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Use of nitrification inhibitor DMPP to improve nitrogen recovery in irrigated wheat on a calcareous soil

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Abstract

The 3,4-dimethyilpyirazole phosphate (DMPP), commercialized as Entec, is a nitrification inhibitor developed by BASF (Germany) that may help to minimize N losses and to obtain a higher profit from N fertilizers. A two-year field trial was established in 2001 in the Northeast of Spain to assess the effects of DMPP on N use efficiency (NUE) and to determine the economic returns. Seven treatments have been carried out comparing the effect of DMPP on pig slurry and on mineral fertilizers. The application of DMPP resulted in better efficiency indexes on mineral fertilizers. An apparent nitrogen recovery of 0.465 kg kg^{-1}, on average, was obtained for the Entec treatment. A net benefit of € 809 ha^{-1}, on average, was obtained for the Entec treatment compared with € 607 ha^{-1} for the control treatment. The results of this study suggest that the nitrification inhibitor could improve farmer profit in irrigated wheat on a calcareous soil.

Additional key words: ammonium sulphate-nitrate; apparent nitrogen recovery; Entec; nitrogen efficiency indexes; pig slurry.

Resumen

Utilización del inhibidor de la nitrificación DMPP para mejorar la recuperación del nitrógeno en trigo de regadío en un suelo calcáreo

El 3,4-dimetilpirazol fosfato (DMPP), comercializado como Entec, es un inhibidor de la nitrificación desarrollado por la compañía BASF (Alemania) que puede ayudar a minimizar las pérdidas de nitrógeno y a conseguir un mayor rendimiento económico de los fertilizantes nitrogenados. En el año 2001 se estableció en el Noroeste de España un experimento de campo de dos años de duración para evaluar los efectos del DMPP en la eficiencia en el uso del nitrógeno (NUE) y para determinar los rendimientos económicos. Se llevaron a cabo siete tratamiento para comparar el efecto del DMPP en los purines de cerdo y en los fertilizantes minerales. La aplicación de DMPP proporcionó mejores índices de eficiencia en los fertilizantes minerales. Para el tratamiento con Entec se obtuvo de promedio una recuperación aparente de nitrógeno de 0,465 kg kg^{-1}. El beneficio neto del tratamiento con Entec fue en promedio de 809 € ha^{-1}, comparado con los 607 € ha^{-1} del tratamiento control. Los resultados de este estudio sugieren que el inhibidor de la nitrificación puede mejorar el beneficio del agricultor que cultiva trigo en regadío en un suelo calcáreo.

Palabras clave adicionales: Entec; índices de eficiencia de nitrógeno; nitrosulfato amónico; purín de cerdo; recuperación aparente de nitrógeno.

Introduction

Farmer profit in Mediterranean cropping systems could be improved using more efficient N fertilizers. Environmental N losses could also be reduced by improving N fertilization management. To reconcile economic and environmental issues, we need to establish field experiments in order to define the best strategy to manage N. Food production must increase to meet the increasing population needs. Cassman *et al*. (2002) suggest that nearly all of this increase must come from producing higher yields on existing agricultural land.

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Abbreviations used: AE (agronomic efficiency), ASN (ammonium sulphate-nitrate), DCD (dicyandiamide), DMPP (3,4-dimethyilpyirazole phosphate), EC (electrical conductivity), ETo (reference evapotranspiration), NI (nitrification inhibitors), NP (nitrapiryn), NREC (apparent nitrogen recovery), NUE (nitrogen use efficiency), NUpE (nitrogen uptake efficiency), NUtE (nitrogen utilization efficiency), PE (physiological efficiency), PS (pig slurry), STK (soil test K), STP (soil test P).

Management options for improving the use of nitrogen is one of the goals. Nitrogen recovery and agronomic nitrogen use efficiency are important issues for sustainability of agricultural systems. Sustainability is related to both economical and environmental aspects. Some strategies could be established to improve the utilization of applied N to maximize yields and to minimize N losses in a cereal cropping system. Among these strategies, enhanced-efficiency fertilizers are the most innovative. These products include urease inhibitors, coated N fertilizers, condensed urea and urea-formaldehyde and the nitrification inhibitors (NI). The release of commercial fertilizers that potentially reduce nitrate leaching from agricultural lands needs validation on farmland scale with field observation data.

This research is focused on the use of nitrification inhibitors. Controlling the microbiological process of nitrification, nitrate losses (leaching and denitrification) could be reduced, increasing the assimilated N (Subbarao *et al*., 2006). Nitrification inhibitors are compounds that delay the bacterial oxidation of ammonium ions to nitrite (and subsequently to nitrate) by suppressing the activity of *Nitrosomonas* spp. in the soil (Prasad and Power, 1995; Trenkel, 1997). NIs have been used to increase yields and to reduce nitrate leaching in several crops (Malzer and Randall, 1985; Frye *et al*., 1989; Malzer *et al*., 1989; Walters and Malzer, 1990; Corré and Zwart, 1995; Davies and Williams, 1995; Martin *et al*., 1997; Trenkel, 1997; Ball-Coelho and Roy, 1999; Serna *et al*., 2000; Pasda *et al*., 2001; Díez-López *et al*., 2008; Li *et al*., 2008). A large number of chemicals have been reported as nitrification inhibitors, but only three of them are commercialized worldwide. These are nitrapiryn (NP), dicyandiamide (DCD), and 3,4-dimetilpirazolphosphate (DMPP). Disadvantages of DCD and nitrapyrin have been described by Zerulla *et al.* (2001). DMPP has been demonstrated to be effective at very low application rates. DMPP has also a low solubility in water and it is not phytotoxic (Zerulla *et al.*, 2001).

Di and Cameron (2002, 2007) demonstrated that using an NI such as DCD significantly decreased nitrate leaching and N_2O emissions in grazed pastures in New Zealand.

The 3,4-dimethyilpyirazole phosphate (DMPP) is a nitrification inhibitor developed by BASF (Germany) in cooperation with universities and other research institutes (Zerulla *et al*., 2001). DMPP has a high potential of inhibition, with a duration of between 6-8 weeks depending on soil temperature, and a bacteriostatic effect on *Nitrosomonas*. DMPP is incorporated in solid mineral fertilizer (Entec® stabilized fertilizers).

