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Abstract

The long-term effect of conventional plough tillage (PT) and conservation minimum tillage (MT) on soil N (0-5, 5-15, 15-30, 30-45 and 45-60 cm), recovery efficiency of ^{15}N-fertilizer (REN), plant N concentration and N exported with crops was evaluated during two years in a 14-year-old ryegrass-maize forage rotation. Adjacent PT (n=9) and MT (n=9) plots were randomly assigned in triplicate to three treatments to which $^{15}NH_4{}^{15}NO_3$ (10 atom % ^{15}N) was applied in one of the three first fertilizations ($^{15}N_{October}$-, $^{15}N_{March}$- and $^{15}N_{May}$-fertilizer), the others being done with unlabelled N. Plant N concentration (% N) was affected ($p< 0.001$; n=18) by the crop [80 % of variance explained: ryegrass-1 (2.6 ± 0.9 %) > ryegrass-2 (1.9 ± 0.4%) > maize-2 (1.4 ± 0.1 %) > maize-1 (1.1 ± 0.2 %)] and the crop-tillage interaction (22 % of variance explained). Jointly considering all data, more ^{15}N-fertilizer was recovered in the MT (25 ± 4 %) than in the PT soil profile (19 ± 6 %) at the end of the experiment whereas the N exported with the crops was unaffected by the tillage system and varied from 5-6 % ($^{15}N_{October}$-fertilizer) to 45-49% ($^{15}N_{March}$-fertilizer) and 52-53 % ($^{15}N_{May}$-fertilizer; despite only three instead of four subsequent crops were studied).The ^{15}N unaccounted for in the case of $^{15}N_{October}$-fertilizer (72 ± 5 %) was more than twice that in $^{15}N_{March}$- (34 ± 7 %) and $^{15}N_{May}$-fertilizer (25 ± 14 %). Considering soil, site and weather conditions, denitrification and nitrate leaching during the ryegrass-1 crop were the most likely processes explaining the high losses of the $^{15}N_{October}$-fertilizer. Results suggested a higher initial immobilization of the applied ^{15}N in the soil organic matter (SOM) of MT, that reduces ^{15}N availability to the first crop, followed by an increase of the residual availability of the fertilizer ^{15}N to the subsequent 2-3 crops.

Key-words: corn; Italian ryegrass; *Lolium multiflorum*; plough layer; soil N; subsoil; *Zea mays*.

1. Introduction

The mineral nutrition is one of the most important factors that affect plant growth and, among essential nutrients, N occupies a key position as a widespread limiting element which, moreover, is prone to strong losses in the soil-plant systems. Due to the rarity of N-bearing minerals, most plant available reserves are concentrated in the SOM (Haynes, 1986) and, consequently, the agricultural production can not be sustained if nutrients exported with the crops are not replenished or, alternatively, adequate management practices are implemented to maintain or increase the SOM.

Worldwide, annual N inputs to cropland were estimated to be 169 x 10^6 Mg of which 46 % is supplied by inorganic fertilizers, that are essential to maintain and improve crop production (Cassman et al., 2002; Smil, 1999) and that are rutinarily applied to around half the cultivated land. As the N-fertilizer use efficiency for crops is usually in the range from 50 to 70 % (IAEA, 2008), and only 33% in rain-fed cereal crops (Fageria and Baligar, 2005), an excessive fertilization can exacerbate NO_3^--N losses by leaching and increase the reactive N (Galloway et al., 2004), with strong environmental consequences. Moreover, there are sound evidences of an inexorable decline in the soil organic C and N pools due to the use of N fertilizers, especially when applied beyond crop requirements, that threaten soil productivity, food security and environmental conservation (Mulvaney et al., 2009). An increase in the N use efficiency and a reduction in the fertilization rates could notably improve both air and water quality (Shoji et al., 2001), being fundamental for a sustainable agriculture and also biosphere conservation in the 21st century. As Cassman et al. (2002) stated, the match between N supply and crop demand (both in time and amount) is the key to achieve an acceptable compromise among the conflicting goals of maximizing yield, profit and environmental protection; according to these authors "the global challenge of meeting increased food demand and protecting environmental quality will be won or lost in cropping systems that produce maize, rice, and wheat".

As they reduce soil erosion and compaction caused by plough-based conventional tillage systems, conservation tillage practices help to preserve soil quality and fertility and are increasingly used around the world (Peigné et al., 2007). Most of the world surface under conservation tillage (95 x 10^6 ha) is concentrated in North (47%) and South America (39%), followed at distance by Australia (9%) and with less than 0.05% in Europe, where there is still much scepticism about the suitability of conservation agriculture for the European climate conditions and cropping systems (Brennan et al., 2015; Brennan et al., 2014; Stagnari et al., 2010; Van Den Bossche et al., 2009). In the Spanish temperate humid zone, the most common rotation under conservation tillage is the maize-Italian ryegrass due to the economic and timeliness advantages without detrimental effect on yields (Bueno et al., 2007), as also reported for other Atlantic climates (Hansen et al., 2011). For this forage rotation, improvements of the physical, chemical and biological properties in the topsoil layer have been reported after adopting conservation tillage management (Bueno et al., 2006; Díaz-Raviña et al., 2005; Gómez-Rey et al., 2012; Gómez-Rey et al., 2014).

By minimizing soil disturbance and keeping crop residues on the soil surface, conservation tillage reduces residue decomposition and leads to organic matter accumulation in the upper soil layer (Balesdent et al., 2000). At the short-term, the adoption of conservation practices may increase N immobilization and reduce plant available N (Doran, 1987), but in the long-term conservation tillage improves N availability to plants (Fageria, 2002; Rice et al., 1986) through increasing soil N retention and a labile N pool in the upper soil layers (Franzluebbers et al., 1994; Jacobs et al., 2009; Kaiser et al., 2014; McCarty and Meisinger, 1997), although not in all cases (Liang et al., 2004). According to Giacomini et al. (2010), N fertilization must be adapted to the chemical, physical and biological changes in soil after cessation of ploughing. With regard to differences in NO_3^--N leaching between conventional ploughed and conservation tillage, contradictory results have been reported: higher losses under zero-tillage (Dowdell et al., 1987; Eck and Jones, 1992; Edwards et al., 1993), no differences (Lamb et al., 1985; Sharpley et al., 1991) or lower losses under conservation tillage (Jordan et al., 2000).

