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Maize and cover crop sequence in the Pampas: effect of fertilization and water stress on the fate of nitrogen

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Abstract:	Cover crops are well known for their positive effects on erosion processes, soil organic matter, soil physical properties, weed populations and nitrate leaching. In this work, we evaluated the fate of nitrogen (N) from fertilizer in maize (Zea mays) and then in ryegrass (Lolium multiflorum) as cover crop, in the conditions of the Argentine Pampas. To this end, a field experiment was carried out at the School of Agriculture, University of Buenos Aires, Argentina (34°36´ S, 58°29´ W). The design of the experiment was a factorial with three replications. We applied to maize 2 levels of N (0 and 140 kg N ha-1 (125 lb N ac-1) (ammonium nitrate target with 15N) and two levels of water (50 and 100% of crop evapotranspiration). 15N was determined in both the soil and plants. Maize plants and the soil organic fraction were the main sinks of fertilizer N, depending on the water treatment. The N from fertilizer remaining as nitrates in the soil (0 to 1.50 m [0 to 4.92 ft] depth) at maize harvest was 8% in plots subjected to water stress compared to 3% in the non-water stressed. Nitrogen losses due to volatilization were minor. Total N (soil and fertilizer) accumulated in ryegrass tissues plus nitrates remaining in the soil were higher in cover crop plots than in bare soil (130 vs. 51 kg N ha-1 [116 vs. 45.5 lb N ac-1]). The N in the soil organic matter originating from fertilizer significantly decreased between maize harvest and cover crop harvest. This soil organic N that originated from fertilizer mineralized at high rate (around 47% in 6 months), suggesting it was in more labile. This mineralized N can be subjected to potential losses during following months.
Response to Reviewers:	Answers to Reviewer comments line 80 should the average be referred as "regional" instead or "areal"? After an analysis about the differences between "regional" and "areal", we accepted to change to "…. weather characteristics were around regional average." line 96 replace the "2 levels of N and two levels of water" with "two levels of N and two levels of water" Replaced.

	line 262 replace "denitrificación" with "denitrification" Replaced, the word remained in Spanish. line 268 replace "data not showed" with "data not shown" Replaced. Thanks for the attention

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Drs. Jorge Delgado
Editor
Journal of Soil and Water Conservation

Dear Dr. Delgado

I am submitting you the manuscript entitled **Fate of nitrogen and nitrate leaching in maize and cover crop sequence in the Pampas**, asking you to consider it for publication in the **Journal of Soil and Water Conservation**, The authors are H. Rimski-Korsakov, M.S. Zubillaga, M.R. Landriscini and myself

Thank you very much for your attention

Sincerely

Raul S. Lavado
Professor

Fate of nitrogen and nitrate leaching in maize and cover crop sequence in the Pampas

H. Rimski-Korsakov, M.S. Zubillaga, M.R. Landriscini and R.S. Lavado

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Maize and cover crop sequence in the Pampas: effect of fertilization and water stress on the fate of nitrogen

Abstract: Cover crops are well known for their positive effects on erosion processes, soil organic matter, soil physical properties, weed populations and nitrate leaching. In this work, we evaluated the fate of nitrogen (N) from fertilizer in maize (*Zea mays*) and then in ryegrass (*Lolium multiflorum*) as cover crop, in the conditions of the Argentine Pampas. To this end, a field experiment was carried out at the School of Agriculture, University of Buenos Aires, Argentina (34°36´ S, 58°29´ W). The design of the experiment was a factorial with three replications. We applied to maize 2 levels of N (0 and 140 kg N ha^{-1} (125 lb N ac^{-1}) (ammonium nitrate target with ^{15}N) and two levels of water (50 and 100% of crop evapotranspiration). ^{15}N was determined in both the soil and plants. Maize plants and the soil organic fraction were the main sinks of fertilizer N, depending on the water treatment. The N from fertilizer remaining as nitrates in the soil (0 to 1.50 m [0 to 4.92 ft] depth) at maize harvest was 8% in plots subjected to water stress compared to 3% in the non-water stressed. Nitrogen losses due to volatilization were minor. Total N (soil and fertilizer) accumulated in ryegrass tissues plus nitrates remaining in the soil were higher in cover crop plots than in bare soil (130 vs. 51 kg N ha^{-1} [116 vs. 45.5 lb N ac^{-1}]). The N in the soil organic matter originating from fertilizer significantly decreased between maize harvest and cover crop harvest. This soil organic N that originated from fertilizer mineralized at high rate (around 47% in 6 months), suggesting it was in more labile. This mineralized N can be subjected to potential losses during following months.

Key words: cover crop–maize–nitrate losses–nitrogen balance–tagged nitrogen

The recovery of fertilizer nitrogen (N) in crops total biomass does not generally exceed 50%, but is even lower after the occurrence of different stresses during the crop cycle (Ma et al. 1995; Gardner and Drinkwater 2009). Water stress, for instance, decreases N uptake by a crop, irrespective of the source of nitrates (fertilizers, soil organic matter, manure), and some nitrates remain in the soil after crop harvest. These nitrates are usually called "residual nitrates". This not recovered N can go into the soil organic fraction, remain as residual nitrates, or be lost by processes such as leaching, volatilization and denitrification. Nitrogen losses have economic consequences and environmental risks. In some agricultural systems, nitrate leaching cause contamination of aquifers (Spalding and Exner 1993). This process is favored by prolonged periods with the soil free of live vegetation, during which nitrates are not taken up by crops (Di and Cameron 2002). Thus, in most agricultural systems, bare fallow is a period susceptible to suffer this kind of losses (Drury et al. 1996; Cambardella et al. 1999). This is typical of production systems dominated by maize, which leaves long fallow periods (Dinnes et al. 2002). If during fallow the soil is covered by growing vegetation, i.e. cover crops (CC), or even weeds, the risk of nitrate leaching is usually reduced (Di and Cameron 2002; Tonitto et al. 2006).

Cover crops are defined as crops seeded between cash crops which are not harvested, not incorporated into the soil as green manures, and are not intended to be grazed, such as annual forages (Glossary of Soil Science Terms). They are well known for their ability to control erosion processes (Langdale et al. 1991; Kaspar et al. 2001), increase the soil organic matter content and thus improve the soil physical properties (Reicosky and Forcella 1998; Ding et al. 2006), and reduce weed populations (Williams et al. 2000). Cover crops are also used to decrease the risk of contamination of aquifers by nitrates coming from fertilizers and organic matter mineralization (Meisinger et al. 1991; Di and Cameron 2002; Dinnes et al., 2002; Meisinger and Delgado 2002; Tonitto et al. 2006). The effect of CC seems to occur as a two-stage process: i) the absorption of the nitrates of any origin present in the soil and ii) the release of such N during the next crop cycle (Collins et al. 2007). Cover crops can also reduce nitrate leaching because their high transpiration rate reduces water percolation (Thorup-Kristensen at al. 2003).