On our planet, nitrogen research is an important activity because it is extremely necessary for crop production and to maintain the increasing rate of food and feed supply. Environmental impact is also an aspect of major concern. Recently, at the 16th N Workshop celebrated in Turin (Italy), more than 300 works were presented with more than 1,000 authors (Grignani *et al*., 2009). In Spain, a scientific network on nitrogen use efficiency (www.ruena.csic.es) involving more than 20 institutions and 100 researchers is currently working on and conducting research into all aspects relating to nitrogen, including fertilizer development and legislation measures. The behavior of these nitrification inhibitors is one of the topics being investigated. The use of animal manure (pig and cattle slurries, litter manure and farmyard manure, for example) and other waste products as a source of nitrogen in crop production is also arousing the interest of the leading fertilization management teams.

Catalonia (NE Spain) is the leading region in Spain and the 6th in Europe for pig production. For this reason, the study of pig slurry management is of great interest. Based on Catalonian agricultural census data, it is estimated that about 10 million m^3 of pig slurry (PS) are produced every year. Most of this pig slurry is spread in agricultural soils at rates that usually exceed crop nutrient requirements. Contamination of soils by nutrients and heavy metals, atmospheric emissions of ammonia and nitrous oxide, odor and airborne pathogens, and groundwater pollution are produced as a consequence. One approach to partially reduce this pollution is to preserve the mineral N in the soil as ammonium instead of in its nitric form (Ball-Coelho and Roy, 1999). The addition of nitrification inhibitors (NIs) to pig slurry can help maintain soil mineral N in the ammoniacal form (inhibiting *Nitrosomonas* bacteria activity) and reducing the N losses to the environment. The value of N in pig slurry in relation to mineral fertilizer has been analyzed in a 4-year field experiment in the same area of study (Guillaumes *et al*., 2006). The addition of NIs to different types of slurry has also been experimentally tested. The effect of the DCD controlling the nitrification process was demonstrated on pig slurry by Tittarelli *et al.* (1997). The evaluation of the effect of DMPP added to either N mineral fertilizer or pig slurry showed between 17 and 20% reduction of N leaching in a pot experiment (Guillaumes and Villar-Mir, 2004; Guillaumes *et al*., 2007). It was also observed

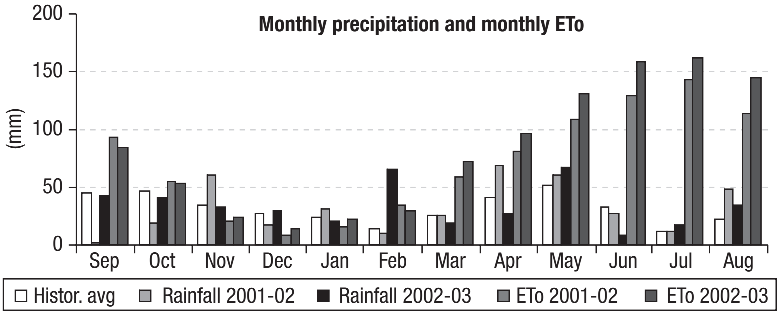

Figure 1. Monthly total precipitation (mm) and average monthly ETo (FAO P-M, mm) during the growing season at El Poal (Spain). Reference evapotranspiration as reported by Allen *et al.* (1998).

on Ray-grass forage that NI DMPP added to pig slurry increased agronomic efficiency (Villar *et al.*, 2008). Fangueiro *et al.* (2009) studying the influence of DCD and DMPP, concluded that DMPP combined with cattle slurry is more efficient as a NI than DCD to prevent nitrate formation and led to grater ryegrass yields.

The objective of this study was to assess the effects of DMPP added to either pig slurry or mineral fertilizer on soil N mineral profiles, yield, nutrient uptake and N recovery, as well as to demonstrate if these effects produce environmental, productive and economical advantages in irrigated winter wheat on a widespread calcareous soil.

Material and methods

Site and soil

A field experiment was conducted during two wheat (*Triticum aestivum* L. cv. Bancal) growing seasons (2001/02 and 2002/03) in the irrigated area served by the Urgell Channel (Northeast Spain) at a commercial farm (41° 40' 11" N, 0° 55' 30" E; elev. 239 m). The mean annual rainfall is 377 mm, mean annual temperature is 13.9°C and mean annual ETo (Penman-Monteith) is 928 mm. Weather conditions during the survey were recorded by an automated station (Campbell Sci., Logan, UT) located at El Poal (41°40' N, 00° 51' E; elev. 227 m) (Weather stations network, DAR, *Generalitat of Catalunya*) located within 5.5 km of the experimental site. Monthly total precipitation and average monthly reference evapotranspiration are given in Figure 1.

The soil type was a Linyola silty clay loam series (Herrero *et al.,* 1993). The soil moisture regime is xeric, and the soil temperature regime is mesic (Soil Survey Staff, 1998). Some of the soil properties are shown in Table 1. The soil is deep and without gravels. The experimental design was a randomized complete block with three replications. Individual plots were 10 m wide and 30 m long. The seven treatments were: (1) unfertilized control (Control), (2) pig slurry before planting (PS) (15, 20 m^3 ha^{-1}, for the 2001-2002 and 2002-2003 growing seasons, respectively), (3) pig slurry plus DMPP before planting (PS-DMPP), (4) pig slurry before planting plus ammonium sulphate-nitrate top-dress (PS + ASN), (5) pig slurry plus DMPP before planting

Table 1. Soil properties

	0-30 cm	30-60 cm	60-90 cm	90-120 cm
pH (soil/water ratio of 1:2.5)	8.4	8.0	8.0	8.0
EC (dS m^{-1} 25 C) (1:5)	0.63	3.23[a]	3.25[a]	2.59[a]
Organic matter (g kg^{-1})	17	10	9	6.5
CO_3Ca equivalent (g kg^{-1})	190	190	190	140
P (Olsen method, mg kg^{-1})	16	14	17	9
K (extracted with 1 *M* ammonium acetate, mg kg^{-1})	192	192	190	158
NO_3-N (mg kg^{-1})	7	4	5	3
Clay (g kg^{-1})	447	452	423	373
Sand (g kg^{-1})	75	83	105	59

[a] EC values are due to gypsum content.