Following Eickhout et al. (2006), in the 1995-2030 period total reactive N loss will grow strongly in the world's increasingly intensive agricultural systems and, therefore, further increase of N use efficiency and improvement of agronomic management must remain a priority. Besides the obvious interest in studying the N dynamics and fertility in the plough layer, Mulvaney et al. (2009) highlighted the urgent need of extend the evaluation of soil fertility and organic matter to the subsoil.

Accordingly, we hypothesized that conservation minimum tillage could lead to N stratification in soil and changes in the N-related plant variables. Therefore, the present study aimed to evaluate the long-term effect of two tillage practices (conventional and minimum tillage during 14 years) on soil N (plough layer and subsoil), recovery efficiency of N, plant N concentration and amount of N exported with crops in a ryegrass-maize forage rotation.

2. Material and methods

2.1. Site description

The experimental field was located in the Gayoso-Castro farm (43° 06' N, 7° 27' W, 420 m a.s.l.) at Castro de Ribeiras de Lea (Galicia, NW Spain). The area has a temperate and rainy climate. During the study period (October 2006-October 2008), at the meteorological stations of As Rozas, Rubiás and Lugo, placed within a radius of 17 km from the farm and at similar altitude, rainfall mainly occurred in the October to June period (Fig.1) and the wettest month was October 2006. The soil is a Phaeozem Gleyic (IUSS Working Group, 2014) developed over sandy-clayey deposits, with sandy loam topsoil (700 g kg^{-1} of sand in the 0-5 cm), acidic pH_{H2O} (about 5.5) and an organic C content of 3.1-7.9 g kg^{-1}.

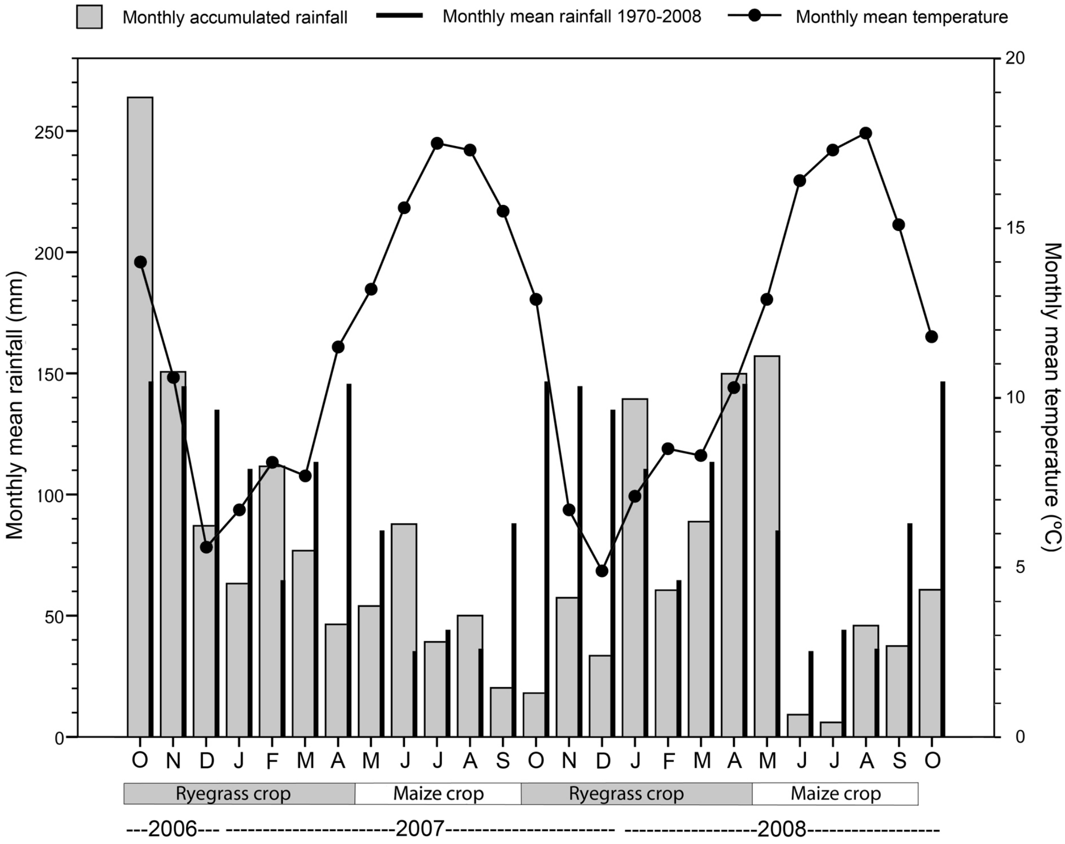

Fig. 1. Monthly mean temperature (ºC, points connected by a line) and rainfall (mm) during the growing season of ryegrass (dark grey bars) and maize (light grey bars) in comparison with the annual mean precipitation for the 1970-2008 period (dark lines).

Since 1994, a rotation of silage maize (*Zea mays* L.) and Italian rye-grass (*Lolium multiflorum* L.) has been annually cultivated in two adjacent areas with different tillage system: conventional plough tillage (PT) and conservation minimum tillage (MT). Maize was sown in rows 0.75 m apart (approx. 95,000 plants ha^{-1}, 4 rows per plot) in late May and harvested in late September, while rye-grass was sown in rows 0.17 m apart (40 kg ha^{-1}, 17.5 rows per plot) in late October and harvested in early May. In the MT treatment, before maize sowing, the adventitious vegetation was destroyed with glyphosate (36 %, at a dose of 5 L ha^{-1}). In the MT system, after 8 years of no-tillage (no-till drilling on stubble of the preceding crop), the management was changed to minimum tillage and during the last 6 years the soil was annually loosened with a bent-leg subsoiler to a depth of 30 cm before maize seeding aiming to revert the problem of increasing soil compaction and decreasing emergence of maize seedlings. In the PT treatment, the soil was ploughed to a depth of 25-30 cm with a reversible plough twice a year (May and October), to incorporate crop residues and to prepare the seed bed. Further agrochemical treatments were similar for both tillage systems. During the maize cultivation, the plots were treated with herbicides (33 % acetachlor and 16.5 % atrazine, 4 L ha^{-1}), insecticide (48 % clorpiriphos, 0.33 L ha^{-1}) and N (63 kg ha^{-1}), P (55 kg ha^{-1}) and K (157 kg ha^{-1}) fertilizer which was applied in sowing at 10 cm of depth side. During the ryegrass cultivation, plots received NPK fertilizer in early October (N: 27 kg ha^{-1}; P: 23 kg ha^{-1}; K: 67 kg ha^{-1}) and NH_4NO_3 fertilizer in early March (N: 81 kg ha^{-1}), in both occasions the fertilizer was applied on the soil surface.

