Effect of dried pig manure fertilization on barley macronutrients and sodium in a nitrate vulnerable zone

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Abstract

Purpose: Dried pig manure (DPM) may be valorized as a fertilizer suitable for barley crop in nitrate vulnerable zones (NVZs). The aim of this study was determine the macronutrients and sodium contents in barley (*Hordeum vulgare* L.) resulting from its application in a NVZ in Fompedraza (Valladolid, Spain).

Methods: DPM was applied at three rates (85, 133 and 170 kg $N\cdot ha^{-1}\cdot year^{-1}$), the mineral fertilizer with a nitrification inhibitor was applied at two rates (90 and 108 kg $N\cdot ha^{-1}\cdot year^{-1}$) and these were compared with the control treatment (without fertilization) in a randomized complete block design. Nutrients were monitored in four different plant growth stages and in grain over a three-year period.

Results: DMP-based fertilization increased P and Na contents in plant and decreased those of Ca and Mg. These changes were only translated into a P increase in grain. The Na content in plant should not affect the final crop yield, making this waste management strategy viable even in NVZs. However, N content in plant in tillering and stem elongation stages was lower for DPM-based fertilization than for mineral fertilization, and so was the C content, both in plant and in grain.

Conclusions: Since N content is a limiting factor for crop development, supplementary mineral fertilization would be advised to compensate for N immobilization if this organic waste material is to be valorized as a fertilizer.

Keywords: cogeneration plant; DPM; ENTEC; NVZ; sodium; waste valorization.

Abbreviations:

DMPP: 3,4-dimethylpyrazol phosphate

DPM: dried pig manure

NVZ: nitrate vulnerable zone

Introduction

Barley (*Hordeum vulgare* L.) is commercially used to produce malt, for seed, for animal feed and for human food applications, since it is rich in carbohydrates, proteins, dietary fibers, vitamins and minerals (Ullrich 2011). The study of alternatives to the use of conventional mineral fertilizers for its cultivation, with a view to a more sustainable development, should thus be regarded as a priority, provided that it is one of the leading cereal crops in the world and the second most important crop in Europe.

Agricultural practices have disrupted the natural equilibrium of soils, leading to a decrease in organic matter and thereby to a decline in the agricultural soil quality (Zornoza et al. 2008). In this context, organic waste materials, such as animal manure, may play an important role in agronomic management (Anwar et al. 2018; Ding et al. 2014). The application of animal manure (e.g., pig manure) to agricultural soil has been recommended as the first choice for the management of this type of waste materials (Makara and Kowalski 2018).

Agricultural practices have also occasioned high concentrations of nitrates in surface and sub-surface water sources in certain areas, which—in accordance with European Regulation (91/676/CEE)—have been declared nitrate vulnerable zones or NVZs (when nitrates concentration exceeds 50 $mg{\cdot}L^{-1}$). In these areas, farmers should follow a series of measures on fertilizer application to minimise the risk of nitrates polluting ground or surface water.The treatment of pig manure in cogeneration plants generates an organic waste suitable for application in nitrate vulnerable zones, thanks to the reduction of moisture content and of the leaching capacity of nitrates.

However, the use of organic waste as fertilizers in agricultural soils can immobilize the nitrate content due to their organic matter content (Sánchez-Báscones et al. 2019), and may increase the Na content, thus increasing soil salinity (Li-Xian et al. 2007) and negatively affecting crop yield.

The application of organic waste as fertilizers must be well understood with regard to the provision of nutrients for crop growth, which is frequently restricted by availability from post-anthesis assimilation as well as by translocation from vegetative organs (Simpson et al. 1983). Aforementioned increase in Na content can reduce the uptake of nutrients such as K and Ca, and those of other elements such as P. In the case of barley, the critical limit for plant growth in terms of Ca concentration would be 0.25% (Dang et al. 2016), and an insufficient concentration of P and K in grain may negatively affect seed germination (Agegnehu et al. 2016; Hejcman et al. 2013).

The content of macro- and micronutrients in barley has been reported to be influenced by the type of fertilization treatment (Agegnehu et al. 2016; Jākobsone et al. 2015; Maleki-Farahani et al. 2011; Shepherd et al. 2017; Wilczewski 2014), genetics (Dang et al. 2016; Dick et al. 1985), environmental conditions (Cossani et al. 2011; Maleki-Farahani et al. 2011), biomass growth (Greenwood et al. 2008), and the type and characteristics of the soil (Agegnehu et al. 2016; Dang et al. 2016; Jākobsone et al. 2015; Rutkowska 2013). Nonetheless, studies on the variation of the content of N, P, K, Ca, Mg and Na during plant growth for barley are scarce, being only relatively well-documented for grain composition.

