doi: 10.17221/105/2016-PSE

The nitrification inhibitor 3,4-dimethylpyrazole phosphate decreases leaf nitrate content in lettuce while maintaining yield and N_2O emissions in the Savanna of Bogotá

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ABSTRACT

The use of nitrogen (N) fertilizers in crops increases their yield but can modify their quality and lead to environmental problems by the emission of greenhouse gases (GHG). One of the strategies for mitigating this emission is the use of nitrification inhibitors (NI) as 3,4-dimethylpyrazole phosphate (DMPP). Additionally, the increased persistence of N after the application of NI can reduce the amount of fertilizer applied. A field experiment with lettuce was conducted in the Savanna of Bogotá. N was applied as ammonium sulphate nitrate (ASN 26%) at a rate of 70 kg N/ha and as the combination of ASN with DMPP (ENTEC® 26) at 50 and 70 kg N/ha rates. GHG emissions, soil parameters, lettuce yield, its components, N, nitrate and mineral elements contents were measured. With high soil nitrate contents, a standard dose of N fertilizer with DMPP maintained the yield and N content of lettuce, while it had no effect on GHG emissions. A reduction of 20 kg N/ha using DMPP was able to keep the yield meanwhile improving the quality of the crop due to a lower nitrate accumulation in lettuce leaves.

Keywords: CH_4 emissions; CO_2 emissions; N_2O emission factor; nitrogen content; yield-scaled N_2O emissions

Vegetables crops are adapted to a wide range of climatic conditions. In Colombia, leafy vegetables production area in 2013 was 4000 ha, reaching a production of 83 637 t (Agronet 2013). The use of the nitrate (NO_3^--N) form in agriculture can lead to its accumulation in leaves when the rate of plant NO_3^--N uptake exceeds the rate of its metabolic reduction to ammonium (NH_4^+-N). Crops such as lettuce and spinach are prone to contain high levels of nitrates. In the case of lettuce, very high NO_3^--N contents, reaching 4500 mg/kg of fresh matter, were reported (Umar and Iqbal 2007). Plant NO_3^--N itself is not toxic for human beings, but during digestion it is reduced to nitrite, which oxidizes the ferrous iron present in haemoglobin producing methahaemoglobin, which decreases the oxygen-carrying ability of haemoglobin, particularly in infants (Weitzberg and Lundberg 2013). Colombia lacks any legislation regarding NO_3^--N permitted levels in vegetables; thus, acceptable daily NO_3^--N intake limits of 3.7 mg/kg bodyweight (Weitzberg and Lundberg 2013) recommended by authorities such as the Joint Food and Agriculture Organization, World Health Organization (WHO), Expert Committee on Food Additives and the European Food Safety Authority can be adopted as reference.

Moreover, N fertilization generates environmental pollution, mainly as leaching and the release of N gases to the atmosphere; agriculture is thus

Supported by the Spanish Government, Projects No. AGL2012-37815-C05-02 and AGL2015-64582-C3-2-R MINECO/FEDER; by the Basque Government, Project No. IT526-10 and by the EuroChem Agro Iberia S.L., Project No. UPV/EHU 2012.0007. Ximena Huérfano held a grant from the Spanish Ministry of Economy and Competitiveness.

doi: 10.17221/105/2016-PSE

responsible for 84% of anthropogenic nitrous oxide (N_2O) emissions (Krey et al. 2014). Regarding greenhouse gases (GHG) emissions, N_2O can be produced by both nitrification and denitrification processes; carbon dioxide (CO_2) is emitted mainly through the decomposition of organic matter and heterotrophic respiration (Ball 2013); methane (CH_4) is produced by methanogens under anaerobic conditions, while methanotrophic bacteria oxidize CH_4 under aerobic conditions (Aronson and Helliker 2010).

