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Controlled-release and stabilized fertilizers are equivalent options to split application of ammonium nitrate in a double maize-oats cropping system

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ABSTRACT
The application of fertilizers as a topdressing in maize raises serious concerns because too much fertilizer is retained in the upper leaves, causing burning to the tissues. In this study, the use of a controlled-release and a stabilized fertilizer (with 3, 4-dimethylpyrazole phosphate) was compared with the application of a conventional fertilizer split into two equivalent applications in a forage maize-oats cropping system. In maize, 100 and 200 kg N ha^{-1} of different fertilizers were used in addition to an unfertilized control. The oat crop was not fertilized, since it served only as a winter catch crop. Maize dry matter (DM) yield increased significantly with N rate only in 2019, being the second growing season, with the control showing the lowest average value (7.1 t ha^{-1}). The most fertilized treatments (200 kg N ha^{-1}) gave the highest DM yields, ranging between 14.2 and 16.7 t ha^{-1}, but with no significant differences between them. Oats had a relevant role as a catch crop recovering residual N that could have potentially been lost from the soil. Stalk nitrate concentration proved to be very sensitive to N fertilization (varying from 150.4 to 1945.6 mg kg^{-1} in 2018 and 494.9 to 1574.9 mg kg^{-1} in 2019), showing great potential as a tool of N management. These three fertilization strategies seem to be valid options that farmers can consider, after incorporating technical-economic information related to equipment suitability and the price of fertilizers.

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Zea mays; *Avena sativa*; slow-release fertilizers; dry matter yield; stalk nitrate test; nitrogen use efficiency

Introduction

Nitrogen (N) management in agricultural fields requires great attention, especially in highly demanding crops where high N rates are used. In addition to its role in crop growth and yield, N is easily lost from the soil to the environment, particularly as nitrate (NO_3^-)-N, causing the eutrophication of water bodies (Mulla and Strock 2008), or as greenhouse gases to the atmosphere (Coyne 2008). Several strategies can be adopted to improve N use efficiency and to reduce losses to the environment, namely the use of moderate rates and splitting N applications (Havlin et al. 2014). The use of some fertilizers with mechanisms to reduce the solubility or mobility of N in the soil can also have the potential to reduce N losses from agricultural soils. Some of the most prominent of these are controlled-release fertilizers (CRF) and stabilized fertilizers (SF).

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In CRF, soil nutrient availability is regulated by coating materials, namely sulfur, polymers and other natural or synthetic products of varied properties (Arrobas and Rodrigues 2013; Mehmood et al. 2019; Xiao et al. 2019). These fertilizers can be simple, containing only N among the macronutrients, such as sulfur-coated urea, or encapsulated NPK compound fertilizers in which N can be present in nitric and ammoniacal forms. A great number of CRFs have been used under different conditions and have been shown to be effective in increasing N use efficiency and crop growth, especially in potted and containerized plants in greenhouses and nurseries (Arrobas et al. 2011; Adams, Musk, and Blake 2017; Li et al. 2019). In the field, positive results have also been reported (Sun et al. 2019; Xiao et al. 2019), although the benefits of using CRF have not always been recorded (Rodrigues et al. 2010; Chilundo et al. 2016).

Stabilized fertilizers are prepared with the incorporation of a urea hydrolysis inhibitor, or more frequently, a nitrification inhibitor (Trenkel 2010). Nitrification inhibitors act on *Nitrosomonas*, which slows the oxidation of ammonium (NH_4^+) to NO_3^- during nitrification, reducing the risks of NO_3^- leaching and denitrification. Among the used nitrification inhibitors, 3, 4-dimethylpyrazole phosphate (DMPP) is one of the most widespread. In several studies it has been shown that the use of DMPP reduced the emissions of nitrous oxide (N_2O) from agricultural soils (Kong, Eriksen, and Petersen 2018; Nauer et al. 2018; Wu et al. 2018), and improved N use efficiency and crop productivity (Martínez et al. 2017; Vogel et al. 2020).

