Residual effect of nitrification inhibitors enhances NUE in a cropping system

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Abstract
Nitrification inhibitors (NI) may increase the recovery of N fertilizer applied to a crop, but little is known about the effect on the soil N supply capacity over time and the recovery by the subsequent crops. During two seasons, a field experiment with maize was fertilized with ammonium sulfate nitrate (ASN) and DMPP blended ASN (ENTEC®) at two levels (130 and 170 kg N ha^{-1}). A control non-fertilized treatment was included. Maize yield, grain quality, nutritional state and N use efficiency were evaluated. During a third experimental season, a non-fertilized sunflower was planted in the same plots to study the cumulative effect. Laboratory determinations were performed to elucidate possible sources of residual N. The second year, DMPP application allowed a 23% reduction of the fertilizer rate without decreasing crop yield or grain quality. In addition, the non-fertilized sunflower scavenged more N in treatments previously treated with ENTEC® than with ANS, increasing N use efficiency. After DMPP application, N was conserved in non-ready soil available forms during at least 1 year and subsequently released to meet crop demand. The potential N mineralization obtained from aerobic incubation was higher for soils from the ENTEC® treatments. A higher $\delta^{15}N$ in the soil indicated larger non-exchangeable NH_4^+ fixation in soils from the plots treated with ENTEC® or ASN-170 than from the ASN-130 or the control. These results open the opportunity to increase N efficiency in crop rotations by taken advantage from the effect of NI on the soil residual N.

Key Words
Fertilizer use efficiency, DMPP, N recovery, ASN, ENTEC®, non-exchangeable ammonium.

Introduction

The application of nitrification inhibitors (NI) is a strategy to increase the efficiency of nitrogen (N) in farming systems. The reduction of N losses with NI has been widely documented, while the effect on crop yield or N use efficiency (NUE) is still not clear (Abalos et al., 2014). In fact, the opportunity of saving N-fertilizer, reducing the number of applications, or increasing the productivity are the main advantages that would justify the higher price of NI-blended fertilizers to farmers as a viable alternative over conventional fertilizers. Therefore, identification of cropping systems or environmental conditions in which NI enhances NUE and crop yield may contribute to the best practice of this fertilizer technology.

A cumulative effect of NI could be explained through N immobilization by microorganisms and fixation by soil clay minerals in non-exchangeable forms (Ma et al., 2015). However, this cumulative effect has been mostly studied as the annual effect of NI on crop yield or NUE, and rarely as the effect on the subsequent crop following the application (Sharma and Prasad, 1996).

We hypothesized that the application of NI could increase the soil N supply capacity over time and contribute to an enhancement of crop recovery in the cropping system. During two seasons, a maize crop was planted and fertilized with ASN, with and without DMPP, in order to determine the effect of NI fertilizers on grain yield, N content and NUE, compared to conventional fertilizers. A non-fertilized sunflower crop was planted in the same plots to study the cumulative effect during a third experimental season.

Methods

Field experiment

The experiment was located in Aranjuez (Central Spain). The soil was silty clay loam (*Typic Calcixerept*) with pH~8 and the climate Mediterranean semiarid with high inter-annual variability (Gabriel et al., 2011).
Fifteen plots (12 x 6 m^2) were randomly distributed in five treatments, with three replications. In two treatments, ASN (26 % N) together with the nitrification inhibitor DMPP (3, 4-dimethylpyrazole phosphate) was applied either at the recommended rate in the area of 170 kg N ha^{-1} (ENTEC-170), or with a reduced rate of 130 kg N ha^{-1} (ENTEC-130). In two other treatments, ASN conventional fertilizer was applied with the same rates (ASN-170, ASN-130). A non-fertilized control was included. In 2013 and 2014 fertilizers were applied over a maize crop (*Zea mays* L) at the end of May. In 2015, a sunflower crop (*Helianthus annuus* L.) was planted in the same plots but no N-fertilizer was applied, therefore, the sunflower was used as a test for

the cumulative effect of the previous treatments. Both maize and sunflower were sowed in April at a seeding rate of 80,000 seeds ha^{-1}, and harvested in September. Irrigation water was delivered using sprinklers. The irrigation schedule and doses were calculated from the daily values of crop evapotranspiration (ETc). In 2013 and 2014, total water input was ~8% larger than the ETc to ensure a leaching fraction and avoid an increase on soil salinity, while in 2015 the irrigation was adjusted to sunflower needs.