The study presented herein, conducted in real conditions over a three-year period, fills a research gap by assessing the effect of the application of two fertilization sources (organic and mineral) at different doses in various stages of the vegetative cycle of the barley plant and their influence on grain. A bibliographical revision has been carried out in order to establish intervals of the content of these nutrients in plant and grain. Starting from the hypothesis that DPM organic waste can be valorized as a fertilizer for NVZs, its suitability has been surveyed and an analysis of possible anomalous increases or deficits resulting from the application of DPM as a new type of organic fertilizer is provided.

Materials and methods

Location

The experiment was carried out over a three-year period in a NVZ located in Fompedraza (Valladolid, Spain), at an altitude of 889 m.a.s.l. The Autonomous Community of Castilla y Leon designated Fompedraza as a NVZ in 2009 (Decree 40/2009, Official Journal of the Junta de Castilla y León). The climate in the region is semiarid continental Mediterranean, with low annual precipitation (425 mm), a minimum average air temperature of 3 ºC and a maximum average air temperature of 18 ºC. The mean temperatures and rainfall during the period of study are depicted in Fig. 1 (MAPAMA 2017).

[FIGURE 1]

Soil characteristics

The soil physicochemical properties are summarized in Table 1. The soil was rich in carbonates (28.7%). Phosphorus content, extracted by the Olsen method, was also high (22.1 mg·kg^{-1}), provided that a high content in carbonates promotes the formation of insoluble calcium phosphate. Exchange cations saturated 100% of the cation exchange capacity, as one would expect for a basic pH soil.

[TABLE 1]

Organic and mineral fertilizers

Two types of fertilizers were assayed. Dried pig manure (DPM) was supplied by a cogeneration plant located in Fompedraza. Its physicochemical properties are presented in Table 2. A high electrical conductivity (EC) was found, indicative of a high salt content, as well as a high phosphorus content (so the application of DPM should generally meet the plants needs without mineral supplements).

[TABLE 2]

ENTEC® 24+8+7 was used as a mineral fertilizer (purchased from EuroChem Agro Iberia, Barcelona, Spain), which contains 24% N (10.5% NO_3^--N and 13.5% NH_4^+-N), 8% P_2O_5, 7% K_2O, and 5% SO_3, together with 3,4-dimethylpyrazol phosphate (DMPP) nitrification inhibitor (0.8%).

Experimental design

A randomized complete block design was followed in the study: the experimental plot was divided into 4 blocks, which were subdivided into 6 subplots, one per assayed treatment. Each subplot measured 6×10 m^2, and a 2 m-spacing was kept between them.

Barley (*Hordeum vulgare* L.), planted as an annual crop, was chosen for the study. For non-irrigated land, its nitrogen needs were estimated at 30 kg N·t^{-1} and its production at 3 t·ha^{-1}. Seeding was conducted in early March and harvesting in July. Organic fertilizers and mineral fertilizers application was carried out one month before seeding and one week before seeding, respectively, using a minimum tillage (i.e., conservation tillage) system.

The following treatments were applied during the three-year period: mineral fertilization at the usual rate applied in the region (T1, Table 3); mineral fertilization according to crop needs (T2); DPM at 85 kg N·ha^{-1}·yr^{-1} application rate (T3); DPM at 133 kg N·ha^{-1}·yr^{-1} application rate (T4); and DPM at 170 kg N·ha^{-1}·yr^{-1} application rate (T5). T0 corresponded to the control soil without fertilizer.

The application rates of DPM were chosen taking into account the maximum amount of livestock manure that can be applied to an area declared as NVZ according to Spanish legislation (Spanish Ministry of the Presidency 1996): they ranged from 50% of the maximum legal dose (85 kg N·ha^{-1}·yr^{-1}) for T3 to 100% of the maximum legal dose (170 kg N·ha^{-1}·yr^{-1}) for T5.

As shown in Table 3, no additional mineral fertilization was required in the subplots to which the DPM-based treatments were applied, provided that the crop P and K needs could be met with the organic waste alone. On the other hand, a K supplement (60% KCl) had to be added to T2 subplots in order to meet crop needs.

[TABLE 3]

Methodology for plant and grain analyses

Samples of barley plant were collected at four stages of the vegetative period (viz. tillering, SI (1-5); stem elongation, SII (6-9); heading, SIII (10.1-10.5); and ripening, SIV (11.1-11.4), according to Feekes scale), in three different spots in each subplot. The grain was obtained at full maturity by hand harvesting of 0.5 m^2 subplots. Once the entire plants had been harvested, they were throughly washed, first with tap water and then with deionized water (three times). Plant samples were dried in an oven at 60 °C until constant weight was achieved. Grains were separated from straw once the whole plants had been dried in a stove. Plant and grain samples were ground with a Retsch ZM-100 (Retsch GmbH, Haan, Germany) stainless steel grinding mill.

