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Soil Properties for Predicting Soil Mineral Nitrogen Dynamics Throughout a Wheat Growing Cycle in Calcareous Soils

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Abstract: A better understanding of the capacity of soils to supply nitrogen (N) to wheat can enhance fertilizer recommendations. The aim of this study was to assess the soil mineral N (N_{min}) dynamics throughout the wheat growing season in crucial stages for the plant yield and grain protein content (GPC). To this aim, we evaluated the utility of different soil properties analyzed before sowing: (i) commonly used soil physicochemical properties, (ii) potentially mineralizable N or N_o (aerobic incubation), and (iii) different extraction methods for estimating N_o. A greenhouse experiment was established using samples from 16 field soils from northern Spain. Wheat N uptake and soil N_{min} concentrations were determined at following growing stages (GS): sowing, GS30, GS37, GS60, harvest, post-harvest, and pre-sowing. Pearson's correlation analysis of the soil properties, aerobic incubations and chemical extractions with the soil N_{min} dynamics and N uptake, yield and GPC was performed. In addition, correlations were performed between N_{min} and the N uptake, yield, and GPC. The dynamics of soil N_{min} throughout the cropping season were variable, and thus, the crop N necessities were variable. The soil N_{min} values in the early wheat growth stages were well correlated with the yield, and in the late stages, they were well correlated with GPC. N_0 was correlated with the late N uptake and GPC. However, the chemical methods that avoid the long periods required for N_0 determinations were not correlated with the N uptake in the late wheat growth stages or GPC. Conversely, clay was positively correlated with the late N_{min} values and GPC. Chemical methods were unable to estimate the available soil N in the later stages of the growing cycle. Consequently, as incubation methods are too laborious for their widespread use, further research must be conducted.

Keywords: soil N supply; soil N mineralization; N fertilization; potentially mineralizable N; humid Mediterranean climate

1. Introduction

Few agroecosystems supply enough nitrogen (N) to sustain satisfactory crop production without fertilizers. Throughout agricultural history, agriculturists have attempted to maintain fertility levels in the soil, depending on biologically fixed N, through the application of organic amendments and the decomposition of soil organic matter (SOM) to provide N to crops. In cereal cropping systems, N is one of the most important elements controlling crop development [1]. Thus, to assure that the potential yield is achieved each year, N is frequently applied in excessive amounts without determining the appropriate N fertilization rate, which usually leads to N losses. To comply with economic and ecological regulations in recent years, concerns about the need for improving nitrogen use efficiency

(NUE) in cereal production have increased. The N fertilizer demand is dependent on the plant available N supplied by soils and the potential yield, which varies from year to year. Available soil resources should be taken into account for the determination of appropriate N fertilization rates to enhance the efficiency of agricultural systems and ecosystem health.

It is necessary to provide better insight into the capacity of soils to provide N to crops (soil N supply) to understand the factors that control N mineralization in soils and therefore improve N fertilization recommendations for cereal [2]. In the field, different climatic and agronomic parameters affect wheat yield production, and among them, the contribution of soil N dynamics is very relevant. Nitrogen cycling in the soil–plant system is very complex and involves interactions between soil and plant factors. Only a small portion of N is biologically active, serving as a substrate for N mineralization [3]. To determine the rates of N fertilizer application, it is necessary to take into account the inorganic N of the soil and the organic N mineralized during crop growth [4,5].

The method used most in Western Europe for N fertilization application in cereals is the N_{min} method, which is based on the measured amount of soil mineral N in the main rooting depth before N fertilizer application at the beginning of the rapid period of crop growth. The calculation of the N fertilizer recommended rate is made using the predicted N demand for the target yield minus the measured soil N_{min} value. However, with this method, the N that has been mineralized during the remainder of the growing cycle is not taken into account. In addition, taking soil samples in a narrow period of time and analysing the mineral N in each individual field and each season is not practical. Therefore, with the aim of measuring the N supply capacity of the soil during the entire growing season, the potentially mineralizable N (N_o) or bioavailable N is measured [6]. The standard method for measuring N_o was defined by Stanford and Smith [7], who developed a method based on long-term aerobic incubations, in which soil was maintained under optimum conditions (35 °C and field capacity). However, this method is impractical for routine laboratory analysis due to the long incubation periods required (32 weeks). With the purpose of avoiding the long period required, several chemical methods have been developed to estimate N_o, such as extractions with different saline solutions. Different methodologies have been used to build a global strategy with the aim of estimating crop N availability [4,8–11]. However, no one method has yet obtained general approval [12]. In a previous article, in the area where this study was carried out (Araba, Basque Country, northern Spain), with calcareous soils and under humid Mediterranean conditions, Villar et al. [13] determined that the most appropriate laboratory technique to estimate the amount of available N that soils are able to provide to wheat was hot KCl extraction.