Nitrification inhibitors (NI) like 3,4-dimethylpyrazole phosphate (DMPP) are compounds that delay the microbial oxidation of NH_4^+-N to NO_3^--N, decreasing thus soil NO_3^--N content (Pasda et al. 2001). Starting from the hypothesis that applying NIs induces a greater availability of soil ammonium for plant nutrition and gives the possibility of improving the N use efficiency (NUE), the decrease of the nitrate content in lettuce leaves can be achieved through a proper NO_3^--N/NH_4^+-N ratio in the soil solution. Many crop species show sensitivity to ammonium nutrition; in lettuce, Cruz et al. (2006) reported a decreased biomass yield when plants grew under ammonium nutrition. Thereby, the aim of this study was to determine in a field trial the effect of DMPP on crop yield, leaf N content and NO_3^--N accumulation in an irrigated lettuce crop under the Savanna of Bogotá conditions. Given that NIs have been described to reduce N_2O emissions in crops (Abalos et al. 2014) and that in Colombia there are no data on N_2O emissions in annual crops, the soil fluxes of GHG were also measured to evaluate the effect of DMPP application under these edaphoclimatic conditions.

MATERIAL AND METHODS

An irrigated crop of lettuce (*Lactuca sativa* L. cv. Grandes Lagos), with broccoli (*Brassica oleracea* L. cv. Italica) as preceding crop was conducted at the Colombian Corporation of Agricultural Research in the Savanna of Bogotá (4°42'N, 74°12'W) at an altitude of 2516 m a.s.l. with an average annual precipitation of 660 mm and average temperature of 15°C. The soil was a Typic Haplustands with a loamy texture (39% sand, 43% silt and 18% clay); pH 5.3; 4.2% organic carbon; 0.41% total N; 122 $mmol_+$/kg effective cation exchange capacity; 93.2 ppm P; 1635 ppm Ca; 281 ppm Mg and 269 ppm K in the upper layer (0–30 cm).

Drip irrigation began at the same time as fertilization and was applied daily to ensure a soil moisture level close to 60% of soil water filled pore space (WFPS). The experiment began with the seedlings transplanted (19th September 2013), and finished with the harvest (18th December 2013). Fertilizers were applied on 4th October as ammonium sulphate nitrate (ASN 26% N and 32.5% SO_3) and the combination of ASN with DMPP, available in the market as ENTEC®26 (registered trademark of EuroChem Agro Mannheim, Germany). N content in ASN and ENTEC was 7.5% in NO_3^--N form and 18.5% in NH_4^+-N form. The DMPP content in ENTEC is 0.8% of the total NH_4^+-N. A complete randomized block design with four replications and a plot size of 20 m^2 was established. Four treatments were applied: control without fertilizer application, treatment with 70 kg ASN-N/ha (70-ASN), treatment with 70 kg ENTEC-N/ha (70-E) and treatment with 50 kg ENTEC-N/ha (50-E).

Yield and quality. The marketable yield (fresh weight) was determined for lettuce of head diameter higher than 30 cm after removing spoilt leaves. A 5-plant subsample was mixed and dried to determine dry matter content (biomass) and leaf contents of N (Kjeldahl method); NO_3^--N, phosphorus, sulphur and boron (colorimetry). Provided that DMPP was described to be a possible chelator of cations such as copper (Ruser and Schulz 2015), potassium, calcium, magnesium, iron, manganese, copper and zinc (atomic absorption spectrophotometer – contrAA® 300 Jena, Germany) were also determined. NUE was calculated as:

$$(\text{kg DW}_F - \text{kg DW}_C)/\text{kg N}_{APP}$$

Where: DW – weight of dry matter harvested (kg DW/ha) in control ($_C$) and fertilized ($_F$) treatments, and N_{APP} – total N applied per ha.

Soil determinations. GHG fluxes were assessed using two PVC chambers per plot (34 cm in diameter and 20 cm in height), following the closed chamber method described by Menéndez et al. (2006). Sampling was carried out between 9:00 and 12:00 a.m. After fertilizer application, gas fluxes measurements were taken every two days during the first week, every three days in the second week, then weekly or fortnightly. Daily and cumulative GHG emissions were determined and calculated with a gas chromatograph as described in Huérfano et al. (2015). The global warming potential (GWP)

doi: 10.17221/105/2016-PSE

was calculated was calculated using a factor of 310 to N_2O and 21 to CH_4 (Krey et al. 2014). Soil temperature (0–10 cm depth) and gravimetric soil moisture (expressed as WFPS) content (0–30 cm depth) were also determined.