Table 2. Treatments and nutrient (kg ha^{-1}) amounts applied each year

Treatment	2001-2002				2002-2003				Cumulative NPK applied		
	N before planting	N top-dressing	P	K	N before planting	N top-dressing	P	K	N	P	K
Control	0		0	0	0		0	0	0	0	0
PS	29	0	5	69	145	0	22	55	174	27	124
PS-DMPP	29	0	5	70	126	0	48	118	155	53	188
PS+ASN	29	75	5	69	145	75	22	55	324	27	124
PS-DMPP + Entec	29	75	5	70	126	75	48	118	305	29	128
ASN	50	75	0	0	75	75	0	0	275	0	0
Entec	50	75	0	0	75	75	0	0	275	0	0

plus ammonium sulphate-nitrate top-dress with NI (Entec®) (PS-DMPP + Entec), (6) ammonium sulphate-nitrate before planting plus ammonium sulphate-nitrate top-dress (ASN), (7) Entec® before planting plus top-dress Entec®) (Entec). The amounts of N, P, and K applied in each treatment are shown in Table 2**.**

Pig slurry was surface applied and immediately incorporated to the soil before sowing. Pig slurry contained 2.6% dry matter the first year, and 6.9 % dry matter the second year. The DMPP was added to the pig slurry at the moment of its application. One liter of DMPP solution (25%) per hectare was mixed with the amount of pig slurry to be applied. Ammonium sulphate-nitrate (26%N, 15%S) and Entec® (26%N, 13.1%S) were broadcasted and incorporated before planting on November 23rd, 2001, and October 10th, 2002. Top-dressed mineral fertilizer was applied on March 11th, 2001 and March 18th, 2002 as ammonium sulphate-nitrate and Entec®. The amount of N applied before planting as mineral fertilizer was 50 kg N ha^{-1} the first year and 75 kg N ha^{-1} the second year. The amount of top-dressed N was always 75 kg N ha^{-1}. Planting date was November 30th, 2001, and November 16th, 2002. Harvesting date was July 5th, 2002 and June 26th, 2003.

Measurements

The variables measured in this study were grain yield, biomass production (3 stages), N uptake (3 stages), soil temperature at different depths, and NO_3-N and NH_4 -N in the soil profile during the wheat growing season. All plant samples were dried at 65°C for 2 days in a forced-air dryer and then weighed. Harvested plant samples were separated into leaves, stems and grain for subsequent total nutrient analysis. Grain yields were adjusted for moisture of 120 g kg^{-1}. Annual nutrient removal was calculated by multiplying dry mass by nutrient concentration. N concentration was determined by Kjeldahl digestion and P, K, Ca, Mg, S, Mn, Cu, Zn, Na and B were analyzed by ICP method. Soil was sampled to determine initial soil fertility and nitrate and ammonium content (5 cores for 0-30 cm and 2 cores for 30-60, 60-90 and 90-120 cm). All samples were extracted with water (1:5 soil/water ratio per solution) and colorimetrically analyzed for NO_3-N using a Technicon Autoanalyser (Anasol 4P2S1BM2P, ICA Instruments, Tonbridge, Kent, UK). Soil NH_4-N was extracted with KCl 1 M (1:2.5 soil /water) and analyzed with a UV-VIS spectrophotometer. Organic matter was determined by Walkley-Black procedure. Soil pH was measured at a soil/water ratio of 1:2.5. Soil P was extracted with $NaHCO_3$ (Olsen method). Soil K was extracted with 1 M ammonium acetate ($NH_4C_2H_3O_2$). During the second year soil temperature was monitored at 5, 10, 20, 30 and 40 cm below the soil surface between Nov 10th and Mar 19th with a 10TCRT temperature probe using a data logger (CR10X, Campbell Sci, Logan, UT).

Soil fertility in autumn 2001 in the upper 30 cm had 17 g organic matter kg^{-1}, a typical value in the irrigated semiarid soils of the area, and 7 mg NO_3-N kg^{-1}, a value under the critical level for wheat. Soil texture is silty clay (Soil Survey Staff, 1998) in all the horizons (Table 1). The soil had a pH of 8.4 and the CO_3Ca equivalent content was 190 g kg^{-1}, characteristic of a calcareous soil. Soil test P (STP) was normal (16 mg P kg^{-1}), while soil test K (STK) was interpreted as normal-high (192 mg K kg^{-1}). The nutrient application rate criterion of maintenance for P and K could be established for those soil fertility levels. High levels of EC (1:5 soil/water ratio) in subsurface horizons are due to gypsum ($CaSO_4.2H_2O$) (Table 1). The soil samples were analyzed in the soil testing laboratory (Applus, Sidamon) participated by the University of Lleida.

Agronomic indicators

The indices for nitrogen use efficiency used to assess the effect of the different treatments are described below to avoid excessive use of acronyms and definitions.

Apparent nitrogen recovery (NREC), also named N recovery efficiency, is the additional N uptake per unit of added nutrient (kg kg^{-1}) and was calculated as described by Greenwood and Draycott (1989) on the basis of the following relationship:

$$\mathrm{NREC} = \frac{\mathrm{N\ uptake}_{\mathrm{fertilised\ \ crop}} - \mathrm{N\ uptake}_{\mathrm{unfertilised\ \ crop}}}{\mathrm{N\ applied}} \times 100$$

Some authors, such as Thomason *et al.* (2000), refer to NREC as nitrogen use efficiency (NUE). Apparent nitrogen recovery with respect to available N ($NREC_{avail}$) was calculated based on the following relationship:

$$\mathrm{NREC}_{\mathrm{avail}} = \frac{\mathrm{N\ uptake}_{\mathrm{fertilised\ \ crop}} - \mathrm{N\ uptake}_{\mathrm{unfertilised\ \ crop}}}{\mathrm{N\ available}} \times 100$$

Soil available nitrogen was calculated as NO_3-N at the beginning of the growing season (0-120 cm) plus N applied. N mineralized from organic matter was not included in the calculations.

Agronomic efficiency (AE) is the additional grain yield per unit of added nutrient and was calculated as follows:

$$\mathrm{AE} = \frac{\mathrm{Yield}_{\mathrm{fertilised\ \ crop}} - \mathrm{Yield}_{\mathrm{unfertilised\ \ crop}}}{\mathrm{N\ applied}}$$

Agronomic efficiency with respect to available N (AE_{avail}) was calculated as follows:

$$\mathrm{AE}_{\mathrm{avail}} = \frac{\mathrm{Yield}_{\mathrm{fertilised\ \ crop}} - \mathrm{Yield}_{\mathrm{unfertilised\ \ crop}}}{\mathrm{N\ available}}$$

Physiological efficiency (PE), or internal utilization efficiency, is the ratio of grain yield to aboveground nutrient uptake, as defined by Yadvinder-Singh *et al.* (2004), and was calculated as follows:

The data for the PE are not shown since they can be easily estimated from the relationship:

$$\mathrm{AE} = \mathrm{NREC} \times \mathrm{PE}$$

Nitrogen utilization efficiency (NUtE) is the efficiency of utilization of N defined as grain yield (kg) per unit N uptake. Nitrogen Use Efficiency (NUE) is the grain yield produced (kg) per N applied (kg) and Nitrogen uptake efficiency (NUpE) is the N uptake (kg) per N fertilizer applied (kg).