Total carbon and nitrogen were determined using a CNH Leco-2000 (Leco Corp., St. Joseph, MI, USA) analyzer. Na, K, Ca and Mg were determined using flame atomic absorption spectrometry (FAAS) with a Varian AA240FS spectrometer (Agilent Technologies, Santa Clara, CA, USA). Whole plant and grain samples (0.3 g) were digested in an Ethos Touch Control Advanced Microwave Labstation (Milestone, Sorisole, BG, Italy) with 10 mL of HNO_3 (65% PA-ISO) and H_2O_2 (30% w/w PA) (5:3). Once it was cool, the digested solution was filtered -using Whatman (n^o 1) filters- and the filtrate was then diluted with deionized water for the determination of cations (K, Na, Ca and Mg) by atomic absorption spectrophotometry. P content was analyzed from the microwave-digested samples by the molybdate-blue colorimetric method (MAPA, 1994), using dual-wavelength ultraviolet spectrophotometry. A Shimadzu (Kyoto, Japan) UV-2450 UV-Vis spectrophotometer was used for this characterization. All analyses were done in triplicate.

Statistical analyses

Statistical analyses were conducted with a mixed model for the analysis of variance (MIXED procedure in SAS software v.9.2, SAS Institute Inc., Cary, NC, USA). The statistical analysis of the plant composition values of C, N, P, K, Ca, Mg and Na was conducted taking into account the effects of the treatment (T, 6 levels), year (Y, 3 levels), stage of growth (S, 4 levels) and their interactions. The statistical analysis of the grain composition values of C, N, P, K, Ca, Mg and Na was conducted taking into account the effects of the treatment (T, 6 levels), year (Y, 2 levels) and their interactions. Data normality and variance homogeneity were verified prior to the analysis. Tukey's multiple range test at 0.05 probability level ($p<0.05$) was chosen for the *post hoc* comparison of means.

Results

Macronutrients and sodium contents in barley plant

The content of macronutrients (C, N, P, K, Na, Ca and Mg) and sodium in barley plant was determined during crop development at the tillering (SI), stem elongation (SII), heading (SIII) and ripening (SIV) stages, over a three-year period, for six different fertilization treatments. Average values with their corresponding deviations are depicted in Fig. 2. Significant differences between treatments in the same plant growth stage, and differences by plant growth stage in the same treatment are summarized in Table S1.

Among the elements analyzed in barley plant throughout its growing state, only the C content, with an average value of 6%, increased from SI to SIV stage. The average concentration of the rest of the elements decreased: by 67% for N; by 63% for Na and K; by 49% for Ca and Mg; and by 42% for P.

The highest average plant content in the ripening stage (SIV) of the crop corresponded, for all treatments, to C (42.2±0.54%), followed by N (1.17±0.17%), K (1.09±0.22%), Ca (0.44±0.15%), P (0.22±0.06%), Mg (0.10±0.01%), and Na (0.07±0.05%). The C and Mg content varied by 1% and 10%, respectively, while Na presented the highest variability (with 71% in the ripening (SIV) stage) across the three years of the field test and the six different fertilization treatments.

[FIGURE 2]

Significant differences were found for the values of N, P, K and Na with respect to the Year * Treatment * Stage interaction, and for the values of C, Ca and Mg with respect to the treatment factor (Table 4).

[TABLE 4]

As noted above, the content of macronutrients and sodium analyzed in the plant was found to present a higher variability in its initial growth stages (SI and SII) than in subsequent ones (SIII and SIV), in which the composition values were generally quite stable.

Carbon

The C content in the plant for the organic fertilization treatments (T3 and T5) was significantly different from those of the control (T0) and T1 mineral treatment, and so were the values between the two mineral fertilization treatments (T1 *vs*. T2). The highest carbon content in plant (42.6%±0.7) was determined for T1 in SIV stage, while the lowest value (38.8%±1.4) was found for T5 in SII stage. The highest values at the end of the crop cycle were observed for the mineral fertilization treatments (T1 and T2), followed by the control (T0) and finally by the treatments with dried pig manure (DPM, T3-T5).

Nitrogen

The N content in the plant showed significant differences between the mineral fertilization treatments (T1 and T2) and the rest of treatments (i.e., control and organic) in SI stage in the first and in the second year, and in SIII stage in the third year. Likewise, significant differences were also found in SII stage in the first year of the experiment between mineral (T1 and T2) and two of the organic fertilization treatments (T4 and T5). The highest values in the initial growth states (tillering and stem elongation) were obtained for the mineral fertilization treatments (T1 and T2), followed by the DPM treatments (T3-T5), and finally by the soil without fertilization (T0). The N content in plant in the subplots treated with DPM (T3-T5) was similar to that of the control subplots.