Maize is a highly demanding crop for nutrients due to its potential for biomass production. To increase N use efficiency and reduce the risk of N losses to the environment, or potential damage to the seed, the N rate is usually split (Rodrigues et al. 2006; Wasaya et al. 2017). In agricultural systems where fertigation is implemented, the application of nutrients can be multi-fractioned with several benefits. However, in many regions of the world, fertilization is carried out by applying the fertilizers to the soil, splitting the rate into two applications, just before sowing and during the growing season (Garcia 1990; Arrobas, Aguiar, and Rodrigues 2016). The application during maize growing season may present technical difficulties, requiring farm implements that can apply the fertilizers close to the soil (side-dressing). The application as a topdressing may be problematic as the cones formed by the upper leaves take up the N fertilizer inside, causing burning to the leaves due to a saline effect. Here, CRF and SF can help to overcome the problem, delaying the availability of N for plant uptake, thus eliminating the need for splitting the N fertilization.

The objective of this study was to compare the application of a conventional N fertilizer at two rates (100 and 200 kg N ha^{-1}), both split into two fractions (50% on sowing and 50% at the four unfolded leaf stage), with the same rates of a CRF and a SF applied at pre-plant in forage maize. The cropping system included two annual crops, fodder maize as the main crop grown in the summer season and oats grown in the winter season. The oat crop was not fertilized to better assess the effects of residual N applied to the maize. Thus, this work hypothesized that the CRF and SF can replace the application of N as a topdressing and avoid its drawbacks.

Materials and methods

Site characterization

The field experiment was carried out in Bragança (41° 47′ N; 6° 46′ W; 750 m a.s.l.), NE Portugal, from May 2018 to May 2020. The experiment field has eight-year rotation, where four years of a double crop, forage maize in summer and oats in winter, is followed by a temporary (four-year) pasture.

The region of Bragança has a Mediterranean climate, where average annual air temperature and accumulated precipitation are 12.7 °C and 772.8 mm, respectively. Data of average monthly

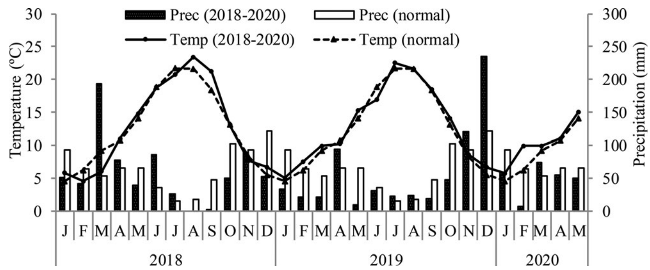

Figure 1. Monthly temperature and precipitation during the experimental period and data of the climate normal of the region.

temperature and precipitation were recorded during the experimental period as shown in Figure 1.

The soil is a Eutric Fluvisol (WRB 2015), developed in a fluvial deposit, sandy clay loamy textured (540 g kg^{-1} sand, 250 g kg^{-1} silt and 210 g kg^{-1} clay). Primary soil analysis showed that it had $pH_{(H2O)}$ 5.54, organic carbon (C) 12.6 g kg^{-1}, extractable phosphorus (P) 26.0 mg (P_2O_5) kg^{-1}, extractable potassium (K) 63.0 mg (K_2O) kg^{-1} and cation exchange capacity 17.6 $cmol_c$ kg^{-1}.

Experimental design

The experiment was arranged as a completely randomized design with seven N fertilizer treatments and three replicates. The fertilizer treatments were a conventional N fertilizer, CRF and SF types applied at two N rates (100 and 200 kg N ha^{-1}) plus a non-fertilized control. These seven treatments were abbreviated as N0, N100, N200, CRF100, CRF200, SF100 and SF200. Each experimental unit consisted of five rows with 0.7 m in between and 3 m long, and was spaced on the sides and top by outer rows of 0.7 and 0.5 m, respectively.

The conventional fertilizer consisted of ammonium nitrate 27% N (13.5% NH_4^+-N and 13.5% NO_3^--N). The CRF used was a compound NPK (12-10-18) fertilizer where part of the fertilizer granules was encapsulated by polyurethane (the fractions of N, P and K encapsulation were, respectively, 73.3%, 15.3%, and 55.0%). The SF contains 26% N (7.8% NH_4^+-N and 18.5% NO_3^--N) and incorporates the molecule DMPP as a nitrification inhibitor. All the treatments, including the control, received equal rates of P and K as the CRF200 treatment (166.7 kg P_2O_5 ha^{-1} and 300 kg K_2O ha^{-1}) by supplementing the fertilization plan with superphosphate (18% P_2O_5) and potassium chloride (60% K_2O). CRF, SF, half the rate of conventional fertilizer and superphosphate and potassium chloride were applied at pre-plant and incorporated into the soil during seedbed preparation. As side-dressing they were applied the remaining rates of conventional N fertilizer.