The relationship between these indexes is:

$$\mathrm{NUE} = \mathrm{NUpE} \times \mathrm{NUtE}.$$

Economic indicators

To assess the economical suitability of the different treatments some economical concepts have been used. Gross benefit was defined as the product of yield and price. The price of soft wheat considered was €0.15 kg^{-1}. Total variable cost consists of fertilizer cost (product of amount of N applied and cost of N). We considered market prices paid by farmers at the beginning of 2009 (€1 kg^{-1} N). The economic threshold is the value of grain yield that equals the kg N applied (1/0.15 = 6.7 kg grain kg^{-1} nitrogen).

The cost of spreading fertilizer or pig slurry is not included. The marginal product is the difference between grain yield from treated plot (kg) and grain yield from unfertilized control plot (kg). The marginal return is the product of marginal product and wheat price. The marginal benefit is the difference between the marginal return and the total variable cost.

Analysis of variance was performed using the Statistical Analysis System (SAS Inst., 2002). Means were separated using Duncan's multiple range tests with 0.10 of significance level.

Results and discussion

Meteorological conditions and soil temperature

Meteorological conditions were different during the 2-year experiment. Although total rainfall was similar to the normal distribution; some remarkable differences in the monthly distribution had an important effect on winter temperatures and on spring temperatures. In the first year, November and April were wetter than normal. In the second year February had a higher precipitation, but April was very dry and ETo values were really high. The first year was the most suited for wheat production (Fig. 1).

Soil temperature at different depths and air temperatures was registered during the second campaign and are shown in Figure 2. Soil temperature shows a typical distribution. As expected, daily temperature amplitude decreases with depth. At a depth of 40 cm maximum and minimum temperatures are almost identical. The lowest temperatures are reached at 5 cm from the soil surface. No temperatures of less than 0°C were registered. Soil temperature amplitude began to increase in February. The maximum temperature registered at 5 cm is always below 15°C until February 12th.

According to Irigoyen *et al.* (2003), soil temperature is one of the most remarkable factors affecting the efficacy of the nitrification inhibitors. Theses authors demonstrated that at 10°C, the N-NH$_4^+$ soil content was constant, suggesting that these inhibitors were capable of preventing nitrification completely during the 105-day incubation period. Nevertheless, in our experiment, this temperature factor was not expected to limit the efficacy of the nitrification inhibitor because wheat is a winter crop.

Evolution of soil nitrate and ammonium

The evolution of soil nitrate and ammonium are presented in Tables 3 and 4. Few statistical differences

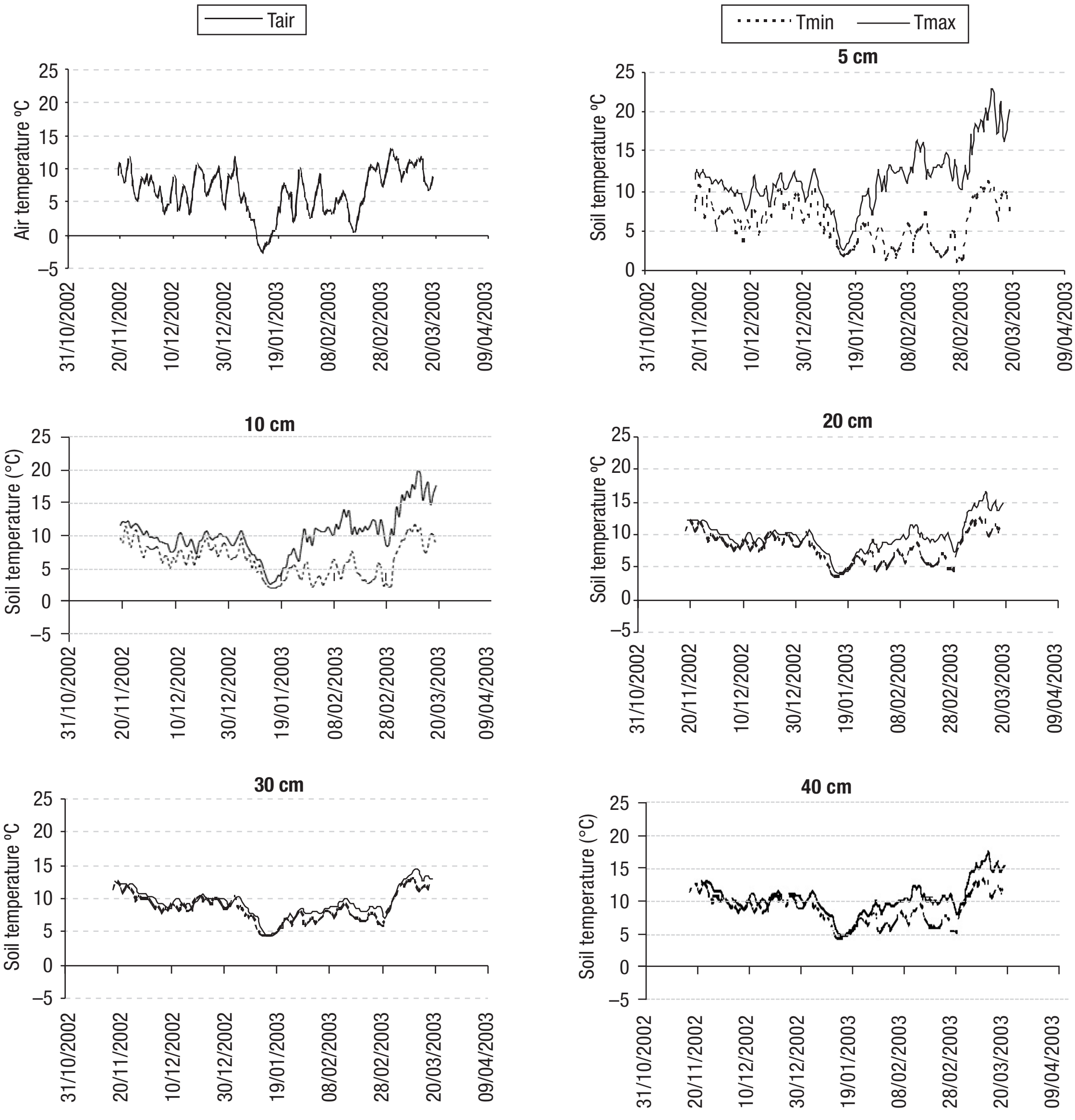

Figure 2. Air and soil temperatures at different depths during the second year of the experiment.