Variables measured

For the 2013 and 2014 maize crop the grain yield, N grain concentration, N content and N uptake were determined at harvest. In 2015, anticipating the sunflower damage by birds before harvest, a sampling was performed at the full flowering stage when maximum N uptake was expected. At this time, biomass, N concentration and content in sunflower head, stem and leaves were determined. Each year, the crop nutritional state was evaluated with SPAD® at various growth stages, from stem elongation to full flowering.

Two components of the NUE were calculated for each experimental season: the agronomic efficiency (AE_N) that refers to the kg of crop yield increase obtained per kg of N applied, and the N recovery efficiency (RE_N) that refers to the kg of crop N uptake per kg of N applied. The total RE_N during the whole experimental period was also calculated for each treatment based on the crop N uptake during the three seasons.
Before the crop sowing and after harvesting each year, soil NO_3^- and NH_4^+ contents were determined in each plot to 1m depth by 0.2 intervals.

In order to elucidate and locate the hypothetical residual effect of ENTEC® fertilizers, laboratory determinations were performed two years after the starting of the experiment. The soil N mineralization potential (N_0) was determined after a 10-week aerobic incubation in samples from the top layer (0-20 cm). The soil non-exchangeable NH_4^+ ($NH_4^+{}_f$) was determined after a potassium hypobromite-dry soil extraction in samples from 0-60 cm depth (in 20 cm interval). In all subsamples, the non-exchangeable N-NH_4^+ concentration and the delta ^{15}N were determined.

Results

Grain yield, N content and uptake by crops were affected by treatments (Table 1). The second experimental year, significant differences were found between treatments. ENTEC® treatments and the conventional fertilizer at the recommended rate (170 kg N ha^{-1}) achieved greater maize yield. As well, these treatments had a higher grain N content. Therefore, ENTEC® allowed a fertilizer rate reduction without decreasing yield or grain quality. These results were supported by optical readings, indicators of the crop N nutritional status: in 2014, the ASN-170 treatment showed greater SPAD® values than the ASN-130 at the flowering stage. However, no differences were found between ENTEC® treatments, and values were similar to ASN-170.

Table 1. Yield, grain N concentration, grain N content, N uptake, and the N agronomic efficiency (AE_N). Within year, treatments followed by letters show significant differences (P≤0.05, Duncan test).

Treatment	Grain			Crop	
	Yield Mg ha^{-1}	N concentration %	N content kg N ha^{-1}	N uptake kg N ha^{-1}	AE_N kg grain kg^{-1} N
			2013		
Control	6.4 b	1.16 b	74.6 b	107.9 b	
ASN- 130	8.6 ab	1.34 a	115.8 a	168.2 a	16.72 a
ASN- 170	10.0 a	1.34 a	134.4 a	212.3 a	21.18 a
ENTEC-130	9.1 ab	1.37 a	125.3 a	191.6 a	20.68 a
ENTEC-170	9.7 ab	1.34 a	129.4 a	190.4 a	19.24 a
			2014		
Control	5.0 c	1.14 b	55.8 b	69.1 c	
ASN- 130	7.6 b	1.12 b	85.9 b	114.1 bc	20.73 c
ASN- 170	10.7 a	1.15 b	123.4 a	162.8 ab	33.93 b
ENTEC-130	11.3 a	1.33 ab	150.7 a	186.4 a	48.93 a
ENTEC-170	10.6 a	1.46 a	154.8 a	196.2 a	33.07 b

The last year, plots that were fertilized with ENTEC-170 in the previous years had the highest biomass in sunflower heads at the time of full flowering, and those previously fertilized with ASN-170 and ENTEC-130 had also higher biomass than the control (Table 2). The sunflower N uptake of plots fertilized in previous years with ENTEC-130 or with the ASN at the recommended rate was larger than in the control and ASN-130 treatments. The ENTEC-170 treatment had the highest sunflower N uptake. At the sunflower heading stage, SPAD® readings were greater for ENTEC® treatments than for the control. At full flowering, when N uptake had mostly occurred, ENTEC® with the recommended rate showed the greatest N uptake.

