Fertilizer absorption efficiency δ^{15}N -labelled applied to maize crop (Zea mays) in Central Chile.

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Abstract: - Nitrogen stable isotopes (δ^{15}N) help to understand the nitrogen (N) uptake in different crop parts. This study investigates the contribution and distribution of N absorption within the different fractions/organs (leave, stem, root and grain) of the crop plant (Zea mays), and labelled N remaining into the soil (0–50cm). An experiment was carried out in an Inceptisol soil type located at Botalcura (35º 18' 26" S; 71º 47' 22''W), a rural area at Maule region, Central-Chile. Three microplots of (2.5m x 1m) were fertilized with urea $(NH_4)_2SO_4$ tagged with δ^{15}N (10% excess atom) and three intercalated microplots were located as controls. Treatments were control, 50, 100, 100 and 100 kg N ha^{-1}. Results indicate that on averages the maize store N in the leaves with 333 kg N ha^{-1} and less of 106 kgNha^{-1} came preferably from the root. From 350 kg N ha^{-1} as an applied fertilizer to the studied microplots, 6.3% was recovered from the leaves, 6.8% came from the stem, 7.1% came from the root, and 9% came from grain. However, most of the N store in the organs plant came from the soil reaching 409 kgNha^{-1} in stems and grains. Therefore, considering the recovery efficiency of N in the plant and soil a total of 95.3%; 100%; 30.3% and 100% were stored for leave, stem, root and grain, respectively. This study demonstrates the recovery efficiency of N from the fertilizer is moderate to low and remained N from the soil widely supplies the demand of the plant.

Key-Words: - Maize recovery, nitrogen isotope, δ^{15}N, isotopic composition, crop parts, N absorption

1 Introduction

Among the fertilizers, nitrogen (N) is one of the most common and needed nutrients for several crop growth [1]. However, due to excessive nitrogen fertilizer application, high nitrogen concentrations can be accumulated in the edible parts of the crop [2, 3]. In presence of excessive fertilization, crops may store high nitrogen concentrations leading to dangerous human health problems. Basically, due to long-term consumption of high levels of nitrate in crops are associated with illnesses such as infant methemoglobinemia or cancer of the digestive tract [4, 5] For this reason, the Joint Expert Committee of the Food and Agriculture (JECFA) Organization of the United Nations/World Health Organization indicate an acceptable daily intake for nitrate of 0–3.7 mg kg^{-1} body weight [3].

According to [6], studies in dairy animals feed by cereals indicated that nitrate toxicity increases with exposure time. In fact, [7] indicated in humans, approximately 80% of dietary nitrates are derived from vegetable consumption and cereals. Therefore, the reduction of fertilizer application is an important issue for sustainable agricultural practices because it can reduce the negative effects of farming on the surrounding environment. Relative to this, cereal crops are important sources of nitrate to dietary nutrition [8] of animals and humans, mainly inorganic N fertilization of intensive crops, such as maize (*Zea mays L.),* potatoes (*Solanum tuberosum L.*), and wheat (*Triticum aestivum L.)* [9].

Maize is the second most important annual crop after wheat in Chile and represents 24% of the total planted area [10]. The average yield of maize production in Chile surpasses 20 Mg ha^{-1} that is considered one of the highest average annual yields in the world [11]. Nitrogen is a determinant nutrient for maize grain yield, and for their biological processes such as absorption of water and minerals, vacuole storage, and xylem transport [12]. However, large amounts of yield exhibit a risk is the excessive use of fertilizers on human health and the environment that depend on the dosage, types and procedures of fertilization [5]. It is well known that the amount and timing of the application of nitrogen fertilizers are two important factors in nitrogen efficiency [13], and the use of a large number of synthetic fertilizers in corn is a common practice. The current problems are mainly associated on the excessive application of synthesized fertilizers, unbalanced application of nutrients, and deficiency of nutrients for crop growth [5].

Through isotopic techniques is possible to provide information on the processes involved in the N cycles in fertilization practices [14]. The $\delta^{15}N$ tracer technique has been widely used to distinguish the N plant from fertilizer or soil N and the N fluxes in agro-ecosystems [1]. Therefore, this study aimed to quantify N absorption and distribution within the plant to determine the effects of fertilization practice and rate of N fertilizer on maize production, fate of urea-$\delta^{15}N$ and N efficiency in an Inceptisol soil in Central Chile, during summer season of 2017–2018.

