



# Winter wheat genotype ability to recover nitrogen supply by precedent crops under combined nitrogen and water scenarios

M.D. Raya-Sereno<sup>a</sup>, J.L. Pancorbo<sup>a</sup>, M. Alonso-Ayuso<sup>b</sup>, J.L. Gabriel<sup>a,c</sup>, M. Quemada<sup>a,\*</sup>

<sup>a</sup> Department of Agricultural Production, CEIGRAM. Universidad Politécnica de Madrid, Madrid, Spain

<sup>b</sup> Soil and Water Department, Estación Experimental de Aula Dei (EEAD), Spanish National Research Council (CSIC), Zaragoza, Spain

<sup>c</sup> Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, CSIC), Madrid, Spain

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## ABSTRACT

Legumes offer the opportunity to reduce the nitrogen (N) fertilizer requirements of the crop rotation but the capacity of the subsequent crop to recover the N provided by the legume determines the fertilizer saving. Therefore, breeding for genotypes capable of take up N under different limiting conditions is essential for understanding crop performance and optimizing fertilization. The objective of this work was to test the ability of four wheat (*Triticum aestivum* L.) genotypes with different drought-tolerance (Cellule, Apache, Allez-y, and Nogal) to take up N from the precedent legume under different water scenarios. Two field experiments with pea-wheat and barley-wheat crop rotations, two irrigations levels, and three N treatments were conducted in central Spain over 3 years. The biomass, N uptake, and nitrogen nutrition index (NNI) were determined at flowering, and the grain yield (GY), grain N concentration (GNC), and N output at harvest. The precedent legume crops enabled a reduction in N fertilizer, but its effect depended on the genotype and climate conditions. The drought-tolerant genotypes (Apache and Cellule) had the highest NNI, biomass, GY, and N output under low water and N inputs, whereas Allez-y and Nogal obtained better performance under non-limiting conditions. Therefore, this study confirms differences between wheat genotypes, with different root system, in N uptake from the precedent legume in a crop rotation under different water levels and highlights the potential of NNI to assess wheat N status.

## 1. Introduction

Conventional agriculture, which is strongly dependent on external nitrogen (N) mineral fertilization, represents 75% of agricultural land available in the European Union (Eurostat, 2019). However, the N mineral fertilizers increase CO<sub>2</sub> emissions together with soil and environmental degradation as a result of their production, distribution, and application (Peoples et al., 2004). To reduce fertilizer application, it is time to reconsider the legume-based crop rotations, given that only 1.9% of arable land in Europe is allocated to legumes (Eurostat, 2019). However, a crucial aspect in making certain that legumes contribute to the sustainability of the cropping system is to ensure that the subsequent crop in the rotation can use the N introduced by the previous legume into the soil by the atmospheric biological fixation (Quemada et al., 2020).

The possibility of using genotypes capable of improving N recovery has received considerable attention in recent years (Noureddin et al.,

2013). Wheat is a major source of vegetable protein for humans worldwide, and it is important to search for the best-performing genotypes that can yield high levels of production by increasing the efficiency of N use. Generally, plant breeders assume that direct selection for grain yield (GY) will indirectly select varieties with the optimum root system that make it possible to overcome common growth limitations, such as N and water availability (Wasson et al., 2012). Evidence for this comes from several experiments in wheat (Li et al., 2019b), maize (Gao and Lynch, 2016), and barley (Robinson et al., 2018) showing that deeper roots improve capture of N and water, and consequently increase the GY. However, there is no guarantee that a genotype with high GY under non-limiting N or water conditions also performs well in low-input or stressed environments (Bustos-Korts et al., 2018). Therefore, efforts in breeding simultaneously for N and water need to be strengthened as the relevance of colimitation has been emphasized, particularly in Mediterranean environments (Cossani and Sadras, 2018; Quemada and Gabriel, 2016).

\* Corresponding author.

E-mail address: [miguel.quemada@upm.es](mailto:miguel.quemada@upm.es) (M. Quemada).

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The colimitation principles state that GY is higher when N and water are both equally available (Cossani et al., 2010). Drought may cause nutrient deficiencies, even in fertilized fields, as the physicochemical properties of the soil can lead to reduced mobility and absorbance of individual nutrients (Ammann and Blatt, 2009). In this sense, numerous studies have been conducted to improve drought resistance in wheat by identifying quantitative trait loci (QTLs) and increasing their frequency in breeding germplasm. Touzy et al. (2019) used modeling and statistical approaches to identify the specific drought-tolerant QTLs in a panel of 210 elite European genotypes in 35 fields. The assessment of water stress dynamics in each environment led to clustering of four water stress scenarios: optimal condition with no water stress, post-anthesis water stress, moderate-anthesis water stress, and high pre-anthesis water stress. The genotypes were tested in all scenarios, and the authors reported that the environmental clustering improved our understanding of the impact of drought on wheat GY, which explained 20% of the genotype (G)  $\times$  environment (E) interaction. Moreover, they claimed that their results enable breeders to introduce drought-resistant genotypes to specific environmental conditions in which other limitations, such as nutrients, could be present. Therefore, in this research we explored four bread wheat genotypes based on different drought-tolerance scenarios according to the results reported by Touzy et al. (2019) to test the adaptability of the genotypes to using N under various conditions of water and N availability. The genotypes selected were Apache as tolerant, Cellule as intermediate, and Allez-y and Nogal as susceptible to water stress.

It is difficult to predict at the field scale the N available after a legume or other precedent crop because the available N is constantly changing due to agricultural management, soil properties, and environmental conditions (Raya-Sereno et al., 2022). Measuring soil inorganic N before fertilizing is not sufficient for assessing the N provided by the previous crop, since the mineralization of N over the growing season continues and contributes to N uptake of crops (Quemada et al., 1997). Given the relevance of the N supplied for GY and grain N concentration (GNC), indicators of the N status of crops have been developed to assess optimal N fertilization and crop performance under different N-availability conditions. Among these indicators, the nitrogen nutrition index (NNI) is the most widely used. It compares the N concentration in the leaves and shoots (%N) with the critical N concentration ( $N_c$ ) at a given biomass (Justes et al., 1994). The  $N_c$  is calculated based on the critical dilution curve (CDC) that relates the %N to the biomass (Greenwood et al., 1990), and CDC equations were developed for many crops including bread wheat (Justes et al., 1994; Hoogmoed and Sadras, 2018; Pancorbo et al., 2021). Based on the CDC, the N status of wheat can be monitored by continuous determination of %N in a sample of aerial biomass.

The main objectives of this work were to test the ability of four wheat genotypes with different drought-tolerance to take up N from the precedent legume under two water levels. The specific objectives focus on (i) comparing the effect of a legume versus a non-legume precedent crop on the agronomic performance of different wheat genotypes, and (ii) detecting which genotypes have a greater capacity to take up N from precedent legumes.

## 2. Materials and methods

### 2.1. Field experiment

Two field experiments were carried out over three consecutive years: 2018–2020 and 2019–2021 (hereinafter referred to as “Exp 1” and “Exp 2”, respectively) at the research station La Canaleja (40°31'N, 03°18'W, 600 m a.s.l.) located in Alcalá de Henares (Madrid, Spain). The experiments were conducted in the same field, but in plots separated by 50 m. Plots were fallow for a minimum of 1 year and had not received organic amendments or N fertilizer during the 3 years before the start of the experiment. According to Köppen, the climate of the area is classified as

hot-summer Mediterranean climate (Csa). The mean annual temperature is 13.5 °C and the mean annual rainfall is 440 mm (1950–2015). Precipitation occurs mainly in autumn and spring. Meteorological data were recorded by a weather station located near the experimental field (< 1 km). The soil of the experimental field is Calcic Cambisol (WRBRSR, 2014) and has loam-sandy texture in the two upper horizons (0.4 m), loamy down to 1 m, and sandy below. The topsoil (0.20 m) has 4.18 g organic C kg<sup>-1</sup>, 0.45 g organic N kg<sup>-1</sup> of soil, and a pH of 8.1.