were found between treatments. Carrasco and Villar (2001), comparing the effect of ASN and urea with and without NI, reported significant amounts of ammonium in the soil as an effect of the DMPP. Nevertheless, there was a high spatial variability of these values, especially in the subsurface horizons due to soil sampling procedures. Variation coefficients were often higher than 30%. Last sampling day (17th Oct 2003) represents the residual N after the 2 year experiment. There are no significant differences between treatments, and the final nitrate content (between 10 and 14.7 mg kg^{-1}) is similar to the initial values (13.9 mg kg^{-1}). These values could be considered normal-high. There is a sharp increase of soil N concentration after fertilizer applica-

Table 3. NO_3-N content (mg kg^{-1}) in the soil over time

Treatment	11/22/01	01/18/02	04/10/02	07/10/02	10/17/02	10/31/02	11/07/02	11/18/02	07/03/03	10/17/03
0-30 cm										
Control	13.9	16.7^{b}	2.0	8.0^{a}	12.0^{a}	17.0^{c}	15.0^{d}	21.7^{b}	6.0	12.0^{a}
PS	13.9	19.3^{b}	1.5	7.7^{a}	14.7^{a}	35.0^{ab}	34.3^{bc}	35.7^{a}	8.0	13.3^{a}
PS-DMPP	13.9	24.0^{b}	1.6	7.0^{a}	15.0^{a}	29.7^{b}	29.0^{c}	36.7^{a}	8.0	11.3^{a}
PS + ASN	13.9	20.0^{b}	2.1	6.3^{a}	11.7^{a}	31.0^{b}	40.0^{abc}	39.7^{a}	8.0	10.0^{a}
PS-DMPP + Entec	13.9	29.3^{ab}	1.8	6.0^{a}	16.0^{a}	42.3^{a}	30.7^{bc}	36.7^{a}	7.3	14.7^{a}
ASN	13.9	40.7^{a}	1.2	6.0^{a}	15.0^{a}	24.7^{bc}	45.0^{ab}	41.3^{a}	10.0	11.0^{a}
Entec	13.9	28.7^{ab}	1.3	7.3^{a}	18.0^{a}	28.7^{b}	51.7^{a}	39.3^{a}	11.0	10.7^{a}
0-120 cm										
Control	12.4	14.1^{ab}	1.4	7.7^{ab}	8.6^{ab}	NA	NA	NA	5.0	7.6^{a}
PS	12.4	10.0^{b}	0.8	7.4^{ab}	8.2^{ab}	NA	NA	NA	5.0	9.3^{a}
PS-DMPP	12.4	12.3^{b}	1.1	4.8^{b}	9.9^{a}	NA	NA	NA	5.5	7.5^{a}
PS + ASN	12.4	10.2^{b}	1.5	8.4^{a}	6.7^{b}	NA	NA	NA	6.5	7.8^{a}
PS-DMPP + Entec	12.4	12.8^{ab}	1.6	4.7^{b}	9.4^{ab}	NA	NA	NA	3.6	7.7^{a}
ASN	12.4	17.4^{a}	0.8	5.6^{ab}	8.5^{ab}	NA	NA	NA	6.0	9.3^{a}
Entec	12.4	12.7^{ab}	1.6	5.3^{ab}	10.6^{a}	NA	NA	NA	6.0	9.3^{a}

Within columns, means followed by the same letter are not significantly different from each other at the 0.10 probability level using Duncan's multiple range test. NA: not analyzed.

Table 4. NH_4-N content (mg kg^{-1}) in the soil over time

Treatment	11/22/01	01/18/02	04/10/02	07/10/02	10/17/02	10/31/02	11/07/02	11/18/02	07/03/03	10/17/03
0-30 cm										
Control	NA	3.8^{a}	1.3	NA	NA	5.2^{a}	9.3^{b}	3.0^{a}	NA	9.9^{a}
PS	NA	3.2^{a}	1.4	NA	NA	6.0^{a}	12.2^{ab}	4.1^{a}	NA	6.9^{a}
PS-DMPP	NA	2.2^{a}	5.0	NA	NA	4.7^{a}	15.8^{ab}	3.2^{a}	NA	7.6^{a}
PS + ASN	NA	2.6^{a}	0.5	NA	NA	5.6^{a}	13.4^{ab}	2.9^{a}	NA	8.2^{a}
PS-DMPP + Entec	NA	2.6^{a}	0.4	NA	NA	5.9^{a}	11.0^{b}	3.3^{a}	NA	7.2^{a}
ASN	NA	2.5^{a}	0.3	NA	NA	5.9^{a}	21.8^{a}	3.2^{a}	NA	7.7^{a}
Entec	NA	2.6^{a}	0.9	NA	NA	6.8^{a}	15.9^{ab}	2.3^{a}	NA	8.4^{a}
0-120 cm										
Control	NA	2.8^{ab}	1.3	NA	NA	NA	NA	NA	NA	9.1^{a}
PS	NA	1.9^{b}	0.7	NA	NA	NA	NA	NA	NA	6.3^{b}
PS-DMPP	NA	2.0^{b}	1.7	NA	NA	NA	NA	NA	NA	8.0^{ab}
PS + ASN	NA	2.2^{b}	1.2	NA	NA	NA	NA	NA	NA	6.6^{b}
PS-DMPP + Entec	NA	2.1^{b}	0.6	NA	NA	NA	NA	NA	NA	6.3^{b}
ASN	NA	3.2^{a}	0.7	NA	NA	NA	NA	NA	NA	7.9^{ab}
Entec	NA	2.1^{b}	0.9	NA	NA	NA	NA	NA	NA	7.7^{ab}

Within columns, means followed by the same letter are not significantly different from each other at the 0.10 probability level using Duncan's multiple range test. NA: not analyzed.

