Nitrogen Losses: Gaseous and Leached Nitrogen Balance

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Abstract

Nitrogen is the element with the greatest influence on plant production and on protein content in the case of grain crops. Nevertheless, nitrogen over-fertilization produces environmental problems such as water pollution and global warming, which has led to the declaration of vulnerable zones to nitrate pollution in the European Union and to the adhesion of many countries to the Kyoto protocol. In the case of wheat there is a demand for producing quality grain, which is primed with a bonus price. Under these both economical and environmental circumstances, arose the need for a rational system of nitrogen fertilization which enabled the optimization of nitrogen use under the specific edaphoclimatic of Northern Spain. In order to cope with this objective a net of nitrogen fertilization assays was established by means of which a series of fertilization strategies together with some associated diagnosis tools were evaluated. Thus, N losses occurring both by nitrate leaching and by $N_2O$ emissions to the atmosphere were quantified, as well as plant N extractions regarding the different nitrogen fertilizer treatments applied.

Keywords: gaseous N losses, leached N

1. Introduction

Nitrogen (N) plays a key role in the growth and development of wheat; thus, wheat’s growth and quality might be modified through N fertilization [48]. However, cultures use N inefficiently, and, in general, 50% of the applied N is not used by plants [12, 41]. Therefore, N losses take place, both gaseous and leached, which cause economic and environmental costs.
Within the economic costs, that caused due to diminished N use efficiency stands out. Among the environmental costs, the contribution of some N-based gaseous compounds that play a role in the greenhouse effect [10], acid rain [23, 24] and the contamination and eutrophication of waters due to nitrate leaching further than the root zone [1, 17] are notable. In this sense, in Álava, Arrate et al. [6] describe a series of changes in the management of arable land in the years 1967–1997 (wetland drainage, application of large quantities of fertilizers, phytosanitary products etc.) that progressively increased the concentration of N compounds in subsurface waters. Due to such reasons and by application of the European Directive 88/778/EEC related to water for human consumption [13], the zone related to the Eastern sector of the quaternary aquifer of Vitoria was designated as vulnerable to nitrate pollution in the year 1999 [14]. This zone comprises 38% of the area where wheat is grown in Álava, 9500 ha approximately. In this zone, the N fertilization is as much as 140 kg N ha\(^{-1}\) depending on the previous culture and soil richness; N fertilization is not allowed at a distance closer than 3 m of any water course.

Nitrous oxide (N\(_2\)O) is not a very reactive gas; it persists in the atmosphere for as much as 150 years [47]. This gas adsorbs electromagnetic radiation in various wavelengths in the infrared region between 7.7 and 17 μm [35] and its greenhouse effect per mass unit is some 300 times larger than that of CO\(_2\) [36]. In this sense, it is estimated that in the last 100 years, N\(_2\)O has contributed approximately 5% to the warming up of the planet [42, 43]. The origin of 90% of the N\(_2\)O emissions is anthropogenic, and agriculture is its main source [22]. N\(_2\)O in soils can be produced both due to nitrification and denitrification (Figure 1). Nitrification is a microbial aerobic process in which ammonium first oxidizes to nitrite and then to nitrate. In this ammonium to nitrate oxidation process, N\(_2\)O can be released into the atmosphere [46]. On the other hand, denitrification is a microbial anaerobic process in which organic carbon is used as the energy source and the nitrate as the last electron acceptor so that it reduces to the last nitrogenous gaseous compounds N\(_2\)O and N\(_2\). The nitrification and denitrification processes

![Figure 1. Transformations of mineral nitrogen in the soil](image-url)
can occur simultaneously in the soil since the aerobic and anaerobic conditions can simultaneously take place at the same soil aggregate [26].

The N balance allows the knowledge of the N evolution at the soil–plant system in a settled period of time. It also enables the knowledge of the sources of N other than fertilization, and the rate of transfer between the different components of N, the main mechanisms of N loss, and what amount of N is not likely to be recovered [30]. Therefore, determining the balance of N in a culture system helps explain certain parameters that determine the dose of N needed by the cereal [28, 49], optimizing the nitrogenous nutrition of the plant and reducing the danger of contamination.

The goal of this work was to ascertain the quantity of N lost by leaching or by being released to the atmosphere and to study the factors that have an effect on such losses. Also, the N balance in the soil–plant system was to be determined.