Table 2. Head biomass, N concentration, N content and N uptake in sunflower at flowering in 2015. Treatments followed by letters show significant differences (P≤0.05, Duncan test).

Treatment	Head biomass	Head N concentration	Head N content	Crop N uptake
	$Mg\ ha^{-1}$	%	$kg\ N\ ha^{-1}$	
Control	0.7 d	1.77	12.4 d	25.6 d
ASN- 130	1.4 cd	1.77	23.1 cd	50.5 c
ASN- 170	2.4 ab	1.63	38.3 ab	79.2 b
ENTEC-130	2.1 bc	1.64	33.9 bc	72.5 b
ENTEC-170	3.3 a	1.55	51.1 a	100.0 a

Both components of the NUE showed the effect of the treatments in the second year (Table 1, Figure 1). In 2014, the AE_N of the ENTEC-130 was the highest, and no differences were observed when the recommended rate was applied. By reducing the rate from 170 to 130 kg N ha^{-1} the kg of maize per kg of N applied with ENTEC® increased in 44%. The effect of the fertilizer type on RE_N was significant on 2014 and for the whole experiment period. In 2014, the RE_N in the maize fertilized with ENTEC® was >74% whereas the average of conventional fertilizer treatments was ~45%. Values in 2014 were higher than the reported in the literature suggesting a residual effect of NI. The average RE_N during the whole experimental period for the ENTEC treatments was 91%, whereas the ASN treatments recovered 63% of the N applied with fertilizers the previous years. Therefore, the cumulative effect of NI was clearly showed.

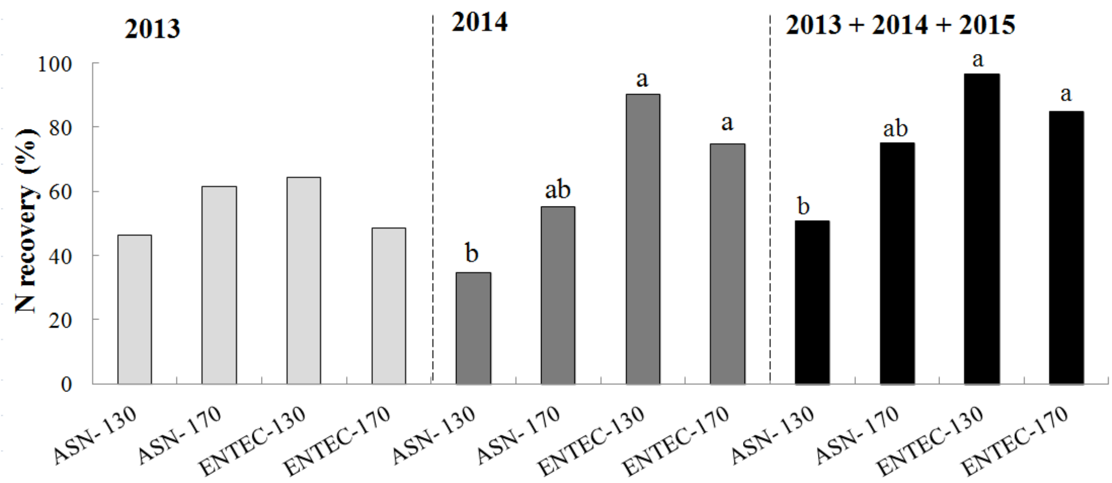

Figure 1. Nitrogen recovery efficiency (RE_N) of the fertilizer treatments in 2013, 2014; and cumulative N recovery over the three experimental seasons. Letters show differences between treatments (P≤0.05. Duncan test)

Soil inorganic N results confirmed the capacity of sunflower to scavenge the N and endorse the calculation of RE_N: at sunflower harvest the soil was depleted (~41 kg N ha^{-1}) and no differences in mineral N were observed between treatments.