In Araba (Basque Country, northern Spain), traditionally, two applications of N fertilizer are supplied at following growing stages (GS): GS21 (beginning of tillering) and GS30 (stem elongation) for wheat, according to the Zadoks scale [14]. As soils differ in their composition and mineralization patterns, the N rate applied in these two main periods should be specific with respect to the available N [15]. Furthermore, as in many other wheat-producing countries, the demand for high grain protein content (GPC) has also increased. Therefore, it is essential to estimate the amount of N mineralized from SOM to adjust the rate of N fertilizer required to optimize crop yield and quality, reducing the negative impacts of excessive N on the environment.

The main objective of this study was to assess the soil N_{min} dynamics throughout the wheat growing season at crucial stages for plant yield and GPC. To this aim, we analyzed the utility of different characteristics of the soil before sowing: (i) the commonly used routine soil physicochemical analyses (SOM, Ntot, pH, $CaCO_3$ and texture), (ii) potentially mineralizable N (N_o), analyzed using aerobic incubation, and (iii) different extraction methods to estimate N_o.

2. Materials and Methods

2.1. Experimental Setup

A greenhouse experiment was established in Derio (Bizkaia, Basque Country, Spain) at NEIKER-tecnalia experimental facilities using 16 field soils collected from Villanañe, Soportilla, Lantaron, Arangiz, Betolaza, Gauna, and Tuesta (Araba, Basque Country, Spain) from the 0–30 cm layers (Table 1). No organic amendments had been applied to the studied soils for several years before sample collection or N fertilization in the previous months. Moreover, no leguminous crops were grown in the fields preceding soil collection. Soils were air-dried and sieved though a 2 mm mesh and poured into pots (height of 30 cm, and diameter of 22 cm). Before sowing, 300 mL of a nutrient solution without N [10] was added to the soil to ensure that there were no nutrient limitations to the plants. Twenty seeds of soft red winter wheat (*Triticum aestivum* L., var Soissons) per pot were sown (30 May 2011), and after germination, the number of plants was reduced to 14 seedlings to simulate the common sowing dose of 240 kg ha^{-1}. There were three replicates per soil that were distributed in a completely randomized experimental design. Pots were kept at field capacity during the whole experiment. The experiment was extensively described in a previous article [13].

Table 1. Location, and physical and chemical characteristics, of the soils from Araba (Basque Country, northern Spain) used in the greenhouse experiment.

Soil	Location	Soil Texture	Sand [a] %	Silt [a] %	Clay [a] %	SOM [b] %	Ntot [c] %	pH [d] %	$CaCO_3$ [e] %
1	Villanañe	Clay-loam	30.5	37.1	32.4	1.8	0.12	8.3	29.0
2	Soportilla	Loam	35.9	39.3	24.8	1.0	0.07	8.5	29.4
3	Lantaron	Loam	31.7	43.0	25.4	1.3	0.09	8.4	56.5
4	Arangiz	Silty-loam	19.4	55.9	24.6	1.6	0.12	8.3	56.3
5	Arangiz	Clay-loam	26.1	45.6	28.3	2.0	0.16	8.3	21.7
6	Arangiz	Silty-clay-loam	16.2	56.9	27.0	2.0	0.13	8.3	53.0
7	Betolaza	Silty-clay-loam	12.1	58.6	29.3	3.1	0.24	8.3	29.3
8	Gauna	Sandy-clay-loam	47.4	24.9	27.6	2.0	0.15	8.1	8.0
9	Tuesta	Silty-loam	18.8	54.8	26.4	1.4	0.11	8.3	55.8
10	Arangiz	Silty-clay-loam	17.5	55.3	27.2	2.0	0.12	8.3	56.8
11	Arangiz	Silty-clay-loam	18.0	52.8	28.5	1.8	0.11	8.4	40.9
12	Gauna	Clay-loam	38.5	32.6	29.0	2.0	0.12	8.1	9.2
13	Arangiz	Silty-loam	19.8	54.8	25.4	1.5	0.10	8.4	53.8
14	Tuesta	Clay-loam	36.8	28.4	34.8	1.9	0.15	8.2	16.4
15	Gauna	Sandy-clay-loam	47.6	24.5	27.9	2.2	0.17	8.0	12.1
16	Gauna	Clay loam	44	25.9	30.1	2.1	0.16	8.0	7.2