2 MATERIAL AND METHODS

2.1 Description of the study site. A maize production experiment was conducted in the Pencahue valley, Maule Region, Central Chile (35º 18’ 26” S; 71º 47’ 22’’W) during the spring-summer seasons from November 2017 to March 2018, in a private land authorized previously by the owner to conduct this biogeochemical study (Fig. 1a). The experimental site has semi-arid climatic characteristics, with maximum and minimum average temperatures of 30 and 4.4 °C, respectively. Annual average rainfall of 618 mm most of it accumulated in the winter period (Fig. 2; [15, 16]. The soil is classified as Typic Xerochrepts, Inceptisol, belonging to Los Puercos series, with a clay loam texture [17]. Initial values of soil physicochemical analysis are shown in Table 1. The soil had a loam-clay texture with a bulk density of 1.60 ± 0.02 g cm^{-3}, phosphorus level of 18.50 ± 0.94 mg kg^{-1} (Table 1). The main crops in the area are maize (Zea mays), alfalfa (Medicago sativa L.) and vineyards.

Tables

Table 1. Physical and chemical characterization of soils at every microplot (n=5, 19/10/17 for Botalcura), (± standard error of median), of the sampling area.

2.2 Experimental design. The experimental design consisted of six microplots (2.5 m x 1 m), three microplots as the experimental unit and three controls. Microplots were bound to wood frames to avoid water runoff (Fig. 1b). The experiment was seeded manually on 23st October 2017. A hybrid corn of the variety "Caliber" (Pioneer) was sown. Plants seeds were sown in rows 0.12 m apart with a 50 kg ha^{-1} seed rate. Every experimental unit consisted on: control, 50, 100, 100 and 100 kg N ha^{-1}. It was consisted of after the fertilization base, it was followed by three fertigation, every three weeks, which also added 100 kg N ha^{-1}. Three Microplots of (2.5m x 1 m) were fertilized with $(NH_4)_2SO_4$ tagged with $\delta^{15}N$ (1.5% abundance) and three intercalated microplots were located as controls. Additionally, during the season approximately 20 irrigations of pure water are seen. A fertigation scheme was applied between 15-20 mm of water to the crop. The crop was irrigated with a central pivot system (irrigation efficiency > 90%) in accordance with producer management practices. Irrigation (quantity and frequency) was managed by farmers according to the typical programming for the area that consisted of irrigation every five days, during November and December, and every three days during summer (January). During February and March, the irrigation was decreasing to allow the maturity of the crop. Water flux (30 psi approx.,) used in the irrigation was determined measuring the irrigation time and irrigation discharge.

Data manipulation and analysis. Relative to the plant and soil $\delta^{15}N$ analysis, the isotopic $\delta^{15}N$ analysis was used to identify the amount of N fertilizer resulting in the corn plant, grain and root. In three microplots, urea fertilizer enriched with $\delta^{15}N$ isotope (10% $\delta^{15}N$ excess atoms) was applied to the centre of each microplots (Fig. 1b). Isotopic analyses were analyzed at the Laboratory of Environmental Chemistry of the Chilean Commission of the Nuclear Energy (CCHEN), Chile, to determine the N concentration and ^{15}N natural abundance (expressed as $\delta^{15}N$ or parts per thousand [‰]) using an elemental analyzer connected to an Isotope Ratio Mass Spectrometer. Plant biomass and Total Nitrogen concentration were determined at physiological maturity of the crop and $\delta^{15}N$ was determined in six fractionated samples, with a total of 18 samples, $\delta^{15}N$ was used to determine the amount of N fertilizer that results of the maize plant that includes the root, stem, leaves and grain. To evaluate the maize $\delta^{15}N$ recovery, three maize plants were collected from the central row of the treatment (microplots; Fig. 1b). For the determination of $\delta^{15}N$, the maize plants were taken at ground level and divided into shoots (grain, stem, leaves and root). From the plants sampled, the roots were unearthed and collected using rectangular trenches that were up to 0.4 m deep below the central row. The roots were carefully sifted (5 mm) and washed. The samples of plants and residues collected from the microplots were dried for 48 h in an oven at 60 °C to determine the dry mass. They were placed in hermetically sealed bags for further isotopic analysis. Soil samples were also collected from 0 to 20 cm for chemical analysis.