Four bread wheat (*Triticum aestivum* L.) genotypes (Cellule, Apache, Allez-y, and Nogal) were tested on their capacity to recover N supply after cropping a legume (pea, *Pisum sativum* L.) or a non-legume (barley, *Hordeum vulgare* L.). The precedent crops were randomly distributed in 16 plots (20  $\times$  12.5 m each plot) and their residues were buried into the soil at the end of May in both seasons. A four-replicated split-plot experiment with 192 plots (2.8  $\times$  4.2 m each plot) was designed considering precedent crops as the main factor and wheat genotype, N fertilization, and irrigation as secondary factors. The wheat genotypes selected were sown at the beginning of November on the same plots as the precedent crops at a seed rate of 160 kg ha<sup>-1</sup>. Borders were included between precedent crops and irrigation treatments. At the end of January, wheat plots were split into three N fertilization levels, and half of the experiment was divided into irrigated plots and the other half into rainfed plots. Thus, an interaction of N and water levels was created for each genotype (Fig. 1).

Nitrogen fertilizer was split into two applications and hand-broadcast to wheat plots in two growth stages (GS) (Meier, 1997): at tillering (GS22; 23/01/2020 and 29/01/2021) and at the beginning of stem elongation (GS32; 04/03/2020 and 11/03/2021). Each subplot received no N (N0), conventional fertilizer calcium ammonium nitrate (27% N) at the reduced rate (N1: 25 kg N ha<sup>-1</sup> after pea and 75 kg N ha<sup>-1</sup> after barley precedent crops), or the recommended rate (N2: 75 kg N ha<sup>-1</sup> after pea and 125 kg N ha<sup>-1</sup> after barley precedent crops) (Fig. 1). To calculate the recommended and reduced N rates, composite soil samples (two soil cores per plot) were taken before the first fertilization at depths of 0–0.2, 0.2–0.4, and 0.4–0.6 m. Soil subsamples were extracted with 1 M KCl (~30 g of soil: 150 ml of KCl), centrifuged, and stored in a freezer until later analysis. Nitrate (NO<sub>3</sub>-N) concentration was measured with the Griess-Ilosvay method (Keeney and Nelson, 1982) in the extracts, and ammonium (NH<sub>4</sub><sup>+</sup>-N) via the salicylate-hypochlorite method (Crooke and Simpson, 1971). The soil NO<sub>3</sub>-N and NH<sub>4</sub><sup>+</sup>-N content was determined accounting for the bulk density (1.3 g cm<sup>-3</sup>) and summed to obtain the inorganic N content ( $N_{min}$ ) for each layer and plot. The recommended fertilizer rates were calculated in Exp 1 as the expected GY (5000 kg ha<sup>-1</sup>) multiplied by an extraction coefficient (30 kg N/Mg grain), and were corrected by the  $N_{min}$  in the top 0.6 m of soil before the first fertilizer application and by the N supply from the mineralization of the precedent crop residues (2/3 N content in the aboveground residue). Fertilizer rates in Exp 2 were the same as in Exp 1. Additionally, soil  $N_{min}$  was determined following the same methodology in samples taken before wheat sowing to identify differences in the N provided by the two precedent crops. Soil tests showed that P and K levels were suitable for crop development.

Irrigation water was delivered using a mobile cannon sprinkler system to match crop evapotranspiration calculated using daily local climate data. In Exp 1, half of the plots received 15 mm of water on 12 March and 30 mm on 18 and 25 May. In Exp 2, half of the plots were irrigated in seven events with 10 mm for each one (on 26 and 30 March, 7 April, and 17, 18, 24, and 31 May). Total water input was 449 mm during the 2019–2020 season and 399 mm during the 2020–2021 season for the wheat crop (Fig. S1). Weed control was achieved by herbicide (Intensity + Broadway) application for the control of broad leaves and grassy weed. The barley-wheat and pea-wheat rotations of each experiment and the management plans are presented in Fig. 2.

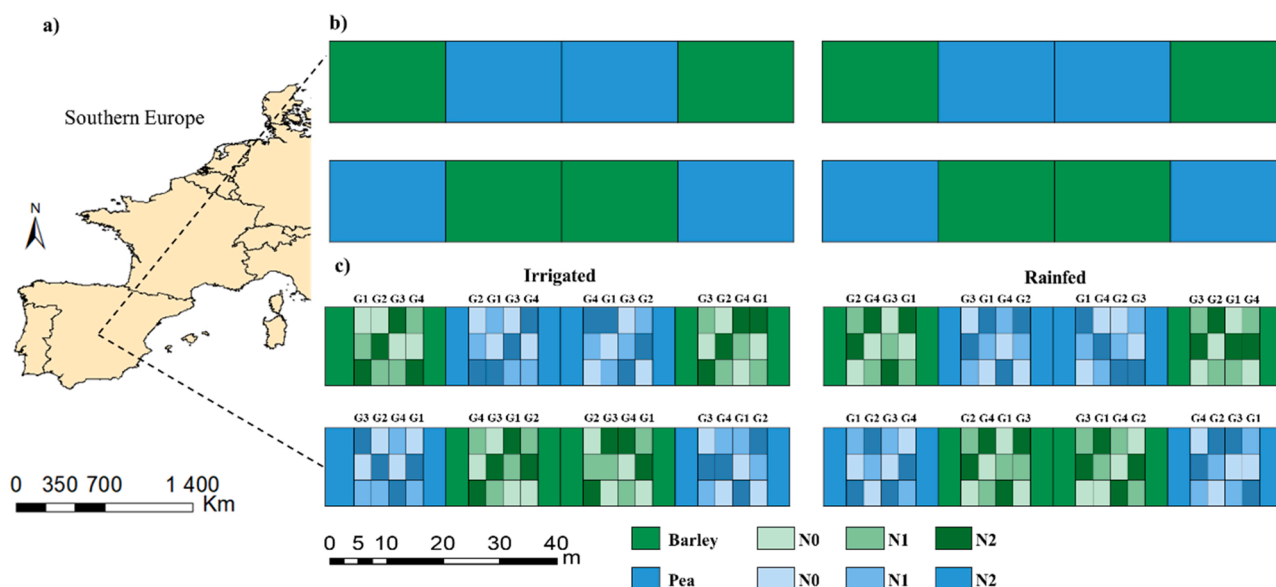


Fig. 1. Location of the experiment (a), different precedent crops established in 2018 and 2019 (b), and split-plot experimental design with four different wheat genotypes (G1, G2, G3, G4), three different N rates (N0, N1, N2), and two water levels (Irrigated, Rainfed) in 2020 and 2021 (c).