All these facts contribute to the general favorable consensus about the ability of CC to reduce nitrate losses. However, it has also been reported that CC are not efficient or can even increase nitrate leaching in the long term (Berntsen at al. 2006). Their incorporation within a crop sequence can also show negative consequences, reducing the yield of the following crop (Williams et al. 2000; Salmerón et al. 2011). This usually happens due to the competition between the commercial crop and the CC for water and nutrients (Dabney et al. 2001). The most common species used as CC belong to the family Poaceae and, to a lesser extent, to the family Fabaceae. Among Poaceae, annual ryegrass (*Lolium multiflorum*) is one of the species used as CC in winter fallows because it is characterized by its fast growth and good adaptation to clayey, wet soils (Clark 2007). Our objective was to evaluate the fate of N from fertilizer in aboveground biomass, soil organic matter, soil nitrates and its distribution in the soil profile, and ammonia volatilization, in a maize production system subjected to water stress with ryegrass as a CC, in the conditions of the Argentine Pampas.

Materials and Methods

A field experiment was carried out in the campus of the School of Agriculture, University of Buenos Aires, Argentina, located at 34°36´ S, 58°29´ W. The soil was a fine, illitic, thermic Vertic Argiudoll (USDA 2006), whose main characteristics in the plow layer, determined using standard techniques (Sparks et al. 1996), were: electrical conductivity 0.08 dS m^{-1}; pH in water 7.3; organic carbon (Walkley and Black) 1.75%; total Kjeldhal N 0.16%; and available phosphorus (Bray #1) 17.1 mg kg^{-1} (ppm). The experiment was carried out in maize

(*Zea mays* cv FAUBA 209) followed by annual ryegrass (local commercial population of *Lolium multiflorum*) as CC. This was a short term experiment, like most studies using ^{15}N (Gardner and Drinkwater 2009). The main reason in present experiment was that water was provided by irrigation and the drought was not related to climate variability. Besides, temperature and other weather characteristics were around regional average. Rainfall and other meteorological data during the length of the experiment were taken from a Meteorological Station from the National Weather Service, located less than 500 m (1640 ft) from the experiment location. The annual mean temperature registered in the Station was 16.6 ºC. January is the hottest month (mean 24.9ºC) and July the coldest (mean 11.0 ºC). The annual rainfall amounts 1146 mm, with no define seasonality but with great variations among years.

Maize Cropping. Maize was seeded on November 15, 2005, following conventional tillage, and harvested on May 16, 2006. The plot had maize as a previous crop. Maize was seeded manually in 50 cm (19.7 in) rows with 20 cm (7.9 in) between plants for a total population of 100,000 plants ha^{-1} (40,469 plants ac^{-1}). Each plot was 2.5 x 7.5 m (8.2 x 24.6 ft) and a "microplot" (1.5 x 1.2 m [4.9 x 3.9 ft]) was installed in the center of each fertilized plot (figure 1). To cover maize P requirement, two days before seeding, triple superphosphate (30 kg P ha^{-1} [26.8 lb P ac^{-1}]) was broadcasted in all plots. Weeds, insects and diseases during the crop cycle were controlled when necessary.

The experimental was a factorial (2x2) in randomized complete block design with three replications. We applied to maize two levels of N and two levels of water: ***N0:*** not N fertilizer was applied; ***N140:*** N fertilization doses of 140 kg N ha^{-1} (125 lb N ac^{-1}) and -W***:*** subjected to water stress; ***+W:*** not subjected to water stress.

Nitrogen fertilization was carried out in the phenological stage V6 (Ritchie and

Hanway 1982), applying ammonium nitrate (140 kg N ha^{-1} [125 lb N ac^{-1}]) manually in bands at 3 to 4 cm (1.2 to 1.6 in) depth. Within the microplots, the same N doses were applied using ^{15}N tagged fertilizer (1.5% abundance; $^{15}NH_4{}^{15}NO_3$) (figure 1). To regulate rain water input in the experiment, a plastic structure was installed on all plots. When rainfall was forecasted, this structure was unfolded and all spaces between the seeding lines were covered by the plastic strips. The structure had a slope toward a ditch, to remove the rain water from the plots. This simple mechanism limited most rain water from reaching the soil.

The potential evapotranspiration was calculated to estimate crop water requirement (Penman 1948). A drop irrigation system was installed, which delivered 100% and 50% of the calculated maize water requirement (621 mm [24.4 in and 310 mm [12.2 in]), respectively. During the critical water period for maize (15 days before and after flowering), no water was added to the ***-W*** plots. Clearly, the plastic cover did not prevent total flow of rainfall into the soil, but its effect on the water treatments was minor, as tested before the experiment. Water is the main variable factor affecting plant behavior between years. In the present experiment water was provided by irrigation, reasonably solving the lack of environmental replications. Besides, temperature, radiation and other weather factors that affect crop growth were around average. Maize was manually harvested at physiological maturity. Plant material (stems+leaves+cobs+husks and grain) were collected and dry weights measured after drying in an oven at 60°C (140ºF) to constant weight. Total N and the proportion of ^{15}N were determined in plant samples by the Kjeldahl method (Bremner and Mulvaney 1982) and optical emission spectrometry (Fiedler and Proksch 1975), respectively. Soil samples at 0.30 m (0.98 ft) intervals up to 1.50 m (4.92 ft) depth were taken at maize seeding and harvest. Nitrate concentration was determined in all soil samples by extraction with 2M KCl and distillation with MgO and Devarda alloy (Keeney and Nelson 1982). The proportion of NO_3-^{15}N was also determined. Total N and the proportion of ^{15}N were determined at harvest in top soil samples by the above mentioned techniques. The N derived from fertilizer assimilated into the soil organic fraction was calculated by subtracting the NO_3-^{15}N from the total ^{15}N. Ammonia volatilization was determined following the chamber method proposed by Nommik (1973). Ammonia was trapped in sponges with sulfuric acid-glycerol solution, subjected to airstreams distillation and determined using a colorimeter (Sparks et al. 1996). Volatilization of ammonia was determined 4, 15, 34 and 150 days after N fertilization.