2. Materials and methods

2.1. Assay establishment

A nitrogen fertilization experiment was carried out in Gauna, Álava (average annual rainfall of 779 mm and average annual temperature of 11.5°C) from November 2001 to February 2004 in three consecutive seasons. The assay was conducted in the Western Sector of the quaternary aquifer of Vitoria, adjacent to the area vulnerable to the contamination of nitrates of agricultural origin. The trial was organized in random blocks with four repetitions in which each elementary plot covered an area of 50 m². The soil on which the trial was established was classified as Aquertic Eutrudept [40] and was planted with wheat. Some of the soil properties are shown in Table 1. Data regarding when sowing, the first, second, and third N broadcastings, and the harvest took place are shown in Table 2. Before sowing, 90 kg ha⁻¹ of P₂O₅ ha⁻¹ and 90 kg ha⁻¹ of K₂O ha⁻¹ were applied as 0-14-14. Nitrogen doses of 0, 140, and 220 kg N ha⁻¹

| Depth (cm) | 0–30 | 30–60 |
|-----------|------|-------|
| Sand (%)  | 45.18| 48.25 |
| Silt (%)  | 27.14| 39.21 |
| Clay (%)  | 27.67| 12.49 |
| pH        | 7.98 | 8.13  |
| Organic matter (%) | 2.12 | 1.52  |
| Phosphorous (P) (mg kg⁻¹) | 43.30 | 32.53 |
| Potassium (K) (mg kg⁻¹) | 135.00 | 93.00 |
| Carbonates (%) | 11.90 | 22.00 |

Table 1. Soil properties of the experiment in Gauna (Álava).
were applied as ammonium nitrate (33.51% N g/g) in two or three broadcasts as described in Table 3. A control treatment in which no N was applied was included. The treatment in which 140 kg N ha\(^{-1}\) were broadcast was applied in two or three amendments (Table 3) to observe the splitting effect and to evaluate a low fertilization strategy in three broadcasts suggested as a possible N fertilization management in vulnerable zones.

### 2.2. Mineral nitrogen (N\text{min})

The first N\text{min} measures, (ammonic and nitric N), took place at start of tillering and at the end of winter 2001. They were determined from a mixture of eight samples throughout the trial taken at the depths 0–30 and 30–60 cm. Subsequently, all N\text{min} values were determined from a mixture of two samples per treatment, block and depth. These samples were taken before sowing, at the end of winter, at Z20, and also after harvest in all years and all treatments except at the end of winter of year 2003, when only samples of the control treatment were taken.

The soil samples were shredded manually. Stones, roots, and any other type of plant material were discarded. Then, the humidity of the samples was gravimetrically ascertained.

200 mL of 1 M KCl were added to 100 g of soil and the mixture was stirred for 30 min. After filtering, the nitrogen of nitric origin in the filtrate was analyzed by segmented flow injection [3, 4]. The calculation of N\text{min} per hectare was corrected according to the content of coarse elements of the soil (Table 4).

| Total dose (kg N ha\(^{-1}\)) | Treatment | Broadcasts (kg N ha\(^{-1}\)) | Start of tillering (Z20) | Start of jointing (Z30) | Flag leaf (Z37) |
|-------------------------------|-----------|-----------------------------|------------------------|-----------------------|----------------|
| 0                             | 0         | 0                           | 0                      | 0                     | 0              |
| 140                           | 40 + 100  | 40                          | 100                    | 0                     |                |
| 140                           | 40 + 60 + 40 | 40                        | 60                     | 40                    |                |
| 220                           | 80 + 140  | 80                          | 140                    | 0                     |                |

\(Z20, Z30\) and \(Z37\) correspond to Zadoks’ scale [50].

Table 3. Dose and broadcasts of the N fertilization treatments.
2.3. Leached nitrate

Two ceramic cups were inserted per block and treatment. They were buried at a depth of 60 cm (Figure 2). As a test, in the year 2003, a hole in the soil was performed after harvest. It was observed that wheat roots reached a depth of 60 cm as a maximum; so the depth at which the ceramic cups were inserted was considered appropriate and it can be considered that the gathered nitrogen was not profitable for plants. Liquid samples were collected from ceramic cups when it had rained 20–40 mm or every fortnight. The first liquid sample after every insertion was extracted but then discarded. The sampling data occurred between December 20, 2002 and September 18, 2003 and between January 9 and September 27, both data in 2004. At both periods, the ceramic cups were removed at harvest and then inserted again. After every sampling session, a vacuum of approximately 50 kPa was performed with a manual pump. Afterwards, the nitrate concentration in the sampled water was analyzed through segmented flow injection [3, 4].

The water balance was determined in layers 0–20, 20–40, and 40–60 cm depth following Campbell’s [11] simplified waterfall method. In this method, it is considered that waters fills

| Depth (cm) | Soil apparent density (g cm⁻³) | Coarse elements (% g/g) | Stone free apparent density (g cm⁻³) |
|------------|-------------------------------|-------------------------|-------------------------------------|
| 0–20       | 1.57                          | 20.8                    | 1.4                                 |
| 20–40      | 1.59                          | 21.5                    | 1.4                                 |
| 40–60      | 1.93                          | 42.6                    | 1.6                                 |

Table 4. Soil apparent density (g cm⁻³) and coarse elements (% g/g) at 0–20, 20–40, and 40–60 cm depths.