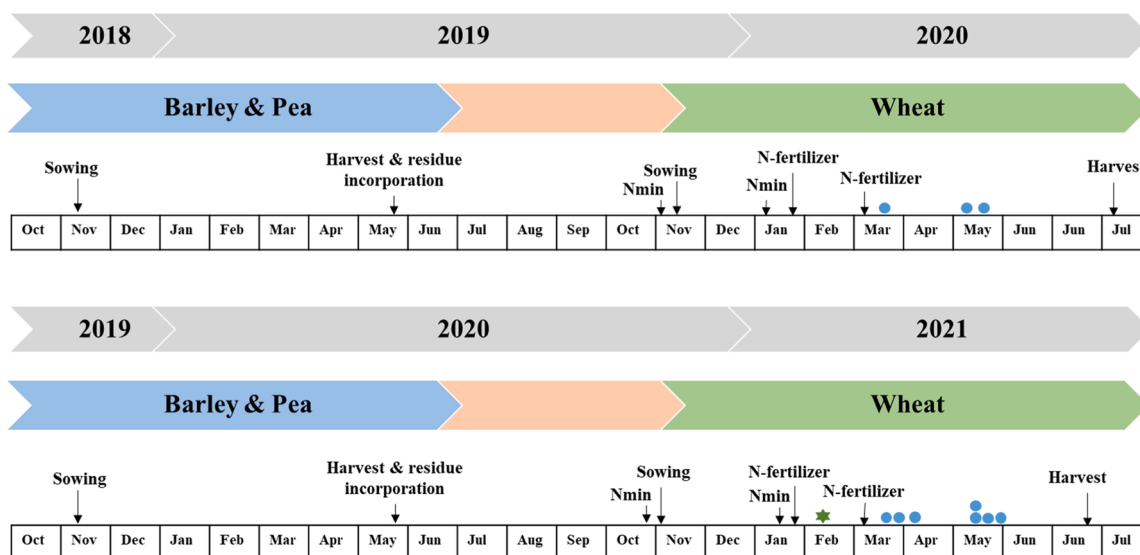


Fig. 2. Barley–wheat and pea–wheat rotations and management plans established in both seasons. The green star represents the herbicide application, and the blue circles the irrigation events.

## 2.2. Crop analysis

At the end of spring in both experimental seasons, a subsample per plot ( $0.35 \times 0.35$  m) of precedent crops (pea and barley) was hand-harvested before being buried into the soil, oven-dried ( $65^\circ\text{C}$ ), weighed, and ground for later analysis. In subsequent wheat genotypes, the effect of precedent crop background on biomass, N uptake, GY, GNC, and N output was examined. At flowering, a sample of wheat plants ( $0.25 \times 0.25$  m) was hand-harvested in unfertilized plots (N0) and in the plots that received  $75\text{ kg N ha}^{-1}$  in both precedent crops (N1-barley and N2-pea), and a subsample of each aerial plant component (spikes and the rest of the aboveground biomass) was oven-dried, weighed, and ground. In summer (10/07/2020 and 29/06/2021), a 1.4-m-wide central strip was harvested from all plots with an experimental combiner, and the wheat yield was recorded. A grain subsample from each plot was oven-dried, weighed, ground, and saved for analysis.

The total N concentration (%N) of the precedent crops and the wheat components was determined through the Dumas combustion method (LECO FP-428 analyzer, St. Joseph, MI, USA). The N content of each precedent crop and wheat component was calculated by multiplying its dry biomass ( $\text{kg ha}^{-1}$ ) by its N concentration. The total wheat crop N uptake ( $\text{kg N ha}^{-1}$ ) was calculated by adding the N content in both plant components. The NNI was calculated as the ratio between the actual crop N concentration and the critical N concentration that enables maximum growth for a given biomass (Justes et al., 1994). The critical N concentration was calculated following the N dilution curve developed by Pancorbo et al. (2021) for winter wheat under similar environmental conditions. At harvest, wheat N output ( $\text{kg N ha}^{-1}$ ) was calculated as the product of GY ( $\text{kg ha}^{-1}$ ) multiplied by GNC.

Agronomy efficiency ( $\text{AE}_\text{N}$ ) was calculated at harvest as the ratio of the difference between the GY of a treatment and the average GY of the control, to the N fertilizer applied (Ladha et al., 2005; Alonso-Ayuso

et al., 2016). This component of the N use efficiency refers to the kg of crop yield increase obtained per kg of N applied.

### 2.3. Statistical analysis

Statistical analysis was carried out to assess the potential of different genotypes to take up N from precedent crops and fertilization. For this, after verification of data normality and variance homogeneity, different wheat agronomic variables were analyzed using a linear mixed model. For each experiment, precedent crop, water, genotype, and N treatments, as well as the interaction between them, were considered as fixed factors, whereas the subplot was considered as a random effect for the analysis of variance. The mean was separated by Tukey's test at the 0.05 probability level ( $P \leq 0.05$ ). Additionally, as all the interactions including the experiment factor were significant for most of the variables analyzed, the statistical analysis of each experiment (Exp 1 and Exp 2) was performed separately using the same methodology. All statistical analyses were performed using the software R (version 4.1.1; R core Team, 2021).

## 3. Results

### 3.1. Precedent crop and soil $N_{min}$

The total dry matter of the precedent crop at harvest was lower in Exp 1 (2.6 Mg ha<sup>-1</sup> for pea and 3.2 Mg ha<sup>-1</sup> for barley) than in Exp 2 (5.9 Mg ha<sup>-1</sup> for pea and 5.8 Mg ha<sup>-1</sup> for barley), probably due to the low rainfall (194 mm) during the 2018–2019 season compared to that of 2019–2020 (374 mm), which limited crop growth (Fig. S1). Similarly, the N content in the precedent crop residues was lower in Exp 1 (69.2 kg N ha<sup>-1</sup> for pea and 19.8 kg N ha<sup>-1</sup> for barley) than in Exp 2 (131.1 kg N ha<sup>-1</sup> for pea and 41.5 kg N ha<sup>-1</sup> for barley), and the precedent pea crop reached higher values than barley in both experiments (Fig. 3).

Before wheat sowing, the  $N_{min}$  in the upper 0.6 m was higher after the precedent pea crop than after barley, with larger differences in Exp 2 (Fig. 4a). In Exp 1, the  $N_{min}$  after the precedent pea crop was 56.7 kg N ha<sup>-1</sup> and after barley it was 42.1 kg N ha<sup>-1</sup>. In Exp 2, the  $N_{min}$  after the precedent pea crop was 111 kg N ha<sup>-1</sup> and after barley it was 39.1 kg N ha<sup>-1</sup>. The differences in  $N_{min}$  between precedent crops remained until the first wheat topdressing, but the data suggested that in Exp 2 wheat sown over pea plots took up more N than in Exp 1 (Fig. 4b). In this sense, the  $N_{min}$  was similar in both experiments (49.6 kg N ha<sup>-1</sup> in Exp 1 and 58.8 kg N ha<sup>-1</sup> in Exp 2 after precedent pea crop, and 31.8 kg N ha<sup>-1</sup> in Exp 1 and

24.9 kg N ha<sup>-1</sup> in Exp 2 after barley).

### 3.2. Wheat analysis

The analysis of variance indicated significant ( $P \leq 0.05$ ) interactions between the main factor precedent crop and the experiment in all variables studied, whereas other main factors such as genotype, water level, and N fertilization showed an interaction with the experiment in some of the variables analyzed (Table S2). Since all significant interactions included the experiment, the results from Exp 1 and Exp 2 were examined separately.

#### 3.2.1. Effects of main factors

The impact of the precedent crop was observed at wheat flowering and harvest for all variables studied in Exp 2, except for GNC at harvest: The biomass, N uptake, NNI, GY, and N output were higher in wheat sown over precedent pea than over precedent barley (Table 1). However, no differences were found in Exp 1.

The irrigated treatment led to higher biomass and N output than the rainfed condition in both experiments, and also to higher GY in Exp 2 (Table 1). This indicated that wheat suffered higher water stress under rainfed conditions, although precipitation in both seasons was higher than average (Fig. S1). No effect of water level on N uptake, NNI, or GNC was observed in any of the experiments.

Increasing N levels had a positive effect on all wheat variables studied in the two experiments (Table 1). At flowering, all variables distinguished between the two N levels sampled: unfertilized (N0) and fertilized treatment (N1-barley and N2-pea fertilized with 75 kg N ha<sup>-1</sup>). The three N rates were differentiated by GY in Exp 1, by GNC in Exp 2, and by N output in both experiments. However, GY in Exp 2 only distinguished between unfertilized (N0) and fertilized treatments (N1 and N2).