The N derived from the fertilizer (NdfF) in the soil or plant compartment (%) and the N derived from the fertilizer (kg N ha^{-1}) were calculated using equation 1 and equation 2, respectively. The natural abundance of ^{15}N in the commercial ammonium nitrate used to dilute the tagged ammonium nitrate was estimated as 0.366% (IAEA 2001). For the plant and soil compartments of the fertilized treatments, we used the natural abundance of ^{15}N in the equivalent N0 treatment compartment. The N volatilized from the fertilizer was calculated using equation 3.

$$NdfF\ in\ plant\ or\ soil\ (\%) = \frac{\%\ atom\ ^{15}N\ in\ plant\ or\ soil - \%\ atom\ ^{15}N\ natural\ in\ plant\ or\ soil}{\%\ atom\ ^{15}N\ in\ fertilizer - \%\ atom\ ^{15}N\ natural\ in\ fertilizer} * 100$$

(Equation 1)

$$NdfF\ in\ plant\ or\ soil\ (kg\ N\ ha^{-1}) = NdfF\ in\ plant\ or\ soil\ (\%) * N\ in\ plant\ or\ soil\ (kg\ N\ ha^{-1}) / 100$$

(Equation 2)

$$NdfF\ volatilized = NH_4\text{-}N\ volatilized\ in\ treatment\ N140 - NH_4\text{-}N\ volatilized\ in\ treatment\ N0\ (kg\ N\ ha^{-1})$$

(Equation 3)

The recovery of fertilizer in each compartment was estimated following equation 4.

$$Fertilizer\ recovery\ (\%) = \frac{NdfF}{N_{fertilizer}} * 100$$

(Equation 4)

Where:

NdfF = N derived from the fertilizer in the plant, soil organic N, soil NO_3-N, NH_4-$N_{volatilized}$ (kg N ha^{-1})

$N_{fertilizer}$ = Nitrogen applied by fertilization (kg N ha^{-1})

The total recovery of the fertilizer applied was calculated using equation 5.

$$N\ fertilizer\ (100\%) = NdfF\ plant\ (\%) + NdfF\ volatilized\ (\%) + NdfF\ nitrate\ (\%) + NdfF\ soil\ organic + \\ + NdfF\ unaccounted\ (\%)$$

(Equation 5)

Where:

$N_{fertilizer}$ = N applied by fertilization (100%).

$NdfF_{plant}$ = N from the fertilizer taken by the whole plant at physiological maturity (%).

$NdfF_{volatilized}$ = N from the fertilizer volatilized (%).

$NdfF_{nitrate}$ = N from the fertilizer remaining as nitrates in the soil (%).

$NdfF_{organic}$ = N from the fertilizer remaining in the soil as organic N (%).

$NdfF_{unaccounted}$ = N from the fertilizer not detected in any of the compartments studied (%).

Cover Crop. The plastic cover to control rainfall was removed after maize harvest and each maize plot was divided in two. A new factorial design with three treatments was defined: Nitrogen fertilization applied to maize (N0 and N140), water applied to maize (-W and +W) and cover crop (with or without CC). Annual ryegrass (*Lolium multiflorum*) was seeded on May 20, 2006, by broadcasting the equivalent to 30 kg seeds ha^{-1} (26.8 lb seeds ac^{-1}) in one half of the plots (figure 1). The other half remained without vegetation, weeds were controlled manually and pulled weeds were left in the plot. The rainfall and evapotranspiration during the CC cycle amounted to 468 and 448 mm (18.4 and 17.6 in), respectively. During the last 30 days of the experiment, the rainfall was very high (277 mm [10.9 in]) and of great intensity.

On November 5, 2006, the ryegrass was harvested by cutting 0.25 m^2 (2.7 ft^2) in the center of all half plots, both where the maize was fertilized with ^{15}N and where it was not. At the same time, soil samples at 0.30 m (0.98 ft) intervals up to 1.50 m (4.92 ft) depth were taken in the center of all half plots. Total N and the proportion of ^{15}N were determined on the plant material and top soil samples; nitrates and the proportion of ^{15}N were determined in the soil samples taken to 150 cm depth. The above-described methods were followed in all cases. The apparent mineralization of the soil organic N coming from the fertilizer (^{15}N) was estimated as the difference between the soil organic ^{15}N content at maize harvest and at the end of the experiment (ryegrass harvest). The immobilization of the ^{15}N released by the decomposition of maize stubble during the study period in the soil organic fraction was not measured. This could have affected the mineralization quantities estimated.

Statistics. The effects of the treatments were statistically analyzed using factorial ANOVA, and the interactions between them and their effects were analyzed. When the interaction was significant between treatments (W and N), comparisons were made between all combinations of N and water application, whereas when the interaction was not significant, comparisons were made between treatments. The least significant difference (LSD) was used to differentiate means. Differences were considered to be significant at the 5% (p=0.05) probability level.

Results and Discussion

Maize plants. Maize vegetative and total aboveground biomass was significantly higher when high N doses were applied and water supply was enough for crop requirements (N140+W) as compared with the remainder treatments (tables 1 and 2). The other treatments showed no significant differences between them. The water-N interaction agrees with that found by Pandey et al. (2000) but not with that found by Gheysari et al. (2009), who found additive effects on total maize biomass when N doses increased with different water conditions. It is possible this behavior was caused by the lower water stress imposed in that experiment (15 to 30 % of non-stressed treatment), as compared with our water conditions (50%). On the other hand, the grain yield increased significantly and additively when water supply and N were adequate.

Nitrogen concentration in the vegetative biomass (0.45%) and in grains (1.21%) was not significantly different between treatments. Nitrogen accumulation in the total aboveground biomass (vegetative biomass+grains) and grains alone was higher with high fertilizer doses and without water stress (additive effects) (tables 1 and 2). Nitrogen coming from the soil and located in total aboveground biomass was not affected by the treatments applied and was on average 74% of the total N accumulated in that biomass (tables 1 and 2). Treatment affected the accumulation of N coming from fertilizer in total aboveground biomass, which was significantly higher when maize was not subjected to water stress (tables 1 and 2). The fertilizer recovery in total aerial biomass was then 24 vs. 46%, of the N applied via fertilization, when maize was or not subjected to water stress, respectively.

Nitrogen in soil at maize harvest. The total content of soil nitrates (derived from the soil + derived from fertilizer) at 0 to1.50 m (0 to 4.92 ft) depth at maize harvest was significantly higher in fertilized treatment than in non-fertilized plots (119 vs. 105 kg NO_3-N ha^{-1} [106 vs. 94 lb NO_3-N ac^{-1}]; figure 2A). Plots subjected to water stress showed a trend (p=0.067) to a higher concentration of nitrates than non-subjected to water stress plots (118 vs. 106 kg NO_3-N ha^{-1} [105 vs. 95 lb NO_3-N ac^{-1}] respectively). Lower N uptake by maize and lack of nitrate movement due to dry conditions can explain this behavior (Gheysari et al. 2009). The soil derived nitrates (non-tagged nitrates, NO_3-^{14}N) from 0 to 1.50 m (0 to 4.92 ft) presented no effects caused by the treatments imposed. The N derived from fertilizer (tagged nitrates, NO_3-^{15}N) in the water stress treatments was responsible for the trend shown in figure 2B. The content of nitrates derived from the soil plus derived from fertilizer and the nitrates from fertilizer was significantly higher only at 0 to 30 cm (0 to 11.8 in) depth in non-subjected to water stress plots, without effects of N fertilization (N0 or N140). No differences in total nitrates were found at the other soil depths studied.