Phosphorus

The P content in plant presented significant differences between the control (T0) and one of the mineral treatments (T1) in the SII stage in the first year of the experiment; and between the organic fertilization treatments (T3-T5) and the rest of treatments in the SII stage in the third year of the experiment. The range of values for the P content varied between 0.55% (SII, T5) and 0.22% (SIV, T3) for the organic fertilization treatments; between 0.36% (SI, T1) and 0.18% (SIV, T1) for the mineral fertilization ones; and between 0.35% (SI) and 0.24% (SIV) for the control soil.

In the first two years, P levels in the plant did not show significant differences due to the type of fertilization treatment. On the other hand, in the last year of the experiment, the content of P was higher in SII for treatments with DPM (T3-T5), with a positive trend between the applied amounts of organic waste material and the amount of P in plant (0.56% for T3, 0.61% for T4 and 0.75% for T5).

Potassium

The K content in the plant showed a very small variability between different treatments, years and growth stages. Significant differences were only found in the SI stage in the first year between T2 mineral treatment and T3 organic treatment (smallest dose of DPM). The treatments with mineral fertilization resulted in the highest K contents in plant in the SI stage. The values of K content in plant ranged from a maximum value of 3.34%±1.20 in SI for T2 mineral treatment to the lowest value of 0.96%±0.10 in SIV for T0.

Sodium, calcium and magnesium

The Na content in plant showed significant differences between the control treatment (T0), the organic fertilization treatments (T3-T5) and T2 mineral treatment in the SI stage in the first year of the experiment, and between the organic fertilization treatments (T3-T5) and the rest of treatments in SI and SII stages in the third year of the experiment. The highest Na content in plant (0.26%±0.11) was found for the highest dose of DPM (T5) in SI stage, while the lowest value (0.07%±0.06) corresponded to T2 mineral fertilization treatment in SIV stage.

Concerning the Ca and Mg contents in barley plant, T0, T1 and T2 showed significant differences compared to T4 for Ca, and to T4 and T5 for Mg. The highest Ca content in the plant was found for T2 in SII stage (1.38%±0.43) and the lowest for T4 in SIV stage (0.38%±0.20). In the initial growth stages (SI and SII), Ca concentrations were higher for the mineral fertilization treatments and the control than in the DPM-treated subplots. In the SII stage, there was an inverse trend between the Ca content in the plant and the application rate of DPM (1.09% for T3, 0.96% for T4 and 0.94% for T5).

The highest Mg content in plant was determined for T1 and T2 treatments in SI stage (0.20%±0.03) and the lowest values corresponded to T5 in SIV stage (0.10%±0.01). Magnesium was the element that showed the lowest variability among treatments and growth states.

Macronutrients and sodium content in barley grain

The average contents of C, N, P, K, Na, Ca and Mg analyzed for the different fertilization treatments are summarized in Fig. 3.

[FIGURE 3]

The average contents of Ca, K, Na and C in grain decreased by 70%, 49%, 31% and 4% with respect to the contents in plant in SIV growing stage, while the average contents of P, N and Mg increased by 39%, 31% and 13%, respectively. This implies that N, P and Mg accumulated in larger amounts in the grain than in the plant, while Ca, K, Na and C were mainly concentrated in the plant.

The statistical analysis evidenced significant differences in the C content in grain for the interaction of the year and treatment factors (Y*T), in the content of N and P for the treatment (T) and year (Y) factors, and in the content of Mg for the year factor (Y). K, Ca and Na contents in the grain did not present variations associated with the type of treatment, year and their interaction (see Table 5).

[TABLE 5]

The C content in grain was significantly different between mineral fertilization treatments (T1 and T2) and the control (T0).

The N content in grain ranged from 1.35±0.08% in the control soil to 1.85±0.24% in the soil with T2 mineral fertilization treatment. The nitrogen content in grain in the soils treated with mineral fertilization (T1 and T2) was significantly different from those obtained for the rest of treatments.

The P content in grain showed significant differences between the treatments with DPM (T3 and T4) and the control (T0). The percentage of P in grain varied between 0.27±0.02% (T0) and 0.33±0.04% (T3 and T4).

Neither the content of K in grain nor those of Na, Ca or Mg presented significant differences as a function of the type of fertilization treatment. Potassium content varied between 0.53±0.04% for the mineral fertilization treatment (T2) and 0.59±0.08% for the highest application rate of DPM (T5). Nonetheless, the content of K in grain showed a positive response to the increase in the amounts of applied dried pig manure. The Na content in grain varied between 0.04±0.04% for T2 mineral fertilization treatment and 0.07±0.08% for the highest DPM application rate (T5). The Ca content varied between 0.11% (T1) and 0.18% (T4). In relation to the Mg content, it varied between 0.10 and 0.11%.