Finally, the response of wheat genotypes varied between Exp 1 and Exp 2 (Table 1). At flowering, differences among genotypes were found in biomass and N uptake in Exp 1. The genotypes Cellule and Apache achieved higher biomass than the Allez-y and Nogal genotypes. The N uptake was again higher in Apache than in Allez-y and Nogal, while the uptake for Cellule was in between. At harvest, differences were found in GY for both experiments, with Cellule, Apache, and Allez-y obtaining higher GY than Nogal in Exp 1; whereas in Exp 2, Cellule obtained the maximum GY followed by Apache, Nogal, and Allez-y. A similar response to GY was found for N output in Exp 2, whereas no differences between genotypes were shown in Exp 1. In contrast, the GNC response was inverse to GY and N output, with Nogal obtaining the highest values in both experiments (Table 1). Additionally, Exp 1 experienced N deficiency ( $NNI \leq 0.80$ ) in both water levels and all genotypes, while in Exp 2  $NNI$  was  $\geq 0.92$  in all genotypes in rainfed and irrigated treatments. Overall, Cellule and Apache presented a higher response to N fertilizer application in most of the variables studied (except in GNC) than Allez-y and Nogal. However, the interaction between factors was significant and therefore it was analyzed.

#### 3.2.2. Effects of precedent and water factors with genotypes

A significant interaction was observed among genotypes, water level, and experiment factors in all variables studied at flowering (Table S2). In rainfed plots, Apache obtained the highest biomass (12 Mg ha<sup>-1</sup>) in Exp 1 and Cellule in Exp 2 (22 Mg ha<sup>-1</sup>), whereas Nogal was the genotype with the lowest biomass in both experiments (Table 2). In irrigated plots, a clear difference among genotypes was observed in Exp 1, forming two groups, with Cellule and Apache having greater biomass than Allez-y and Nogal, while no differences between genotypes were found in Exp 2 (Table 2). When analyzing N uptake and NNI, a similar response was found in Exp 1: the Apache genotype presented the highest values and Nogal the lowest. By contrast, in Exp 2 no differences among genotypes were found in N uptake, whereas NNI was the highest in the Nogal genotype.

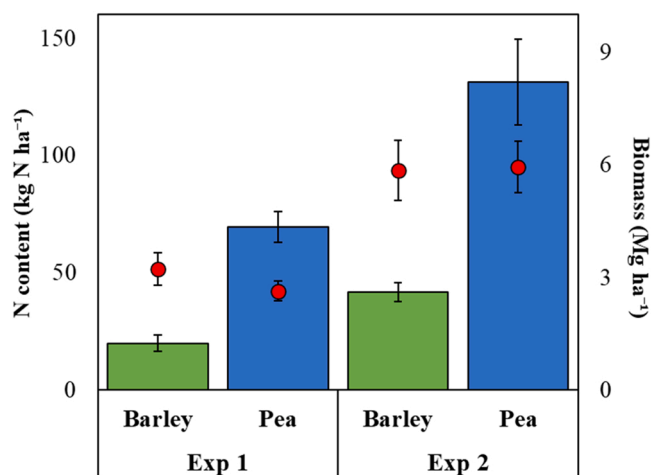


Fig. 3. Nitrogen content (bars, kg N ha<sup>-1</sup>) and biomass accumulated (red circles, Mg ha<sup>-1</sup>) at harvest for barley and pea precedent crops in both experiments. Error bars in red circles and in bars represent standard error.

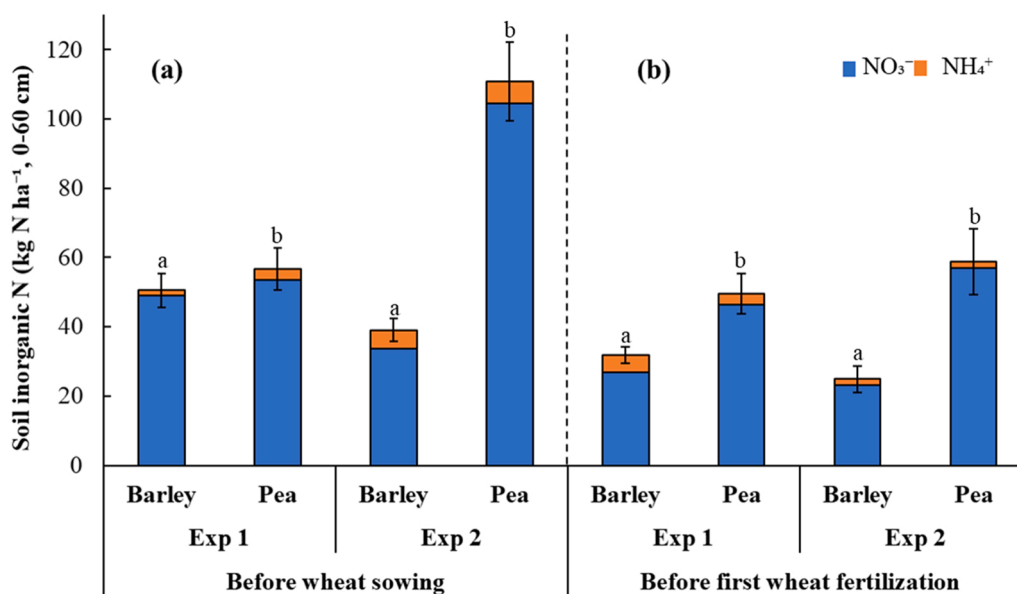


Fig. 4. Soil inorganic N content ( $N_{min}$ ) in the 0–60-cm layer (a) before wheat sowing and (b) before first wheat fertilization measured in both experiments. Treatments on the x-axis correspond to precedent crops used in the previous season. Letters above bars indicate significant differences between previous crop treatments in the same experiment according to Tukey’s test ( $P \leq 0.05$ ). Small bars above bars indicate the standard error.

Table 1

Wheat total biomass ( $Mg\ ha^{-1}$ ), N uptake ( $kg\ N\ ha^{-1}$ ), and nitrogen nutrition index (NNI) at flowering as well as and grain yield ( $Mg\ ha^{-1}$ , 14% moisture), grain N concentration (GNC, %), and N output ( $kg\ N\ ha^{-1}$ ) at harvest according to precedent (barley and pea), water (rainfed and irrigated), nitrogen (N0, N1, and N2), and genotype (Cellule, Apache, Allez-y, and Nogal) factors in both experiments. Treatment N0 received  $0\ kg\ N\ ha^{-1}$  as fertilizer, N1  $75\ kg\ N\ ha^{-1}$  (after barley) or 25 (after pea), and N2  $125\ kg\ N\ ha^{-1}$  (after barley) or 75 (after pea).