Table 5. Wheat grain yield response to fertilizer treatments

Treatment	Grain yield (kg ha^{-1})			Increment respect to unfertilized control (%)		
	2001-2002	2002-2003	Avg. 2001-2003	2001-2002	2002-2003	Avg. 2001-2003
Control	4,943 (541)^{e}	3,730 (423)^{d}	4,336^{e}	—	—	—
PS	5,492 (62)^{cde}	4,695 (576)^{bcd}	5,232^{cd}	11.1	25.9	20.7
PS-DMPP	5,252 (920)^{de}	4,469 (485)^{cd}	4,861^{de}	6.3	19.8	12.1
PS + ASN	6,368 (457)^{bc}	5,313 (334)^{abc}	5,840^{bc}	28.8	42.4	34.7
PS-DMPP + Entec	6,140 (697)^{bcd}	5,454 (279)^{abc}	5,797^{bc}	24.2	46.2	33.7
ASN	6,596 (127)^{ab}	5,603 (1226)^{ab}	6,100^{ab}	33.4	50.2	40.7
Entec	7,349 (320)^{a}	6,099 (451)^{a}	6,724^{a}	48.7	63.5	55.1

Within columns, means followed by the same letter are not significantly different from each other at the 0.10 probability level using Duncan's multiple range test. Values in parenthesis are standard deviations of replicate analyses (n = 3).

tion and a significant decline during the period of maximum N crop absorption. During summer there is also an increase in the soil-N content due probably to soil organic matter mineralization.

Soil texture is an important factor affecting the efficiency of nitrification inhibitors. The relative NO_2^- formation decreased and the efficiency of DMPP increased when soils were higher in sand content (Barth *et al.,* 2001). In the present experiment soil texture was silty-clay, very different from the loam texture in previous experiments (Carrasco and Villar, 2001).

Wheat grain yield and nutrient uptake

Grain yields were significantly affected by N treatment (Table 5). Two-year average grain yields ranged between 4.3 and 6.7 Mg ha^{-1}. The highest average grain yield was obtained for mineral fertilized treatment with NI (Entec treatment). On average, the increment for this treatment with respect to unfertilized control was 55.1%. Pig slurry treatments without mineral fertilizer top-dressing showed yield increments between 12.1 and 20.7% with respect to the unfertilized control, but these yields were not significantly different. No effect was observed when DMPP was added to pig slurry on wheat yields. The second year, wheat yields (5,052 kg ha^{-1}) were significantly lower than the first growing season (6,020 kg ha^{-1}), nevertheless the amount of N applied was higher.

Less favorable meteorological conditions during the second year (lower winter temperatures, and higher May temperatures) produced generalized lower grain yields in the area. Higher ETo and lower precipitation during the second half of May (grain filling period) enhanced that effect (Fig. 1).

Total nutrient uptakes in grain are given in Tables 6 and 7. The total amount of N removed with the wheat grain during the 2 year period ranged between 139.6 and 238.2 kg N ha^{-1}. The total amount of P removed in

Table 6. N, P and K grain uptake (kg ha^{-1})

Treatment	Grain uptake 2001-2002			Grain uptake 2002-2003			Grain uptake during the 2-year period		
	N	P	K	N	P	K	N	P	K
Control	78.8^{d}	17.9^{c}	19.7^{c}	60.7^{d}	15.0^{c}	15.8^{d}	139.6^{d}	32.9^{d}	35.5^{d}
PS	86.5^{cd}	19.8^{bc}	22.0^{bc}	79.3^{bcd}	18.9^{b}	20.2^{bc}	169.6^{cd}	39.5^{bc}	43.0^{bc}
PS-DMPP	83.6^{cd}	18.8^{bc}	20.2^{c}	73.0^{cd}	18.0^{bc}	19.1^{cd}	156.7^{cd}	36.8^{cd}	39.3^{cd}
PS + ASN	101.8^{bc}	23.0^{ab}	25.6^{ab}	112.3^{a}	20.4^{ab}	22.1^{bc}	214.1^{ab}	43.4^{b}	47.7^{b}
PS-DMPP + Entec	99.0^{bcd}	21.7^{bc}	24.0^{bc}	93.7^{abc}	20.6^{ab}	22.5^{abc}	192.6^{bc}	42.3^{bc}	46.5^{b}
ASN	111.9^{ab}	22.4^{ab}	25.4^{ab}	105.4^{ab}	20.8^{ab}	23.4^{ab}	217.3^{ab}	43.3^{b}	48.7^{b}
Entec	131.4^{a}	26.3^{a}	29.1^{a}	106.8^{ab}	23.4^{a}	26.3^{a}	238.2^{a}	49.7^{a}	55.4^{a}

Within columns, means followed by the same letter are not significantly different from each other at the 0.10 probability level using Duncan's multiple range test.

Table 7. Secondary macronutrients and some micronutrients wheat grain uptake (kg ha^{-1})

