

Agricultural sustainable production in vulnerable zones to nitrate pollution

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WHO (World Health Organization) establishes an illustrative threshold for nitrates in drinkable water of 50 mg L⁻¹. In order to reach this objective, Council Directive 91/676/EEC indicates that countries should define vulnerable zones, which are or could be affected by high nitrate levels and eutrophication. The aim of this research is to determine different fertilization treatments impact on yield and quality for two legume crops cultivated in a rotation system in vulnerable zones to nitrate pollution of La Rioja, northern Spain. Four fertilizing treatments were tested in two commercial plots for two years. One of them was the control treatment usually performed by the farmer, and the other treatments were designed to reduce N fertilization requirements looking to increase crop sustainability. Moreover, N amount in soil, in plant were measured weighing crop biomass and yield to analyse the effect of fertilizing treatment. Furthermore, harvest quality was assessed specifically for each crop such as tenderness for pea or seed to pod ratio for green bean. Each crop was monitored using Sentinel-2 satellite imagery and UAV multispectral imagery acquired during crop development. Results showed no significant differences between treatments in pea, whose yield varied from 9.4 ± 0.4 t ha⁻¹ to 13.4 ± 0.9 t ha⁻¹. Tenderness degree showed a bit high values which oscillated from 85 ± 4 to 122 ± 10 . Green bean crop provided larger but scattered yield values which changed from 6.6 ± 2.9 t ha⁻¹ to 18.2 ± 3.6 t ha⁻¹. Seed to pod ratio showed slight but opposed differences to yield varying from 2.8 ± 0.4 % to 6.0 ± 3.4 %. In conclusion, legume crops will be over fertilized even if nitrogen application is reduced between 59.6 % to 86.3 % during a one-year rotation.

1. Introduction

In the European Union, several policies have been developed and implemented to address nutrient pollution from agriculture. The Council directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources was adopted to protect water quality preventing nitrates from agricultural sources polluting by promoting the use of good farming practices. Pure and clean water is necessary to life development, for instance, it is recommended to consider guideline values for drinkable water not to exceed 50 mg L⁻¹ for nitrate and 3 mg L⁻¹ for nitrite (WHO, 2017). In the past years, water pollution from nitrates has been improving quickly, considering that in 1991 in Valencia several towns showed nitrate values over 150 mg L⁻¹ (Vitoria and Arias, 2000) while in 2016, only 1,6 % of analysed samples exceeded 150 mg L⁻¹ (Palau, 2016). However, other countries have a greater incidence of nitrate pollution in drinkable water (Vitoria et al., 2015) and desirable situation should avoid all pollution caused by nitrates from agricultural sources. Furthermore, nitrates could be present in different vegetables as contaminant in food. Although nitrates are relatively low toxic for humans, they can be reduced to nitrites within body causing lack of oxygen (Ranasinghe and Marapana, 2018). For this reason, European Commission in the regulation No 1258/2011 limit nitrate content, especially

for baby and young children food which should contain less than 200 mg nitrate kg⁻¹, which sometimes is exceeded in some leafy vegetables, although they accomplish limits for adult food (Pérez-Urrestarazu et al., 2019).

Nitrogen phosphorus and potassium are essential for modern farming to keep high productivity in order to feed the world increasing population. However, when nitrogen was not taken up by plants, it could cause environmental pollution through gaseous losses and leaching. In addition, nitrogen fertilizers help to feed around 48 % of the global population although a large portion of N fertilizers is lost (Erisman et al., 2008). To avoid these undesirable effects meeting both the Sustainable Development Goals related to agriculture and food demand projected by the Food and Agriculture Organization (FAO) new methods to manage nitrogen fertilization should be developed (Zhang et al., 2015).

Currently, herbaceous crops are grown in rotation systems that sometimes included more than one crop in only a year. Therefore, it is advisable to manage N fertilizing considering the whole rotation instead of looking at only one crop. Recommendations to reduce N leaching are the control of N surplus at the rotation level, use catch crops to reduce N leaching (De Notaris et al., 2018), avoid N fertilizing before winter rainfalls (Libutti and Monteleone, 2017) and prevent farmers from N fertilizer overusing (Muratoglu, 2020).

The aim of the present research is to determine different fertilization treatments impact on yield and quality for two legume crops (pea and green bean) cultivated in a rotation system in vulnerable zones to nitrate pollution, assessing the impact on crops production, quality, and soil nitrogen content by sampling and by imagery analysis.

2. Materials and methods

During 2019 and 2020, nitrogen fertilization was monitored in 2 different commercial plots in La Rioja, northeast Spain. These commercial plots were planted with an herbaceous crop rotation that included pea and green bean in the same year (Table 1).