Related to N plant uptake (Nplant), it was calculated considering dry matter (kg ha^{-1}) and total nitrogen concentration.

$$Nplantf = DM\ (kg\ ha^{-1}) * \%N / 100 \quad (1)$$

The N derived from fertilizer (Ndff) indicates the amount of N fertilizer in the corn or in soil (1), expressed by kg N ha^{-1} [1].

$$Nddf = [\alpha-\beta]/[\gamma-\beta] * Total\ Nitrogen \quad (2)$$

In the case of Nddf is the amount of N derived from fertilizer (kg ha^{-1}), α is the abundance of $\delta^{15}N$ atoms in the sample (‰), β is the natural abundance of $\delta^{15}N$ atoms (0.366‰), γ is the abundance of $\delta^{15}N$ atoms in the fertilizer (10% atoms), and total Nitrogen is the total nitrogen contained in the measure sample (kg ha^{-1}). The presence of the control treatment without fertilizer incorporation was used to calculate the efficiency of fertilizer use by the difference method [18, 1].

$$Ndds = Nplantf - Nddf \quad (3)$$

Where Ndds is the quantity of mineral N (kg N ha^{-1}) take from the soil.

$$NRE\% = Nddf / N\ dose * 100 \quad (4)$$

Nitrogen recovery efficiency (NRE) [19, 20], was used to express the percentage of the total N fertilizer recovered by maize crops.

The data were subjected to ANOVA one-way analysis ($p \leq 0.05$) previous test of normality (Shapiro-Wilks test and homogeneity (Bartlett´s test). All statistical analysis and graphs were performed using Rstudio program. A posteriori test such as Tukey analysis was applied if significant differences were found between variables.

3 Results and Discussion

Physical-chemical parameters distribution. Statistical differences of dry matter, N plant, $\delta^{15}N$ and grain yield of maize during the harvest as shown by Table 2. Dry matter and total nitrogen content were not different between fertilized treatments ($p > 0.05$). This response was the expected, since in the three treatments the same total dose of nitrogen fertilizer was used but showed significant differences with respect the control (Fig. 1). Moreover, total content of dry matter (10622; 10585; 2931 and 5172 Kg ha^{-1}) and total nitrogen (3.2; 4.0; 3.6 and 2.9 Kg ha^{-1}) for every organ showed significant differences ($p<0.05$) between them (leaves, stem, root and grain), respectively. Highest total N concentration and significant differences ($p< 0.05$) were found in the leaves, contrariwise, N concentration in root biomass showed no significant differences between microplots. Total nitrogen level from the soil was similar to the sum of leaves, stem, grain and root biomass but the N absorption by maize from the fertilizer varied. The grain yield of maize for stem, leave, root and grain were significantly lower at the control treatment (20 kg ha^{-1}) than the fertilized treatments (31, 34 and 37 kg ha^{-1}, respectively). These are lower to major grain yields reported [21] in other Central Chilean studies, with high N dose (around 350 to 600 kg ha^{-1}). The three fertilizers microplots treatment showed significant biomass than the two control microplots. These yields were lower in comparison with other studies with similar conditions [5], which are probably related to water stress in the crop [22], mainly because during spring and summer highest temperatures are present in the area reaching 35 ºC. Some differences were found in total absorbed Nitrogen (Nplantf), N derived from fertilizer (Nddf) and N derived from the soil (NRes) in the maize components (Fig 3; Table 2).

In relation to $\delta^{15}N$ isotope, differences between $\delta^{15}N$ treatments were found with $\delta^{15}N$ ranges from 1.35‰ for the leave at M1 to 5.26‰ $\delta^{15}N$ for the grain at M2. The values found in this study were higher than the natural content of $\delta^{15}N$ in corn (0.3692%) that indicates a good use of the N applied in the harvest stage. The relative availability of $\delta^{15}N$ in crop plants decreases with respect to the effects of dilution with soil N. For this current study, fertilizer N applications did not impact negatively grain yield and presented a moderate, positive and linear influence on biomass and plant N content (Fig. 4). The total amount of N derived from fertilizer (Nddf) was 32.8. The greater significant absorption of total N in the leaves, stems and grain, is explained by the Nddf content (8.3%; 10.9% and 10.9%), respectively. The root showed the less Nddf values with 2.8%. In the case of Nddf showed high relationship with the plant N content (Fig. 4), potentially related to N timing with later application increasing plant N content and Nddf [20]. Relative to Nitrogen derived from soil (Ndds), a high absorption of N from the soil is observed, with a significantly higher Ndds in the leaves (325 ± 22.8 kg N ha^{-1} for the three microplots) and in stems (409 ± 45.3 kg N ha^{-1} for the three microplots; Table 2).