The content of total soil organic N including the ^{15}N derived from the fertilizer at 0 to 30 cm (0 to 11.8 in) depth was not affected by the treatments imposed. Conversely, the N fraction in the soil organic matter derived from fertilizer was higher in the ***-W*** treatment than in the +***W*** treatment (78 and 51 kg N ha^{-1} [70 and 46 lb N ac^{-1}]), which means 56 and 37% of the applied fertilizer, respectively (figure 3). Stressed plants, with low N recovery efficiency, left a high concentration of N in the soil, which was immobilized by soil biota and mainly accumulated in the soil organic fraction. Our data were relatively greater that those found by Reddy and Reddy (1993) and Portela et al. (2006), who found 10 to 30% of the applied N in the soil organic matter at harvest of maize not subjected to limiting conditions.

Volatilization during maize. Accumulated volatilization during the maize cycle was significantly higher when fertilizer was applied, with no differences between water treatments. Volatilization in non- fertilized treatments was the natural volatilization from the soil (3.2 kg NH_3-N ha^{-1} [2.9 lb NH_3-N ac^{-1}]) while fertilized treatments lost 7.1 kg NH_3-N ha^{-1} (6.3 lb NH_3-N ac^{-1}) on average. Based on these data, we estimated that the N volatilized from fertilizer was 3.9 kg NH_3-N ha^{-1} (3.5 lb NH_3-N ac^{-1}) during the maize cycle. These losses were lower than other data observed in the Pampas region (Palma et al. 1998) and could be attributed to the N source (ammonia nitrate), the depth fertilizer applied and/or the occurrence of adequate humidity in the soil.

Fate of N from fertilizer during maize cycle. Maize biomass was the main sink of N from fertilizer in plants not subjected to water stress, being the soil organic fraction the second most important sink: 47% and 37%, respectively (figure 4). When plants were subjected to water stress, we observed the opposite trend: the soil organic fraction was the main sink for the N from fertilizer (56%) followed by maize plants (24%). The N from fertilizer remaining as residual nitrates (0 to 1.50 m [0 to 4.92 ft] depth) was higher in ***-W*** plots (8%) than in +***W*** plots (3%). Volatilization was a minor sink of N (3%), with no differences between water treatments. Around 10% of the N applied was not recovered. Roots were also a sink of this unrecovered fraction. Although roots were not quantified in the present study, in an experiment carried out near the experimental site, the amount in roots was around 3% of the applied N (Rimski-Korsakov et al. 2012). The remaining unrecovered N can be attributed to losses by denitrification, leaching below the studied depth, or inaccuracies of the methods applied. Nitrogen losses via denitrification are currently low in the area (<0.6% of applied N) (Palma et al. 1997; Sainz Rozas et al. 2001).

Cover crop plants. Nitrogen fertilization and water stress applied to maize showed little effect on the biomass produced by the CC (table 3). Only a trend (p=0.067) to higher biomass production of ryegrass when previous maize was fertilized was found (table 3). The N concentration (1.89% on average) (data not shown) and total N accumulation in the CC crop aerial biomass was not significantly affected by maize fertilization or water status (table 3). No significant differences were observed in the N accumulated derived from the soil or from the fertilizer in the CC (table 3). The N accumulated in CC aerial biomass was 78 kg N ha^{-1} (70 lb N ac^{-1}) being 4.22 kg N ha^{-1} (3.77 lb N ac^{-1}) derived from the fertilizer applied to the maize (table 3).

Nitrogen in soil at cover crop harvest. The total nitrates (derived from soil [non-tagged]+ derived from fertilizer [tagged]) from 0 to 1.50 m (0 to 4.92 ft) depth present a trend to show interaction (p=0.068) between the water treatments (+***W*** and –***W***) on the previous maize and the subsequent CC (+CC and –CC) (tables 4 and 5). The lowest nitrate content in the soil was found when previous maize non-subjected to water stress and the CC was grown; the other treatments showed no differences between them. The nitrates derived from fertilizer in the whole soil profile (0 to 1.50 m [0 to 4.92 ft]) or at any depths showed no significant differences caused by the treatments, being on average 2.6 kg NO_3-^{15}N ha^{-1} (2.32 lb NO_3-^{15}N ac^{-1}) (table 4). About 1.27 kg (2.80 lb) of the NO_3-^{15}N derived from the fertilizer was found in the top soil (0 to 0.30 m [0 to 0.98 ft] depth) after the CC harvest (figure 5). At the end of the experiment, the nitrate concentration in the soil covered by the CC and in the bare soil was similar. Working in contrasting rainfall years, Willumsen and Thorup-Kristensen (2001) found results similar to those of the present experiment. These authors attributed this result to nitrate leaching in the wet period and the opposite in the dry year, when the uncovered soil showed high nitrate content. A month before the end of the present experiment, high rainfall was recorded (277 mm [10.9 in]). Such precipitation could have displaced the residual nitrates deeper in the profile, mainly in uncovered soil. The CC retained 78 kg N ha^{-1} (70 lb N ac^{-1}) in their biomass (table 3), which would be not available to suffer leaching losses. The CC acted as an efficient trap, avoiding losses of residual nitrates.

The content of N derived from fertilizer in the soil organic fraction at CC harvest showed no significant differences between treatments, although it was significantly lower than N coming from fertilizer at maize harvest (p=0.001) (figure 3). On average, 35 kg (77 lb) of the N derived from fertilizer was found at the end of the experiment retained in the soil organic fraction. This immobilized N could be taken by the next crop after soil organic matter mineralization. The apparent mineralization of the fertilizer retained in soil organic N during the CC cycle, was on average 47%. Even taking the inaccuracies into account, this amount was order of magnitude greater than the mineralization of soil organic matter in the Pampas's soil (4-5 % yearly) (Alvarez and Alvarez 2000). The N from fertilizer applied to maize remains in the labile fractions of the soil organic matter, thus being more easily mineralized (Alvarez and Alvarez 2000). Then, the N from fertilizer retained in the soil organic fraction could attenuate nitrate leaching but only in the short term. This high mineralization rates show that this N would be released swiftly and that when no other crop retains it, it can be leached.

The N retained in CC plants (table 3) plus nitrates remaining in the soil (table 4) showed that the CC treatment accumulated significantly more N ($p<0.001$) than the nitrates remaining in the uncovered soil during the fallow (130 vs. 51 kg N ha^{-1} [116 vs. 45 lb N ac^{-1}] respectively). The N derived specifically from the fertilizer remaining in plants plus nitrates remaining in the soil was significantly higher in the CC treatment ($p=0.03$). When N in the soil organic fraction was included, no significant differences in the soil-plant system were recorded among the studied factors, probably due to the organic component magnitude which dilutes the magnitude of other sinks.

Summary and Conclusions

In the conditions of present research, results showed that the main sinks of fertilizer N were maize plants in non-water stressed treatments and the soil organic fraction in the water stressed. Residual nitrates were low (8% vs. 3%, according to water stress). Volatilization was always a minor sink. The CC took up nitrates from the soil leaving fewer nitrates subjected to losses (i.e. leaching) and the N accumulated in CC will be available for the subsequent crop. The N from fertilizer retained in the organic matter is quickly mineralized (around 47% in 6 months), releasing N subjected to possible losses in the months ahead.