[a] Texture using a pipette method [16]; [b] Soil organic matter [17]; [c] Soil total N (dry combustion using a LECO TruSpec CHN); [d] pH (1:2.5 soil:water); [e] $CaCO_3$ (NH_4AcO; [18]).

2.2. Plant Sampling

Wheat aboveground biomass was sampled at GS30 (stem elongation), GS37 (leaf flag emergence), and harvest (19 December 2011). Fresh biomass samples were weighed and oven dried, and the dried biomass samples were again weighed for dry matter content determination. Biomass was estimated, and the N concentration was determined using dry combustion with LECO equipment (TrueSpec® CHN-S, LECO Corporation, Michigan, USA). At harvest, grain and straw were separated and dried at 70 °C for two days to obtain the dry matter content. Grain yield was measured, and grain and straw N concentration were determined using dry combustion with LECO equipment (TrueSpec® CHN-S). Nitrogen uptake was calculated, and GPC was determined by multiplying the total grain N concentration by 5.7 [19].

2.3. Soil Samples

Soil was sampled with a soil sampling rod (full depth from each pot) at sowing, GS30 (stem elongation), GS37 (leaf flag emergence), GS60 (beginning of flowering), harvest, post-harvest, and pre-sowing to determine the ammonium and nitrate levels. NH_4-N and NO_3-N were spectrophotometrically determined [20,21]. N_{min} was calculated as the sum of NH_4-N plus NO_3-N.

2.4. Aerobic Incubation

Aerobic incubation was performed following the method described by Stanford and Smith [7] and modified by Campbell et al. [22]. Fifteen grams of each soil sample was air-dried and sieved (2 mm mesh) and then mixed with an equal amount of quartz sand. Soils were incubated aerobically at field capacity for 32 weeks in a culture chamber at 35 °C. Samples were leached every 2 weeks during the first 8 weeks and every 4 weeks thereafter with a 0.01 M $CaCl_2$ solution. Mineral N was determined in each sample spectrophotometrically, and N_o was estimated by fitting the accumulated N_{min} against time to a first-order kinetic exponential model. N mineralized in the first two weeks (N2wk) and accumulated after 30 weeks of incubation (N30wk), and potentially mineralizable N (N_o) were calculated. The procedure of the experiment was extensively described in a previous article [13].

2.5. Chemical Extractions

2.5.1. The 0.01 M $CaCl_2$ Extraction

Calcium chloride extraction was performed using the method described by Houba et al. [23] and modified by Velthof and Oenema [10]. Soil samples were divided into three parts: the first part was air-dried (30 °C), the second part was dried at 40 °C, and the last part was dried at 105 °C. After drying, 6 g of soil was extracted with 60 mL of 0.01 M $CaCl_2$ via shaking for 2 h. After extraction, the samples were centrifuged for 5 min at 2328× *g*. Nitrate and ammonium were determined spectrophotometrically. Two mineralization indices were calculated: MI-$CaCl_2$ I and MI-$CaCl_2$ II. MI-$CaCl_2$ I was calculated as the difference between the ammonium extracted at 105 °C and the ammonium extracted from the air-dried samples. MI-$CaCl_2$ II was calculated as the difference between the ammonium extracted at 105 °C and the ammonium extracted at 40 °C.