Table 1: Plot location and crop rotation in each plot and year.

Plot	Nearest village	UTM coordinates (x; y) (m)	2019 crop	2020 crop
1	Bañares	506,168; 4,700,856	Pea Green bean	Wheat
2	Castañares	507,322; 4,705,331	Wheat	Pea Green bean

Plots were placed nearby each other, all of them in a Mediterranean climate. Daily weather data were obtained from a weather station placed at Garu-Cooperative (504,346.3 X; 4,698,650.7 Y) which provided air temperature, relative humidity, rainfall, solar radiation, wind speed and Penman-Monteith reference evapotranspiration. All plots were formed on an Entisol order soil according to U.S. Soil Taxonomy without differentiated layers. Soil samples were taken and analysed before tests started to describe soil properties. Despite soil did not show differentiated layers, two samples at two different depths were analysed per sampling point in order to look for any physical or chemical difference that may determine nitrogen movement through soil (Table 2).

Table 2: Soil analysis results at the beginning of the tests.

Plot	Depth (cm)	Texture (USDA)	pH	EC ¹	N total (‰)	C/N	CEC ² (meq 100 g soil ⁻¹)	OM ³ (%)
1	0-30	Loam	6.0	0.2	0.1	8.3	5.7	1.9
	30-60	Loam	5.8	0.1	0.1	7.3	4.3	1.7
2	0-30	Clay loam	6.3	0.1	1.4	8.3	8.0	2.1
	30-60	Clay loam	6.4	0.1	1.5	7.7	8.3	1.9

¹ Electrical Conductivity (dS m⁻¹)

² Cation Exchange Capacity

³ Organic Matter

2.1 Experimental design

For each considered plot, 3 decreasing nitrogen fertilization treatments (T1, T2 and T3) were applied and the farmer decision-based treatment (T0) was also considered to compare the potential variation of results. In each plot, 1152 m² were selected and divided into 16 experimental units or plots of 72 m² to obtain 4 replications for

plot and treatment. Treatments were distributed following a strip block design to enhance the statistical significance of results. Pea and green bean were irrigated using sprinklers spaced 12 x 18 m. Different nitrogen granular fertilizers were applied to the soil surface at different dates and quantities for each plot and crop (Table 3). Statistical analysis was performed using SPSS (SPSS Inc., Armonk, USA.) including one data per variable, experimental unit, and sampling date.

Table 3: Nitrogen amount applied for each plot, crop, and date for 3 decreasing nitrogen fertilization treatments (T1, T2, T3) and farmer decision-based treatment (T0).

Plot	Year and crop	Nitrogen application	Date	T0 kg N ha ⁻¹	T1 kg N ha ⁻¹	T2 kg N ha ⁻¹	T3 kg N ha ⁻¹
1	2019 pea	1 st Fertilization ¹	16/02	44	44	44	44
		Total		44	44	44	44
	2019 green bean	1 st Fertilization ²	20/07	65	24	24	0
		2 nd Fertilization ²	8/08	0	24		
		Total		65	48	24	0
	2020 wheat	1 st Fertilization ^{3, 2}	27/02	65	48	35	21
		Total		65	48	35	21
	2019 wheat	1 st Fertilization ⁴	6/01	71	71	71	71
		Total		71	71	71	71
	2020 pea	1 st Fertilization ^{1, 2}	14/02	34	27	20	13
		Total		34	27	20	13
2	2020-green bean	1 st Fertilization ²	13/07	61	40	20	0
		Total		61	40	20	0

¹ 1,5 % nitric, and 6.5 % ammoniacal nitrogen

² 13.5 % nitric, and 13.5 % ammoniacal nitrogen. In 2020, when compound fertilizer was applied to T0, in order to apply same amount of phosphorus and potassium, T1, T2, and T3 were fertilized using a mix between fertilizer 2 (straight nitrogen fertilizer), phosphorous straight fertilizer and potassium straight fertilizer.

³ 5.1 % nitric, and 7.9 % ammoniacal nitrogen

⁴ 1,5 % nitric, and 4.5 % ammoniacal nitrogen

2.2 Soil and plant measurements

For the 2 plots, 4 soil samples were taken from seeding to harvesting of each crop in order to analyse nitrate, ammonia and gravimetric humidity content. Each replication was sampled separately considering two soil layers: 0 – 0.3 m depth and 0.3 – 0.6 m depth, considering that crop rotation included shallow and medium rooting plants. 1 M KCl was used for ammonia extraction by spectrophotometric analysis using an enzymatic multianalyzer equipment (Analyzer Y15, Biosystems, Barcelona, Spain), Nitric nitrogen was measured using reflectometry (Nitrachek 404, Eijkelpamp, Giesbeek, The Netherlands) and plant nitrogen content was measured by Kjeldahl method (Harwitte,1990).