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Figure 1

Diagram showing a plot boundaries and the microplot location, and within them the fertilized band, the place of the cylinders for volatilization determination, the sampling plants, the sampling soil site and the rows of maize

Figure 2

Nitrate content from 0 to 150 cm (0 to 59 in) depth at maize harvest. The experiment was carried out in the Argentinean Pampas. Maize was seeded and 2 nitrogen fertilizer doses 0 and 140 kg N ha^{-1} were applied. ^{15}N tagged fertilizer was used. The crop was subjected to water stress or not subjected to water stress by drop irrigation. Bars: standard error

Figure 3

Nitrogen located in the soil organic matter at 0 to 30 cm (0 to 11.8 in) depth, derived from the fertilizer at maize and cover crop harvest. Maize was seeded and 2 nitrogen fertilizer doses 0 and 140 kg N ha^{-1} were applied. ^{15}N tagged fertilizer was used. The crop was subjected to water stress or not subjected to water stress by drop irrigation. After the maize a cover crop was seeded. Bars: standard error.

Figure 4

Sinks of the N from the fertilizer in each treatment relative to the N applied via fertilization. Plant: N accumulated in the total aboveground biomass of maize at harvest; Volatilized: ammonia-N volatilized from fertilization to crop harvest; Nitrates: nitrates-N from 0 to 150 cm (0 to 59 in) depth, at maize harvest; Organic N: ^{15}N measured in the soil organic pool, 0 to 30 cm (0 to 11.8 in) depth; Unaccounted: fraction of the applied N, non-recovered.

Figure 5

Nitrates derived from fertilizer from 0 to 150 cm (0 to 59 in) depth at cover crop harvest. The experiment was carried out in the Argentinean Pampas. After maize harvest, annual ryegrass was seeded. It was not fertilized and received only rainfall water.

Bars: Standard error.

Table 1

Maize biomass production at harvest, total N absorbed (production x N concentration), N derived from the soil (NdfS) and N derived from fertilizer (NdfF), in aerial vegetative biomass (stems+leaves+cobs+husks) and grains. N0: not N fertilizer was applied; N140: N fertilization doses of 140 kg N ha-1 and -W: subjected to water stress; +W: not subjected to water stress. The standard error is given between parentheses.

Treatments	**Biomass** (kg DM ha^{-1})	**N total** (kg N ha^{-1})	**NdfS** (kg N ha^{-1})	**NdfF** (kg N ha^{-1})
Aerial vegetative biomass				
N0-W	7,490 (419) b	31.2 (1.8)	31.2 (1.8)	--------
N0+W	6,669 (255) b	27.6 (1.0)	27.6 (1.0)	--------
N140-W	7,767 (933) b	38.7 (7.9)	31.1 (5.5)	7.6 (2.3)
N140+W	10,421 (100) a	51.5 (4.2)	34.9 (5.0)	16.6 (0.9)
Grains				
N0-W	6,799 (543)	77.9 (10.8)	77.9 (10.8)	--------
N0+W	8,058 (1,011)	94.5 (12.6)	94.5 (12.6)	--------
N140-W	9,153 (838)	114.9 (12.5)	88.8 (8.0)	26.0 (5.9)
N140+W	13,301 (577)	171.1 (9.4)	122.4 (11.5)	48.6 (6.4)
Total aboveground biomass				
N0-W	14,289 (838) b	109.1 (12.5)	109.1 (12.5)	--------
N0+W	14,727 (1,267) b	122.2 (11.6)	122.2 (11.6)	--------
N140-W	16,921 (1,762) b	153.6 (20.3)	119.9 (13.2)	33.7 (8.1)
N140+W	23,723 (663) a	222.6 (5.8)	157.3 (6.5)	65.2 (5.8)

Letters indicate significant differences between treatments ($p<0.05$) and the occurrence of a positive interaction.

Table 2

Analysis of variance probability values for the effects of water stress (W) and nitrogen (N) availability. Differences were considered to be significant at the 5% (p=0.05) probability level.

	Treatments		**Interaction**
	W	N	WxN
	p value	*p* value	*p* value
Aerial vegetative biomass			
Biomass production	0.142	0.010	0.018
N concentration	0.953	0.103	0.934
Total N absorbed	0.342	0.013	0.112
N absorbed from soil	0.970	0.384	0.367
N absorbed from fertilizer	0.107	-------	-------
Grains			
Grains production	0.009	0.002	0.090
N concentration	0.575	0.127	0.949
Total N absorbed	0.032	0.005	0.180
N absorbed from soil	0.073	0.145	0.490
N absorbed from fertilizer	0.032	-------	-------
Total aboveground biomass			
Biomass production	0.023	0.003	0.037
Total N absorbed	0.038	0.003	0.122
N absorbed from soil	0.091	0.118	0.369
N absorbed from fertilizer	0.038	-------	-------

Table 3

Ryegrass aerial biomass production and total N absorbed (production x N concentration), N derived from the soil (NdfS) and N derived from fertilizer (NdfF), in aerial biomass at ryegrass harvest and analysis of variance probability values for the effects of water (W) and nitrogen (N) availability. The water and N treatments are those applied to the preceding maize. N0: not N fertilizer was applied; N140: N fertilization doses of 140 kg N ha-1 and -W: subjected to water stress; +W: not subjected to water stress. The ryegrass was neither fertilized nor irrigated. The standard error is given between parentheses. Differences were considered to be significant at the 5% (p=0.05) probability level.

Treatments	**Aerial Biomass** (kg DM ha^{-1})	**Total N** (kg N ha^{-1})	**NdfS** (kg N ha^{-1})	**NdfF** (kg N ha^{-1})
N0-W	2,560 (370)	48.53 (2.68)	48.53 (2.68)	--------
N0+W	4,053 (352)	83.06 (9.06)	83.06 (9.06)	--------
N140-W	4,677 (806)	80.82 (17.39)	77.54 (16.65)	3.27 (0.77)
N140+W	5,429 (1,021)	100.09 (19.64)	94.93 (18.29)	5.16 (1.52)
ANOVA p values				
W	0.201	0.144	0.134	0.475
N	0.067	0.174	0.228	--------
WxN	0.652	0.651	0.594	--------

Table 4

Content of total nitrates, nitrates derived from the soil (Nitrates dfS) and nitrates derived from the fertilizer (Nitrates dfF) from 0 to 150 cm (0 to 59 in) to depth (kg NO_3-N ha^{-1}) at ryegrass harvest. N0: not N fertilizer was applied; N140: N fertilization doses of 140 kg N ha-1; -W: subjected to water stress; +W: not subjected to water stress; +CC: with cover crop; -CC: without cover crop. The standard error is given between parentheses.