The field experiment was designed taking into account the recommendations of Powlson and Barraclough (1993) about dose, enrichment and application of ^{15}N-fertilizer, as well as plot size and sampling and processing of soil and plant material. Nine replicate plots (4 m x 3 m; with 1-m wide buffer zones between them) were setup in the PT area and randomly assigned in triplicate to the three treatments that differed on N fertilization in the first year: $^{15}NH_4{}^{15}NO_3$ 10 atom % ^{15}N fertilizer applied in the first

(October 2006, $^{15}N_{October}$-fertilizer), second (March 2007, $^{15}N_{March}$-fertilizer) and third (May 2007, $^{15}N_{May}$-fertilizer) date of application. The same was done in the adjacent MT area. The goals were to elucidate the effects of tillage system on soil N and the recovery efficiency of N fertilizers applied during the whole cropping cycle, as well as on plant N concentration and the amount of N exported with crops. In October 2006 and March 2007 a solution with $^{15}NH_4{}^{15}NO_3$ was uniformly applied around the plot, while in May 2007 powdered $^{15}NH_4{}^{15}NO_3$ was applied at a 10 cm depth along the seeding lines previously open. The other fertilizations were done with commercial unlabelled fertilizer.

2.2. Soil and plant sampling

Soil samples were collected just after rye-grass (May 2007 and 2008) and maize (October 2007 and 2008) harvesting. In each plot, soil was taken at 0-5, 5-15, 15-30, 30-45 and 45-60 cm depth with a stainless steel probe (4 cm internal diameter) from 8 points uniformly distributed between the rows; afterwards it was thoroughly mixed to obtain a composite sample per plot for each depth, sieved (< 2 mm) and air-dried.

For calculating the aboveground biomass, all plants of the plot were cut at the base in May (rye-grass) and October (maize) of 2007 and 2008 dried and weighted. In 2007, no tillage effect was observed for maize (6613 and 6641 kg ha^{-1} for PT and MT, respectively) and ryegrass yield (3158 and 3114 kg ha^{-1} for PT and MT, respectively); however, in 2008, both crops yielded more in MT (7903 and 7313 kg ha^{-1} for ryegrass and maize, respectively), than in PT (4425 and 4755 kg ha^{-1} for ryegrass and maize, respectively) (Gómez-Rey et al., 2014). For N and ^{15}N analysis, only plants from the plot centre (75 cm inward from the edge) were considered, which were homogenized and crushed *in situ*, and a subsample was taken for chemical analysis. The subsample was dried at 60 ºC for 10 h and crushed again (< 4 mm).

2.3. Chemical analysis

The dry matter content of soils and plant material was assessed by oven-drying subsamples at 105 ºC to constant weight. Soil and plant total N and ^{15}N were measured on ground samples (< 100 µm) with an elemental analyser (Carlo Erba CNS 1508) coupled on-line with an isotope ratio mass spectrometer (Finnigan Mat, delta C).

The inorganic N content was analyzed by a modified diffusion method (Khan et al., 1997). In order to sequentially liberate the soil inorganic N pools as gaseous NH_3, 25 ml aliquots of soil extracts were placed in a 500 ml wide-mouth glass jar and first added with MgO (0.2 g) for diffusing (24 h at 50 ºC) the NH_4^+-N. Then, the extracts received a second dose of MgO (0.2 g) plus Devarda's alloy (0.4 g) for NO_2^--N and NO_3^--N reduction and diffusion (24 h at 50 ºC). The evolved NH3 was recovered in an acid trap into 10 ml of 0.005 M H_2SO_4 in a Teflon bottle attached to the glass jar and measured by back titration of the excess of H_2SO_4 with 0.01 M NaOH. After titration, the resulting $(NH_4)_2SO_4$ solutions were acidified with 1 mL of 0.005 M H_2SO_4 and dried at 60 ºC in a vacuum oven (Memmert VO400, PM400). To accelerate the drying process, the oven was alternatively under vacuum (15 kPa) and atmospheric pressure; in order to trap possible traces of atmospheric NH_3, the incoming air was passed trough a column of activated charcoal. The $(NH_4)_2SO_4$ salts were finally packed into tin capsules and analyzed for ^{15}N in an elemental analyser (Carlo Erba CNS 1508) coupled on line with an isotope ratio mass spectrometer (Finnigan Mat, delta C).

All analyses were carried out in duplicate and the mean of both analyses was used in the statistical procedure.

2.4. Interpretation of ^{15}N data

The percentages of plant N derived from soil (% Ndfs) and fertilizer (% Ndff) were calculated as follows:

$$mg\ N_{plant}\ x\ \%E_{plant} = (mg\ N_{soil}\ x\ \%E_{soil}) + (mg\ N_{fertilizer}\ x\ \%E_{fertilizer})$$

where N_{plant} = plant total N, N_{soil} = plant N derived from soil, $N_{fertilizer}$ = plant N derived from fertilizer, $\%E_{plant}$ = atom % excess ^{15}N of plant N, $\%E_{soil}$ = atom % excess ^{15}N of soil N taken up by plants and $\%E_{fertilizer}$ = atom % excess ^{15}N of fertilizer applied. As $\%E_{soil}$ = 0, then:

$$mg\ N_{plant}\ x\ \%E_{plant} = mg\ N_{fertilizer}\ x\ \%E_{fertilizer}$$

$$\%\ Ndff = \frac{mg\ N_{fertilizer}}{mg\ N_{plant}}\ x\ 100 = \frac{\%E_{plant}}{\%E_{fertilizer}}\ x\ 100$$

$$\%\ Ndfs = 100 - \%\ Ndff$$

The recovery efficiency of fertilizer N (REN) was calculated as follows:

$$REN = \frac{mg\ N_{fertilizer\ assimilated}}{mg\ N_{fertilizer\ applied}}\ x\ 100$$

$$REN = \frac{\%Ndff\ x\ mg\ N_{plant}}{mg\ N_{fertilizer\ applied}}\ x\ 100$$

The percentage of fertilizer-N recovered in the fine earth (< 2 mm) of each soil layer for each sampling date was calculated as follows:

$$\%Ndff_{layer\ A} = \left[\frac{\left(\dfrac{mg\ N}{kg_{soil}}\right)_{layer\ A} x\ kg_{soil\ layer\ A}\ x\ \%E_{soil\ layer\ A}}{mg\ N_{fertilizer\ applied}\ x\ \%E_{fertilizer}} \right] x\ 100$$