Each crop was monitored through a sampling schedule based on the phenological stages from emergence to harvesting. Plant and soil samples were taken at the same date to determine soil nitrogen content along to plant nitrogen content. Fresh and dry biomass production was also measured excluding roots by uprooting 1 m² of plants, weighting, drying the sample in a stove at 65 °C up to weigh stop decreasing. Moreover, nitrogen content in plant samples was analysed considering pods and rest of plant separately. Only in the last sampling date, just before harvesting, pods were manually separated from the sample. They were weighted and dried to determine fresh and dry yield along with nitrogen content for each plant part.

Each crop was monitored using Sentinel-2 satellite imagery and UAV multispectral imagery acquired during crop development. Acquired images were processed to determine the Normalized Difference Vegetation Index (NDVI) and Normalized Difference Red Edge Index (NDRE).

2.3 Commercial harvesting measurements

Harvesting was carried out according to Garu Cooperative guidelines when each product quality was optimal. A sample was taken from each experimental unit, harvesting 1 m² for pea and green bean. Using these data, crop yield was calculated in t ha⁻¹. Harvest quality was also assessed by measuring tenderness degree for peas using a tenderometer (4001, FMC, Philadelphia, USA) and seed to pod ratio for green beans.

3. Results and discussion

Despite the highly variable results, for pea and green bean marketable yield (Figure 1) did not provide significant differences ($\bar{A} < 0.05$) neither did quality. Results indicated that a reduction in nitrogen fertilization of 56.6 % (from 109 kg N year⁻¹ to 44 kg N year⁻¹) in 2019 and 86.3 % (from 95 kg N year⁻¹ to 13 kg N year⁻¹) in 2020 did not significantly influence pea nor green bean yield (Table 4). Pea yield showed stable values, while green bean yield provided high variability for both marketable yield and seed to pod ratio, which did not show significant differences. In 2020, one hailstorm affected each crop, 9th of May for pea and 9th of July for green bean which were emerging. Therefore, this accident enlarged marketable yield variability, mainly for pea and reduced yield for the green bean.

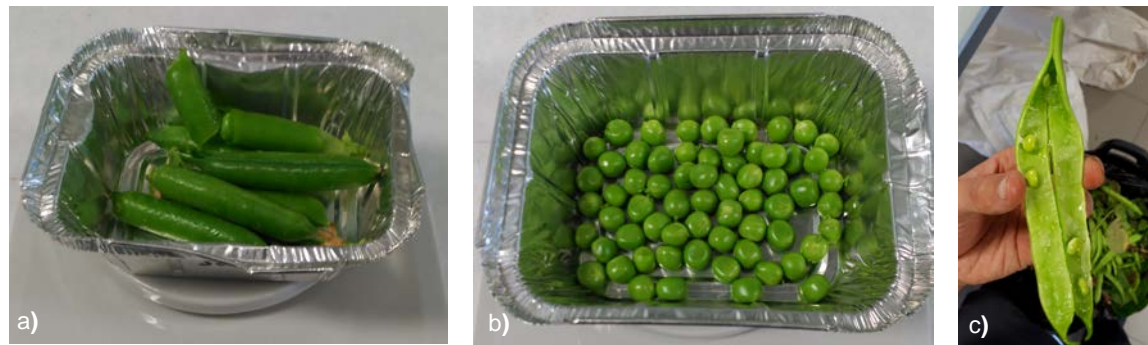


Figure 1: Pea pods (a), pea seeds which were considered as marketable yield (b) and unfold green bean with very few and little seeds (c). For green bean, the whole pod was considered as marketable yield.

Table 4: Marketable harvest and quality measurements on pea and green bean rotation. Different letters indicate significant differences ($\bar{A} < 0.05$) between treatments for the same plot and the same year according to Tukey's test.