Figure 2. Ceramic capsule in the soil for leached liquid sampling.
up every layer of the soil before it flows to the following layer (Eqs. (1)–(3)). Every time a sample was taken, the humidity of each of the three layers was measured so that the variation of the water reservoir was measured. For every layer, the humidity was measured gravimetrically as well as with an IMKO TDR (time domain reflectrometry) so as to calibrate the later. However, no relation between both measures was observed; thus, only the gravimetrical measures were considered afterward.

The water balance in the 0–20 cm deep layer was calculated as specified in Eq. (1):

\[ D_{20} = Pr - ETc \pm VR_{20} \]  

(1)

where \( D_{20} \) is drainage (mm) below 20 cm, \( Pr \) stands for rain, \( ETc \) is the culture’s evapotranspiration (mm) determined according to FAO methodology [2] and \( VR_{20} \) (mm) is the variation of the water reservoir in the 0–20 cm layer.

Drainage below 40 and 60 cm was, respectively, ascertained as described in Eqs. (2) and (3):

\[ D_{40} = D_{20} \pm VR_{40} \]  

(2)

where \( VR_{40} \) is the water reservoir variation in the 20–40 cm (mm) layer and \( D_{40} \) is drainage beyond 40 cm (mm).

\[ D_{60} = D_{40} \pm VR_{60} \]  

(3)

where \( VR_{60} \) is the water reservoir variation in the 40–60 cm (mm) layer and \( D_{60} \) is drainage beyond 60 cm (mm).

Finally, the N mass drained during the sampling period was calculated with Eq. (4).

\[ N_i = D_{60} \cdot [N]_i \cdot 10^{-2} \]  

(4)

where \( N_i \) is the N mass of nitric origin drained per period and treatment (kg N ha\(^{-1}\)), \( D_{60} \) is the water loss due to deep percolation in the period between sampling days (L m\(^{-2}\)) and \([N]\) is the nitric nitrogen concentration in the leachate sampled at the end of the period \( i \) (mg L\(^{-1}\)).

Finally, the values obtained per day in Eq. (4) for each treatment and sampling day in the period of time comprehended between the days the capsules were inserted and when they were removed were summed up so as to assess the N mass leached per hectare in that period.

A piezometer was installed next to the assay so as to detect the moments when the level of water was beyond 60 cm. This occurred on the days February 5 and May 7, 2003 and March 11, and April 1 and 30, 2004.

2.4. Gaseous losses

2.4.1. \( N_2O \) emissions

To assess the fertilizer application effect on \( N_2O \) emissions (\( N_2O_{em} \)), in the year 2002, those emissions were measured after the N fertilizer applications, from March 4 to May 17. During
this period of time, samples were taken every 2 days in treatments 0, 40 + 100, 40 + 60 + 40, and 80 + 140. In the year 2003, more exhaustive measures were taken so as to assess not only the differences in the emissions after N applications but also to study the N$_2$O emissions of the field after harvest and laboring and to determine the effect of the temperature and humidity on emissions. Therefore, that year, samples were taken every 2 days after N broadcasting and every fortnight from January 20, 2003 to February 4, 2004 in treatments 0, 40 + 100, and 80 + 140. Measurement frequency intensified around laboring time.

To perform such measurements, 3 L polyvinyl chloride (PVC) hermetic chambers with a rubber septum were inserted in the soil in every block at a depth of 2 cm (Figure 3). Before their placement, four 10 mL samples from the atmosphere of the essay were taken at a height of 2 m. After 45 min, four 10 mL samples of the air of each chamber were taken and kept in Vacutainer® blood sampling tubes. Then, the N$_2$O in the samples was analyzed with a gas chromatograph (Unicam 8925) equipped with an electron capture detector (ECD). When the described measurements were performed in the field, the temperatures of the air at a height of 2 m and that of the soil at a depth of 10 cm were also measured. In 2003, the humidity of the first 30 cm soil layer was also gravimetrically measured at 4 points of the essay.

With the soil humidity measure, the percentage of soil water-filled pore space was assessed using Eq. (5).

$$WFPS = \frac{H \rho_{sfs}}{1 - \rho_{sfs}/2.65}$$

(5)

where WFPS stands for the water-filled pore space (% ml/ml), H, the water percentage in dry soil for the 0–30 cm soil layer (% g/g), and $\rho_{sfs}$: stone free soil apparent density (g cm$^{-3}$).

Soil apparent density is the quotient between the weight of the soil solid particles and the in situ total volume of the soil. The in situ volume of the soil was assessed through the excavation method [9] with the adaptation of using polyurethane resin (Wolf, [45]) as the average value obtained at four holes dug in the essay at depths 0–20, 20–40, and 40–60 cm. Due to the
fact that the soil was quite stony, the apparent density required a correction which was executed considering the percentage of gross elements (those that did not pass through a 2 mm sieve) (Table 4) and their density, which is acknowledged as 2.65 g cm\(^{-3}\) [37].