Treatment	Ca	Mg	S	Mn	Zn	Cu	Fe	Na	B
2001-2002									
Control	1.9^{c}	6.7^{c}	8.6^{b}	0.17^{b}	0.21^{a}	0.006^{ab}	0.23^{a}	0.69^{a}	0.02^{b}
PS	2.2^{bc}	7.5^{bc}	9.2^{b}	0.19^{b}	0.22^{a}	0.001^{c}	0.21^{a}	0.60^{a}	0.02^{b}
PS-DMPP	2.7^{ab}	6.9^{bc}	9.9^{ab}	0.18^{b}	0.21^{a}	0.003^{ab}	0.26^{a}	1.22^{a}	0.04^{ab}
PS + ASN	3.2^{a}	8.6^{ab}	10.3^{ab}	0.20^{ab}	0.27^{a}	0.007^{a}	0.27^{a}	1.03^{a}	0.04^{ab}
PS-DMPP + Entec	2.2^{bc}	8.3^{abc}	10.3^{ab}	0.19^{ab}	0.22^{a}	0.002^{bc}	0.29^{a}	1.23^{a}	0.03^{ab}
ASN	2.3^{bc}	8.5^{ab}	12.0^{a}	0.20^{ab}	0.23^{a}	0.002^{bc}	0.23^{a}	1.34^{a}	0.03^{ab}
Entec	2.8^{ab}	9.9^{a}	12.3^{a}	0.24^{a}	0.28^{a}	0.006^{ab}	0.36^{a}	1.02^{a}	0.05^{a}
2002-2003									
Control	1.3^{c}	5.4^{d}	8.2^{d}	0.12^{c}	0.21^{b}	0.006^{a}	0.18^{c}	0.37^{c}	0.02^{ab}
PS	2.9^{a}	6.9^{bc}	11.3^{bc}	0.16^{ab}	0.34^{a}	0.008^{a}	0.35^{ab}	0.91^{a}	0.02^{ab}
PS-DMPP	1.6^{bc}	6.4^{cd}	10.0^{cd}	0.14^{bc}	0.20^{b}	0.007^{a}	0.29^{abc}	0.51^{bc}	0.02^{ab}
PS + ASN	2.2^{abc}	7.3^{bc}	11.9^{bc}	0.15^{ab}	0.23^{b}	0.006^{a}	0.28^{abc}	0.55^{bc}	0.02^{b}
PS-DMPP + Entec	2.4^{abc}	7.5^{bc}	12.8^{ab}	0.16^{ab}	0.23^{b}	0.008^{a}	0.23^{bc}	0.51^{bc}	0.02^{b}
ASN	2.1^{abc}	7.7^{ab}	13.5^{ab}	0.15^{ab}	0.24^{ab}	0.008^{a}	0.30^{abc}	0.64^{b}	0.03^{a}
Entec	2.7^{ab}	8.8^{a}	15.2^{a}	0.18^{a}	0.27^{ab}	0.009^{a}	0.40^{a}	0.90^{a}	0.02^{ab}

Within columns, means followed by the same letter are not significantly different from each other at the 0.10 probability level using Duncan's multiple range test.

wheat grain during the 2 year period ranged between 32.9 and 49.7 kg P ha^{-1}. Grain P content was on average 0.41 %. The total amount of K removed in wheat grain during the 2 year period ranged between 35.5 and 55.4 kg K ha^{-1}. Grain K content was on average 0.45% and 0.48% for the first and second season respectively. Similar values of P (0.31%) and K (0.48%) content in the grain were reported by Greaves and Hirst (1929). The Entec treatment produced the highest and significantly different cumulative P and K grain uptake. The Entec treatment produced, in general, the highest grain nutrient uptake.

Apparently the NI DMPP in the mineral fertilizer improved the absorption of P and K. This is an indirect effect observed in other pot experiment (Guillaumes and Villar-Mir, 2004) and explained by a decreasing of the pH at the rizosphere level (Pasda *et al.*, 2001). Nevertheless, this effect was not observed when DMPP was mixed with pig slurry.

The total amount of sulphur (S) removed in wheat grain during the 2 year period ranged between 16.8 (for the unfertilized treatment) and 27.5 kg S ha^{-1} (for the Entec® treatment). In all cases, S content in the grain was higher than 0.12%, the threshold for deficiency according to Havlin *et al.* (2005). The highest values were for the treatments with mineral fertilizers (that include S in their composition), significantly different from the control and PS treatments. The highest value was for the Entec® treatment during the second year (15.2 kg S ha^{-1}). This effect is also shown for other nutrients such as Mg, Fe, Cu and Zn. The grain Cu concentration increased during the second season with respect to the first. There was also an increment of the average grain Zn and Fe concentration changing from 44 to 58 mg kg^{-1} and from 48 to 62 mg kg^{-1} respectively. The B and Na concentration presents a high variation coefficient of more than 20% probably due to the analytical method.

The total amount of N removed from wheat grain and straw (Table 8) during the two-year period was 184.5 kg N ha^{-1} for the unfertilized treatment. Pig slurry treatments (with and without DMPP) had higher aboveground N uptake than unfertilized control and significantly lower than PS + ASN, ASN and Entec treatments because the lower amount of N applied. During the first growing season a faster response to total N uptake relating to mineral fertilizers could be identified compared with pig slurry. In the pig slurry treatments plus side-dress fertilizer there was a faster response with ASN, whereas this did not occur with Entec treatment. During the second growing season the same effect occurred with the side-dress treatments, though at a diminished rate. The highest N uptake obtained was for the Entec treatment. N concentration in the grain exceeded 1.79%, necessary for an acceptable quality for bread. During the first year the two mineral

Table 8. Total aboveground N uptake (kg ha^{-1}). Values are means of grain stem and leaves

Treatment	Total uptake 2001-2002	Total uptake 2002-2003	Total aboveground N uptake during the 2-year period	Increment and percentage with respect to the unfertilized treatment
Control	108.5^{e}	76.0^{d}	184.5^{d}	
PS	128.0^{cde}	105.3^{bc}	236.0^{c}	51.5 (27.9%)
PS-DMPP	123.5^{de}	93.2^{cd}	216.7^{cd}	32.2 (17.5%)
PS + ASN	151.6^{b}	141.6^{a}	293.2^{ab}	108.7 (58.9%)
PS-DMPP + Entec	133.2^{bcd}	125.2^{ab}	258.4^{bc}	73.9 (40%)
ASN	147.1^{bc}	134.4^{a}	281.5^{ab}	97.0 (53%)
Entec	172.0^{a}	140.4^{a}	312.3^{a}	127.8 (69.2%)

Within columns, means followed by the same letter are not significantly different from each other at the 0.10 probability level using Duncan's multiple range test.

fertilizer treatments exceeded 1.9%. In the second year, probably due to lower yields, higher protein content in the grain was produced.

Nitrogen use efficiency indexes

Nitrogen efficiency indexes are given in Table 9. Apparent N recovery was higher, on average, for mineral fertilizer treatments. Average N recovery over the 2 year experiment ranged between 20.8 and 46.5%. An efficiency of 42% is the average for cereal production in developed countries (Raun and Johnson, 1999). The highest value was for Entec treatment and the lowest for pig slurry plus DMPP. The effect of DMPP appears to be positive in mineral fertilizers, but the effect is less clear when NI is added to pig slurry.

The N recovery in pig slurry was always lower than in mineral fertilizers. The effect of the N residual from the first year, available at the beginning of the second year, is clear. $NREC_{avail}$ is always lower than NREC and reduces substantially the differences between treatments. Nevertheless, this is probably not a useful index because of the high spatial variability in the determination of mineral N.