2.5. Calculation and statistical analysis

Plant and soil data were examined by two-way ANOVA after verifying the fulfilment of the assumptions of normal distribution (Shapiro-Wilk's W test) and equality of variances among groups (Levene's test). Tillage system and crop were the factors considered for data on N exported with the crop and N concentration in plant biomass (n=9), as well as for the percentage of plant N derived from the ^{15}N-fertilizer and the recovery efficiency of fertilizer N (n=3). For data on soil variables (NH_4^+-N, NO_3^--N and ^{15}N fertilizer immobilized at the end of the study) the factors considered were the tillage system and soil depth (n= 9). In the case of departure from normality or heteroscedasticity the original data of the latter variables were subjected to Tukey's ladder of powers or Box-Cox transformations to yield normal distribution and equality of variances. The Bonferroni's test was used to detect significant differences between the group means, at $p<0.05$. The proportion of the variation accounting for each factor or interaction in the ANOVA was determined by the partial eta-squared (η^2) statistic. Statistical procedures were performed using SPSS 15.0 for Windows.

3. Results

3.1. Soil N

Irrespectively of the sampling date, the soil inorganic N pool was largely dominated by the nitrates ($NO_3^-/NH_4^+ = 6.7$, mean value across all depths and sampling dates) and neither the NH_4^+-N (Fig. 2a) nor the NO_3^--N (Fig. 2b) content was affected by the tillage system, while the soil depth accounted for a quarter (NO_3^--N) and a third (NH_4^+-N) of its variation and the tillage-depth interaction was not significant (Table 1).

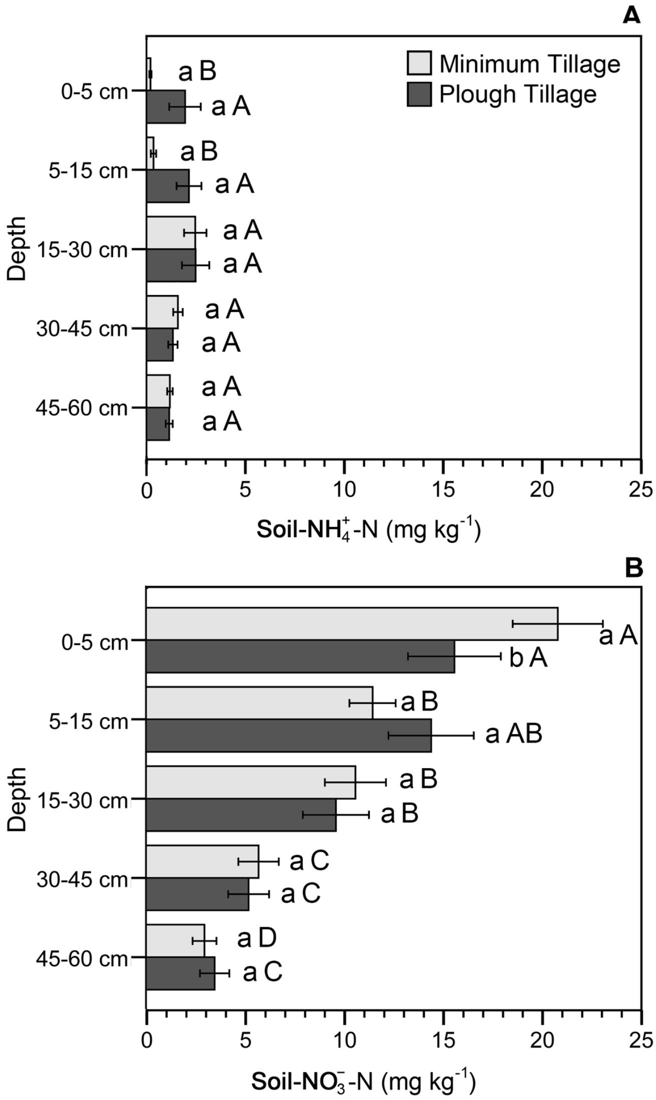

Fig. 2. Mean contents across sampling dates of NH_4^+-N and NO_3^--N in each layer of soil under conservation minimum tillage and plough tillage. Lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n= 9, p< 0.05).

At the end of the study period, the percentage of $^{15}N_{October}$-fertilizer that was recovered in the soil (PT: 20.1 ± 6.6 %; MT: 25.4 ± 4.9 %) did not differ between tillage systems and decreased with depth (85 % of variance explained; p< 0.001; see Table 1). However, the tillage*depth interaction, which explained 42 % of variance (p< 0.05), showed a stronger stratification of the $^{15}N_{October}$-fertilizer under MT than under PT (Fig. 3a), mainly due to differences in the uppermost soil layer. For the $^{15}N_{March}$-fertilizer, the percentage recovered in the soil at the end of the study was affected by the tillage system, soil depth and the interaction of both factors (39 %, 84 % and 51 % of variance explained, respectively): the recovery was slightly higher (p= 0.08) under MT (25.9 ± 4.3 %) than under PT (15.6 ± 6.5 %) and decreased significantly with depth, this decrease being much stronger in MT (Fig. 3b). Finally, the percentage of $^{15}N_{May}$-fertilizer recovered in the soil at the end of the study was unaffected by the tillage system (PT: 20.6 ± 5.9 %; MT: 24.7 ± 4.6 %) and the tillage-depth interaction, most of its variance (86 %) being explained by soil depth (Fig. 3c). Jointly considering the three fertilization dates (n=9), the percentage of ^{15}N fertilizer recovered in the 0-60 cm soil layer at the end of the experiment was significantly higher in MT than in PT (25.3 ± 4.0 % and 18.8 ± 6.0 %, respectively; p < 0.05).