Evolution of N:P ratio during plant growth

The N:P ratio values in the different growth phases are represented in Fig. 4. The N:P ratios for all treatments decreased during the growth cycle until the final stages (SIII and SIV), in which the values were practically constant. The N:P ratio ranges were similar within each type of fertilization treatment: 10-5 for T0 (control), 13-6 for mineral fertilization (T1 and T2) and 8-5 for DPM fertilization (T3, T4 and T5).

[FIGURE 4]

Discussion

Variations in macronutrients and sodium content between stages in barley plant have also been observed by other authors, such as Alessi and Power (1969) and Hoppo et al. (1999) for P; and by Rutkowska (2013) for P, K and Mg. The generation of a greater amount of structural carbohydrates throughout its growth cycle implies an increase in the C content and a dilution of the rest of the elements (Bishop 1930).

Barley has a high capacity for the remobilization of macro- and micronutrients, but it is somewhat lower for Mg, which presents very similar content values of grain and plant (Maillard et al. 2015). Moreno et al. (1996) observed the same trend for N, P, K, Ca and Na between straw and grain, although the Mg content was higher in straw. Ostrowska and Porębska (2017) observed that after the retranslocation of the elements, Ca content in grain was reduced by 80%, while Mg content remained at similar levels.

Both mineral fertilization and mixtures with organic fertilization have been reported to have a positive effect on the contents of N, K and P in grain, observing no differences for Ca and Mg contents (Hejcman et al. 2013). Nonetheless, in this study, the content of K in grain did not present any sort of influence, as the soil was able to provide a sufficient amount of it.

According to Gonzalez et al. (1992), in wheat the application of organic waste favored the increase of P, K and Na in grain, whereas mineral fertilization increased N content. In barley, Moreno et al. (1996) found that the application of sewage sludge increased the content of all macronutrients and Na with respect to the control soil.

The interval of variation in the contents of macronutrients and sodium in barley, analyzed in the plant throughout the growth cycle, was found to decrease in the final stages of the vegetative cycle, minimizing the possible effects of the type of fertilization treatment as well as the impact of the environmental conditions in the different years of the study. This can be ascribed to the fact that barley presents a behavior in terms of its composition that is remarkably influenced by a genetic component, presenting a low variability at the end of its growth stage (Maleki-Farahani et al. 2011).

Carbon

Carbon translocation is higher in early stages for wheat and barley (Kuzyakov and Domanski 2000). The high C content for the mineral fertilization treatments in the ripening stage and in grain may be related to the high N availability, since there is a positive correlation between the dry matter content and the accumulation of nitrogen until the anthesis (Przulj and Momcilovic 2003). Nitrogen has a positive effect on the development of the plant root system, allowing an increased mineral uptake from the soil (Wilczewski 2014). For wheat, it has been reported that the roots with a larger availability of nitrates are able to translocate more carbohydrates, probably because of their greater development (Lambers et al. 1982).

Although this explanation can explicate the results obtained for the mineral fertilization treatments, it does not shed light on why the organic fertilization treatments (with DPM) showed lower C contents than the control treatment in the initial growth stages. A tentative explanation would be that this is due to a C immobilization effect associated with the application of organic matter in the DPM treatments, but barley is a crop that mainly uptakes C from atmospheric CO_2 (Kuzyakov and Domanski 2000). Consequently, a more plausible explanation would be a dilution of C content due to the increase in P and Na in plant, and a damage of the structure of the chloroplast for low levels of N supply (Shah et al. 2017).

References on the C content in barley grain are scarce. Agegnehu et al. (2016) did not observe any effect on C content as a result of different fertilization treatments in one year.

Nitrogen

The effect of the fertilization treatments on the grain nitrogen content was similar to that obtained in the first stages of the crop (SI and SII), in which the N content in plant was higher for the mineral fertilization treatments. The availability of N at the beginning of the vegetative cycle of the plant would thus be reflected in the final N content in the grain, as well as in the crop yield. Both in wheat (Gonzalez et al. 1992) and in barley (Agegnehu et al. 2016), the N accumulated in the plant is transferred to the spike and to the grain for the synthesis of proteins, which leads to a reduction in the N content at the end of the vegetative cycle of the plant, mostly in straw. Greenwood et al. (1986) found that, for spring barley and other arable crops, the growth rate of plant dry weight is approximately proportional to % N in the plant. Moreover, they noted that, for cereals, restricted growth as a result of temporary N deficiency at an early stage (or indeed at any other stage) cannot be compensated for by delaying the harvest date.

In this experience, it becomes apparent that the nitrogen applied by DPM was not efficiently assimilated: the crop reduced the amount of plant developed, resulting in a lower grain yield (Sánchez-Báscones et al. 2019). Gonzalez et al. (1992) also reported that mineral fertilization increased N content in the plant with respect to the application of organic waste –such as pig slurry compost– in the case of wheat, and the capacity of nitrogen uptake for barley is known to be higher for mineral fertilization than for its organic counterpart (Maleki-Farahani et al. 2011; Sørensen et al. 1994). Also in the case of wheat, in order to obtain an optimum crop yield, Jahan and Amiri (2018) advised to supplement organic fertilization with N and P mineral fertilizers.