Management of the field trial

Soil was plowed using a moldboard plow, followed by a pass of cultivator to level the ground during the first season. Subsequently, the fertilizers were applied manually in the respective plots and incorporated into the soil with a final pass of cultivator. Soil preparation and fertilizer application was done at May 15th 2018. On May 16th, maize (hybrid Monero, mid-season FAO 500) was sown by using a seeding density of 80,000 seed ha^{-1}, with the seeds spaced at 0.70 m and 0.18 m between and in the rows, respectively. The crop received an herbicide treatment in the phenological stage 14 (four leaves unfolded) (Meier 2001), on July 7th 2018. The herbicide contains isoxadifen-ethyl (22 g L^{-1}) and tembotrione (44 g L^{-1}) as active ingredients and was applied at a concentration of 0.5 L hL^{-1} (2 L ha^{-1}). N was also side-dressed at July 7th 2018. During

Summer maize was sprinkled irrigated with a central pivot. The harvest took place on 7th September 2018 in the growth stage 73 (early milk).

Oat crop (cv. Boa Fé) was sown using a centrifuge broadcaster after brief soil preparation with cultivator on October 23rd 2018. Oats crop was not fertilized. The sowing rate was 130 kg seed ha^{-1}. No other cropping operations were carried out on oats crop until harvest, which was performed on May 07th 2019 at full flowering (growth stage 65).

In 2019 the date of maize sowing was on May 27th. The applications of herbicide and N as a side-dress were performed on July 17th. Maize was harvested at September 19th. Oats was sown on October 30th 2019 and harvested on 12th May 2020.

Field measurements and plant and soil sampling

The greenness of the leaves was determined with the portable SPAD (Soil and Plant Analysis Development)-502 Plus chlorophyll meter (Spectrum Technologies, Inc.). Thirty readings were taken from the middle of the blade of the youngest fully expanded leaves to create an average reading of a given experimental unit. The measurements were performed at August 03rd 2018 and August 08th 2019, at growth stage 14 (4 leaves unfolded).

A normalized difference vegetation index (NDVI) was determined using the FieldScout CM 1000 (Spectrum Technologies, Inc.). The meter senses and measures the ambient light at the wavelength of 660 nm and the reflected light (non-absorbed by leaf chlorophyll) at 840 nm wavelength. The measurements were taken on the same leaf part and dates as SPAD readings.

Chlorophyll *a* fluorescence and OJIP transient were determined by using the OS-30p + chlorophyll meter (Opti-sciences, Inc.), through the dark adaptation protocols F_V/F_M, F_V/F_0 and the advanced OJIP test, where F_M, F_0 and F_V are, respectively, maximum, minimum and variable fluorescence from dark adapted leaves. The variables F_V/F_M and F_V/F_0 were estimated as $F_V/F_M = (F_M\text{-}F_0)/F_M$ and $F_V/F_0 = (F_M\text{-}F_0)/F_0$. The OJIP test gives origin fluorescence at 20 μs (O), fluorescence at 2 ms (J), fluorescence at 30 ms (I) and maximum fluorescence (P, or F_M). Measurements were taken from the middle of the blade of the youngest fully expanded leaves, after a period of dark adaptation longer than 35 minutes, on the dates mentioned above.

On August 3rd 2018 and August 8th 2019, samples of the youngest fully matured leaves were also taken to assess crop nutritional status, and carried out to the laboratory, oven-dried at 70 °C and analyzed for elemental composition.

On September 7th and 19th in 2018 and 2019 (at early milk stage), maize was harvested by cutting the plants at the ground level in a 1 m linear (0.7 m^2) in the central line of the plots. The samples were weighed in fresh in the field. Still in the field, representative fresh sub-samples were weighed again and sent to the laboratory. After oven-drying at 70 °C, the sub-samples were weighed dry, to allow estimating the DM yield per unit area. From the initial maize samples, basal maize stalks, 15 to 35 cm above ground, were also taken and sent to the laboratory, dried and ground, and analyzed for NO_3^- concentration.

