1} Foskal® [contains 7, 20, 1, 4, and 2 kg ha^{-1} phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S), respectively] and 20 kg N ha^{-1} as ammonium nitrate were applied to all the plots before sowing. No additional fertilizer N was applied to the half plots, while 250 kg N ha^{-1} as ammonium nitrate (NH_4NO_3) were added to the other plots, split into four applications: 90 kg N ha^{-1} at sowing, 40 kg N ha^{-1} at stem elongation (BBCH stage 30; Lancashire et al., 1991), 60 kg N ha^{-1} at heading (BBCH stage 50) and 60 kg N ha^{-1} at flowering (BBCH stage 60). The fertilizer was broadcasted by hand on the entire surface of the lysimeters.

The Swiss spring wheat cultivars 'Albis', 'Toronit' and the experimental line '94491' ('L94491') were bred by the Swiss Federal Research Station in Reckenholz and were grown in the three seasons. Characteristics of the genotypes used in our experiments are compiled in Table 1. The yield potential and protein content of 'Albis' is average. A higher yield potential and a somewhat lower protein content than 'Albis' characterize 'Toronit'. 'L94491' was included in the experiment because of its high grain protein concentration, despite its lower yield potential.

Sowing dates were 30 March 1998, 15 March 1999, and 23 March 2000. The seeding rate was 420 seeds m^{-2} in seven rows, 0.14 m apart, and the sowing depth was 20 to 30 mm.

Agrochemicals were applied when necessary during the growth periods to keep the experiments free of pests, diseases and weeds. Moddus (Syngenta Agro AG, Basel, Switzerland) was applied at the 1st node stage (BBCH stage 30–32) at a rate of 0.5 L ha^{-1} to prevent lodging.

The lysimeters were irrigated with 20 mm and with 10 mm during early dry seasons in 1998 and 1999 respectively and with 10 mm during grain filling in 1998, and 35 mm during grain filling in 2000.

Data on temperature and precipitation were obtained from a local automatic weather station and are compiled in Figure 2.

TABLE 1 Characteristics of the genotypes used in the experiments

	Genotype		
Characteristic	'Albis'	'L94491'	'Toronit'
Price class / baking quality	VH	—	MH
Protein remuneration	MH	H	MH
Grain yield	M	M	H-VH
Lodging resistance	MP	MP	H-VH
Mean plant height (m)	1.00	~0.90	0.82
Year of release	1983	Bänziger et al. (1992)	1996

VH = very high; H = high; MH = medium high; M = medium; MP = medium poor.

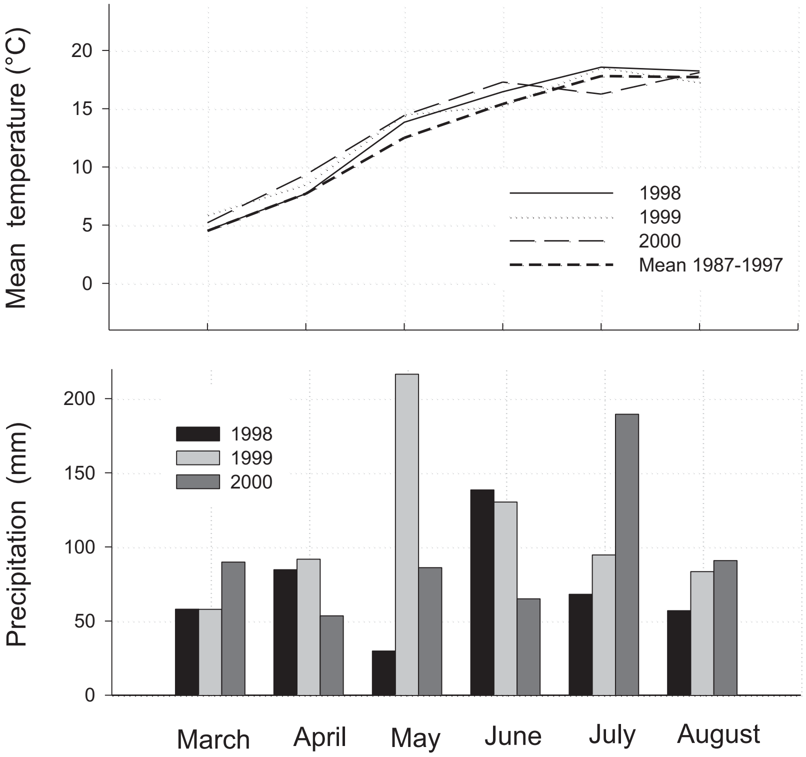

FIGURE 2 Monthly temperature and precipitation at the experimental site in the three growing seasons.