3.2. Crop N

Whatever the fertilization considered, the two-way ANOVA showed that the tillage system had no significant effect on the Recovery Efficiency of Nitrogen (REN; see Table 1). For the $^{15}N_{October}$-fertilizer, the factor crop explained 84 % of REN variance, with a progressive decrease from the first to the fourth subsequent crop; the significant tillage-crop interaction (40 % of variance explained) showed that the decrease was initially faster, and then slower, in MT than in PT (Fig. 4). In the cases of $^{15}N_{March}$- and $^{15}N_{May}$-fertilizers the REN decreased suddenly from the first to the second crop after the fertilization and then further declined slowly. Most part of the REN variance (98 % and 89 %, respectively) was explained by the factor crop (Fig. 4). Jointly considering the four crops during the study, the REN of the $^{15}N_{October}$-fertilizer was very low (5.78 ± 1.42 % in PT; 4.76 ± 1.07 % in MT), while for the $^{15}N_{March}$-fertilizer the values were almost one order of magnitude higher (45.73 ± 3.67 % in PT; 44.90 ± 8.20 % in MT plots). Despite only three subsequent crops were studied, the global REN reached the highest values for the $^{15}N_{May}$-fertilization: 53.06 ± 11.28 % in PT and 51.7 ± 17.2 % in MT.

Table 1. Proportion of the variation (partial eta-squared statistic, η^2) of the N-related variables accounted for by each factor or interaction in the two-way ANOVA: a) soil NH_4^+-N and NO_3^--N during the whole study (n=9); b) percentage of $^{15}N_{October}$, $^{15}N_{March}$ and $^{15}N_{May}$ fertilizer recovered in the soil at the end of the study period (n=3); c) plant Nitrogen Use Efficiency (NUE) and plant N derived from the fertilizer (NDFF) for the $^{15}N_{October}$, $^{15}N_{March}$ and $^{15}N_{May}$ fertilizers jointly considering the four crops during the study period (n=3); and d) N exported with the crops and plant N concentration. (n= 18).

	η^2 Tillage	η^2 Crop	η^2 Tillage x Crop
Soil NH_4^+-N	n.s.	0.267 ***	n.s.
Soil NO_3^--N	n.s.	0.337 ***	n.s.
Soil $^{15}N_{October}$	n.s.	0.853 ***	0.416 *
Soil $^{15}N_{March}$	0.394 **	0.836 ***	0.510 **
Soil $^{15}N_{May}$	n.s.	0.858 ***	n.s.
NUE- $^{15}N_{October}$	n.s.	0.842 ***	0.400 *
NUE- $^{15}N_{March}$	n.s.	0.975 ***	n.s.
NUE- $^{15}N_{May}$	n.s.	0.885 ***	n.s.
NDFF- $^{15}N_{October}$	n.s.	0.846 ***	n.s.
NDFF- $^{15}N_{March}$	n.s.	0.999 ***	0.592 **
NDFF- $^{15}N_{May}$	n.s.	0.991 ***	n.s.
N exported with the crops	0.124 *	n.s.	0.145 *
Plant N concentration	n.s.	0.798 ***	0.222 **

Taking into account the amount of ^{15}N fertilizer exported with all crops and that recovered into the soil at the end of the experiment, the percentage of labelled fertilizer unaccounted for in the case of the $^{15}N_{October}$-fertilizer (72.0 ± 5.2 %) was more than twice that in $^{15}N_{March}$- and $^{15}N_{May}$-fertilizer (33.9 ± 7.0 % and 25.0 ± 14.2 %, respectively).

As for REN, no significant effect of the tillage system on the plant N derived from the fertilizer (Ndff) was found (Fig. 5). The factor crop explained most of Ndff variance (85 %) for the $^{15}N_{October}$-fertilizer and almost all (> 99 %) for the $^{15}N_{March}$- and the $^{15}N_{May}$-fertilizer (Table 1). However, the evolution of Ndff in the successive crops differed among fertilization dates: a) $^{15}N_{October}$-fertilizer, progressive decrease from the first to the fourth crop; b) $^{15}N_{March}$-fertilizer, sudden decrease from the first to the second crop and, to a lesser extend, to the third crop, with little subsequent changes; significant tillage-crop interaction that explained 59 % of the variance; and c) $^{15}N_{May}$-fertilizer, sudden decrease from the first to the second crop and then little reduction.

The crop did not affect the amount of N exported with the harvested biomass, that was slightly influenced (12-15 % of variance explained; $p< 0.05$; n=36; Table 1) by the tillage system, with MT > PT (89.7 ± 30.1 kg ha^{-1} and 71.7 ± 22.7 kg ha^{-1}, respectively; average across the four crops) and the tillage-crop interaction, with significant differences among MT (ryegrass-2 ≈ maize-2 > maize-1; ryegrass-1 having intermediate values; $p < 0.05$; n=9) but not PT crops (Fig. 6a). Plant N concentration (% N) was largely determined by the crop, with ryegrass-1 > ryegrass-2 > maize-2 > maize-1 (2.58 ± 0.88 %, 1.86 ± 0.36 %, 1.35 ± 0.13 % and 1.11 ± 0.17 %, respectively; 80 % of variance explained; $p < 0.001$; n=18), although it was also significantly ($p < 0.001$) influenced by the tillage-crop interaction (Fig. 6b) that explained 22 % of variance.