	Exp 1					Exp 2						
	Flowering			Harvest		Flowering			Harvest			
	Biomass	N uptake	NNI	Grain Yield	GNC	N output	Biomass	N uptake	NNI	Grain Yield	GNC	N output
	$Mg\ ha^{-1}$	$kg\ N\ ha^{-1}$		$Mg\ ha^{-1}$	%	$kg\ N\ ha^{-1}$	$Mg\ ha^{-1}$	$kg\ N\ ha^{-1}$		$Mg\ ha^{-1}$	%	$kg\ N\ ha^{-1}$
<b>Precedent</b>												
Barley	10.7	114	0.73	3.94	1.79	62.2	16.9 a	176 a	0.89 a	3.26 a	1.90	53.8 a
Pea	11.1	116	0.74	3.54	1.64	50.9	24.3 b	291 b	1.24 b	4.77 b	1.93	78.0 b
<b>Water</b>												
Rainfed	10.1 a	101	0.67	3.44	1.64	49.4 a	18.6 a	207	1.01	3.47 a	1.94	57.7 a
Irrigated	11.7 b	129	0.80	4.04	1.79	63.6 b	22.6 b	260	1.12	4.56 b	1.89	74.1 b
<b>Nitrogen</b>												
N0	8.8 a	90 a	0.63 a	2.78 a	1.56 a	37.7 a	17.0 a	179 a	0.90 a	3.40 a	1.77 a	52.5 a
N1	–	–	–	3.97 b	1.62 a	56.0 b	–	–	–	4.29 b	1.87 b	67.9 b
N2	–	–	–	4.47c	1.97 b	75.9c	–	–	–	4.35 b	2.10c	77.4c
N1 Bar & N2 Pea	13.0 b	143 b	0.85 b	–	–	–	24.2 b	288 b	1.23 b	–	–	–
<b>Genotypes</b>												
Cellule	11.9 b	118 ab	0.71	4.0 b	1.62 a	57.8	21.1	202	0.92	5.1c	1.78 a	78.0c
Apache	12.9 b	137 b	0.80	3.9 b	1.68	59.0	21.9	232	1.03	4.2 b	1.86 a	66.8 b
					ab							
Allez-y	9.6 a	107 a	0.72	3.7 b	1.73 bc	57.2	20.0	244	1.11	3.2 a	1.97 b	54.2 a
Nogal	9.0 a	99 a	0.70	3.2 a	1.83c	52.1	19.4	255	1.19	3.6 ab	2.06 b	64.8 ab

Within a column, experiment, sampling date, and variable, the values for each factor studied followed by different letters are significantly different according to Tukey’s test ( $P \leq 0.05$ ). At flowering, measurements were only collected in plots that were fertilized with the same rate ( $75\ kg\ N\ ha^{-1}$ ) in both precedent crops (N1 Bar & N2 Pea).

At harvest, differences between genotypes appeared (Table 2). In Exp 1, the GY in rainfed treatment showed the same performance as biomass at flowering for the genotypes Apache, Allez-y, and Nogal. By contrast, Cellule obtained the highest GY, showing a greater capacity to maintain yield under water stress conditions. Nogal obtained the lowest GY under both rainfed and irrigated conditions. In Exp 2, Cellule obtained the highest GY under rainfed conditions and the highest N output, reinforcing the greater capacity to adapt to water stress conditions observed in Exp 1. Lastly, in the irrigated treatment, two groups were

differentiated, with Cellule and Apache having greater GY and N output than Allez-y and Nogal. Nevertheless, these differences were not evident in N output in the rainfed and irrigated conditions in Exp 1, where  $NNI < 0.9$  for all genotypes (Table 2). Additionally, inverse differences were found in GNC, with Nogal showing higher values than Cellule in irrigated and rainfed treatments in both experiments. Overall, all genotypes obtained higher values in Exp 2 than in Exp 1. Since the water input received by wheat was similar in both experiments, this variation was probably due to differences in the precedent crop effect.

**Table 2**

Wheat total biomass ( $\text{Mg ha}^{-1}$ ), N uptake ( $\text{kg N ha}^{-1}$ ), and nitrogen nutrition index (NNI) at flowering as well as grain yield ( $\text{Mg ha}^{-1}$ , 14% moisture), grain N concentration (GNC, %), and N output ( $\text{kg N ha}^{-1}$ ) at harvest according to the interaction between water levels (rainfed and irrigated) and genotype ( $W \times G$ ) factors in both experiments.

	Exp 1						Exp 2					
	Flowering			Harvest			Flowering			Harvest		
	Biomass $\text{Mg ha}^{-1}$	N uptake $\text{kg N ha}^{-1}$	NNI	Grain Yield (14%) $\text{Mg ha}^{-1}$	GNC %	N output $\text{kg N ha}^{-1}$	Biomass $\text{Mg ha}^{-1}$	N uptake $\text{kg N ha}^{-1}$	NNI	Grain Yield (14%) $\text{Mg ha}^{-1}$	GNC %	N output $\text{kg N ha}^{-1}$
<b>W × G</b>												
<b>Rainfed</b>												
Cellule	10.0 ab	96	0.64	3.8 b	1.55 a	51.7	21.5 b	206	0.94	4.5 b	1.80 a	70.2 b
Apache	12.2 b	118	0.72	3.6 b	1.61 a	50.9	18.9 ab	211	1.02	3.3 a	1.90 ab	54.1 a
Allez-y	9.3 ab	94	0.66	3.4 ab	1.68 ab	50.6	17.4 ab	214	1.05	2.8 a	2.00	47.1 a
											BCE	
Nogal	8.9 a	95	0.67	3.0 a	1.73 b	44.4	16.9 a	197	1.01	3.4 a	2.06c	59.5 ab
<b>Irrigated</b>												
Cellule	13.8 b	139 ab	0.79 ab	4.3 b	1.70 a	63.9	20.8	198	0.92 a	5.6 b	1.76 a	85.7c
Apache	13.7 b	156 b	0.89 b	4.4 b	1.75 a	67.2	24.9	253	1.03 ab	5.1 b	1.81 ab	79.4 BCE
Allez-y	10.0 a	119 ab	0.78 ab	4.0 b	1.78 ab	63.7	22.7	275	1.17 ab	3.6 a	1.93 bc	61.2 a
Nogal	9.2 a	104 a	0.72 a	3.5 a	1.93 b	59.7	21.9	314	1.38 b	3.9 a	2.06c	72.0 ab

Within a column, experiment, sampling date, and variable, the values for each factor studied followed by different letters are significantly different according to Tukey's test ( $P \leq 0.05$ ).

### 3.2.3. Effects of fertilization and N uptake from precedent crops by genotypes

The response to N fertilization became evident in the interaction between precedent crop  $\times$  N level at flowering and harvest (Table 3). At flowering, in both experiments, the biomass, N uptake, and NNI were higher in the fertilized treatments (N1 and N2) than in the unfertilized (N0) treatments (Table 3). At harvest, wheat GY showed a similar response to biomass and NNI over precedent barley in both experiments, indicating differences between unfertilized and fertilized treatments. Nevertheless, no differences in GY between N rates were found in Exp 2 after precedent pea crop, probably because the three N rates presented N sufficiency ( $\text{NNI} > 1$ ) (Table 3). Additionally, three groups were distinguished for N output in Exp 2 after barley and in Exp 1, but in Exp 2 after pea differences between N rates diminished and only two groups were distinguished. Finally, the GNC only distinguished the three N rates in precedent barley in Exp 2, whereas the rest of the treatments highlight the recommended rate (N2) with respect to reduced and unfertilized (N1 and N0) treatments.

Moreover, the response to precedent  $\times$  water  $\times$  nitrogen by different genotypes was observed when comparing similar N rates (N0 versus N1-barley and N2-pea that were fertilized with the same rate of  $75 \text{ kg N}$

$\text{ha}^{-1}$ ) at flowering and harvest (Table 4). In Exp 1, where all N0 treatments had  $\text{NNI} \leq 0.83$ , the GY responded to fertilizer application in both precedent and water treatments (Fig. 5a). By contrast, in Exp 2 the fertilization response was clear after precedent barley, where all N0 treatments had  $\text{NNI} \leq 0.84$ , whereas no response was found after precedent pea as all the genotypes had  $\text{NNI} \geq 0.91$  in the N0 treatment (Table 4; Fig. 5b). In Exp 2 after barley, the GY response to N fertilizer depended on the genotype, as Cellule and Nogal had the same slope under rainfed and irrigated conditions, showing that were able to take up N under water shortage better than Apache and Allez-y. Additionally, Cellule slope was steeper than Nogal, indicating higher capacity to take up N (Fig. 5b). Overall, when differences between genotypes appeared, Cellule showed higher GY than the others in all treatments (Cellule yield like Apache and Allez-y after rainfed and fertilized barley in Exp 1), achieving the highest value in the pea crop with irrigated and fertilized treatment in Exp 2 ( $6.8 \text{ Mg ha}^{-1}$ ) (Table 4).