Oat crop was harvested on May 7th 2019 and May 12th 2020. A square mesh of 0.5 m^2 was used to establish the size of the sample in each experimental unit. The whole samples and a subsamples were weighed fresh, and the subsamples oven-dried at 70 °C and weighed dry. This procedure allowed to estimate the DM yield of the crop, and the dried subsamples the determination of tissue elemental composition.

The soils were sampled shortly before the side-dress N applications at 0-0.3 m depth, to allow performing a pre-sidedress soil inorganic N test (PSNT). At the end of the growing seasons of maize, on October 16th 2018 and October 21st 2019, and at the end of the growing seasons of oats on May 8th 2019 and May 13th 2020, the soil was also sampled with an auger for determination of general soil properties. Each analyzed soil sample consisted of field composite samples by collecting and mixing the soil taken from six random points.

Laboratory analyses

The soil samples were oven-dried at 40 °C and sieved in a mesh of 2 mm. The samples were analyzed for: 1) pH (H_2O, KCl) (soil: solution, 1:2.5); 2) cation-exchange capacity (ammonium acetate, pH 7.0) and exchange acidity (KCl extraction); 3) easily oxidizable C (wet digestion, Walkley-Black method); 4) extractable P and K (ammonium lactate); 5) extractable boron (B) (hot water extraction and azomethine-H methods); 6) extractable iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) (ammonium acetate and EDTA, determined by atomic absorption spectrometry); 7) inorganic N (2 M KCl extraction). In the initial samples there were also determined 8) soil separates (clay, silt and sand fractions) (Robinson pipette method). Methods 1–4, 6 and 8 are fully described by Van Reeuwijk (2002), method 4 by Balbino (1968), method 5 by Jones (2001) and method 7 by Baird, Eaton, and Rice (2017).

Elemental tissue analyses were performed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K) and atomic absorption spectrophotometry (Ca, Mg, Cu, Fe, Zn and Mn) methods after nitric digestion of the samples (Temminghoff and Houba 2004). Nitrate concentration in basal maize stalks was determined according to Baird, Eaton, and Rice (2017) by UV-vis spectrophotometry in a water extract (dry biomass:solution, 10:40).

Data analysis

Data were firstly tested for normality and homogeneity of variances using the Shapiro-Wilk test and Bartlett's test, respectively. The comparison of the effect of the treatments was provided by one-way ANOVA. When significant differences were found ($\alpha < 0.05$), the means were separated by the multiple range Tukey HSD test ($\alpha = 0.05$).

Apparent N Recovery (ANR) was used as an index of N use efficiency. ANR was estimated according to the equation (Ferreira et al. 2020):

$$\text{Apparent N Recovery (ANR, \%)} = 100 \times \frac{\text{N recovered in the fertilized treatments} - \text{N recovered in the control}}{\text{N applied as a fertilizer}}$$

Results

Dry matter yield of maize and oats

In the first growing season of maize significant differences in DM yield between treatments were not found, although an increasing gradient in average values as the N rate increased had been observed, especially between the control and the fertilized treatments (Figure 2). In the second year, significant differences were found between fertilizer treatments in maize DM yield. The treatments of higher N rates of any of the fertilizers under study showed maize DM yields consistently higher than the treatments of lower N rates. Between each fertilization level (N100, CRF100 and SF100 or N200, CRF200 and SF200) significant differences in DM yield due to the type of fertilizer were not found.

Oat DM yield increased significantly with N rate in both growing seasons (Figure 3). In the first season, the higher values were found in the N200 treatment (4.5 t ha^{-1}) and in the second growing season in the CRF200 (4.1 t ha^{-1}) and SF200 (4.2 t ha^{-1}) treatments. The lower average values were found in the control treatment in 2019 (2.6 t ha^{-1}) and in 2020 (1.8 t ha^{-1}).

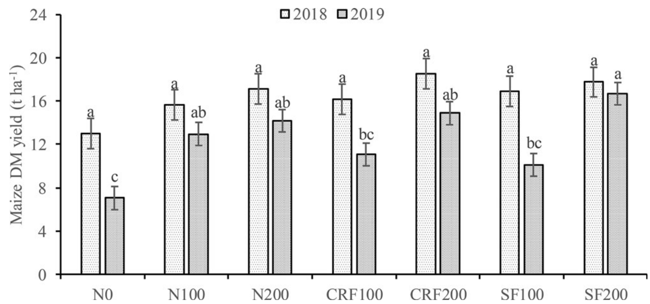

Figure 2. Maize dry matter (DM) yield as a function of fertilizer treatments: N0, N100, N200 (0, 100 and 200 kg N ha^{-1}, as ammonium nitrate, 50% applied at pre-plant and 50% at side-dress); CRF100 and CRF200 (100 and 200 kg N ha^{-1}, as a controlled-release fertilizer applied at pre-plant); SF100 and SF200 (100 and 200 N ha^{-1}, as a stabilized fertilizer applied at pre-plant). Within each year, means associated to the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$). Vertical bars are the standard errors.