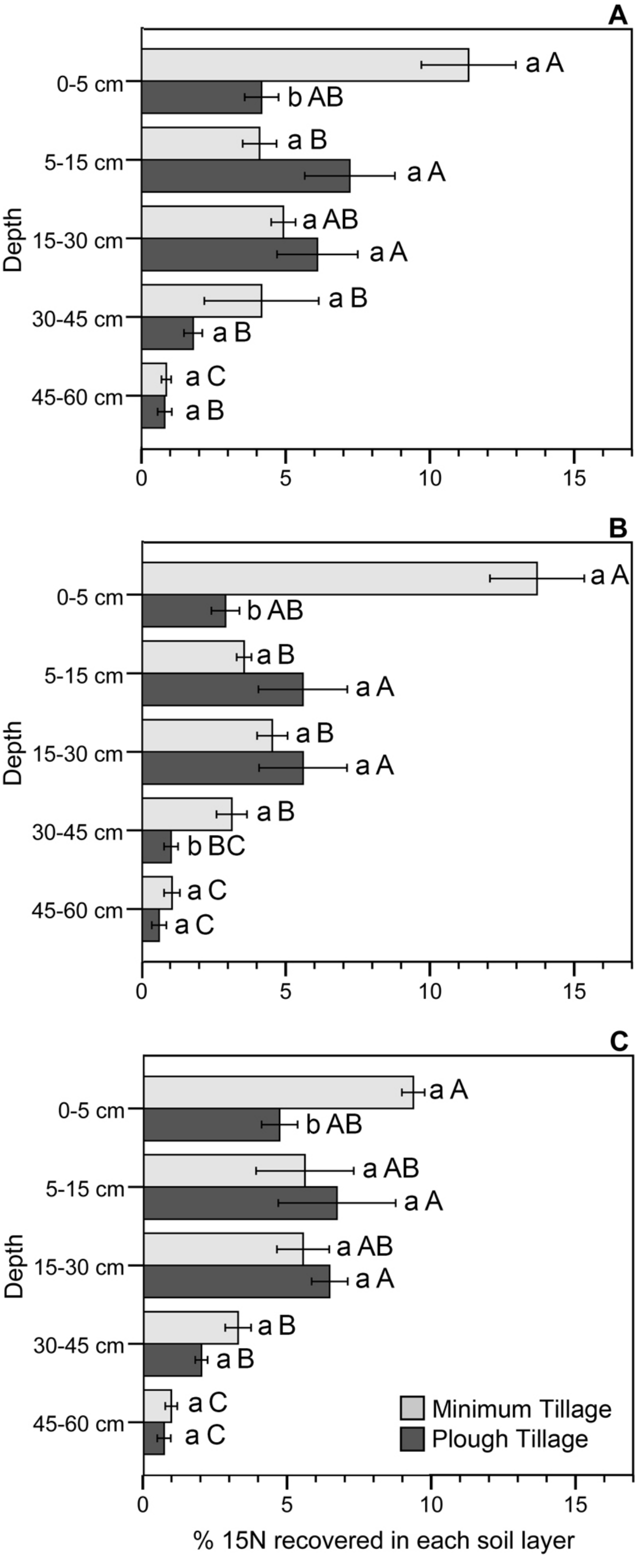

Fig. 3. Percentage of $^{15}N_{October}$-fertilizer (A), $^{15}N_{March}$-fertilizer (B) and $^{15}N_{May}$-fertilizer (C) that was recovered in each soil layer under conservation minimum tillage and plough tillage. Lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n= 3, $p < 0.05$).

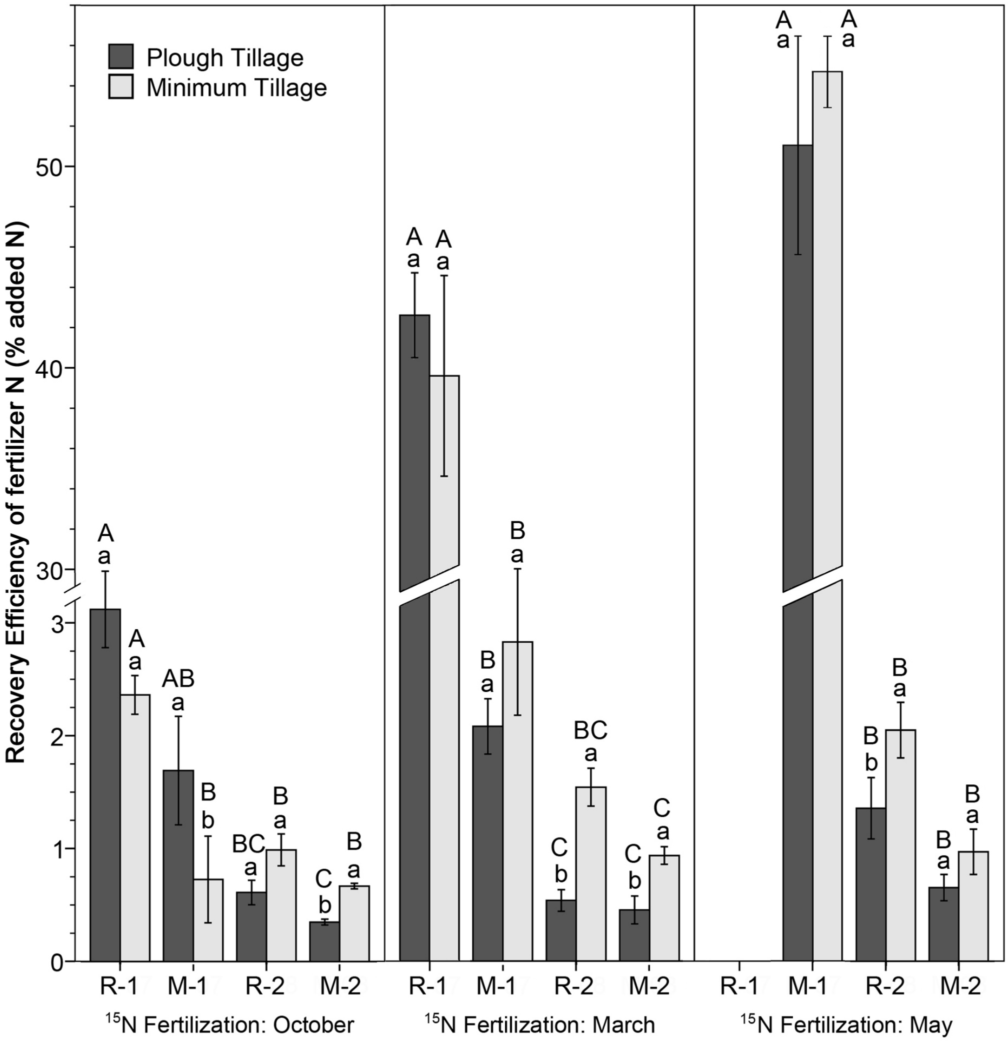

Fig. 4. Recovery efficiency of fertilizer N: percentage of applied ^{15}N taken up by the subsequent crops (R, ryegrass; M, maize; the number indicates the year) under conservation minimum tillage and plough tillage. For each ^{15}N fertilization, lowercase letters indicate significant differences between tillage systems for the same crop and uppercase letters indicate significant differences among crops for the same tillage system (n= 3, $p < 0.05$).