Treatments	Total nitrates (kg NO_3-N ha^{-1})	Nitrates dfS (kg NO_3-N ha^{-1})	Nitrates dfF (kg NO_3-N ha^{-1})
N0-W+CC	47.4 (8.8)	47.4 (8.8)	———
N0-W-CC	44.0 (3.6)	44.0 (3.6)	———
N0+W+CC	40.4 (2.0)	40.4 (2.0)	———
N0+W-CC	39.0 (9.7)	49.6 (9.7)	———
N140-W+CC	76.7 (16.8)	73.6 (16.1)	3.1 (0.8)
N140-W-CC	66.9 (8.8)	63.8 (8.6)	3.1 (0.2)
N140+W+CC	45.8 (14.3)	43.9 (13.9)	1.9 (0.4)
N140+W-CC	61.6 (23.6)	59.2 (22.4)	2.4 (1.3)

Table 5

Analysis of variance probability values for the effects of water stress (W) and nitrogen (N) availability and cover crop (CC) presence for content of total nitrates, nitrates derived from the soil (Nitrates dfS) and nitrates derived from the fertilizer (Nitrates dfF) from 0 to 150 cm (0 to 59 in) depth at ryegrass harvest. Differences were considered to be significant at the 5% (p=0.05) probability level.

Treatments	Total NO_3-N	NO_3-N dfS	NO_3-N dfF
	p value	*p* value	*p* value
N	0.177	0.227	-------
W	0.440	0.447	0.212
NxW	0.473	0.481	-------
CC	0.535	0.533	0.735
NxCC	0.993	0.989	-------
WxCC	0.068	0.063	0.658
NxWxCC	0.498	0.499	-------

Figure 1
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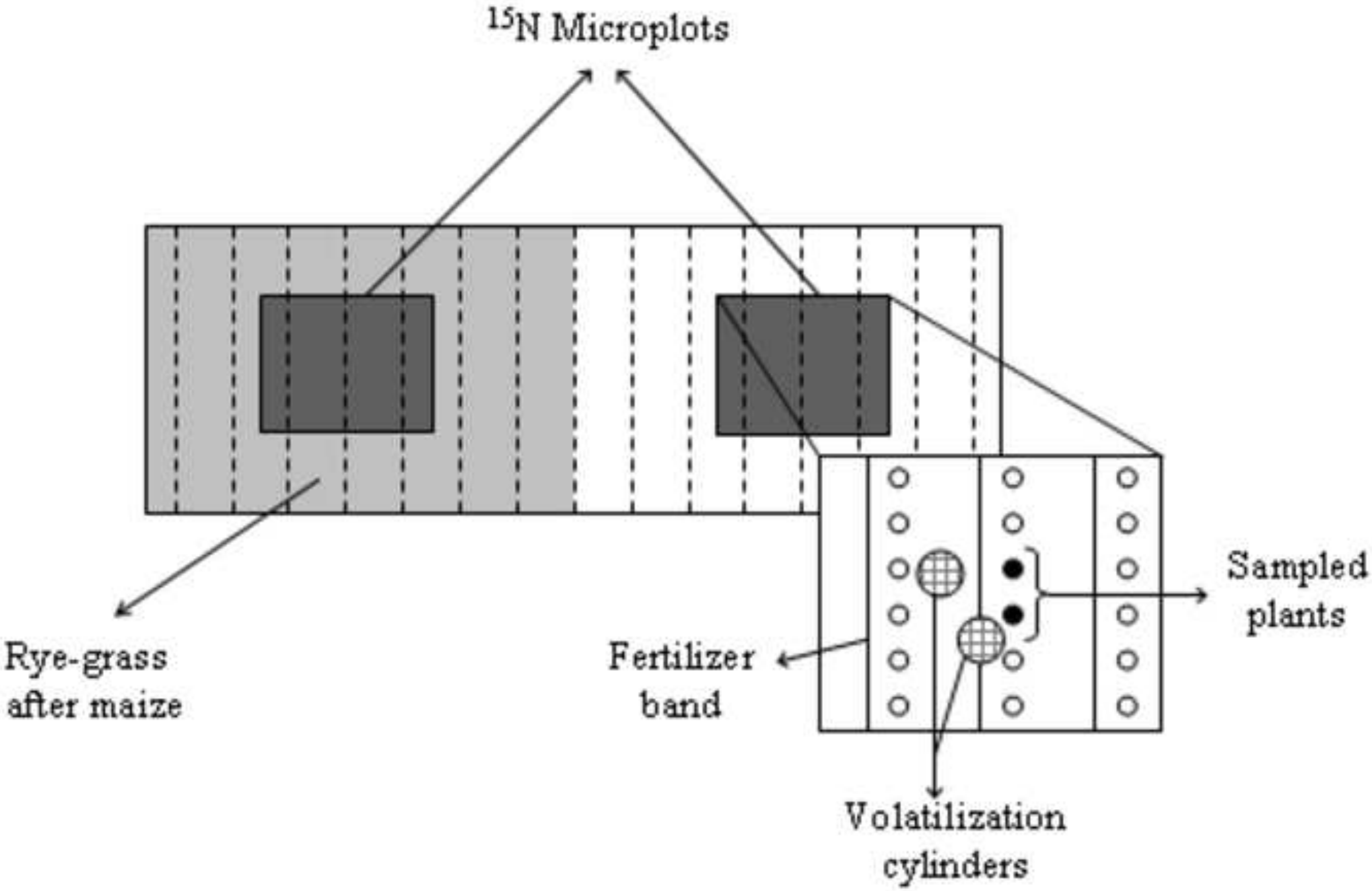