### 2.4.2. \(N_2O\) production and total denitrification at the arable layer

From January 23 to September 18, 2003, the \(N_2O\) production rate (\(N_2O_{prod}\)) was measured in the arable layer at the same treatments and times described for \(N_2O_{em}\). To achieve this, two 30 cm long and 2.65 cm diameter wide cylindrical samples were taken at the plots of the studied treatments. These two samples were transferred to a hermetic 2 L pot with a rubber septum (Figure 4). Then, the pots of the different plots were inserted in a hole next to the studied plot and were covered with the soil of the same hole and kept to incubate for 24 hours. In such a manner, the actual soil temperature and its changes could be mimicked. After 24 hours, 10 mL samples of the atmosphere of each pot were stocked in Vacutainer\(^{®}\) tubes for their ulterior \(N_2O\) determination by gas chromatography (Unicam 8925). The same process was followed for other pots to which 100 mL of acetylene (\(C_2H_2\)) (Air Liquide, SA) were injected making the atmosphere of the pot rich in acetylene by 5% (Figure 4). \(C_2H_2\) blocks \(N_2O\) reduction to \(N_2\) in the denitrification process and the ammonium oxidation in the nitrification process [33]. Therefore, the \(N_2O\) produced in the incubation with an atmosphere of 5% \(C_2H_2\) comprises the joint production rate of \(N_2O + N_2\) due to denitrification (\(N_{deni}\)) [8] (Knowles, [25]).

The cumulated N loss was calculated by the integration of the diary rates of \(N_2O_{em}\), \(N_2O_{prod}\), and \(N_{deni}\) over time.

### 2.5. Plant N extraction

To determine the N extraction of wheat in the aerial part at harvest, a 0.25 m\(^2\) area in each plot was randomly chosen and the plants in that area were cut to the ground. Grain and straw were separated and dried in an oven at 70°C for 48 h at least to determine biomass. Then, the samples

![Figure 4. Incubation pot before it was buried and bag and syringe with acetylene.](image-url)
were ground and sieved through 0.5 and 1 mm sieves respectively. The total N was determined both for straw and grain by Kjeldahl procedure [5] with a Kjeltec Auto sampler System 1035 (Tecator). The N absorption by the aerial part of the plant (Nab) was determined as the sum of the products of the N concentration at grain and straw times their respective biomasses.

The yielded grain quantity datum, needed for the previous calculation was determined by harvesting the central 1.5 m wide aisle of each plot. Yield was referred to a humidity of 120 g kg\(^{-1}\).

### 2.6. N balance

The N balances are based on the application of the mass conservation principle to the cultures. Thus, the variation of N stocked in a system equals the difference among the inputs and outputs to the system [31]. Often, the N balance is inferred considering the inputs and outputs in relation to the main source, the soil Nmin. This is considered available to plants in spite of the fact that it can also be consumed by microorganisms, dissipate as gas, or leach through the soil [34]. The balance was assessed as described in Eq. (6) [29]:

\[
\text{Nmin}_S + \text{Min} + F = \text{Nab} + \text{Nabr} + \text{Nlix} + \text{N}_2\text{Oem} + \text{NminAH} + \text{Nc}
\]

where, \( \text{Nmin}_S \): Nmin quantity in soil before seeding (kg N ha\(^{-1}\)), \( \text{Min} \): soil N fraction in the soil due to the conversion of organic nitrogen to ammonium (kg N ha\(^{-1}\)) (It is calculated from the adjustment of the balance for the control treatment, i.e., 0 (kg N ha\(^{-1}\))), \( F \): fertilizer N applied (kg N ha\(^{-1}\)), \( \text{Nab} \): N absorbed by the aerial part of the plant (kg N ha\(^{-1}\)), \( \text{Nabr} \): N absorbed by the roots of the plant (kg N ha\(^{-1}\)) (It was estimated that this was 25% of \( \text{Nab} \)), \( \text{Nlix} \): leached N (kg N ha\(^{-1}\)), \( \text{N}_2\text{Oem} \): N emitted as \( \text{N}_2\text{O} \) (kg N ha\(^{-1}\)), \( \text{NminAH} \): Nmin in soil after harvest (kg N ha\(^{-1}\)), \( \text{Nc} \): Not accounted for.

Thus, a positive \( \text{Nc} \) means that: (i) the inputs have been overestimated due to an error in its calculation or/and experimental error, (ii) the outputs have been underestimated for the same reasons as in (i), (iii) there are other N outputs that have not been considered, and (iv) a combination of all or some of the situations described here occurs.

\( \text{N}_2\text{Oem} \) was considered in the balance instead of \( \text{N}_2\text{Oprod} \) or \( \text{Ndeni} \) since these last two refer to gases produced in the arable layer and thus may not exit the system.