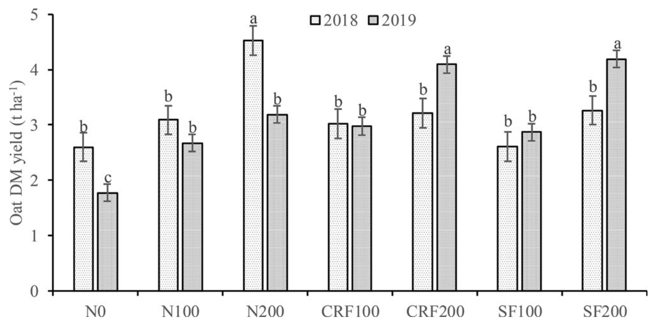

Figure 3. Oat dry matter (DM) yield as a function of fertilizer treatments: N0, N100, N200 (0, 100 and 200 kg N ha^{-1}, as ammonium nitrate, 50% applied at pre-plant and 50% at side-dress); CRF100 and CRF200 (100 and 200 kg N ha^{-1}, as a controlled-release fertilizer applied at pre-plant); SF100 and SF200 (100 and 200 N ha^{-1}, as a stabilized fertilizer applied at pre-plant). Within each year, means associated to the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$). Vertical bars are the standard errors.

Indices of plant N nutritional status and soil available N

Maize leaf N concentration at pre-sidedressing, in the phenological stage 14 (four leaves unfolded), showed an increasing trend with the N rate, although significant differences only occurred in 2019, in which the values in the N200 treatment were significantly higher than in the control treatment (Table 1). The SPAD readings also showed an increasing trend with N rate in 2018, with significant differences between treatments to be observed. The NDVI and the F_V/F_M ratio showed no significant differences between treatments or a clear trend that deserves to be highlighted. At harvest, N concentration in whole plant tissues also did not show significant differences between treatments in 2018, although in 2019 the treatments corresponding to the higher N rates (N200, CRF200 and SF200) have shown significantly higher tissue N levels. The stalk nitrate test proved to be the most sensitive index to N fertilization, with very marked differences between treatments in both the years. In 2018 the values ranged between 150.5 (N0) and 2175.2 (CRF200) mg kg^{-1} and in 2019 between 716.2 (N0) and 1574.9 (N200 and CRF200) mg kg^{-1}.

Soil inorganic N determined in July 2018 at pre-sidedress showed a peak of NH_4^+ associated to the SF200 treatment, which fertilizer was prepared with DMPP as a nitrification inhibitor (Table 2). Soil NO_3^- was significantly higher in the treatments receiving the higher N rates. It should be noted that in this date the plots of conventional fertilizer had only received half of the N rate of each treatment. The results of July 2019 showed a pattern similar to that observed form the date of July 2018. Soil residual inorganic N in October 2018 and 2019, after the harvest of

Table 1. Nitrogen (N) nutritional status and related plant traits as a function of fertilizer treatments: N0, N100, N200 (0, 100 and 200 kg N ha^{-1}, as ammonium nitrate, 50% applied at pre-plant and 50% at side-dress); CRF100 and CRF200 (100 and 200 kg N ha^{-1}, as a controlled-release fertilizer applied at pre-plant); SF100 and SF200 (100 and 200 N ha^{-1}, as a stabilized fertilizer applied at pre-plant). Within each year, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