Three soil cores per plot (0–30 cm depth and 0.25 cm diameter) were taken to determine NO_3^--N and NH_4^+-N contents. Sampling was conducted one day prior to the application of fertilizers and once a week along the subsequent cropping period. Fresh subsamples of 100 g of soil were extracted with 200 mL of KCl (1 mol/L). The extracts were filtered through Sep-Pak C18 (Waters) filters and frozen until their analysis. Concentrations of NO_3^--N and NH_4^+-N were determined by colorimetry, using the Cawse and Berthelot methods, respectively.

Statistical analyses. Data are presented for each parameter as the mean ± standard error of four replicates per treatment. Variance was analysed and separations of means were performed using the (least significant difference) *LSD* or Duncan's test.

RESULTS AND DISCUSSION

DMPP reduces lettuce leaf nitrate content while it maintains yield and total leaf N content. NI use does not always increase NUE (Pasda et al. 2001, Abalos et al. 2014); in the present study, the low NUE observed (Figure 1) was attributed to the high soil NO_3^--N content of around 70 kg N/ha prior to fertilization (Figure 2). Despite N fertilization increased soil NH_4^+-N contents up to 3 or 6-fold along the first month, soil NO_3^--N contents were not significantly different after fertilization. So, the soil's high background NO_3^--N contents could mask the effect of fertilization on yield. However, with respect to treatments with 70 kg/N ha (70-ASN and 70-E), the yield of control was increased from 17–23 t fresh weight (FW)/ha (Figure 1); this increase in yield was maintained with the use of DMPP when the N fertilization rate was reduced in 20 kg N/ha (50-E treatment), which led to increment the NUE in the crop. The biomass production (dry matter harvested), followed the same trend as yield; which demonstrated that lettuce water content was not changed by the application of different treatments. The increase of NH_4^+-N content in soil after fertilization did not provoke any detrimental effect on lettuce, probably because of the simultaneous occurrence of high soil NO_3^--N content.

Leaf NO_3^--N content is an indicator of luxury N fertilization, reflecting the N surplus accumulated in vacuoles. The WHO recommends an intake of 400 g/day of fruit and vegetables, which represent the major contribution to NO_3^--N consumption (60–80%) in the average daily human intake typical in the western diet (Weitzberg and Lundberg 2013). Considering that the maximum intake of NO_3^--N per a 60-kg-weight person should not exceed 200 mg/day, reducing the leaf NO_3^--N content in vegetables is mandatory. In this experiment, the maximum allowed NO_3^--N leaf content of 4000 ppm for lettuce grown in the open air (Umar and Iqbal 2007) was surpassed. However, when ENTEC was used, this content was around 550 ppm lower. Thus, contrary to Pasda et al. (2001), this work demonstrates that DMPP leads to a reduction of NO_3^--N in lettuce leaves.

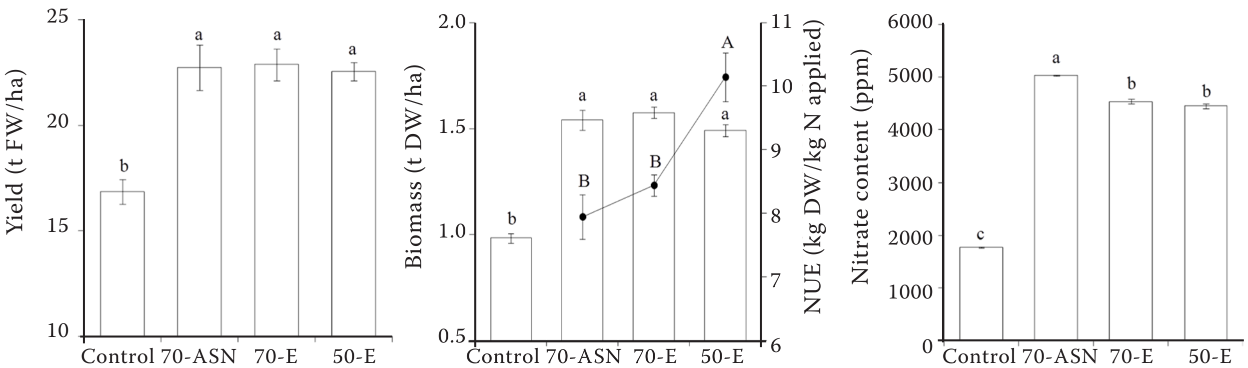

Figure 1. Lettuce yield, biomass, nitrogen use efficiency (NUE) (•) and leaf NO_3^--N content. Different letters indicate significantly different means using the Duncan's test ($P < 0.05$; $n = 4$). Control – without fertilizer; 70-ASN – 70 kg ASN-N/ha; 70-E – 70 kg ENTEC-N/ha; 50-E – 50 kg ENTEC-N/ha