Under normal conditions, as the control treatment, nitrogen recovery efficiency (NRE) was almost zero, mainly at the root, since very similar levels of nitrogen fertilizer can exceed the crop's assimilation capacity [23]. The results showed differences in $\delta^{15}N$ recovered in the crop between the three treatments, with major values at the Microplot 1. The N recovered from the fertilizer application reached a total of 22%, with 6.8% at the stem, 6.3% at leaves, 7.1% at root and 9% at grain, with respect to the 350 kg N ha-1 applied in the fertilizer. These values are relatively low compared to records of other previous Chilean studies that have presented total recoveries from 30 to 80% [24, 25]. In general, low recoveries are associated with poorer soils, with less water or nutrient limitations [17]. However, [25] also indicated that low recoveries of N could be found in soils with high availability of N and with irrigation conditions.

This can be attributed mainly to the higher temperatures that occur during the maize cycle in the area during the summer that are favourable both to the loss of N due to volatilization and nitrate leaching [26]. The recorded average NRE were similar to that reported for Central Chile [24] but was much lower than those reported by [26]. The N recovery or efficiency of fertilizers is an essential matter disturbing NRE, so despite the $\delta^{15}N$ recovered in the crop (NRE) was also estimate the $\delta^{15}N$ recovered in the plant-soil system (NRes) (Table 2). The amount of N recovered ($\delta^{15}N$) in the plant+soil (NRes) evaluated at the harvest was statistically ($p \geq 0.05$) similar between treatments (67.49%; 72.8%; and 76.36) for M1, M2 and M3, respectively.

4 Conclusion

The data obtained in this study allowed the obtaining of the phases of the total N recovered in each organ (leave, stem, root and grain) of the maize crop in the studied microplots. Given the moderate recoveries of the plant, apparently nitrogen remains in the organic matter prior to cultivation. This study suggests that lower concentrations of fertilizer applied to the soil should be applied in this area, due enough nitrogen remains in the soil fraction. The maize grain and the stems were the main N sink from the fertilizer with a 22%. The average recovery for the plant was low with a 23% of the N applied. This would indicate when the maize cannot keep its capacity to accumulate N from fertilizer, the organic sink from soils appears to be of larger importance and could act as a buffer of the system.

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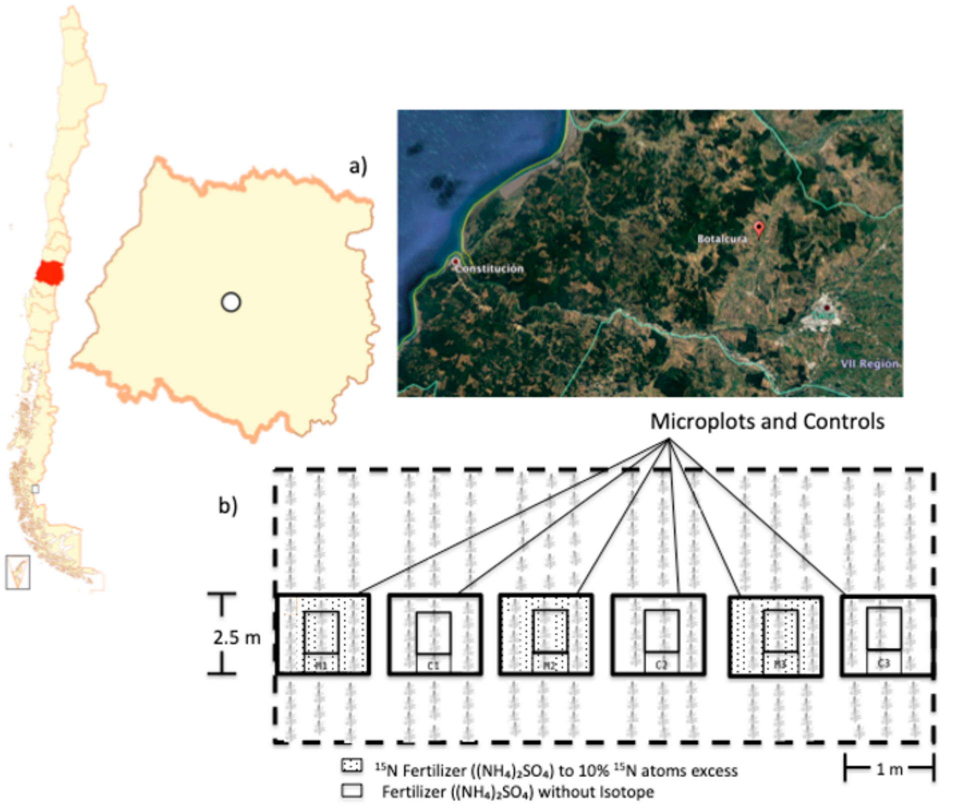