In the third year of the experiment, the rainfall deficit (276 mm vs. normal values of ca. 425 mm) resulted in unfavorable weather conditions, reducing the capacity of nitrogen uptake in the plant (Przulj and Momcilovic 2003), which would explain why the differences between treatments were observed in later stages (SIII), instead of in the initial ones (SI and SII).

With regard to the reported values, they were in good agreement with the ranges found in the literature (Table 6). As expected, the lowest values corresponded to the control soil, i.e., to the absence of N supply by fertilization, and the resulting N content in grain would depend on the native soil fertility (Agegnehu et al. 2016). The application of fertilizers would increase the N content in grain mainly because it is source-limited (i.e., it is controlled by the availability of assimilable N from both soil and fertilizer inputs) (Savin et al. 2006).

The N content in grain is known to increase with its availability (Cossani et al. 2011), in such a way that nitrogen fertilization is the fundamental factor that controls it (Wilczewski 2014). Mineral fertilization treatments would result in the highest values of N content (Maleki-Farahani et al. 2011; Wilczewski 2014), which shows a positive correlation with the amounts of N applied by mineral fertilization (up to 140 kg·ha^{-1}) (Wilczewski 2014). Although organic fertilization treatments typically lead to lower values, because of the greater difficulty to supply an adequate amount according to the needs of the plant (Maleki-Farahani et al. 2011; Sánchez-Báscones et al. 2019), there are exceptions in which organic fertilization treatments have reached similar values to those attained with mineral fertilization (Agegnehu et al. 2016), or even higher values (Moreno et al. 1996) (see Table 6).

[TABLE 6]

Phosphorus

Plant contents in the first SI stage denoted an adequate P supply, with values above 0.3% (Bergmann 1992). In its initial stages of growth, the barley plant presents a wide variation in the P content, which is influenced by the type of soil, the type and amount of fertilizer, as well as by the form of application of the fertilizer (Alessi and Power 1969; Hoppo et al. 1999; Rutkowska 2013; Ylivainio and Peltovuori 2012). These values and the associated variability are then reduced with the development of the crop.

In the literature, values in the same intervals as those detected herein have been reported in different growth stages, both in plant and in leaves (Table 6). However, higher values (up to 1.1%) have been reported by some other authors (Hoppo et al. 1999; Shepherd et al. 2017), and lower values have been found in early stages (0.16-0.22%) by Brod et al. (2016).

The fact that P content in the plant was not influenced by the organic fertilization treatments in the first two years may be ascribed to the high carbonate content of the soil and its alkaline pH (Gonzalez et al. 1992), and to microbial immobilization due to the application of organic carbon (Brod et al. 2016), which have been reported to decrease the uptake by barley, hindering the presence in the soil of P forms available to the plants.

As shown in Table 6, the absence of fertilization would result in lower contents of P in grain (Hoppo et al. 1999; Moreno et al. 1996; Rutkowska 2013), while the application of fertilization would lead to higher values, attaining higher increments when organic fertilizers are used (Maleki-Farahani et al. 2011; Moreno et al. 1996). In a similar fashion to what happens with N, the content of P in grain depends on the ability of the native soil to supply nutrients. The lower values in the P content found in this experiment may be due to the high pH and high amount of carbonates, which would inhibit part of the assimilable P contributed by the soil.

The application of organic waste improves the availability of phosphorus in basic soils: the mineralization of organic material can reduce the pH of the soil, making the phosphorus more assimilable (Souto et al. 2018). Although in this experience the pH did not vary significantly between treatments, the assimilable phosphorus content was significantly higher for the DPM treatment at the highest dose, i.e., T5 (Sánchez-Báscones et al. 2019).

Differences in the content of P between fertilization treatments (mineral *vs*. organic) were not observed in grain. This is in agreement with the findings of other authors (Cruz-Paredes et al. 2017), who concluded that the influence of type of fertilizer and the applied amounts was not reflected in a change in the content of P in grain, having an effect only on its content in plant (straw).

Potassium

The K content in the plant and grain was not significantly different between treatments, suggesting that the soil was able to provide sufficient amounts of K without any sort of influence from the fertilization treatment (Rutkowska 2013). In T2, additional amounts of mineral K were added to meet the needs of the plant not covered by ENTEC fertilizer, which would have led to higher assimilation than the K included in ENTEC. The content of potassium in soil was higher in soils with DPM treatment, but with values very close to those of the control soil (Sánchez-Báscones et al. 2019).

Other authors have reported similar K values in plant and in leaves (Table 6). As in the case of phosphorus, higher values were obtained in the first stages, both in plant (Kováčik et al. 2014) and in leaves (Shepherd et al. 2017), with an upper limit of 8.6%. Potassium content in grain was also similar to those reported by other authors, ranging from 0.40% to 0.63% (Table 6).