doi: 10.17221/105/2016-PSE

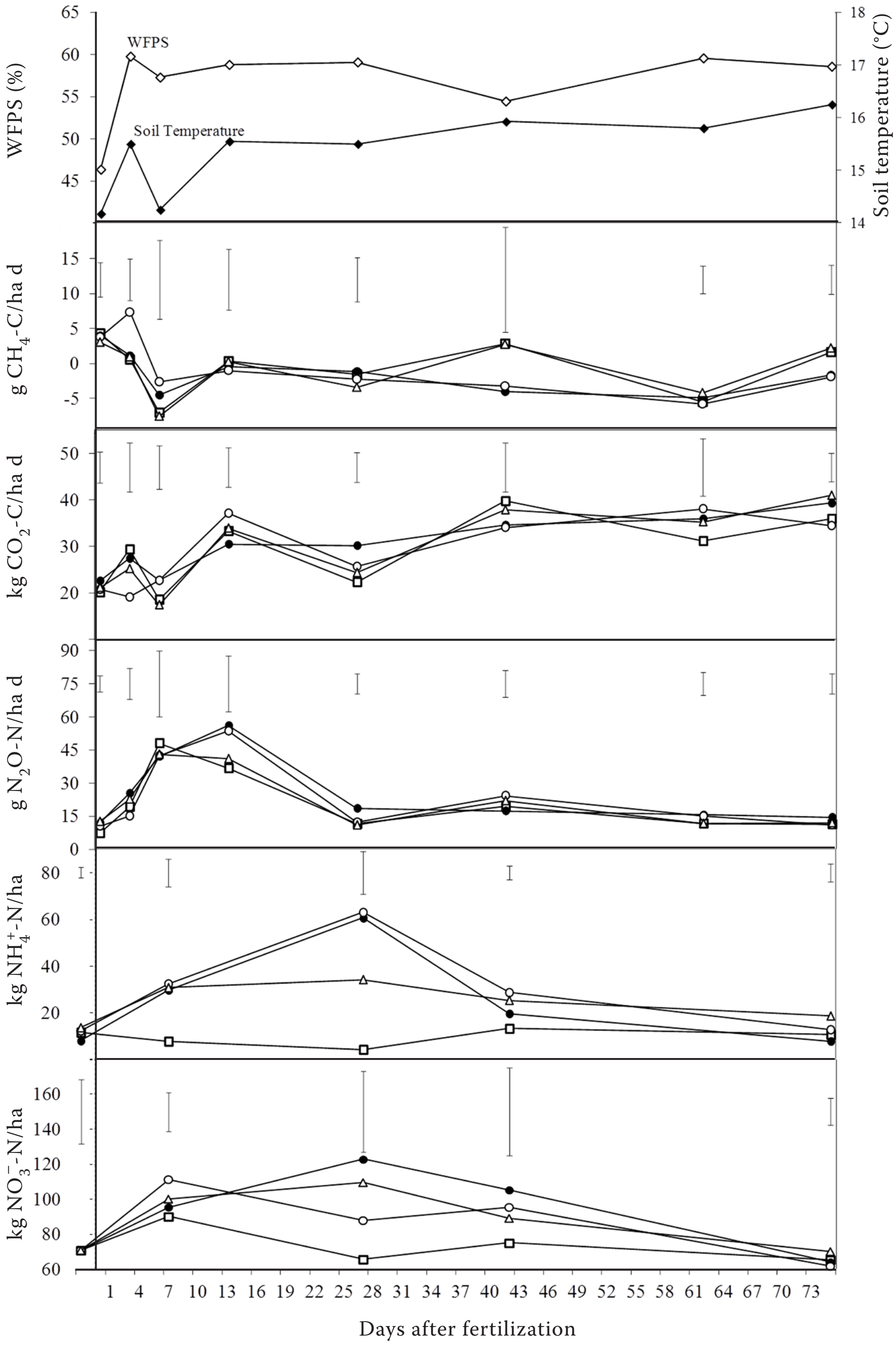

Figure 2. Daily greenhouse gasses (GHG) emissions, water filled pore space (WFPS) (◊) and soil temperature (♦), soil NH_4^+-N and NO_3^--N contents. Control (□) – without fertilizer; 70 ASN (●) – 70 kg ASN-N/ha; 70 E(○) – 70 kg ENTEC-N/ha; and 50 E (Δ) – 50 kg ENTEC-N/ha. Vertical bars indicate least significant difference (*LSD*) ($P < 0.05$; $n = 4$) for each sampling time