4. Discussion

The similarity of the NH_4^+-N and NO_3^--N contents under minimum tillage and plough tillage, irrespectively of the sampling date and soil depth, showed that the tillage practices had little or no effect on the inorganic-N pool. Our results agree with the lack of differences on gross and net N transformation rates found in the same plots by Gómez-Rey et al. (2012), although both a reduction (Doran, 1987; Peigné et al., 2007) and a long term increase of N availability to plants (Kandeler and Böhm, 1996; Rice et al., 1986) after the adoption of conservation practices have also been reported.

To evaluate the efficiency in the use of N fertilizers, both the N exported with the crops and the N immobilized in the rooting soil layer must be taken into account (Ladha et al., 2005). Although no differences were found between tillage systems for any single fertilization date, jointly considering the three fertilizations the recovery of ^{15}N fertilizer immobilized into the 0-60 cm soil layer at the end of the experiment was around one third higher in MT than in PT (25.3 vs 18.8 %), showing a clear advantage of the conservation tillage on maintaining or increasing the soil organic N pool; this result is very important because SON is threatened by the use of N fertilizers, especially when applied beyond crop requirements (Mulvaney et al., 2009). The figures we found were lower than the 28-39 % reported by Glendining et al. (2001), but agree with the increased soil N retention usually reported in the upper soil layers under conservation tillage (Franzluebbers et al., 1994; Jacobs et al., 2009; Kaiser et al., 2014; McCarty and Meisinger, 1997).

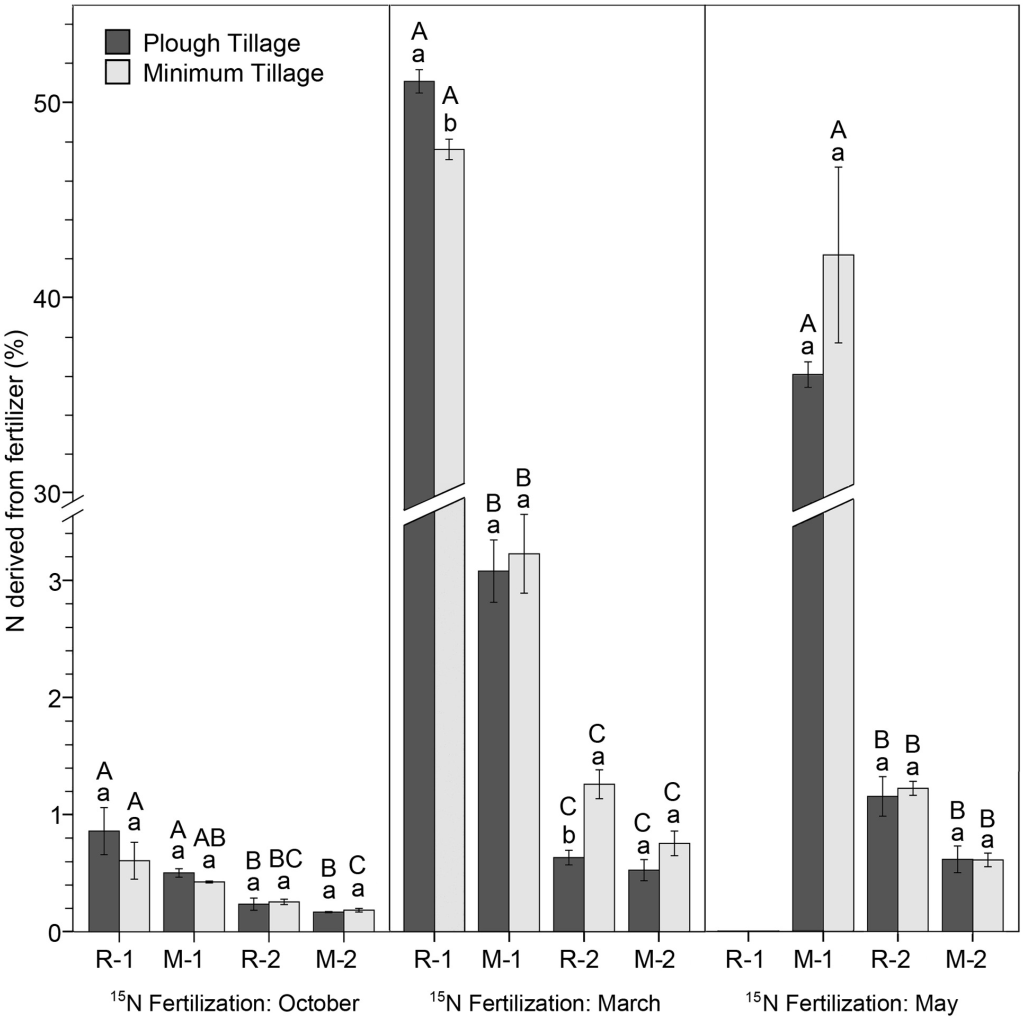

Fig. 5. Percentage of plant N derived from the fertilizer under conservation minimum tillage and plough tillage(R, ryegrass; M, maize; the number indicates the year). For each ^{15}N fertilization, lowercase letters indicate significant differences between tillage systems for the same crop and uppercase letters indicate significant differences among crops for the same tillage system (n= 3, p< 0.05).