Plot	Pea	Green bean			
Year					
	Treatment	Marketable yield (t ha ⁻¹)	Tenderness degree	Marketable yield (t ha ⁻¹)	Seed to pod ratio (%)
	T0	9.581 ± 0.458 a	89 ± 8 a	14.720 ± 2.921 a	2.83 ± 0.44 a
Plot 1	T1	9.418 ± 0.358 a	85 ± 9 a	14.207 ± 7.936 a	4.33 ± 0.53 a
2019	T2	9.590 ± 0.197 a	92 ± 6 a	13.293 ± 3.790 a	4.38 ± 1.27 a
	T3	9.718 ± 0.439 a	85 ± 4 a	18.167 ± 3.550 a	6.02 ± 3.35 a
	T0	13.324 ± 1.678 a	122 ± 10 a	8.872 ± 1.988 a	3.79 ± 0.5 a
Plot 2	T1	13.399 ± 0.939 a	116 ± 6 a	9.421 ± 1.817 a	4.07 ± 0.84 a
2020	T2	11.738 ± 2.485 a	120 ± 13 a	7.264 ± 3.024 a	4.68 ± 0.37 a
	T3	12.927 ± 0.776 a	113 ± 5 a	6.571 ± 2.870 a	4.57 ± 0.76 a

It is remarkable, that in plot 1 for the lowest nitrogen fertilization, which corresponded with 0 kg N ha⁻¹ applied in green beans, seed to pod ratio provided higher values. It should be considered that previously this treatment was fertilized with 44 kg N ha⁻¹ applied in previous rotation crop (pea). In plot 1, it stood out lower seed to pod ratio provided by farmer decision-based treatment, which was almost three times in the lowest nitrogen fertilization. These results are in accordance or slightly lower than seed to pod ratio provided by previous research, which varies from 6 to 10 % while marketable yield was around or slightly lower than the range comprised between 16 t ha⁻¹ to 20 t ha⁻¹ (Ferreira et al., 2006). Further research should be conducted to obtain more consistent results related to crop yield and quality.

Previous research indicates that yield and quality for peas is highly season dependent (Nicolas Tremblay and Desjardins, 2006) in accordance with results shown in table 4. Furthermore, peas showed high phenotypic plasticity (Dener et al., 2016) related to variable nitrogen availability, specially in 2020, when pea nitrogen fertilization was reduced by 61.7 % from farmer decision-based treatment (T0) to lowest fertilization treatment (T3) without showing appreciable yield differences while green bean provide larger differences. This fact may also be due to the fact, that beans have less nitrogen-fixing capacity than peas (Hossain et al., 2016), along with pea crop is able to show less sensibility to a reduction of assimilate availability than wheat or other crops (Sandana et al., 2009). Finally, pea quality was highly linked to tenderness degree which depends on water availability, temperature, and crop phenologic stage. For this reason, climate change could affect future

maturation of pea as it occurs in other crops as vines, for which it is modelled that phenology and quality properties will be affected by climate change (Ramos and Martinez de Toda, 2020).

As it is known, the pea harvest index increases linearly with days after flowering and with thermal time after flowering (Lecoeur and Sinclair, 2001). In the tested plots, harvest index at harvesting did not show significant differences ($\bar{A} < 0.05$) between fertilization treatments for the same plot and year, varying from 0.25 (T3) to 0.33 (T2) for the pea and from 0.43 (T2) to 0.34 (T3) for the green bean. On the other hand, pod biomass, plant biomass and total biomass did not provide any significant differences ($\bar{A} < 0.05$) between fertilization treatments for the same plot and year. Pod biomass oscillated between 3.7 t ha⁻¹ (T0, 2020) and 3.2 t ha⁻¹ (T2, 2020) in pea and between 0.7 t ha⁻¹ (T3, 2020) and 1.4 t ha⁻¹ (T2, 2019) for green beans. Pea total biomass decreased from 11.8 t ha⁻¹ (2019) to 6.1 t ha⁻¹ (2020) mainly due to, the effect of a hailstorm occurred 9th of May, although it did not affect significantly pod biomass nor did affect marketable yield. Finally, plant nitrogen content did not reveal any significant differences ($\bar{A} < 0.05$) between fertilization treatments for the same plot and year. For instance, for pea in 2020 plant nitrogen content varied from 3.5 ± 0.2 % (T0) to 3.7 ± 0.1 % (T1) while for green bean in 2020 plant nitrogen content varied from 1.8 ± 0.3 % (T3) to 2.1 ± 0.5 % (T2).

Soil nitrate-N content did not show significant differences ($\bar{A} < 0.05$) in plot 1 in 2019 due to data scattering because soil nitrate-N content varied from 154 ± 76 (T0) to 377 ± 206 (T2), while in plot 2 in 2020, significant differences were found in shallow layer for the pea and deeper for the green bean. These differences were not completely correlated with nitrogen fertilizer applied but it may describe the nitrate movement from shallow to deeper soil layer (Table 5), including the great amount of pea straw that was laid on the soil after pea harvesting which could mineralize easily due to low C/N ratio (Li et al., 2020).

Table 5: Soil nitrate-N content in the two considered soil layers. Different letters indicate significant differences ($\bar{A} < 0.05$) between treatments for the same plot and the same year according to Tukey's test.