### 2.7. N use efficiency

For the assessment of N use efficiencies, the following parameters were defined (Huggins and Pan, 1993):

**Efficiency in the use of the fertilizer (NUE):** difference between \( \text{Nab} \) of the fertilized treatment and the non-fertilized, divided by the quantity of fertilizer applied.

**Harvest index (HI):** quotient between the N in the grain and the N extracted by the aerial part of the culture (dimensionless).
2.8. Statistics

PROC GLM [39] procedure was used to carry out the variance analysis and then determine the differences between averages with the Duncan procedure.

3. Results and discussion

3.1. Mineral N

Through the Nmin analysis, it was observed that about the 80–95% of the Nmineral (Nmin) analyzed in the floor of the assay was of nitric origin (Table 5). The non-fertilized treatment had the least nitric N percentages at every moment and depth in comparison to the percentages in the fertilized treatments. The nitric N percentage was constant or increased with depth, (i.e., from 0–30 to 30–60 cm) except in the moments after harvest in years 2003 and 2004, when the percentage of nitric N was somewhat inferior in the 30–60 cm layer due to the deep and recent extraction of the culture until harvest, close to the moment “after harvest” referred to in this work.

3.2. Leached nitrate

In Tables 6 and 7, the nitric N leached in the periods comprehended between the beginning of the sampling period until harvest and between the beginning of the sampling period until it finished are, respectively, shown. Statistically significant differences were only observed between treatment 80 + 140 and the rest of treatments at the period of time comprehended between the beginning of the campaign and harvest in year 2003 and all the sampling period in year 2004. Although Webster et al. [44] reported some 19 kg ha$^{-1}$ of N leached for the non-fertilized treatment, a similar figure to that reported in this work in year 2004 (Table 7), the 32 kg N ha$^{-1}$ obtained for treatment 80 + 140 is minor to the 50 kg N ha$^{-1}$ quantities reported by the same authors for treatments with similar dosages, presumably due to variables such as different kinds of soils, etc. By the deduction of nitric N leached at the non-fertilized treatment, the nitric N quantities leached in years 2003 and 2004 were assessed in the other treatments. Until harvest, those quantities ranged between the 4 and the 6% of the applied N and, when all the sampling periods were considered, they comprehended N quantities that ranged between the 8 and the 14% of the N fertilized.

Concurring with the results obtained for treatment 80 + 140, in a study performed in Denmark, Kjellerup and Kofoed [24] observed that N fertilizer dosages around 200 kg N ha$^{-1}$ significantly raised the leached nitrate in drained waters for they exceeded the adsorbing capacity of the crop; the assessed losses were approximately 40 kg N ha$^{-1}$. In the neighbor community of Navarre, Arregui [7] also described the augmenting N leaching trend for dosages larger than 110 or 120 kg N ha$^{-1}$.

Approximately 50% of the leached N-NO$_3^-$ in all the samples in 2004, leached in the period comprehended between the moments after harvest and the end of sampling in September for all treatments (Table 7).
In Tables 6 and 7, the average nitric N in the total quantity of drained water is also shown. It was calculated as the product of the drained total N (kg ha$^{-1}$) throughout all the drainage period and the drained water (mm) in the same period. Thus, it can be observed that the maximum average N-NO$_3^-$ concentration in the sampling period was 7 ppm N in the year 2003 and 14 ppm in the year 2004 for treatment 80 + 140, which was the treatment that regards average N-NO$_3^-$, statistically differentiated from the rest in years 2003 and 2004 except for treatment 40 + 60 + 40 in year 2004 when all the sampling period was considered. The average concentrations in treatments 40 + 60 + 40 and 80 + 140 in year 2004 when all the periods were considered were larger than the allowed 11.3 mg L$^{-1}$ N-NO$_3^-$ limit.

| Moment       | Depth (mm) | Treatment | N-NO$_3^-$/Nmin (%) |
|--------------|------------|-----------|----------------------|
|              | 0–30       | 40 + 100  | 40 + 60 + 40         | 80 + 140 |
| 2002 Before harvest | 0–30       | 82        | 96                   |          |
|                | 30–60      | 78        | 85                   |          |
| End of winter | 0–30       | 78        | 85                   |          |
|                | 30–60      | 85        | 84                   |          |
| After harvest  | 0–30       | 75 89     | 94 81                |          |
|                | 30–60      | 83 89     | 94 84                |          |
| 2003 Before harvest | 0–30       | 82 86     | 79 88                |          |
|                | 30–60      | 90 89     | 89 85                |          |
| End of winter | 0–30       | 67        | 43                   |          |
|                | 30–60      | 43        |                      |          |
| After harvest  | 0–30       | 92 90     | 86 92                |          |
|                | 30–60      | 89 89     | 84 87                |          |
| 2004 Before harvest | 0–30       | 79 84     | 86 83                |          |
|                | 30–60      | 79 83     | 83 88                |          |
| End of winter | 0–30       | 77 87     | 83 89                |          |
|                | 30–60      | 79 83     | 82 91                |          |
| After harvest  | 0–30       | 88 94     | 85 93                |          |
|                | 30–60      | 80 90     | 85 84                |          |