Lettuce leaf N content in this study was 3.25% (Table 1), being unaffected by N fertilization or DMPP use, which was also observed in previous reports by Pfab et al. (2012). The contents of most of the other foliar mineral elements were not affected by the application of different N treatments (Table 1). Only S, Fe and Zn contents were influenced by fertilization. The higher leaf S content observed in the fertilized treatments indicates that the application of NS fertilizers can increase the S content in plant tissues, as described by Lošák et al. (2008). Nevertheless, S content was not af-

doi: 10.17221/105/2016-PSE

Table 1. Mineral elements content in lettuce leaves in different fertilization treatments

Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
	(%)						(ppm)				
Control	3.2 ± 0.01	0.5 ± 0.01	3.3 ± 0.04	0.6 ± 0.04	0.2 ± 0.01	0.2 ± 0.004^{b}	128 ± 0.65^{a}	33 ± 1.86	9.0 ± 0.84	76 ± 1.10^{b}	25 ± 1.39
70-ASN	3.2 ± 0.02	0.6 ± 0.01	2.9 ± 0.20	0.5 ± 0.02	0.2 ± 0.01	0.3 ± 0.008^{a}	124 ± 1.29^{b}	31 ± 0.61	9.3 ± 0.71	85 ± 1.44^{a}	23 ± 0.71
70-E	3.3 ± 0.02	0.5 ± 0.01	2.8 ± 0.07	0.6 ± 0.03	0.2 ± 0.01	0.3 ± 0.004^{a}	121 ± 1.46^{b}	32 ± 0.76	9.3 ± 0.42	80 ± 2.59^{ab}	25 ± 1.54
50-E	3.3 ± 0.01	0.5 ± 0.02	3.0 ± 0.16	0.7 ± 0.04	0.2 ± 0.01	0.3 ± 0.004^{a}	125 ± 1.14^{ab}	33 ± 1.19	9.8 ± 0.82	78 ± 2.19^{ab}	24 ± 1.10

Means followed by the same letter or no letter are not significantly different using the Duncan's test ($P < 0.05$; $n = 4$). Control – without fertilizer; 70-ASN – 70 kg ASN-N/ha; 70-E – 70 kg ENTEC-N/ha; 50-E – 50 kg ENTEC-N/ha

fected by different doses of ASN or the application of DMPP. While the application of ASN induced a slight decrease in leaf Fe content and slight increase in Zn content, the application of DMPP did not induce any significant change with respect to the application of ASN alone. Therefore, as was also reported in other leaf vegetable as spinach (Irigoyen et al. 2006), the use of DMPP seems as a useful additive regarding the maintenance of lettuce yield and quality parameters such as nitrogen and mineral elements contents, while diminishing nitrate content in leaf.

DMPP does not affect GHG emissions in lettuce crop. In the present study, daily N_2O fluxes were of similar rates to those reported by Pfab et al. (2012); under their conditions, the influence of low soil OC (1.8%) could have been compensated by a higher soil temperature (up to 25°C) and water content (up to 80% WFPS). With respect to daily N_2O fluxes from a crop system of vegetables reported by Jia et al. (2012), higher rates of N fertilization (over 5 times greater than ours) and higher WFPS (above 60%), probably had a decisive effect on their significantly higher N_2O emissions. In our study, the influence of soil temperature (Figure 2) on N_2O emissions was insignificant due to the minimal oscillations (14–16°C), while soil OC (4.2%) could be responsible for our N_2O emissions. In fact, it was reported (Harrison-Kirk et al. 2013) in silt loam soils that at high values of WFPS (above 60%), N_2O emissions tended to increase exponentially when the soil OC increased above 4%.