Apart from that, the  $\text{AE}_N$  in Exp 2 was clearly related to NNI and precedent crop, decreasing when NNI at flowering increased. After pea precedent, the NNI was  $\geq 0.91$  in all genotypes and the  $\text{AE}_N$  was lower than after barley precedent. Even negative efficiencies were obtained for Apache and Allez-y after the pea precedent (Fig. 6). After barley ( $\text{NNI} \leq$

**Table 3**

Wheat total biomass ( $\text{Mg ha}^{-1}$ ), N uptake ( $\text{kg N ha}^{-1}$ ), and nitrogen nutrition index (NNI) at flowering as well as grain yield ( $\text{Mg ha}^{-1}$ , 14% moisture), grain N concentration (GNC, %), and N output ( $\text{kg N ha}^{-1}$ ) at harvest according to the interaction between precedent crop and nitrogen (PC  $\times$  N) factors in both experiments. Treatment N0 received  $0 \text{ kg N ha}^{-1}$  as fertilizer, N1  $75 \text{ kg N ha}^{-1}$  (after barley) or 25 (after pea), and N2  $125 \text{ kg N ha}^{-1}$  (after barley) or 75 (after pea).

	Exp 1						Exp 2					
	Flowering			Harvest			Flowering			Harvest		
	Biomass $\text{Mg ha}^{-1}$	N uptake $\text{kg N ha}^{-1}$	NNI	Grain Yield (14%) $\text{Mg ha}^{-1}$	GNC %	N output $\text{kg N ha}^{-1}$	Biomass $\text{Mg ha}^{-1}$	N uptake $\text{kg N ha}^{-1}$	NNI	Grain Yield (14%) $\text{Mg ha}^{-1}$	GNC %	N output $\text{kg N ha}^{-1}$
<b>PC × N</b>												
<b>Barley</b>												
N0	8.2 a	83 a	0.62 a	2.67 a	1.60 a	37.1 a	12.1 a	116 a	0.71 a	2.09 a	1.74 a	32.1 a
N1	13.3 b	145 b	0.85 b	4.51 b	1.69 a	65.5 b	21.7 b	236 b	1.06 b	3.70 b	1.86 b	58.4 b
N2	–	–	–	4.65 b	2.09 b	83.9c	–	–	–	3.99 b	2.11c	71.0c
<b>Pea</b>												
N0	9.4 a	91 a	0.63 a	2.89 a	1.52 a	38.3 a	21.9 a	241 a	1.09 a	4.72	1.80 a	73.0 a
N1	–	–	–	3.43 b	1.54 a	46.5 b	–	–	–	4.88	1.88 a	77.4 ab
N2	12.7 b	141 b	0.84 b	4.28c	1.85 b	67.9c	26.8 b	341 b	1.39 b	4.71	2.10 b	83.8 b

Within a column, experiment, sampling date, and variable, the values for each factor studied followed by different letters are significantly different according to Tukey's test ( $P \leq 0.05$ ). At flowering, measurements were only collected in plots that were fertilized with the same rate ( $75 \text{ kg N ha}^{-1}$ ) in both precedent crops (N1 Barley and N2 Pea).

**Table 4**

Wheat total biomass ( $\text{Mg ha}^{-1}$ ), N uptake ( $\text{kg N ha}^{-1}$ ), and nitrogen nutrition index (NNI) at flowering as well as grain yield ( $\text{Mg ha}^{-1}$ , 14% moisture), grain N concentration (GNC, %), and N output ( $\text{kg N ha}^{-1}$ ) at harvest according to the interaction between precedent, water, nitrogen, and genotype (PC  $\times$  W  $\times$  N  $\times$  G) factors in both experiments. W1 refers to rainfed and W2 to rainfed and irrigated treatments. N0 refers to unfertilized and Fert to N1-barley and N2-pea that were fertilized with the same rate of  $75 \text{ kg N ha}^{-1}$ .

	Exp 1						Exp 2					
	Flowering			Harvest			Flowering			Harvest		
	Biomass	N uptake	NNI	Grain Yield (14%)	GNC	N output	Biomass	N uptake	NNI	Grain Yield (14%)	GNC	N output
$\text{Mg ha}^{-1}$	$\text{kg N ha}^{-1}$		$\text{Mg ha}^{-1}$	%	$\text{kg N ha}^{-1}$	$\text{Mg ha}^{-1}$	$\text{kg N ha}^{-1}$		$\text{Mg ha}^{-1}$	%	$\text{kg N ha}^{-1}$	
<b>PC <math>\times</math> W <math>\times</math> N <math>\times</math> G</b>												
<b>Barley – W1 – N0</b>												
Cellule	7.5	76	0.57	2.4	1.5 ab	31.9	14.8	146	0.80	2.2	1.6 a	32.4
Apache	9.1	78	0.61	2.7	1.4 a	33.1	12.1	134	0.84	2.0	1.7 ab	30.7
Allez-y	7.6	72	0.56	2.0	1.4 a	23.1	9.1	98	0.69	1.5	1.8 ab	22.3
Nogal	6.6	63	0.54	2.1	1.8 b	29.4	12.2	134	0.84	2.3	2.0 b	39.6
<b>Barley – W1 – Fert</b>												
Cellule	14.4 ab	148	0.84	4.7 b	1.5	60.1	19.8	167	0.81	4.8 b	1.7 a	68.6
Apache	16.1 b	164	0.81	4.9 b	1.6	65.7	18.8	186	0.91	2.6 a	1.8 ab	39.2
Allez-y	9.8 a	98	0.68	4.5 b	1.8	68.3	19.8	235	1.09	2.5 a	2.00 ab	39.5
Nogal	10.9 ab	136	0.89	2.8 a	1.8	44.8	17.8	217	1.07	3.2 ab	2.1 b	57.1
<b>Barley – W2 – N0</b>												
Cellule	9.8	91	0.62	2.9	1.7	42.6	7.9	62	0.49	2.3	1.6 a	35.0
Apache	11.7	135	0.83	3.8	1.6	50.3	17.5	133	0.68	3.0	1.7 ab	43.7
Allez-y	6.2	75	0.64	2.9	1.6	41.6	11.9	102	0.63	1.7	1.7 ab	24.9
Nogal	6.8	71	0.59	2.6	1.9	41.9	11.1	122	0.77	2.3	2.2 b	40.4
<b>Barley – W2 – Fert</b>												
Cellule	16.1	160	0.87	5.1	1.6 a	68.9	24.0	188	0.82 a	5.0 b	1.6 a	71.2
Apache	15.4	186	1.02	5.1	1.6 ab	71.4	28.4	283	1.11 ab	4.7 ab	1.9 ab	77.8
Allez-y	12.0	128	0.77	4.8	1.8 ab	72.8	21.2	261	1.18 ab	3.7 ab	1.8 ab	58.5
Nogal	11.5	144	0.91	4.2	2.0 b	71.9	22.3	228	1.53 b	3.1 a	2.1 b	55.3
<b>Pea – W1 – N0</b>												
Cellule	8.0	65	0.50	2.8	1.5	34.8	26.1 b	266	1.11	5.3 b	1.7	79.7
Apache	9.9	87	0.59	3.1	1.2	32.6	21.0 ab	195	0.91	3.9 ab	1.6	53.7
Allez-y	6.7	68	0.57	2.7	1.4	32.9	16.9 ab	199	1.01	3.3 a	1.8	57.6
Nogal	7.5	66	0.52	2.2	1.5	27.9	15.5 a	172	0.95	3.6 ab	1.9	52.7
<b>Pea – W1 – Fert</b>												
Cellule	10.2	97	0.65	4.9 b	1.6 a	66.6	25.1	245	1.06	5.6 b	2.0	95.2
Apache	13.5	144	0.85	3.4 a	2.0 b	58.3	23.6	331	1.42	3.6 a	2.3	71.6
Allez-y	13.0	139	0.85	3.9 ab	1.8 ab	61.5	24.0	278	1.42	3.5 a	2.2	66.3
Nogal	10.8	115	0.74	3.8 ab	1.9 ab	59.9	21.3	252	1.18	3.4 a	2.2	64.6
<b>Pea – W2 – N0</b>												
Cellule	15.6 ab	133	0.74	3.3	1.6	48.5	25.6	266	1.11	6.7 b	1.7	99.7
Apache	16.5 b	108	0.70	3.5	1.5	43.4	21.6	250	1.07	5.8 ab	1.7	85.9
Allez-y	11.7 ab	122	0.80	3.2	1.8	49.5	25.1	290	1.24	4.6 a	1.9	75.8
Nogal	10.6 a	81	0.62	2.5	1.8	39.4	22.2	287	1.28	4.6 a	2.0	79.4
<b>Pea – W2 – Fert</b>												
Cellule	13.9 b	171 ab	0.92	4.9	1.7	69.5	26.3	280	1.14 a	6.8 b	1.9 a	108.9 b
Apache	11.1 ab	193 b	1.02	4.9	2.0	84.5	31.9	347	1.28 a	5.6 ab	2.0 ab	98.3 ab
Allez-y	10.1 ab	152 ab	0.92	4.6	2.0	78.1	32.5	445	1.63 ab	4.2 a	2.1 ab	73.1 a
Nogal	8.1 a	118 a	0.79	3.8	1.9	54.7	31.4	440	1.96 b	4.9 a	2.2 b	92.2 ab