Laboratory determinations carried out two years after the start of the experiment showed that the residual effect was driven by biotic and abiotic processes. After the 10-weeks aerobic incubation under controlled conditions, the soil samples from the ENTEC-170 treatment accumulated more mineral N than samples from the control. No differences were observed in the mineralization rates obtained by fitting the one-pool exponential model, but the soil N mineralization potential was higher for the ENTEC-170 than for the ASN-170 and control treatments (Figure 2).

The $NH_4^+{}_f$ content of the samples ranged between 0.011 and 0.014 %, equivalent to 100-140 mg N kg^{-1}. The detection threshold of the analyzer was 0.005% N, therefore no reliable differences between treatments in the content of $NH_4^+{}_f$ were detected. Delta ^{15}N-$NH_4^+{}_f$ values were higher for ENTEC treatments and the ASN with the recommended rate. These results show that fixation and defixation processes were occurring in the soil. Therefore, the residual effect on ENTEC treatments can be explained by an increase in both pools, microbial biomass and $NH_4^+{}_f$, being the organic more relevant.

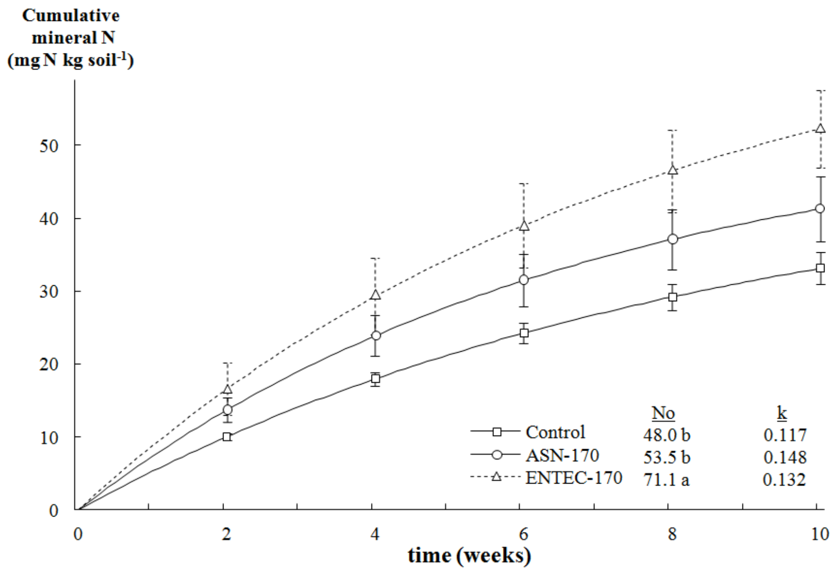

Figure 2. Cumulative N mineralization in soils from the control, ASN-170 and ENTEC-170 during a 10-week aerobic incubation. Soil N mineralization potential (N_0) and N mineralization rate (k) were calculated by fitting a non-linear regression model ($Nt = N_0$ exp (-k t)). Bars represent the standard error.

Conclusion

The ammonium sulfate nitrate blended with the DMPP nitrification inhibitor increased the efficiency of fertilizer N in a three-year crop rotation with respect to conventional ASN. In the following year after application, the recommended rate in the region was reduced 23% by using ENTEC® without decreasing grain yield or quality of maize. In addition, a non-fertilized sunflower planted after the maize was able to scavenge more N in treatments previously treated with ENTEC® than with traditional fertilizers.
After NI fertilizer application, N was conserved in non-ready soil available forms during at least 1 year and subsequently released to meet crop demand, thereby mitigating N losses.

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