Data Collection, Calculations and Analyses

Drainage from the lysimeters was automatically registered. Leachate volume was measured by a gauge, consisting of an outer funnel, a tipping bucket assembly, a base plate, and a housing assembly (Figure 1). The discharge of the tipping bucket was registered using a data logger. Sub-samples of leachate were collected weekly for chemical analysis. Leachate aliquots were analyzed for the concentrations of NH_4^+ and NO_3^- using colorimetry (Evolution II Autoanalyser, Alliance Instruments, Nanterre, France). The calculations of the mass balances of water and N were based on the water quantity lost from the system through deep percolation and the concentration of nitrate and ammonium in the water.

The plants were harvested at physiological maturity (BBCH stage 92 or later) on 4 August 1998, 9 August 1999, and 7 August 2000. All shoots per lysimeter were cut at ground level, dried at 65°C for 48 h and separated into grains and straw. The shoots were then threshed and separated into grains, chaff (rachis plus glumes and awns) and straw. Chaff and straw were mixed thoroughly before weighing and are referred to as straw hereafter. Dry grain and straw weights were determined. The straw was ground in two steps in mills with a 3-mm (Wolf Mühle, Wien, Austria) and a 1-mm sieve (Cyclotec Tecator 1093 Mill, Tecator AB, Höganäs, Sweden). Grains were ground once with an A 10 mill (Janke & Kunkel Labortechnik, Staufen I Br., Germany). The N concentration of the straw and in the grains was determined with a LECO CHN-1000 auto analyzer (LECO Corporation, St Joseph, MI, USA). Shoot biomass, grain yield, shoot N concentration and total shoot N content were measured at physiological maturity due to the limited sample area within the lysimeter plots (1 m^2).

The harvest index was calculated as the ratio of dry mass of the grain to the total aboveground dry mass and the N harvest index as, the ratio of N in the dry grain to the total N in the aboveground dry mass.

Each lysimeter represent one experimental unit (plot). In each year the experimental design was a randomized complete block (RCB) with two treatments (N supply and genotypes) and four replications. The results were analyzed over years with ANOVA and correlation procedures using S-Plus (Venables and Ripley, 2002). The ANOVA showed that there were no significant three-way interactions (C×N×Y) therefore means for NUE and leaching parameters are presented over the years.

RESULTS

Weather Conditions

Monthly temperatures and precipitation during the three growing seasons are compiled in Figure 2. Temperatures were somehow higher in all growing seasons compared to average of a ten-year period. Monthly temperatures for the growing seasons 1998, 1999 and 2000 were 13.2, 13.4, and 13.4°C, respectively, compared to 12.6°C for the ten year period (1987–1997) before the start of the experiment. Among the three growing seasons 1998 was the drier and 1999 was the wettest one. Total rainfall during the growing seasons was 437, 675, and 575 mm in the years 1998, 1999, and 2000, respectively. The corresponding value for the 10-year period (1987–1997) was 652 mm (data not shown). The period most affected by low precipitation was the preanthesis period (March to June) in 1998.

NUE Related Parameters

The statistical analysis showed that the parameters of NUE varied with genotype, level of N fertilization, and year (Table 2). Some genotype effects were modified by N fertilization (grain yield, grain N yield, harvest index) and year (% N in grain and the harvest indices).

As expected from the official Swiss genotype trials, 'Toronit' produced the highest yields of grain. Averaged across years and N levels 'Toronit' produced significantly higher biomass (1753 g m^{-2}) and grain yield (798 g m^{-2}) than the other two genotypes. 'L94491' produced significantly the highest grain N yield (18.4 g m^{-2}) and exhibited the highest concentration of N in the grains (2.53%). 'L94491' had the highest amount of N in the shoot (23.1 g m^{-2}) quite similar to Toronit (23.0 g m^{-2}) despite the considerable differences in total aboveground biomass between the two genotypes. Among genotypes 'L94491'had the highest N harvest index (80.4%) (Table 2).