Within a column, experiment, sampling date, and variable, the values followed by different letters are significantly different according to Tukey's test ( $P \leq 0.05$ ).

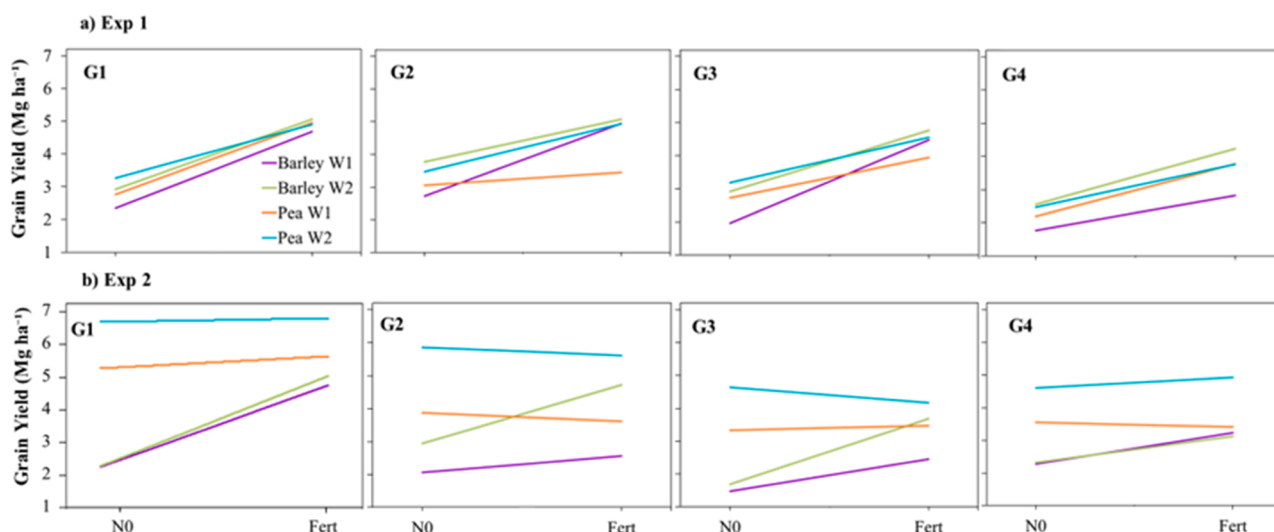
0.84 in unfertilized treatments) and rainfed conditions, Cellule obtained higher  $\text{AE}_N$  than the other genotypes, reinforcing its better performance under limiting conditions.

Overall, irrigated treatments showed higher NNI than rainfed, and wheat after pea precedent had higher NNI than after barley. In accordance, the GY response to fertilizer application was higher after barley than after pea, particularly in Exp 2 (Tables 2 and 4). Additionally, under irrigated conditions in Exp 2 differences between genotypes were highlighted, and Nogal obtained the highest NNI after barley (1.53) and pea (1.96) precedent, followed by Allez-y (Table 4). These high NNI in Nogal at flowering was linked to high GNC at harvest (2.1% after barley and 2.2% after pea) but not to high N output and showed that GNC was affected by genotype (Nogal tended to have higher GNC whereas Cellule

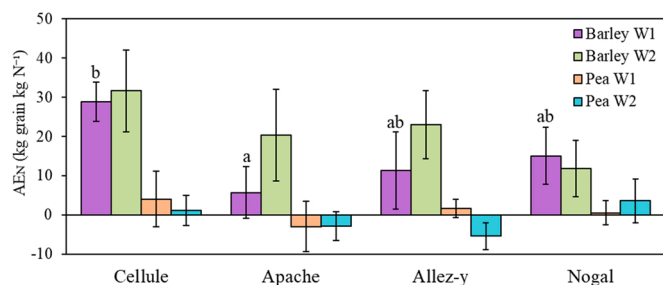
lower). The pattern followed by the N output was similar to the GY for all treatments and genotypes (Fig. S3). Therefore, these results show that the wheat N response depended on the precedent crop and on water level in both experiments, and the response was determined by the genotype in Exp 2.

#### 4. Discussion

The agronomic performance of crops is regulated by the genotype (G), the environment (E), and their management (M), as well as the interactions among these factors (Passioura and Angus, 2010). In the current work, we compared genotypes with a different root architecture, under two environments characterized by the varying weather



**Fig. 5.** Wheat grain yield ( $\text{Mg ha}^{-1}$ ) interaction between precedent crop (barley or pea), water, and nitrogen factors at harvest in all genotypes (G1: Cellule, G2: Apache, G3: Allez-y, G4: Nogal) for both experiments. W1 is rainfed and W2 is irrigated treatment. Treatment N0 received  $0 \text{ kg N ha}^{-1}$  and treatment fertilized (Fert) received  $75 \text{ kg N ha}^{-1}$  corresponding to N1 for barley and N2 for pea precedent treatment.



**Fig. 6.** Wheat agronomy efficiency ( $\text{AE}_N$  ( $\text{kg grain kg N}^{-1}$ )) in all genotypes (Cellule, Apache, Allez-y, Nogal), separated by precedent crop (barley or pea) and water level in fertilized treatment (N1-barley and N2-pea that were fertilized with the same rate of  $75 \text{ kg N ha}^{-1}$ ) at harvest in Exp 2. W1 is rainfed and W2 is irrigated treatment. Letters above bars indicate significant differences between genotypes according to Tukey's test ( $P \leq 0.05$ ). Small bars above bars indicate the standard error.

conditions of both experimental periods, and with management strategies that combined different precedent crops, N fertilizer rates, and water availability. The current experiment shows that, despite the strong interactions associated with the combinations of the various factors, two genotypes (Cellule and Apache) had a higher capacity to take up N from the precedent crop and tolerate water stress, probably due to a more developed root system able to explore a larger soil volume and to make better use of the resources (Touzy et al., 2019).