Figure 1. a) Study area and b) sample fieldwork (M1, M2 and M3) correspond to microplots with $\delta^{15}N$ fertilizer), and (C1, C2 and C3) are related to controls

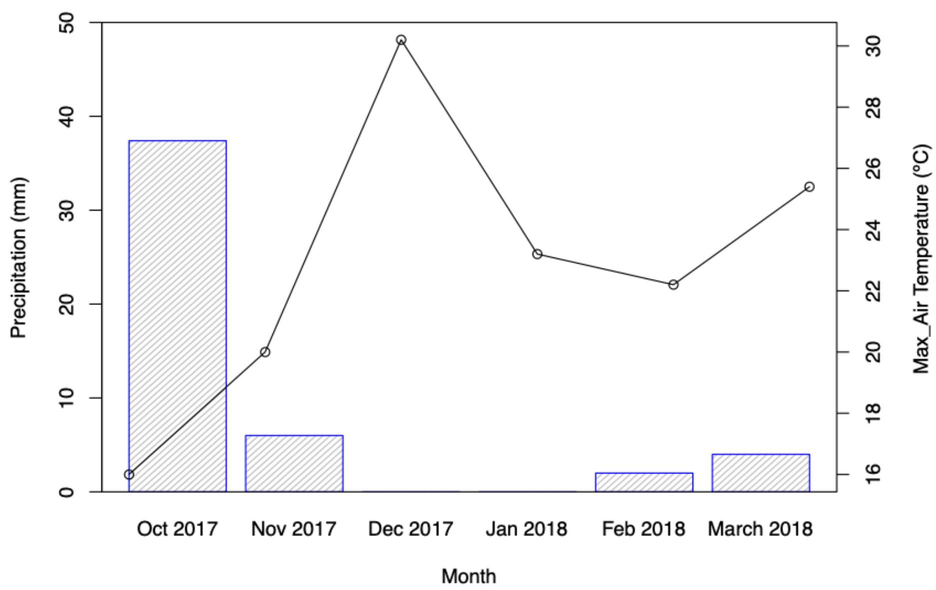

Figure 2. Study Area location and microcosms location with every isotopic labelled plot.

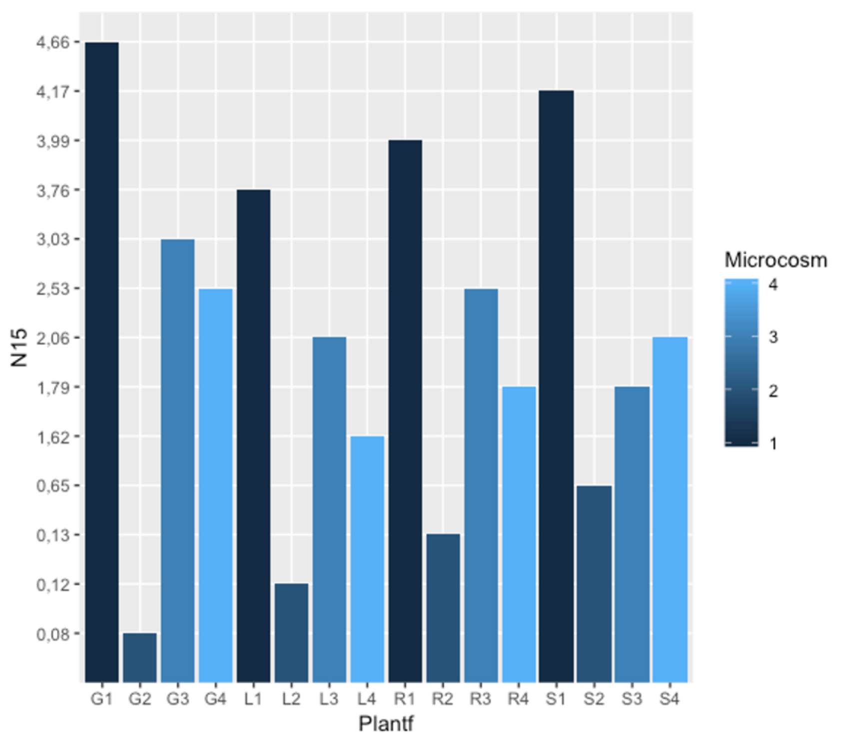

Figure 3. Fate of total nitrogen (Plantf) at every plant organ (leave (L), stem (S), root (R) and grain (G) from $\delta^{15}N$ from fertilizer (N15).