Sodium

Na content values in grain obtained by other authors were in the same range as those reported herein, in the 0.01% to 0.093% interval (Table 6). Authors such as Kováčik et al. (2014) obtained similar values for Na content in plant, whereas Dick et al. (1985) and Dang et al. (2016) obtained values of 0.77% in plant and 0.83% in leaves, respectively, but these later values resulted from the Na content in the soil (Dang et al. 2016). The sodium content of the soil was not affected by the different fertilization treatments (Sánchez-Báscones et al. 2019).

Although, as noted by Dang et al. (2016), the functioning and integrity of the cellular membrane can be affected by a reduction of the uptake of essential nutrients such as K and Ca, due to competition by Na for K binding sites, such deficiencies were not observed in this work.

The application of DPM increased the Na content in plant, but this effect that was not observed in the grain content.

Calcium and magnesium

The plants in the subplots fertilized with DPM increased their Na content (in SI and SII), and reduced their content in Ca (in SII), with respect to the rest of treatments, due to the inhibition induced by the increase of the Na content in the plant. This would be in good agreement with the findings of Dang et al. (2016), who reported that the increase in plant Na content can inhibit the absorption of Ca and K.

Mg, like Ca and K, may suffer from some inhibition due to Na content. In spite of the fact that one would expect that in soils with high carbonate content there would not be an influence of the type of fertilization treatments, as it is the case for wheat (Gonzalez et al. 1992), the application of dried pig manure reduces Mg content. This occurs both by the effect of the increased Na content and because organic matter can complex Ca and Mg, reducing their assimilation capacity for the plant (Moreno et al. 1996). The magnesium content was higher in the soils with DPM treatments, while the calcium content was not significantly different (Sánchez-Báscones et al. 2019).

The Ca content in plant in this work was always higher than the critical limit for plant growth (0.25%) (Dang et al. 2016). Dick et al. (1985) also obtained similar values, although other authors have reported lower contents in plant and in leaves, ranging from 0.09% to 0.28% (Table 6). With regard to Ca content in grain, values close to 0.04% are the most common in the literature (Table 6), but higher values in the 0.28-0.35% range have been reported by Cieślik et al. (2017) and Wilczewski (2014), and exceptionally high values of 0.6-0.7% have been found by Hejcman et al. (2013). In line with the results of other authors (Cieślik et al. 2017; Ciołek et al. 2012; Moreno et al. 1996; Wilczewski 2014), no significant differences were observed between fertilized and untreated soils.

The behavior of Mg composition is very stable for barley between treatments (Rutkowska 2013), presenting always a very similar composition, virtually independent of factors such as the type and amount of fertilization. The contents found were similar to those obtained by other authors in plant and leaves, ranging from 0.10% to 0.34%, and in grain, in the 0.07% to 0.18% interval (Table 6).

With respect to the absence of influence of the different types of treatments on the content of Ca and Mg in grain, it may be explained by the high Ca and Mg contents in the soil, which would minimize the differences resulting from the different types of fertilization (Gonzalez et al. 1992). Taking into consideration the high levels of Ca and Mg in calcareous soils and the basic pH, it is reasonable that the values did not differ between treatments for these elements, in such a way that the soil factor would predominate over the fertilization one (Rutkowska 2013).

To recapitulate, the application of mineral fertilization was found to increase the content of N and C in plant as compared to the rest of treatments, whereas the application of DPM –from the third year of application– would increase the content of P and Na, reducing those of Ca and Mg. Moreno et al. (1996) stated that the application of sewage sludge mainly influenced the content of N, P and Na in straw, which is consistent with the fact that organic fertilizers, such as pig slurry compost and pig solid manure, are sources of P and Na.

Recommendations on optimal nutrient concentrations in spring barley place particular emphasis on the initial growth stages, in which the highest influence of the content of these elements would occur. The limit values would be in the 0.35-0.60% range for P, in the 3.00-5.50% range for K, and in the 0.15-0.30% interval for Mg (Baker and Tucker 2008; Bergmann 1992). Even though in the initial growth stage (SI) it can be assumed that all values were in those intervals, the final crop yield was lower for T0 (control) and for the dried pig manure treatments (T3-T5) (Sánchez-Báscones et al. 2019), so these limits may not be a good indicator of the nutritional status of the crop, in line with the results obtained by Rutkowska (2013).

Evolution of N:P ratio during plant growth

The dynamics of the N:P ratio in different crops show a certain similarity, in such a way that the evolution of the N:P ratio during plant growth remains approximately constant, with values between 11.8 and 5.8 in mass. This relationship is influenced by biomass growth, with lower values when the biomass is higher or the level of nutrients in the soil is lower (Greenwood et al. 2008).