Agronomic efficiency, also know as marginal productivity, was, on average, 15.3 kg extra grain per kg

Table 9. Efficiency indexes for the two-year experiment

Treatment	NREC (%)	$NREC_{avail}$ (%)	AE (kg kg^{-1})	AE_{avail} (kg kg^{-1})	NUtE
2001-2002					
Control					40.1
PS	67.2	3.8	–4.0	–0.5	36.0
PS-DMPP	51.7	6.8	9.3	1.2	37.5
PS + ASN	41.4	14.6	12.0	4.2	37.1
PS-DMP + Entec	23.8	8.3	10.1	3.6	40.9
ASN	30.9	12.2	11.6	4.6	39.5
Entec	50.8	20.0	16.9	6.7	37.8
2002-2003					
Control					43.3
PS	20.2	10.3	5.9	2.9	39.2
PS-DMPP	13.7	5.9	5.2	2.4	42.3
PS + ASN	29.8	19.8	6.3	4.2	33.6
PS-DMPP + Entec	24.5	13.5	7.5	4.2	38.7
ASN	38.9	20.1	11.0	5.7	36.6
Entec	42.9	21.1	13.9	6.3	37.2

NREC: N Recovery respect to applied N. $NREC_{avail}$: N Recovery respect to available N. AE: agronomic efficiency respect to applied N. AE_{avail}: agronomic efficiency respect to available N. NUtE: N utilization efficiency.

N applied for the Entec treatment. The AE for ASN treatment was, on average, 11.3 kg extra grain per kg N applied. The lowest values were for pig slurry treatments without top-dress fertilizer. Pig slurry with top-dress fertilizer showed a similar AE for both ASN and Entec fertilizer.

Physiological efficiency was, on average, higher for all the treatments with NI (data not shown, but easily computed as AE × 100/NREC). The highest value was for Entec treatment (37 kg extra grain per kg extra N uptake). The higher PE values in pig slurry (DMPP) with or without top dress fertilizer, with respect to pig slurry without DMPP, were due to the decrease in N uptake (Table 8). Yadvinder-Singh *et al.* (2004) used the same indexes to analyze the data in wheat crop. These authors obtained slightly higher values for NREC, AE and PE but in the same range.

Economics

The economic impact of NI is partially determined by the use of better nitrogen use efficiency indexes. All economic indexes are given in Table 10. Increasing returns were obtained for all treatments. Marginal return varied between €43 and 337 ha^{-1} and between €103 and 332 ha^{-1} for the first and second growing season, respectively.

The largest net and marginal benefit was achieved under Entec treatment both years. The smallest profit was obtained under the unfertilized control. Farmers should stop applying additional amounts of N when the marginal return is less than the total variable cost. Marginal benefit has to be higher than zero. In all treatments marginal benefit was higher than zero and ranged between €36 and 216 ha^{-1}. The lowest value was for the pig slurry treatment with added DMPP. Adoption of new N management strategies requires them to be environmentally sound and economically profitable. However, a profitable fertilizer recommendation is hard to establish from a scientific point of view, because long term analyses are more appropriate in fertilizer research. Although only a two year experiment is presented in this paper, it is interesting to present this profitability analysis for educational purposes. NIs affect overall nitrogen efficiency indexes as a consequence of their behavior in soil modifying nitrogen dynamics. Although field experiments using NIs are not always consistent, some studies have reported productivity and environmental benefits. A slight improvement in N use, resulting in higher yields using the same rate of N fertilizer, has the potential to increase farm income. When yields are limited by meteorological conditions higher yields could be achieved with smaller amounts of nitrogen, with higher efficiency being a goal in such cases. Ortiz-Monasterio (2002) proposes exploration of a more integrated

Table 10. Profitability of different treatments

Season	Growing season	Marginal product (kg ha^{-1})	Marginal return (€ ha^{-1})	Cost of N (€ kg^{-1} N)	kg N applied per ha	Total variable cost (€ ha^{-1})	Marginal benefit (€ ha^{-1})	Gross benefit (€ ha^{-1})	Net benefit (€ ha^{-1})
2001-2002	Control							692.0	692.0
	PS	549.0	76.9	0.21	29.0	6.1	70.8	768.9	762.8
	PS-DMPP	309.0	43.3	0.24	29.0	6.9	36.4	735.3	728.4
	PS + ASN	1,425.0	199.5	0.38	104.0	39.3	160.2	891.5	852.2
	PS-DMPP + Entec	1,197.0	167.6	0.52	104.0	53.9	113.7	859.6	805.7
	ASN	1,653.0	231.4	0.70	125.0	87.5	143.9	923.4	835.9
	Entec	2,406.0	336.8	0.97	125.0	120.8	216.1	1028.9	908.1
2002-2003	Control							522.2	522.2
	PS	965.0	135.1	0.21	145.0	30.5	104.7	657.3	626.9
	PS-DMPP	739.0	103.5	0.24	126.0	30.0	73.5	625.7	595.7
	PS + ASN	1,583.0	221.6	0.38	220.0	83.2	138.5	743.8	660.7
	PS-DMPP + Entec	1,724.0	241.4	0.52	201.0	104.1	137.2	763.6	659.4
	ASN	1,873.0	262.2	0.70	150.0	105.0	157.2	784.4	679.4
	Entec	2,369.0	331.7	0.97	150.0	144.9	186.8	853.9	709.0

The price of wheat being €0.14 kg^{-1}.

approach to nutrient management and this is the main goal of the research we have been conducting over the last 15 years in this area. Our research has focused on the use of pig slurry (Guillaumes *et al.,* 2006), the use of soil and plant tests (Ferrer *et al.,* 2003), especially soil nitrate before N fertilization, and on the use of new fertilizers that aim to improve the efficiency of N use and minimize environmental impacts.

Conclusion

There is no clear effect of DMPP on pig slurry, but DMPP on mineral fertilizer (Entec treatment) had an apparent effect on grain yield, N, P and K grain uptake, as well as total aboveground N uptake. NI delaying the nitrification process enables better synchronization of nitrogen supply and demand but more research is required on farm conditions to assess this potential method to reduce nitrate leaching.

All treatments exhibited agronomic efficiency higher than the economical threshold. On average, the highest net benefit was for the Entec treatment (€ 908 ha^{-1}). The lowest net benefit was for unfertilized control (€ 522 ha^{-1}). The net profit obtained in all N treatments was higher than for the unfertilized control. In this two year experiment, the net benefit obtained was also higher for top-dressed N after pig slurry than a unique pre-planting pig slurry application.

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