Control without fertilizer treatment is essential to calculate the N_2O emission factor (EF) of N fertilization in the fertilized treatments since the EF of N_2O represents the percentage of N applied lost as N_2O-N. For the lettuce growth period studied, plots fertilized with 70 kg of N/ha with and without DMPP presented EFs of 0.4% and 0.5%, respectively, while the 50-E treatment presented an EF of 0.2% (Table 2). Therefore, if a whole year crop rotation was carried out in the savanna of Bogotá, the annual EF estimated value

Table 2. Cumulative emissions (over 73 days) of greenhouse gases (GHG), N_2O emission factor (EF), yield-scaled N_2O emission and global warming otential (GWP) in CO_2 equivalents (CO_2 eq)

Treatment	N_2O-N (g/ha)	EF (%)	Yield-scaled N_2O emission (g N_2O-N/kg N harvested)	CO_2-C (kg/ha)	CH_4-C (g/ha)	CO_2 eq (t/ha)
Control	1452 ± 220^{a}		45 ± 7^{a}	2330 ± 186^{a}	–193 ± 58^{a}	9.2^{a}
70-ASN	1791 ± 229^{a}	0.5	36 ± 6^{ab}	2444 ± 41^{a}	–75 ± 109^{a}	9.8^{a}
70-E	1729 ± 169^{a}	0.4	34 ± 3^{ab}	2409 ± 95^{a}	–192 ± 72^{a}	9.7^{a}
50-E	1515 ± 139^{a}	0.2	31 ± 2^{b}	2417 ± 122^{a}	–81 ± 111^{a}	9.6^{a}

Means followed by the same the letter are not significantly different using the Duncan's test ($P < 0.05$; $n = 4$). Control – without fertilizer; 70-ASN – 70 kg ASN-N/ha; 70-E – 70 kg ENTEC-N/ha; 50-E – 50 kg ENTEC-N/ha

doi: 10.17221/105/2016-PSE

might also be in the range of the default value of 1% proposed by the IPCC (2006). Provided that N fertilization did not significantly increase N_2O emissions (Table 2) as a consequence of the high soil NO_3^--N content prior to fertilization, DMPP application did not affect total N_2O emissions under the edaphoclimatic conditions studied.

Soil moisture content is a determining factor in oxygen availability and, therefore, in CO_2 emissions. So, the constant soil moisture around 60% WFPS (Figure 2) should not be responsible for different CO_2 emission rates (Figure 2). On the other hand, CO_2 emissions were by far the main ones determining the magnitude of the GWP (Table 2); thus, GWP did not present significant differences between the evaluated treatments.

CH_4 fluxes (Figure 2) were very low in comparison with those reported in lettuce crop by Jia et al. (2012), probably due to the addition of pig manure in their experiment, since organic amendments increase soil CH_4 emissions (Le Mer and Roger 2001). The cumulative CH_4 emissions were negative and not significantly different between treatments, showing that the application of ASN and DMPP did not have a significant effect on the soil's uptake of CH_4.

DMPP as an alternative for the management of N fertilization in lettuce crop. To understand the relation between NUE and GHG emissions, different concepts were suggested. Since the N-input is directly linked with N_2O emissions rather than other gases emissions, Van Groenigen et al. (2010) established the concept denoted as yield-scaled N_2O emissions, which relates total N_2O emissions with the total amount of N harvested. Therefore, this parameter is an index that allows assessing the effect of N fertilization on the relationship between N_2O emissions and crop production. In agreement to Van Groenigen et al. (2010), yield-scaled N_2O emissions (Table 2) showed a negative relation with NUE (Figure 1). Yield-scaled N_2O emissions in 50-E treatment were significantly diminished (around 30% compared with no application of fertilizer); this treatment presented the lowest N_2O emissions per kilogram of N harvested (Table 2). Thus, ENTEC application at low dose of 50 kg N/ha tended to reduce the environmental impact of this crop system without compromising crop yield. This study reveals that under the edaphoclimatic conditions of the Savanna of Bogotá, DMPP use seems as an interesting fertilization strategy, since at least 20 kg N/ha can be saved when it is added jointly with the fertilizer, maintaining lettuce yield and its nutrient contents at the same time that improves crop quality due to the reduction of nitrate accumulation in lettuce leaves.

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Received on February 8, 2016
Accepted on November 3, 2016

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