Fertilization of N increased the grain N yield but reduced the proportion of N partitioned to the grains (N harvest index). There were significant interactions between year and rate of N for all plant-related traits except for the harvest indices and no three way interactions between year, genotype and N rate were found thus results on N related traits are not presented separately for each year.

Leaching Parameters and Their Relation to NUE

The water percolation (volume of leachate) was significantly affected by N fertilization and year but not by genotype. Both the volume of leachate (148–165 l m^{-2}) and the amount of N lost through leaching (0.42–0.52 g

TABLE 2 Effects of genotype (G), rate of N fertilizer (N), and year (Y) and their interactions on parameters of NUE and N leaching

	G	N	Y	G×N	G×Y	N×Y		Genotype			N (g m^{-2})	
Parameter	Pr > F						unit	'Albis'	'L 94491'	'Toronit'	2	27
Biomass	**	**	**	†	†	**	g m^{-2}	1524 b	1511 b	1753 a	1378 b	1814 a
Grain yield	**	**	**	**	NS	**	g m^{-2}	656 c	711 b	798 a	632 b	811 a
Harvest index	**	NS	**	*	**	NS	%	43.3b	46.8a	45.3a	45.5a	44.8a
N in straw	†	**	**	NS	NS	**	%	0.51b	0.56ab	0.60a	0.45b	0.67a
N in grain	**	**	**	†	*	**	%	2.34b	2.53a	2.09c	2.09b	2.55a
Biomass N yield	**	**	**	†	NS	**	g m^{-2}	20.4b	23.1a	23.0a	17.0b	27.4a
Grain N yield	**	**	**	*	NS	**	g m^{-2}	15.6c	18.4a	17.0b	13.5b	20.6a
N harvest index	**	**	**	NS	**	NS	%	77.8b	80.4a	75.5c	79.9a	78.9b
Volume of leachate[1]	NS	*	**	NS	*	*	l m^{-2}	148 a	165 a	159 a	167 a	147 b
Leaching N loss[1,2]	NS	NS	**	NS	NS	NS	g m^{-2}	0.45a	0.42a	0.52a	0.44a	0.48a

[1] Sum over the period from sowing to harvest. [2] Ammonium plus nitrate. NS : non significant at the 0.10 probability level. †, *, **: Significant treatment effects at 0.1, 0.05, and 0.01 probability level, respectively. Within rows treatment means followed by different letters are significantly different from each other at the 0.05 probability level. Three-way interactions (C×N×Y) were not included due to lack of any significance.

m^{-2}) were rather low. The latter trait was affected only by the factor year (Table 2).

When the data from the various years, N rates, genotypes, and replicates were pooled (n = 72), significant correlations (df = 70) between N use and leaching parameters were found. The correlations were always negative, with the exception of the N harvest index (Table 3). The volume of leachate was more closely related to the parameters of NUE than N loss through leaching. However, the relationships between NUE and leaching parameters were less clear within the years (n = 24; df = 22). There were significant correlations between N-related crop parameters and the volume of leachate in 1999 and 2000. The volume of leachate was more closely related to the amount of N in the total above-ground biomass and grain N yield than to biomass and yield, respectively.

DISCUSSION

The highest grain N yields and concentration of N in the grains of 'L94491' confirm results of previous experiments in which 'L94491' consistently outperformed 'Albis' (Bänziger et al., 1992). Irrespective of the levels of N supply and year, 'L94491' had the highest N in the grain (%), grain N yield, biomass N yield and harvest indices. This may indicate the extraordinary ability of this genotype to increase the proportion of N partitioned to the grains at maturity and to accumulate N in the biomass (N off take) due to its lower total biomass compared to other two genotypes. Growing high-protein genotypes to increase the yield and concentration of grain protein