Plot		Pea		Green bean	
Year	Treatment	N-NO ₃ (0-30 cm)	N-NO ₃ (30-60 cm)	N-NO ₃ (0-30 cm)	N-NO ₃ (30-60 cm)
Plot 2 2020	T0	20 ± 3 ab	15 ± 2 a	19 ± 6 a	49 ± 16 ab
	T1	25 ± 7 b	17 ± 4 a	115 ± 63 a	68 ± 17 ab
	T2	18 ± 2 ab	19 ± 6 a	82 ± 74 a	76 ± 20 b
	T3	16 ± 0 a	15 ± 2 a	34 ± 10 a	39 ± 15 a

Finally, plot acquired imagery provide no apparent differences between fertilization treatments neither for NDVI nor for NDRE indexes which only discern cover soil from bare soil (Figure 2). This fact was in accordance with no significant differences neither in yield nor in biomass production, which could be due to over fertilization. Previous research did provide a reduction in nitrogen efficiency use when a legume was included in rotation with other winter crops (Falgowska et al., 2019) which could contribute to 15 % of nitrogen demand in an intercropped cereal (Kakraliya et al., 2018).

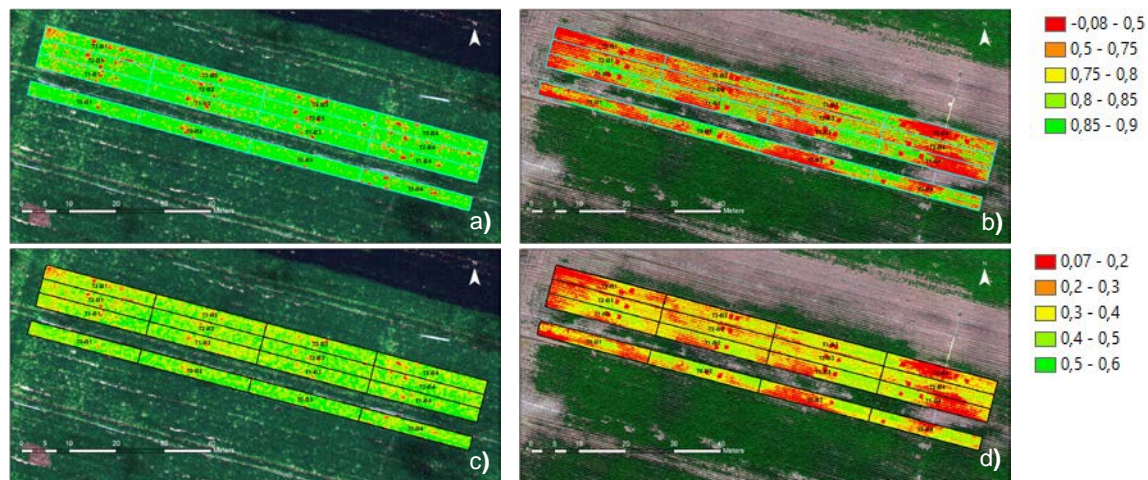


Figure 2: Plot 2 in 2020 image analysis before harvesting. Images were analysed using Normalized Difference Vegetation Index (NDVI) (a and b) and Normalized Difference Red Edge Index (NDRE) (c and d) for pea crop (a and c) and bean crop (b and d).

4. Conclusions

In conclusion, pea and green beans crops will be over fertilized even if nitrogen application is reduced between 59.6 % to 86.3 % during a one-year rotation. To reach this conclusion, four different nitrogen fertilization strategies were assessed in two plots and two different years on a pea and green bean one-year rotation. Significant differences were not found for marketable yield and quality due to nitrogen fertilization. However, soil nitrogen content did provide significant differences in the shallow soil layer at pea harvesting (earlier crop) while at green bean harvesting these differences were moved to the deeper soil layer, indicating nitrogen leaching process. Acquired imagery were also analysed to find differences between treatments but it only provided differences between cover soil and bare soil using both NDVI and NDRE indexes. However, it could be stated that all nitrogen fertilization treatments exceed the optimal value, therefore, differences cannot be found for sampling and imagery analysis. Further research should be conducted to establish optimal nitrogen fertilization to avoid environmental impact achieving a sustainable production on vulnerable zones to nitrate pollution.

Acknowledgments

Authors thanks La Rioja regional government which funds Nitrocon operational group of the European Innovation Partnership (EIP-AGRI). This group was also co-funded by the Spanish Ministry of Agriculture, Fisheries and Food, and the European Union. Authors also acknowledge the collaboration of AIMCRA to perform nitrogen analysis.

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