	Leaf N (g kg^{-1})†		SPAD-readings†		NDVI†		F_V/F_M†		Plant N (g kg^{-1})‡		Stalk NO_3^--N (mg kg^{-1})‡	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
N0	26.8 a	28.8 b	54.6 c	60.2 a	0.82 a	0.79 a	0.78 a	0.77 a	12.3 a	5.8 b	150.5 e	716.2 ab
N100	25.6 a	32.0 ab	61.7 ab	60.7 a	0.82 a	0.81 a	0.78 a	0.76 a	11.2 a	7.8 ab	982.2 cd	796.3 ab
N200	28.3 a	34.2 a	63.3 a	59.9 a	0.83 a	0.79 a	0.79 a	0.78 a	12.1 a	10.6 a	1945.6 ab	1574.9 a
CRF100	25.9 a	31.1 ab	60.9 ab	60.6 a	0.82 a	0.79 a	0.79 a	0.76 a	13.4 a	8.6 ab	1347.3 bcd	1331.6 ab
CRF200	27.0 a	32.0 ab	60.7 ab	61.4 a	0.83 a	0.80 a	0.79 a	0.79 a	12.7 a	10.9 a	2175.2 a	1574.9 a
SF100	26.4 a	29.5 ab	58.5 b	59.0 a	0.83 a	0.78 a	0.76 a	0.76 a	12.8 a	8.3 ab	858.0 d	494.9 b
SF200	29.0 a	32.9 ab	62.8 a	60.4 a	0.85 a	0.80 a	0.76 a	0.77 a	14.2 a	10.2 a	1561.8 abc	998.6 ab
Prob < F	0.1180	0.0330	<0.0001	0.6329	0.1538	0.9261	0.4687	0.2181	0.0739	0.0035	<0.0001	0.0038
SE	0.87	1.38	0.72	2.69	0.01	0.02	0.01	0.01	0.62	0.76	128.9	182.1

†Phenological stage 14 (four leaves unfolded); ‡harvest (early milk)

maize, were found at higher levels in the most fertilized plots, but at similar rates between the different fertilizers within the same N rate. In May soil inorganic N was found at lower levels than in October, and the differences between fertilizer treatments practically have disappeared.

Several other determined soil properties, such as organic C, extractable P and K, the bases of the exchangeable complex and the micronutrients B, Fe, Cu, Zn and Mn, did not vary consistently with the fertilizer treatments and were considered of little interest for assessing the effect of the treatments (data not shown).

Nitrogen recovery in plant tissue

Plant N recovery significantly varied between treatments in each one of the four growing cycles (Figure 4). Total N recovery also varied significantly between treatments. The control treatment showed significantly lower values than any of the other treatments. Within the fertilized treatments no significant differences were found for a given N rate (N100, CRF100 and SF100 or N200, CRF200 and SF200). The higher N rate of each type of fertilizer (N200, CRF200 and SF200) gave significantly higher values of N recovery than the fertilized treatments with a lower N rate (N100, CRF100 and SF100).

The recovery of nutrients other than N in the biomass of maize and oats, particularly the macronutrients, tended to increase with N rate, mostly as the result of the increase in DM yield and less to the effect in tissue nutrient concentration (data not shown).

Apparent N recovery varied from 16.8% in the N100 treatment after the first growing cycle of maize to 62.5% in the CRF100 treatment after the end of the experiment (Table 3). Over the two growing seasons a slight increase in apparent N recovery was observed. In the last growing cycle of oats, which provides the apparent N recovery of the cropping system during the two years of study, the values varied from 44.3% to 62.5%. Overall, the values tended to be higher in CRF and SF treatments than in the treatments corresponding to the conventional fertilizer. It was not clear a tendency to a reduction in apparent N recovery as the N rate increased.

Discussion

The DM yield of maize increased with the N rate. This response is consistent with several previous studies on this crop (Arrobas, Aguiar, and Rodrigues 2016; Córcoles, Juan, and Picornell 2020; Yan et al. 2021), since N has a prominent role in plant nutrition (Hawkesford et al. 2012)

CRF and SF did not show significant differences for conventional N fertilizer treatments whose rates were split into two applications. CRF is based on the encapsulation of nutrients, resulting in

Table 2. Pre-sidedress soil nitrogen test (PSNT) and residual inorganic nitrogen in soil as a function of fertilizer treatments: N0, N100, N200 (0, 100 and 200 kg N ha^{-1}, as ammonium nitrate, 50% applied at pre-plant and 50% at side-dress); CRF100 and CRF200 (100 and 200 kg N ha^{-1}, as a controlled-release fertilizer applied at pre-plant); SF100 and SF200 (100 and 200 N ha^{-1}, as a stabilized fertilizer applied at pre-plant). Within each year, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