Table 5. Nitric origin N percentage in relation to Nmin in the soil at different depths, moments, and treatments.
3.2.1. Gaseous losses

Through the integration in time of the daily emission and production rates, losses due to N\textsubscript{2}O emitted to atmosphere and N\textsubscript{2}O + N\textsubscript{2} due to denitrification in 2002 and 2003 were assessed (Table 8). In spite of the fact that N fertilization has a key role in N\textsubscript{2}O emissions (Bouwman, 1996), no statistical differences were observed between fertilizing treatments for the emission or the N\textsubscript{2}O cumulated production, as described by Menéndez [32] in an essay carried out in Mediterranean climatic conditions. However, when the N\textsubscript{2}Oem quantity corresponding to the non-fertilized treatment is deducted from the other treatments, the N\textsubscript{2}O emitted quantities range from 1.8 to 2.9% of the N broadcast as a fertilizer, superior numbers to those cited by the IPCC [20] when estimating the winter house effect caused by agriculture [21].

In 2003, in spite of the fact that applied N dosage had no effect on the N\textsubscript{2}O emissions to the atmosphere or on its production in the arable layer, it did affect the N\textsubscript{2}O+ N\textsubscript{2} production due to total denitrification—it was larger for the treatment with the largest N dose, i.e., 80 + 140. As mentioned before, N\textsubscript{2}O+ N\textsubscript{2} peaks due to denitrification were only observed until April 2; thus, these largest losses due to denitrification are imputable to the denitrification peaks after fertilization. Due to the same reason, the cumulated values of N\textsubscript{2}O+ N\textsubscript{2} rates due to denitrification until harvest and after it are similar.

| Treatment | N-NO\textsubscript{3} (kg ha\textsuperscript{-1}) | Drainage (mm) | [N-NO\textsubscript{3}] average (mg L\textsuperscript{-1}) |
|-----------|---------------------------------|---------------|-----------------------|
| 0         | 14 b                            | 350           | 4 b                   |
| 40 + 100  | 14 b                            |               | 4 ab                  |
| 40 + 60 + 40 | 12 b                            |               | 3 b                   |
| 80 + 140  | 23 a                            |               | 7:00 AM               |

Statistically different values (α ≤ 0.05) are identified with different letters.

**Table 6.** N-NO\textsubscript{3} leached from January to harvest in year 2003.

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| Treatment | Period | 2004 until harvest | 2004 all the sampling period |
|-----------|--------|--------------------|------------------------------|
|           |        | N-NO\textsubscript{3} (kg ha\textsuperscript{-1}) | [N-NO\textsubscript{3}] average (mg L\textsuperscript{-1}) | N-NO\textsubscript{3} (kg ha\textsuperscript{-1}) | Drainage (mm) | [N-NO\textsubscript{3}] average (mg L\textsuperscript{-1}) |
| 0         | 19 a   | 371                | 5 a                          | 38 b                      | 487           | 8 b                          |
| 40 + 100  | 20 a   |                    | 5 a                          | 41 b                      |               | 8 b                          |
| 40 + 60 + 40 | 31 a   |                    | 8 a                          | 62 ab                     | 13 ab         |
| 80 + 140  | 32 a   |                    | 9 a                          | 70 a                      |               | 14 a                         |

Statistically different values (α ≤ 0.05) are identified with different letters. *from January 9, 2004 to July 1, 2004.**from January 9, 2004 to September 27, 2004.

**Table 7.** N-NO\textsubscript{3} leached from January until harvest in all the sampling period of year 2004.
Although Hussain et al. [19] report in their review, that the emission of greenhouse gases is frequently favored by tilling, such a fact was not observed in this work for N\textsubscript{2}O, Nd\textsubscript{eni}, or N\textsubscript{prod}.

### 3.3. Nitrogen balance

Nitrogen balances for the different campaigns are shown in Tables 9–11. The yield and thus the extraction of the non-fertilized treatment in year 2002 was larger than in the latter two campaigns, due to the fact that in the first year, the field preceding the essay was thoroughly

| Treatment | 2002' | 2003 until harvest’’ | 2003 in all the sampling period’’ |
|-----------|-------|----------------------|---------------------------------|
|           | N\textsubscript{2}Oem | N\textsubscript{2}Oem | N\textsubscript{deni} | N\textsubscript{prod} | N\textsubscript{2}Oem |
| 0         | 2 a   | 5 a                 | 4 b | 7 a | 10 a |
| 40 + 100  | 2 a   | 4 a                 | 5 b | 10 a | 9 a |
| 40 + 60 + 40 | 6 a |                                     | |
| 80 + 140  | 5 a   | 8 a                 | 9a | 11 a | 14 a |

In the year 2003, N\textsubscript{2}Oem accumulation, from the N\textsubscript{2}O production rates (N\textsubscript{2}O\textsubscript{prod}) and N\textsubscript{2}O + N\textsubscript{2}, due to denitrification (N\textsubscript{deni}) from the beginning of the sampling period until harvest and until the end of the sampling period is shown; statistically different values are marked with different letters. From March 5 until May 17, 2002.