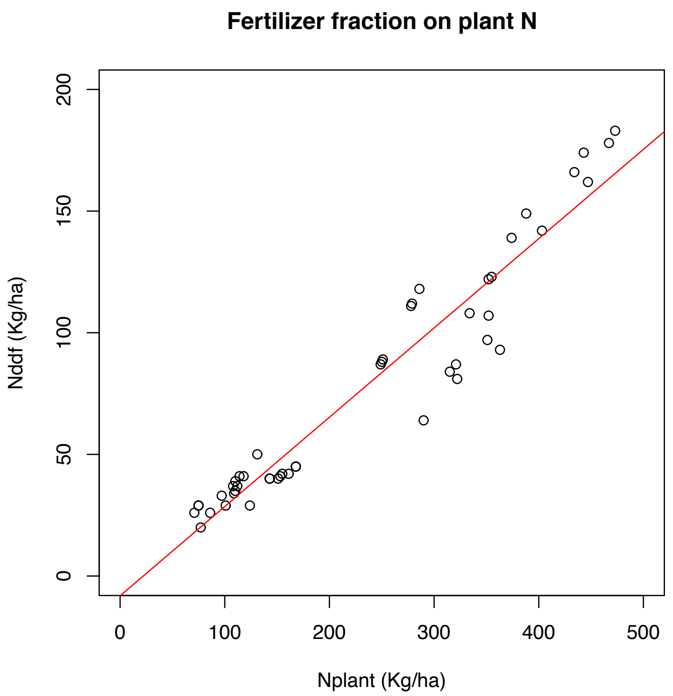

Figure 4. Relationship between Plant N content (NPlantf) is NPlant and Nddf for the fertilizer applied to the maize crop at Botalcura site.

Table 1. Physical and chemical characterization of soils at every microplot (n=5, 19/10/17 for Botalcura), (± standard error of median), of the sampling area.

Microplot	pH	MO	N ext	P sol	K	Ca	Mg	CICE	sand	Silt	Clay
1 (M1)	6.27	2.92	13.57	27.52	334	4.69	1.81	7.54	33.04	49.02	17.89
2 (M2)	6.18	2.48	12.54	16.80	256	5.02	1.91	7.73	38.23	43.88	17.88
3 (M3)	5.49	2.70	22.01	17.35	282	4.84	1.37	7.15	35.78	43.81	20.41
4 (C1)	5.73	2.97	14.27	17.53	305	4.85	1.29	7.11	38.5	43.7	17.80
5 (C2)	5.79	3.08	12.51	14.69	312	5.15	1.48	7.65	38.5	41.2	20.4
6 (C3)	6.08	2.93	12	21.2	262	5.45	1.88	8.15	37.3	41.2	20.6

Table 2. Total Nitrogen content, $\delta^{15}N$, Dry matter, grain yield, Nplantf, Nitrogen derived from the fertilizer, Nitrogen derived from the soil, $\delta^{15}N$ recovery, and not recovered nitrogen.

Crop Organ	Total N	$\delta^{15}N$ %at.exc.	Dry Matter Kg ha^{-1}	Grain Yield	Nplantf	Ndff	Ndds	NRE (%)	Recovered Plant + soil %
Leave	3.2	2.5	10622	-	334	8.3	325	2.4	926
Stem	4.0	2.7	10585	-	420	10.9	409	3.1	1204
Root	3.6	2.8	2931	-	106	2.8	103	0.8	308
Grain	4.0	2.7	10585	-	420	10.9	409	3.1	1204
Microplot									
M1	3.44	4.14	26393	31.05	236	9.56	227	2.73	1021
M2	3.43	2.36	29580	34.8	255	5.46	249	1.56	617
M3	3.37	2.00	31960	37.6	267	5.25	262	1.50	600
C1	-	-	18254	21.5	-	-	-	-	-
C2	3.85	0.14	17000	20	178	0.27	178	0.08	78.2
C3	-	-	18925	22.3	-	-	-	-	-