Figure 2A

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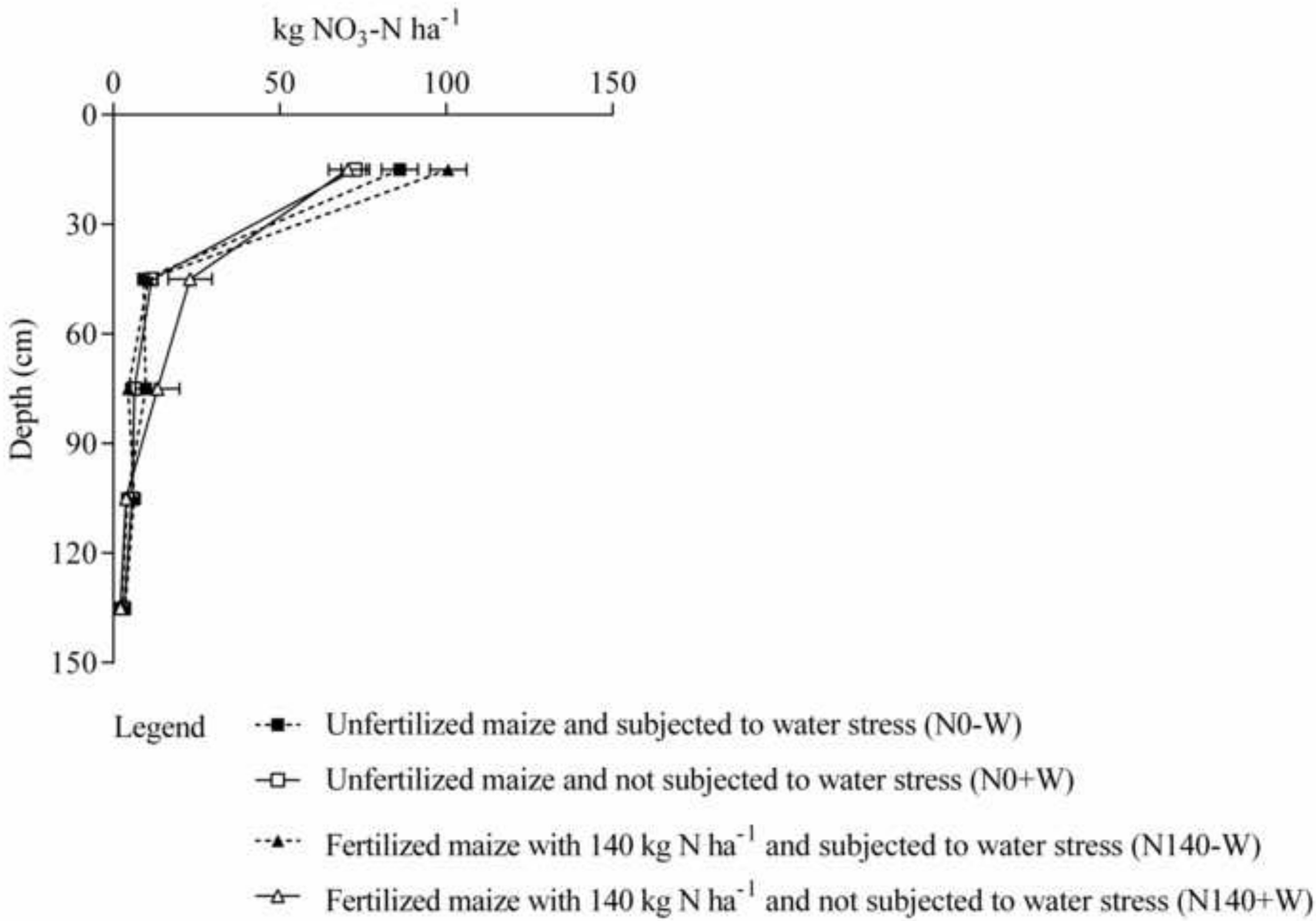

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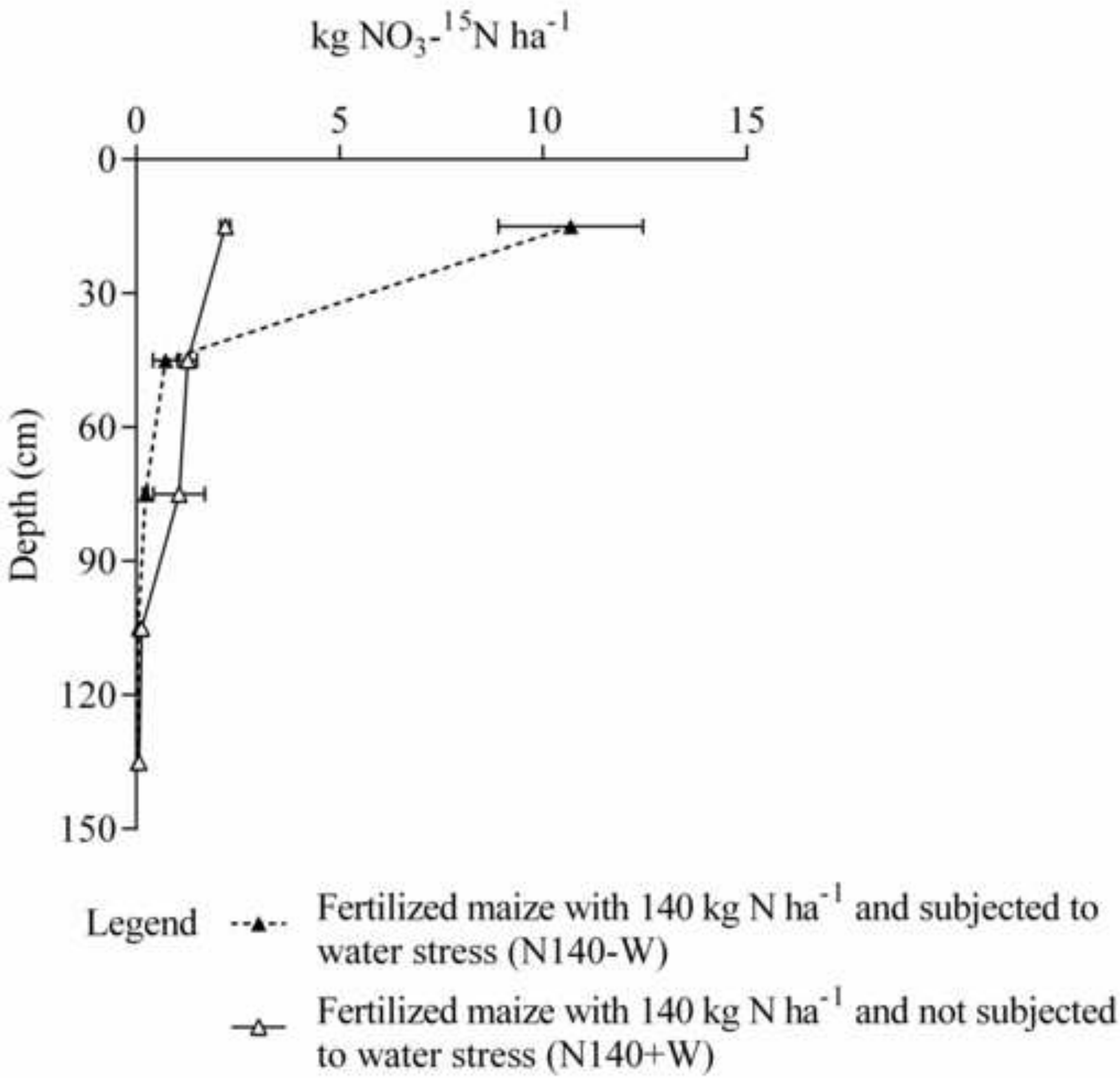