*From January 21 to July 4, 2003.

**From 2003, January 21 to 2004, February 4.

Table 8. Cumulated N\textsubscript{2}O emissions (N\textsubscript{2}Oem) in all the sampling period in year 2002.

Although Hussain et al. [19] report in their review, that the emission of greenhouse gases is frequently favored by tilling, such a fact was not observed in this work for N\textsubscript{2}O, Nd\textsubscript{eni}, or N\textsubscript{prod}.

\textbf{Table 9.} N balance (kg N ha\textsuperscript{-1}) during the 2002 campaign; average values ± standard errors are reported.
fertilized while in the following two campaigns, wheat was again preceded by wheat and it was not fertilized. The extraction by the aerial part at the other treatments ranged between 134 and 188 kg N ha\(^{-1}\). Mineralization rate was between 93 and 123 kg N ha\(^{-1}\) at the three campaigns.

It can be observed that the non-accounted N augmented with the N fertilizer dose, as it happened with the leached N quantities and the N emitted as N\(_2\)O. In general, such an effect was also observed for balances performed in Navarra and Castilla-La Mancha [34]. As Estavillo et al. [15] reported, these facts suggest an N mineralization rate different to that of the non-fertilized treatment and dependent on the broadcast N fertilizer dose. In this sense, Kuzyakova and Stahr [27] observed there was an effect on the mineralization pattern after the fertilizer application and Webster et al. [44] imputed the increasing Nc to short periods of immobilization that increased with the increasing quantity of available Nmin. Another possibility is that the increase of Nc might be due to some other kind of undetermined losses in the study such as those derived from ammonia leaching, which is presumed not to be very large since the ammonia quantity regarding nitrate is around 10% of the Nmin (Table 5). The losses corresponding to ammonia volatilization were not accounted for, but in posterior studies in the zone, they have proved to be dismissible (personal communication).

NUE (nitrogen use efficiency) ranged between 0.35 and 0.60 in the year 2002 and between 0.62 and 0.82 in the years 2003 and 2004 (Tables 9–11). This occurred due to the major extraction of the non-fertilized treatment at the first campaign as compared to the following two, since in the first campaign, the essay was preceded by a fertilized crop, while in the following two campaigns, the

| Dosage (kg N ha\(^{-1}\)) | 0   | 140 | 140 | 220 |
|--------------------------|-----|-----|-----|-----|
| Splitting                |     |     |     |     |
| Nmin initial (NminS)     | 25 ± 5 | 24 ± 1 | 25 ± 3 | 40 ± 10 |
| N mineralized (MIN)      | 123 ± 12 | 123 ± 12 | 123 ± 11 |
| N fertilized (F)         | 0   | 140 | 140 | 220 |
| N TOTAL INPUTS           | 147 ± 13 | 287 ± 12 | 288 ± 13 | 383 ± 15 |
| N absorbed by the aerial part (Nab) | 53 ± 6 | 139 ± 21 | 145 ± 25 | 180 ± 22 |
| N absorbed by the roots (Nabr) | 13 ± 1 | 35 ± 5 | 36 ± 6 | 45 ± 5 |
| N leached (Nlix)         | 14 ± 3 | 14 ± 2 | 12 ± 2 | 23 ± 3 |
| N gas (N\(_2\)O em)      | 5 ± 4 | 4 ± 1 |     | 8 ± 3 |
| Nmin after harvest (NminAH) | 50 ± 4 | 37 ± 4 | 30 ± 3 | 40 ± 1 |
| N TOTAL OUTPUTS 160 ±     | 135 ± 8 | 229 ± 21 | 224 ± 25 | 296 ± 22 |
| N not computed           | 12 ± 16 | 58 ± 24 | 65 ± 28 | 86 ± 27 |
| Fertilizer use efficiency (NUE) | 0.76 ± 0.22 | 0.82 ± 0.21 | 0.72 ± 0.11 |
| N harvest index (HI)     | 0.76 ± 0.77 | 0.77 ± 0.03 | 0.71 ± 0.08 | 0.65 ± 0.04 |

The average values ± standard error are indicated.

**Table 10.** N balance (kg N ha\(^{-1}\)) during campaign 2003.
non-fertilized treatment received no N fertilization. NUE values that range between 0.3 and 0.6 are frequent at the experiments carried out by the ITGA in Navarra [23].