The total amount of water input (irrigation and rainfall) and its distribution were similar over both wheat-growing seasons; however, the low rainfall during 2018–2019 limited the growth of both barley and pea precedent crops and created relevant differences in the soil conditions for the subsequent wheat growth in both experiments. The difference in the availability of N in soil before sowing wheat between precedent pea and precedent barley crops was small in Exp 1 but 2.5-fold greater for the pea crop in Exp 2, and these results may explain the different response in subsequent wheat agronomic variables reported in our study. Most researchers reported that a precedent legume crop increases the performance of subsequent cereals compared to non-legume crops (Angus et al., 2015; Gan et al., 2015). However, the response to N fertilization of the subsequent wheat will greatly depend on the N supply from the precedent crop (Cernay et al., 2018), and the N supply might be low if the precedent crop growth is limited.

Differences in biomass and GY between genotypes were found, both in precedent barley and precedent pea crops. These differences were more noticeable in irrigated than in rainfed treatments in both experiments. When the water  $\times$  precedent interaction occurred, the genotypes Cellule and Apache obtained higher biomass and GY than Allez-y and Nogal in most cases, not only under irrigated but also under rainfed conditions. This is in agreement with the higher tolerance of Cellule and Apache to water stress (Touzy et al., 2019), associated with a deeper root system that explores a larger soil volume (Li et al., 2019). In general, the high water-extraction capacity is also associated with greater nutrient forage (Gao and Lynch, 2016; Robinson et al., 2018; Li et al., 2019); however, the capacity to transform the absorbed N in yield and grain protein depends on other genotypic characteristics (Ortiz-Monasterio et al., 1997). In Exp 2 in which the N provided by the precedent pea crop was high, the Cellule genotype showed its high capacity to translocate the absorbed N to the grain and increase GY, achieving the highest N output and  $\text{AE}_N$  among all genotypes. Non-destructive techniques (i.e., sensors) that allow monitoring crop N status from flowering to grain filling should be implemented to detect different performances between genotypes in the N translocation (Raya-Sereno et al., 2021).

The GNC was higher for the Nogal genotype than for Cellule. The common negative relationship between GNC and GY is due to competition between carbon and N for energy (Munier-Jolain and Salon, 2005) and to a N dilution effect by carbon-based compounds (Acreche and Slafer, 2009). A way to counteract this negative correlation is ensuring N availability after flowering, for instance, delaying the last fertilizer application to the heading stage showed an increase in GNC without a reduction in GY (Bogard et al., 2010). However, this approach is highly dependent on climatic conditions, in particular water availability during the post-anthesis period, and thus it may lead to less efficient N uptake and may boost N losses with multiple environmental consequences (Bogard et al., 2010).

It is important to note that achieving an adequate increase in GY and GNC simultaneously remains a challenge for wheat breeders and producers, and tools that enable the assessment of both variables are needed. However, these agronomic variables do not allow knowing if the different genotypes satisfy their own N demand, which makes it difficult to explore which genotype reaches the maximum performance with the lowest N demand. To overcome this limitation, several authors found that the NNI is the best crop N status indicator to compare different genotypes and crop performance since it includes both N supply and N demand (Lemaire and Ciampitti, 2020; Ciampitti and



Lemaire, 2022). Besides, differences between genotypes could also be explained by the genotypic variability in the tolerance of abiotic constraints. In this regard, Bogard et al. (2010) indicated that the differential access of wheat to soil N may be explained by mechanisms that allow for a higher tolerance or avoidance of water deficit, which may result in a greater N uptake after anthesis.

Water deficit induces a reduction in plant N demand because it reduces biomass growth and affects to metabolic and structural plant components (Sadras and Lemaire, 2014). For this reason, drought induces a depletion in N availability and as consequence a reduction in the crop NNI (Kunrath et al., 2018). This reduction is caused by water deficit through two processes: i) lowering crop N demand linked to the reduction in crop mass accumulation due to plant water stress, and ii) limiting soil N availability associated with soil water restriction (Pandey et al., 2000). In this sense, our experiments agreed with the literature, showing lower NNI in rainfed than in irrigated treatments (Kunrath et al., 2020). Moreover, although in Exp 1 all genotypes suffered N-deficiency (NNI < 1) even in irrigated conditions, the genotypes Cellule and Apache obtained the highest NNI and also achieved the highest biomass and GY. However, in Exp 2 the response was different, obtaining Nogal in irrigated treatment the highest NNI value (1.38) followed by Allez-y (1.17). This disagreement between experiments can be explained by the interaction with the N provided by the precedent crop. Whereas in Exp 1 all genotypes suffered N-deficiency after barley and pea precedent crops, in Exp 2 only the unfertilized barley treatment showed N-deficiency in all genotypes under rainfed and irrigated conditions. As we discussed previously, in Exp 2 more N available from the pea precedent crop was present into the soil, providing a greater N supply. This statement was also emphasized by the  $AE_N$ , since it decreased in wheat after pea precedent. Accordingly, the water  $\times$  precedent interaction highlights that Cellule and Apache were the genotypes that tended to reach the highest NNI under rainfed and low N supply conditions, therefore, they had the lowest N fertilizer demand for reaching their maximum GY. However, the genotypes Allez-y and Nogal achieved their best NNI performance under non-limiting conditions.

Breeding progress in recent decades has been focused on choice genotypes capable of providing the highest biomass or GY under non-limiting conditions. However, this breeding pressure increases crop N demand without corresponding crop N uptake capacity, leading to a high discrepancy between N demand and N supply, and consequently implying large N fertilization application (Ciampitti and Lemaire, 2022). Additionally, as proposed by Bustos-Korts et al. (2018), this breeding methodology does not ensure that a genotype with high performance under non-limiting scenarios also performs well in environments with low inputs. For these reasons, recent studies proposed focusing the efforts on increasing N uptake capacity under limited resources, allowing a sustainable crop improvement (Touzy et al., 2019; Lemaire and Ciampitti, 2020; Ciampitti and Lemaire, 2022). Following this rationale, we can deduce that the higher NNI achieved by Cellule and Apache under stressed conditions was mainly due to their higher capacity to take up N, in turn associated with a deeper root system. This result agrees with those found by Touzy et al. (2019), which showed that Cellule and Apache were tolerant to drought, whereas Nogal and Allez-y were susceptible to water stress. Although complex, breeding research should be following this line, improving root health for increasing plant capacity to take up N.

## 5. Conclusions

Legumes had a positive effect on the biomass, NNI, GY, GNC, and N output of subsequent wheat genotypes, but this depended on the experimental year. Moreover, the crop rotation including pea as precedent crop enriched the soil with inorganic N compared to the rotation that included barley as precedent crop in Exp 2. The crop N status determined through NNI allows separating the variability of the effect of water and precedent interactions. Its determination demonstrated that

Cellule and Apache reached the best performance under low N supply compared with Allez-y and Nogal which performed well under non-limiting conditions of N and water. Moreover, Cellule was the genotype, followed by Apache, that obtained higher biomass, GY, and N output (both over precedent pea and precedent barley) than Allez-y and Nogal in both experiments. These results highlight the capacity of two genotypes previously selected as water stress tolerant, Cellule and Apache, to take up N and therefore to reduce N fertilizer demand. Moreover, Cellule showed a greater ability to translocate the absorbed N to the grain. By contrast, the GNC was lower for Cellule and higher for Nogal in both experiments. Overall, when the growth of the previous legume is appropriate and a relevant N amount is supplied, it is possible to reduce or avoid N mineral fertilization if a genotype with high potential to take up N is used, minimizing economic and environmental impacts.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2022.108758.

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