Figure 3

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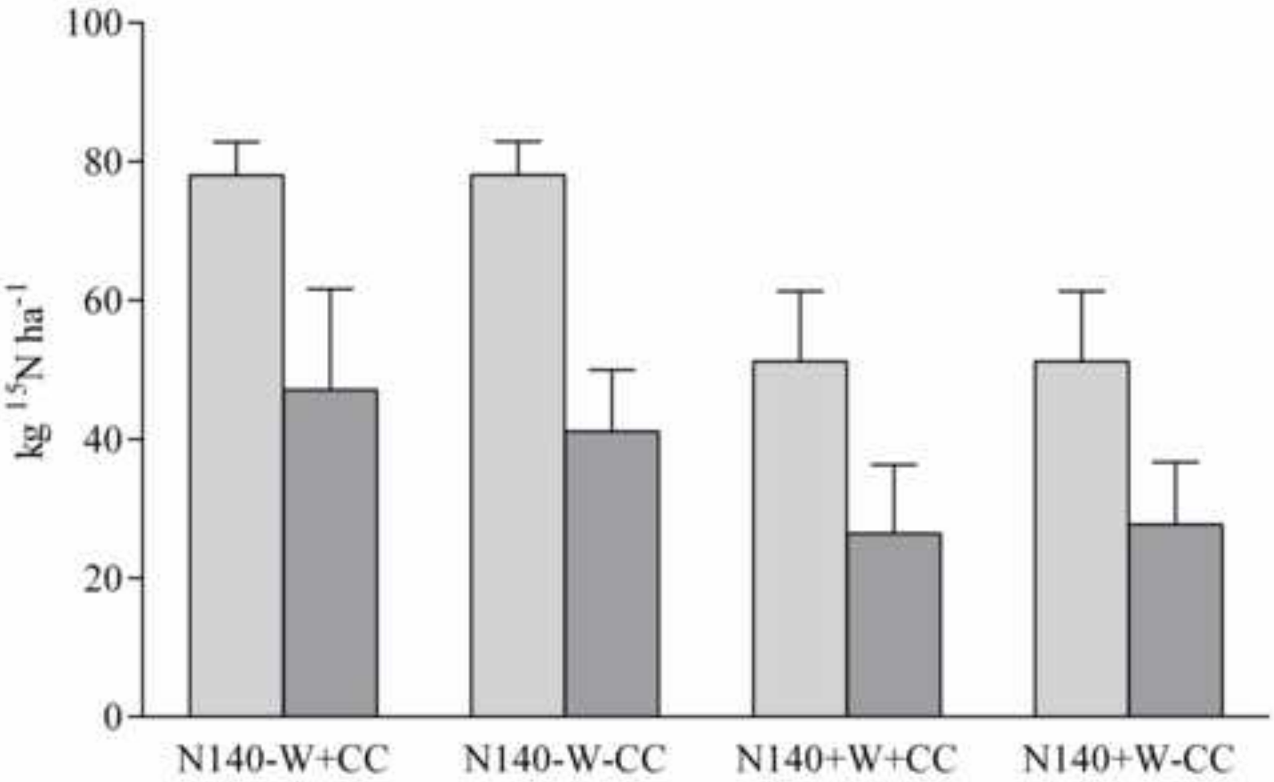

Legend

N140-W+CC = Fertilized maize with 140 kg N ha^{-1} subjected to water stress and with cover crop

N140-W-CC = Fertilized maize with 140 kg N ha^{-1} subjected to water stress and without cover crop

N140+W+CC = Fertilized maize with 140 kg N ha^{-1} not subjected to water stress and with cover crop

N140+W-CC = Fertilized maize with 140 kg N ha^{-1} not subjected to water stress and without cover crop

Maize harvest

Cover crop harvest

Figure 4

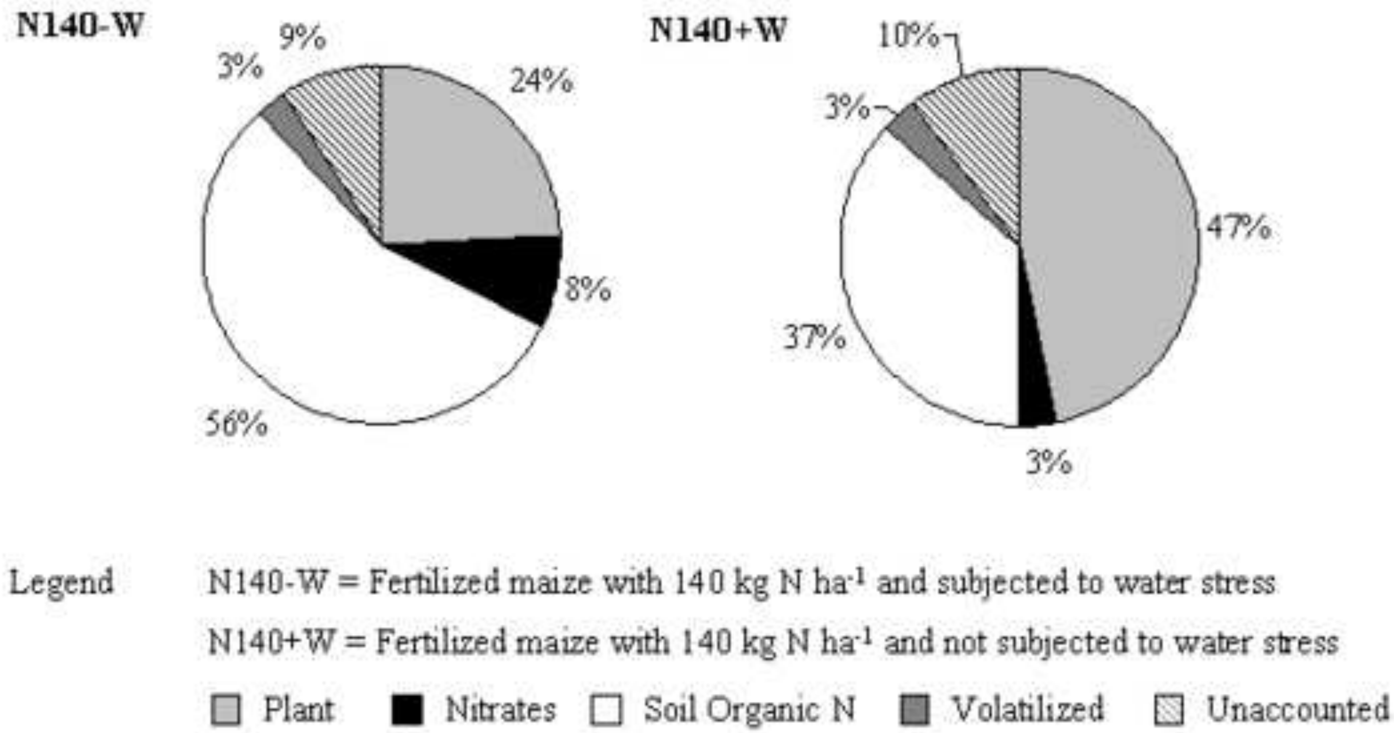
N140-W
9%
3%
24%
8%
56%
N140+W
10%
3%
47%
37%
3%
Legend
N140-W = Fertilized maize with 140 kg N ha-1 and subjected to water stress
N140+W = Fertilized maize with 140 kg N ha-1 and not subjected to water stress
Plant
Nitrates
Soil Organic N
Volatilized
Unaccounted

Figure 5

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