TABLE 3 Correlation coefficients between NUE and N leaching parameters

		Volume of leachate				Leaching N loss		
	Overall	1998	1999	2000	Overall	1998	1999	2000
Parameter	(n = 72)		(n = 24)		(n = 72)		(n = 24)	
Biomass	−0.293*	−0.155	−0.716*	−0.697†	−0.042	+0.346	+0.262	−0.131
Grain yield	−0.403***	+0.179	−0.729*	−0.269	−0.105	+0.533	+0.451	−0.180
Harvest index	−0.337**	+0.661†	+0.379	+0.258	−0.134	+0.466	+0.351	−0.082
N in straw (%)	−0.505***	+0.267	−0.773*	−0.643†	−0.491***	−0.594	+0.403	−0.475
N in grain (%)	−0.467***	−0.438	−0.777*	−0.769*	−0.301*	−0.636†	+0.331	−0.243
Biomass N yield	−0.506***	−0.058	−0.752*	−0.850**	−0.291*	+0.073	+0.364	−0.407
Grain N yield	−0.476***	−0.032	−0.750*	−0.931***	−0.200†	+0.169	+0.402	−0.379
N harvest index	+0.239*	+0.089	+0.726*	+0.374	+0.299†	+0.470	−0.203	+0.276

†, *, **, ***: Significant correlations at 0.1, 0.05, 0.01, and 0.001 probability level, respectively.

is better for the environment than applying higher rates of N fertilizer, because the rate of N fertilizer recovery usually decreases with increasing rate of N application (Gauer et al., 1992; Feil, 1997). In this lysimeter study, only 42% of the fertilizer N applied was recovered by the plants. This proportions of fertilizer N recovery was calculated when the present lysimeter facility was evaluated together with parallel field trials in the study by Noulas et al. (2004). However, the genetic approach is feasible only if it is economically acceptable; in this investigation, the yield of the high-protein line ('L94491') was, on average, 11% lower than that of 'Toronit' (Table 2). Genotypic differences for most NUE parameters presented in this study were also found in the corresponding field experiments that were conducted simultaneously (Noulas et al., 2004). Thus the present lysimeter facility did not exhibit any limitations to the plant growth.

Despite a noticeable variation in the NUE parameters, the genotypes did not differ significantly in N leaching. There were hardly differences between high and low N supplies for leaching N losses. This may have been occurred because of the split (in time) N application at high N supply a strategy which decreases the risk of N leaching and increases NUE (Raun and Johnson, 1999; Fageria and Baligar, 2005).

The effect of N fertilization on the volume of leachate (Table 2) and the negative correlation between the volume of leachate and the biomass (Table 3) probably reflect the increased demand for water of a large biomass. Feil (1997) suggested that breeding for higher above-ground biomass may indirectly improve the uptake of N, because cultivars with high above-ground phytomass are likely to show vigorous root growth.

However, volume of leachate was more closely related to grain yield, N percentage in the straw, N percentage in the grain, N biomass yield, and N grain yield than to above-ground biomass, indicating that factors other than biomass (i.e., soil type, climate, amount of rainfall and intensiveness, irrigation) may also influence the amount of water remaining for free drainage. In interpreting the correlations between NUE parameters and loss of N through leaching, it must be taken into account that the N lost through leaching was extremely low; it was one order of magnitude smaller than the amount of N in the straw. Two factors contributed to this outcome: the volume of leachate was low throughout the vegetation cycle, and the concentration of N in the leachate was low over longer periods of the crop season. Among other factors the volume of leachate may have been low because of the low precipitation recorded during the growth seasons especially in 1998 and in 2000, which was 33% and 12% lower than the average in the region respectively. The low concentration of N in the leachate may reflect the rapid and efficient exploitation of soil mineral N (Feil, 1997). Among other things, denitrification, resulting from the permanently wet soil interface at the bottom of the drainage lysimeter containers, may also have contributed to the low N concentration in the leachate.

CONCLUSIONS

The advantage of the present lysimeter facility to monitor N leaching throughout the cropping season could enable studies of N balance in order to assess the fate of unused fertilizer N linked to the application of high N fertilizer rates. The facility allows performing research on several traits simultaneously something difficult at the field: root turnover and leaching control or root turnover and above ground processes. Under the conditions of the present experiments it was concluded that no genotypic differences existed in the N leaching and the N lost through leaching was extremely low mainly due to low volume of the percolating water. This may indicate a rapid and more efficient exploitation of soil mineral N of all genotypes. The negative correlations between volume of leachate and to the amount of N in the total biomass and grain N yield may indicate that N yields influence the water available for free drainage. The fact that were there genotypic differences in N related parameters but not in the nitrate leaching may also indicate that the genotypes may differ in the quantity or quality of the root exudates that affect N transformation processes such as mineralization of N in the rhizosphere.

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