	PSNT (mg kg^{-1})				Residual soil inorganic nitrogen (mg kg^{-1})							
	Jul 2018		Jul 2019		Oct 2018		May 2019		Oct 2019		May 2020	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
N0	1.5 b	15.5 d	0.8 b	17.4 bc	1.1 c	15.5 c	1.4 a	4.5 a	3.5 b	12.2 b	2.4 ab	7.2 a
N100	1.3 b	22.2 cd	1.9 b	25.3 ab	2.3 bc	27.9 b	0.9 a	4.8 a	3.3 b	12.9 b	1.2 b	7.4 a
N200	2.7 b	29.7 bc	3.2 b	23.8 ab	6.0 a	39.4 a	1.1 a	5.7 a	9.7 ab	31.1 a	2.3 ab	8.1 a
CRF100	2.5 b	32.5 b	3.1 b	14.4 c	1.2 c	21.7 bc	2.3 a	5.8 a	12.0 ab	26.2 ab	3.1 a	7.0 a
CRF200	2.7 b	32.2 b	3.9 ab	29.6 a	4.4 ab	25.1 b	2.1 a	6.1 a	22.0 a	28.5 a	1.5 ab	8.2 a
SF100	1.9 b	20.1 d	1.8 b	24.0 ab	1.6 bc	22.7 bc	1.6 a	4.8 a	11.8 ab	21.1 ab	2.9 ab	10.3 a
SF200	16.0 a	44.5 a	7.9 a	26.4 a	7.1 a	35.8 a	1.7 a	4.5 a	19.3 a	26.0 ab	1.9 ab	7.3 a
Prob < F	<0.0001	<0.0001	0.0014	0.0002	<0.0001	<0.0001	0.0568	0.1121	0.0010	0.0026	0.0262	0.3424
SE	0.82	1.55	0.88	1.66	0.66	1.54	0.357	0.338	2.61	3.04	0.32	0.96

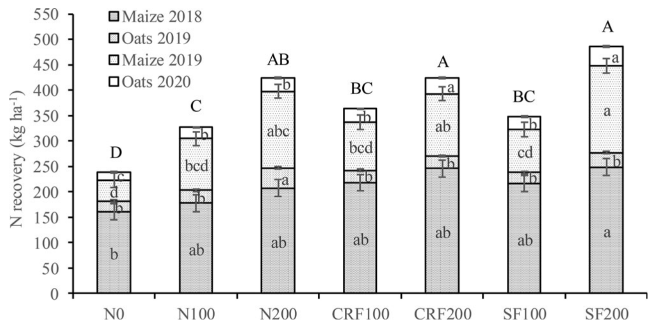

Figure 4. Nitrogen (N) recovery as a function of fertilizer treatments: N0, N100, N200 (0, 100 and 200 kg N ha^{-1}, as ammonium nitrate, 50% applied at pre-plant and 50% at side-dress); CRF100 and CRF200 (100 and 200 kg N ha^{-1}, as a controlled-release fertilizer applied at pre-plant); SF100 and SF200 (100 and 200 N ha^{-1}, as a stabilized fertilizer applied at pre-plant). Within each crop (lowercase letters) and total (uppercase letters), means associated to the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$). Vertical bars are the standard errors.

Table 3. Apparent nitrogen recovery (ANR) after the harvest of the four consecutive crops: N100, N200 (0, 100 and 200 kg N ha^{-1}, as ammonium nitrate, 50% applied at pre-plant and 50% at side-dress); CRF100 and CRF200 (100 and 200 kg N ha^{-1}, as a controlled-release fertilizer applied at pre-plant); SF100 and SF200 (100 and 200 N ha^{-1}, as a stabilized fertilizer applied at pre-plant).

Treatment	Maize 2018*	Oats 2019*	Maize 2019**	Oats 2020**
		——— (%) ———		
N100	16.8	21.7	41.0	44.3
N200	23.2	32.6	43.8	46.6
CRF100	57.2	60.6	57.0	62.5
CRF200	42.6	44.3	42.7	46.6
SF100	55.5	56.3	50.2	54.9
SF200	43.9	47.8	56.5	62.1

ANR (%) = [Average N recovery in fertilized treatments – Average N recovery in N0 treatment)]/N applied as a fertilizer × 100. Estimated taking into account the N rates applied in the *first and **first plus second growing seasons of maize.

a more regular supply to plants, while SF favors the persistence of N for longer in the NH_4^+ form, due to the action of the nitrification inhibitor, which allows NH_4^+ to be adsorbed into the exchangeable complex or fixed in 2:1 type clay minerals (Havlin et al. 2014). Both mechanisms of slow-release can reduce the risk of NO_3^- leaching and denitrification in comparison to conventional fertilizers (Nauer et al. 2018; Li et al. 2019; Sun et al. 2019). The objective of these slow-release mechanisms is to regulate the availability of N throughout the growing season, just as with splitting the N rates, whose effects are widely proven, and the technique has been recognized as having a high potential for improving N use efficiency (Rodrigues et al. 2006; Wasaya et al. 2017).