While the percentages of N immobilized in the soil were in a narrow range (16-21 % in PT; 25-26 % in MT), those exported with the crops varied by a factor of nine, from 5-6 % for the $^{15}N_{October}$-fertilizer to 45-49% for the $^{15}N_{March}$-fertilizer and 52-53 % for the $^{15}N_{May}$-fertilizer. These figures revealed the lack of differences between tillage systems and wide differences among fertilization dates in the recovery efficiency by crops of the applied N, that ranged from very low ($^{15}N_{October}$-fertilizer) to middle ($^{15}N_{March}$- and $^{15}N_{May}$-fertilizer) when compared with the range of 46-65 % reported from researcher-managed experiments for major grain crops (Ghosh et al., 2015; Ladha et al., 2005). The highest REN values of the $^{15}N_{May}$-fertilizer, despite only three instead of four subsequent crops were studied, agrees with the higher efficiency of banded fertilizer application, as usually reported (Beyrouty et al., 1986; Fox and Piekielek, 1987; Ghosh et al., 2015; Peoples et al., 1995), that is common to both MT and PT systems. The differences in the recovery efficiency by crops largely determined those in the global N use efficiency: the recovery in the soil+crops at the end of the study period varied from 26-30 % in the $^{15}N_{October}$-fertilizer, to 60-70 % in the $^{15}N_{March}$-fertilizer and 74-84 % in the $^{15}N_{May}$-fertilizer. These figures were, respectively, very low, similar and high when compared with those of 58-72 % reported by other authors (Delgado et al., 2004; Eagle et al., 2001), and, when compared with the 15-50 % usually reported (Fiez et al., 1995; Foth and Ellis, 1996), they revealed that N losses from our agricultural system were very high for the $^{15}N_{October}$-fertilizer, and normal for the $^{15}N_{March}$- and $^{15}N_{May}$-fertilizers. Denitrification, volatilization and NO_3^--N leaching are the main processes determining N losses (Mosier, 2001; Schlesinger and Bernhardt, 2013), although runoff and erosion can also be important. In our case, the last two processes may be negligible taking into account the flat topography, whereas the acidic pH of soil and weather conditions in autumn and winter likely prevent relevant losses by volatilization. Denitrification takes place when soils are under anaerobic or microaerophilic conditions, either due to heavy rains that lead to excessive soil humidity difficulting its aeration (Delgado et al., 2010) or to drainage restrictions that lead to soil waterlogging. Lixiviation of NO_3^--N is also enhanced by heavy rains, especially in coarse-textured soils which, compared with heavy-textured soils, allowed a faster movement of water below the root layer (Davis et al., 2003; Delgado et al., 2010; Hack-ten Broeke and de Groot, 1998). Therefore, considering the heavy rains during the autumn-winter period after fertilization, the flat topography, the sandy loam soil and the high level of the water table in that period, denitrification and NO_3^--N leaching are the most likely processes explaining the high losses of the $^{15}N_{October}$-fertilizer, that in our case were not mitigated by the conservation tillage management. Taking into account the importance of coupling N fertilization and plant demands to improve N use efficiency (Chien et al., 2009; Dinnes et al., 2002), the losses of $^{15}N_{October}$-fertilizer were likely exacerbated by the low plant uptake due to the delay in ryegrass emergence and growth as a result of the unfavourable weather conditions. Surely, these circumstances were also responsible for the very low contribution of the first fertilization to plant N nutrition (Ndff < 1%), far from those of $^{15}N_{March}$- and $^{15}N_{May}$-fertilizer (around 50 % and 39 %, respectively).

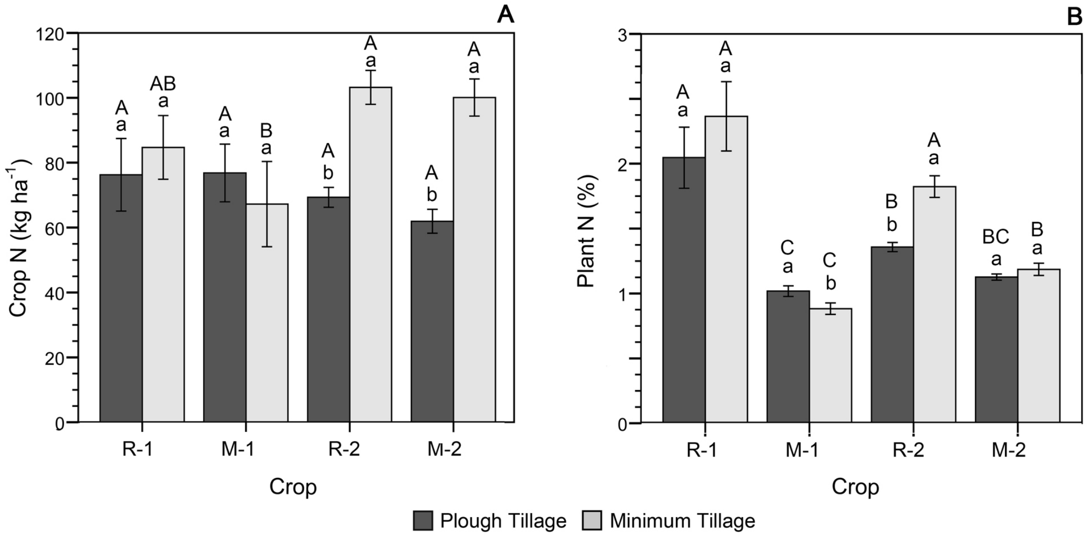

Fig. 6. Amount of N exported with the crops (A) and plant N concentration (B) under conservation minimum tillage and plough tillage (R, ryegrass; M, maize; the number indicates the year). Lowercase letters indicate significant differences between tillage systems for the same crop and uppercase letters indicate significant differences among crops for the same tillage system ($n= 3$, $p< 0.05$).

While no significant effect of the tillage system on the percentage of plant N derived from fertilizer was found in any studied crop, two tillage effects on the recovery efficiency of N were identified: a) slightly lower values in MT than in PT in two out of three first crops; and b) higher values in MT than in PT in seven out of eight subsequent crops, differences being significant in half of cases. These differences could be explained by a higher initial immobilization of the applied N in the SOM of plots under minimum tillage, which consequently: a) suffered a transient reduction in the fertilizer N available to plants (Doran, 1987; Tessier et al., 1990; Van Den Bossche et al., 2009); and b) benefitted from an improved N availability to plants in the long-term (Rice et al., 1986) by increasing soil N retention and the labile N pool (Franzluebbers et al., 1994; McCarty and Meisinger, 1997) in the upper soil layers, that in our case increases the residual availability of the fertilizer ^{15}N to the subsequent 2-3 crops.

5. Conclusions

Tillage practices had little or no effect on the inorganic-N pool at any sampling date. The recovery efficiency of N applied with ryegrass seeding was extremely low due to the autumn-winter weather conditions, which were adverse for ryegrass emergence and growth and favourable for nitrate leaching and denitrification. Conversely, the recovery efficiency of N applied with the second N fertilization of ryegras (two months before harvest) and with maize seeding were within the normal ranges. The highest recovery efficiency was that of banded fertilizer application.

No tillage effects on the recovery efficiency of N by crops were found, but the amount of N immobilized in the rooting soil layer was around one third higher under MT than under PT. Therefore, nourishing the first crop as efficiently as in PT, N losses were 10 percentual points lower in MT and more fertilizer N was retained within the soil profile. Consequently, crops under MT suffered an initial reduction in the amount of fertilizer N available followed by a subsequent improvement of N availability thanks to the build up of a labile pool.

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