The N:P ratio is reduced to a constant value during the growth phase of barley crop. The values obtained by the treatments with mineral fertilization were close to those estimated by Greenwood et al. (2008), while those for the control soil and for organic fertilization treatments were lower in the first stadiums. In the DMP fertilization treatments, the lower N:P ratio would be due to a tendency to absorb more P than necessary to meet immediate needs and store it (Bollons and Barraclough 1999). It is worth noting that the N:P ratio in the treatments with DPM was not influenced by the amount of DPM applied.

Conclusions

Two types of fertilizers suitable for agricultural areas declared as vulnerable to pollution were tested at different doses over a three-year period in a nitrate vulnerable zone, assessing their effect on the content of macronutrients and sodium in barley. A greater variation was found in the initial stages of the growth cycle (tillering and stem elongation). Ca, K and Na concentrated in the plant, P and N concentrated in the grain, and Mg and C had similar concentrations in both grain and plant.

Mineral fertilization resulted in an increase in the N and C content both in plant and in grain, whereas the application of organic fertilization with dried pig manure increased the P and Na contents in plant (within normal values, according to the literature), reduced those of Ca and Mg (also in plant), and increased the P content in grain.

Appropriate fertilizer treatments in areas vulnerable to nitrate contamination should have low nitrate leaching, which may reduce the available nitrogen supply. ENTEC mineral fertilizer with 3,4-dimethylpyrazol phosphate (DMPP) resulted in optimum levels of nitrogen and an optimum crop performance in this agricultural system. On the other hand, the low N contents found in plant and grain for treatments with dried pig manure –similar to those of the control– would advise to complement dried pig manure fertilization with mineral fertilization, in order to compensate for

N immobilization, as N is a limiting factor to crop development. Other essential nutrients for crop development, such as assimilable P and K, were found at suitable levels for all treatments, although the N deficit associated with dried pig manure fertilization led to an increase in P content both in grain and in plant. With reference to the possible inhibition effect on Ca and Mg contents in plant resulting from the increase in Na content, and taking into consideration the values reported in the literature, the application of dried pig manure would not be problematic.

Acknowledgments

This work was supported by Castilla-y-León Regional Government (Regional Ministry of Environment).

Declaration of interest

The authors have no competing interests to declare.

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FIGURE CAPTIONS

Fig. 1 Temperature and rainfall over the three-year period (09/2009-08/2012) in the area of study.

Fig. 2 Average content (%) of C, N, P, K, Ca, Mg and Na in barley plant, over a three-year period, in four stages of its vegetative cycle (viz. tillering (SI), stem elongation (SII), heading (SIII) and ripening (SIV)) as a function of the type of fertilization treatment. T0: control soil; T1: mineral fertilizer at the usual dose applied in the region; T2: mineral fertilizer applied according to crop needs; T3: DPM at an application rate of 85 kg $N\cdot ha^{-1}\cdot yr^{-1}$; T4: DPM at an application rate of 133 kg $N\cdot ha^{-1}\cdot yr^{-1}$; T5: DPM at an application rate of 170 kg $N\cdot ha^{-1}\cdot yr^{-1}$. Average values for three years with 4 replicates per stage and treatment (*n*=12).

Fig. 3 Macronutrients and sodium contents in barley grain as a function of the fertilization treatment. Treatments labelled by the same letter are not significantly different according to Tukey's HSD test (significance at 0.05 level).

Fig. 4 N:P ratio in barley plant, over a three-year period, in four stages of its vegetative cycle (viz. tillering (SI), stem elongation (SII), heading (SIII) and ripening (SIV)) as a function of the type of fertilization treatment. T0: control soil without; T1: mineral fertilizer at the usual dose applied in the region; T2: mineral fertilizer according to crop needs; T3: DPM at an application rate of 85 kg $N\cdot ha^{-1}\cdot yr^{-1}$; T4: DPM at an application rate of 133 kg $N\cdot ha^{-1}\cdot yr^{-1}$; T5: DPM at an application rate of 170 kg $N\cdot ha^{-1}\cdot yr^{-1}$. Average values for three years with 4 replicates per stage and treatment (*n*=12).

TABLE CAPTIONS

Table 1 Soil physicochemical properties before the application of fertilizers.

Table 2 Dried pig manure physicochemical properties.

Table 3 Fertilizer application rates and nitrogen doses for the different treatments (Sánchez-Báscones et al. 2019).

Table 4 Significance of the effects of the year (Y), fertilizer treatment (T), stage of growth (S) and their interactions on the macronutrients and sodium content in barley plant.

Table 5 Significance of the effects of year (Y), fertilization treatment (T) and their interaction on the macronutrients and sodium contents in barley grain.

Table 6 Macronutrients and sodium contents reported in the literature for barley (plant, leaves, straw and grain)