It was observed that the NUE decreased as the fertilizer dose increased, as observed by other authors (Huggins and Pan, [18]). Fischer et al. [16] stated that depending on the quantity and the distribution of N, HI (harvest index) in wheat ranged from 0.64 to 0.85. Similarly, HI comprehended values between 0.65 and 0.87 in our experiment, the most frequent value of HI was about 0.70–0.80 (Tables 9–11).

4. Conclusions

1. The average Nmin values in the soils in years 2002, 2003, and 2004 ranged between 12 and 53 kg N ha⁻¹.

2. Nitrate leaching after harvest in the year 2004 accounted for 50% of the total N leached at every treatment. Thus, it was concluded that a significant mineralization took place after harvest that, together with the absence of crop in summer, causes a Nmin accumulation in summer and its ulterior leaching with the rain in autumn.

3. With treatment 80 + 140, more nitrate was leached than with treatments 0 and 40 + 100; losses up to 70 kg N ha⁻¹ were assessed in all the sampling periods in 2004. In this very treatment and treatment 40 + 60 + 40, the 11.3 mg L⁻¹ N-NO₃⁻ limit was exceeded, calculated as the average concentration in drained water in the sampling period. By the subtraction of the nitric N quantity leached for the non-fertilized treatment, it was assessed that the

Table 11. N balance (kg N ha⁻¹) during campaign 2004; average values ± standard error.

| Dosage (kg N ha⁻¹) | 0  | 140 | 140 | 220 |
|-------------------|----|-----|-----|-----|
| Splitting         | 0  | 40 + 100 | 40 + 60 + 40 | 80 + 140 |
| Nmin initial (NminS) | 28 ± 2 | 35 ±2 | 36 ±7 | 36 ± 3 |
| N mineralized (MIN) | 93 ± 16 | 93 ± 16 | 93 ± 16 | 93 ± 16 |
| N fertilized (F)  | 0  | 140 | 140 | 220 |
| N TOTAL INPUTS    | 121 ± 16 | 268 ± 16 | 269 ± 17 | 349 ± 16 |
| N absorbed by the aerial part (Nab) | 56 ± 14 | 134 ± 7 | 145 ± 10 | 165 ± 5 |
| N absorbed by the roots (Nabr) | 14 ± 3 | 33 ± 2 | 36 ± 2 | 41 ± 1 |
| N leached (Nlix)  | 19 ± 2 | 20 ± 2 | 31 ± 2 | 32 ± 2 |
| Nmin after harvest (Nmin AH) | 32 ± 1 | 33 ± 1 | 42 ± 8 | 53 ± 2 |
| N TOTAL OUTPUTS   | 121 ± 15 | 220 ± 8 | 254 ± 13 | 291 ± 7 |
| N not computed    | 0 ± 22 | 48 ± 18 | 15 ± 22 | 58 ± 18 |
| Fertilizer use efficiency (NUE) | 0.69 ± 0.18 | 0.79 ± 0.2 | 0.62 ± 0.1 |
| Harvest index     | 0.77 ± 0.26 | 0.73 ± 0.03 | 0.87 ±0.04 | 0.74 ± 0.07 |
nitric N quantities leached until years 2003 and 2004 ranged between 4 and 6% of the N broadcast. If the sampling period after harvest in year 2004 was also considered, the losses ranged between 8 and 14% of the N broadcast.

4. N-N\textsubscript{2}O quantities emitted to the atmosphere in all the sampling period in 2003 ranged from 10 kg N ha\textsuperscript{-1} in the non-fertilized treatment to 14 kg N ha\textsuperscript{-1} for treatment 80 + 140. By the subtraction of the N\textsubscript{2}O emitted by the non-fertilized treatment from that emitted by the rest of the treatments, it was assessed that the emitted N\textsubscript{2}O quantities ranged between 1.8 and 2.8% of the N broadcast as fertilizer in the sampling periods in years 2002 and 2003.

5. No effect was observed due to tilling either on the emitted N\textsubscript{2}O or on N\textsubscript{deni} or on N\textsubscript{prod}.

6. Regarding the N balance, it was concluded that the N inputs to the soil–plant system were beyond the outputs. In fact, the N acquainted for as not computed N in the N balance, increased with the raising dosage of fertilizer, as the leached N quantities and the N\textsubscript{2}O emitted quantities did. This fact suggests an N immobilization rate in the fertilized treatments superior to that at the non-fertilized treatments and dependent on the fertilized N dosage.

7. The efficiency in the fertilizer use varied from 0.35 to 0.82 and it was less in year 2002 than in the next 2 years. This was due to a major N extraction at the non-fertilized treatment in the first campaign as compared to the following 2 years, for at the first campaign, the assay was preceded by a fertilized crop, while in the following 2 years, the non-fertilized treatment was not fertilized. The most frequent value for harvest index was around 0.70–0.80.

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