Oats responded to the application of N applied to maize in the previous growing season. This revealed that, of the amount of N that was applied in the summer in the main crop, a significant part remained in the soil as residual N with high potential to be leached in the NO_3^- form or denitrified during the next wet season, respectively due to the water percolation and the creation of conditions of anoxia in the soil (Havlin et al. 2014).

The nutritional status indices corroborated the information obtained from DM yield when comparing the different fertilizers and N rates. The high sensitivity of the stalk nitrate test should be highlighted, revealing differences between treatments that are much more marked than those of other indices, such as the leaf N or SPAD-readings, which have been widely used in monitoring the nutritional status of crops (Schepers et al. 1992; Rodrigues et al. 2006; Afonso et al. 2017). The stalk nitrate test was developed by Binford, Blackmer, and El-Hout (1990) and its relevance as an index of maize N nutritional status has been confirmed in several other studies (Rodrigues et al. 2006; Isla et al. 2015; Yang et al. 2017). The index has the potential to effectively measure the N accumulated in the plant in the form of NO_3^-, which is the main form in which N accumulates in the plant; since NH_4^+ is toxic and is largely assimilated in the roots, NO_3^- may accumulate freely in the vacuoles of the conducting tissues (Hawkesford et al. 2012). Its less frequent use may be due to the fact that it is an end-of-season index which only serves to guide N fertilization in the next growing season (Blackmer and Schepers 1994; Rodrigues et al. 2006; Isla et al. 2015). The PSNT revealed differences in NH_4^+ and generally in NO_3^- in the soil during the growing season. In contrast to the effects of the total amount of inorganic N available in the soil, which increased maize DM yield, fluctuations in the available inorganic N form did not have a significant effect on the crop, perhaps due to the fact that both forms were actually absorbed by plants (Hawkesford et al. 2012).

The results also showed that the higher values of residual soil inorganic N were found in the most heavily fertilized plots, whereas no difference was usually found between treatments of different fertilizers within the same N rate, which corroborate the results of oats DM yield. In this respect, the importance of the winter catch crop in the recovery of residual N should be highlighted, as has been widely shown in previous studies (Rodrigues, Coutinho, and Martins 2002; Valkama et al. 2015; Notaris et al. 2018).

N recovery corroborated the data of DM yield, with greater N recovery in the fertilized treatments with higher N rates. This result also revealed the importance for N in this agro-system, with two grasses cultivated in the same year. The results of apparent N recovery showed values ranging from 44.3% to 62.5% which reflect the high N loss that can occur from agricultural soils, in spite of the use of an unfertilized winter catch crop as was the case in this study. They are however acceptable values taking into account those usually reported on a global scale, which are considered to be between 40 to 60% (Havlin et al. 2014). Oats recovered part of the N potentially lost. However, grasses may not even be the most suitable crops to use as catch crops in these agro-systems, since the highest growth rates and N uptake occur mainly in the spring (Rodrigues, Coutinho, and Martins 2002). For these systems, the inclusion of species of early growth in autumn, such as some brassicas, could be more efficient as a catch crop, but at present these species are less useful for feeding of animals on the farm.

Conclusions

For the same N rates, CRF and SF gave similar DM yields, not dissimilar or even higher plant N nutritional status indices and N use efficiencies, than the conventional fertilizer split into two applications. Thus, the three fertilization strategies compared in this study would seem to be valid options for farmers to consider, having taken into consideration local information of a technical-economic nature, related to the availability of equipment and the price of fertilizers.

The high sensitivity of the stalk nitrate test to N availability in the soil should be noted. Although it is an end-of-season index, it has great potential to help in N management for the next growing season in maize.

The results also reveal the importance of having a winter catch crop in the crop rotation to reduce N loss from the agro-system.

Disclosure Statement

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