2.5.2. The KCl and HotKCl Extraction

Ten grams of soil were extracted with 20 mL of 2 M KCl at room temperature [24]. After extraction, the samples were centrifuged at 2328× *g* for 5 min and analyzed spectrophotometrically to determine the concentrations of NO_3^--N and NH_4^+-N.

The value of N_{min} obtained using hotKCl was determined by heating 1.5 g of soil with 10 mL of 2 M KCl solution on a digestion block set at 100 °C for 4 h. After extraction, the NO_3^--N and NH_4^+-N concentrations were determined as described for the room temperature procedure.

The N mineralization index (MI-hotKCl) was calculated as the difference between the amount of NH_4^+-N extracted using hotKCl and that extracted at room temperature.

2.5.3. The 0.01 M $NaHCO_3$ Extraction

$NaHCO_3$ extraction was carried out using the method described by MacLean [25] and modified by Serna and Pomares [8]. Two grams of soil was mixed with 40 mL of 0.01 M $NaHCO_3$; the samples were then centrifuged at 2328× *g* for 5 min and filtered through Whatman No. 42 filters (Whatman International Ltd., Maidstone, England) and the absorbance of the filtrate was then measured at 205 and 260 nm (205ABS and 260ABS, respectively). The procedure of the experiment was extensively described in a previous article [13].

2.6. Statistical Analysis

For analyzing N_{min} differences among soils, a one-way ANOVA was performed for each growing stage: sowing, GS30, GS37, GS60, harvest, post-harvest, and pre-sowing [26]. For analyzing differences among the soils' final N uptake, yield, and GPC, one-way ANOVA was performed [26]. To separate the means, Duncan's test was used ($p \leq 0.05$) using the R package *agricolae V. 1.2-4* [27].

Pearson's correlation analysis of the soil properties, aerobic incubation and chemical extractions with 1) N_{min} values at sowing, GS30, GS37, GS60, harvest, post-harvest, and pre-sowing and with the

2) nitrogen uptake by the plant between sowing and GS30, GS30–GS37, GS37-harvest, and yield and GPC was performed. In addition, Pearson's correlation analysis was performed between N_{min} and N uptake by the plant, sowing and GS30, GS30–GS37, GS37-harvest, and yield and GPC.

3. Results

3.1. Range of Soil Physical and Chemical Characteristics

The soils varied widely in their physical and chemical characteristics. Sixteen soils were classified into five texture classes: clay-loam (S-1, S-5, S-12, S-14, and S-16), loam (S-2 and S-3), silty-loam (S-4, S-9, and S-13), silty-clay-loam (S-6, S-7, S-10, and S-11), and sandy-clay-loam (S-8 and S-15). The pH values were high (8.0–8.5). Twelve soils were calcareous (>15% $CaCO_3$), and the remaining four soil values varied between 7.0 and 12.1%. Soil organic matter values were low (SOM $\leq$ 1.9%; S-1, S-2, S-3, S-4, S-9, S-11, S-13, and S-14), moderate (SOM 1.9–2.2%; S-5, S-6, S-8, S-10, S-12, S-15, S-16, and S-17), or high (SOM 3.1%; S-7).

3.2. Availability of Mineral N in Soil

Soil mineral N availability values were different among soils and phenological stages (Table 2). Remarkably, N_{min} did not follow the same dynamic pattern throughout the crop cycle in different soils. Soil N_{min} values decreased from sowing to GS37 in the vast majority of the soils, except for S-9, S-3, and S-5, which increased their N_{min} values from sowing to GS30. N_{min} values increased from GS37 to GS60 in each soil and then remained similar. Differences were especially evident at GS30, where S-9 had the highest value and S-2, S-8, S-10, S-12, S-14, and S-16 had the lowest values. Soils with the highest N availability in GS37 were S-1, S-6, S-7, S-8, S-12, and S-14, whereas at GS60, S-7, S-8, and S-14 had the highest N availability.

Table 2. Soil mineral nitrogen (N_{min}; mg kg^{-1}) evolution in 16 soils from Araba throughout the wheat growing season in a greenhouse experiment. Different letters represent significant differences ($p \leq 0.05$) among soils for each growing stage: sowing, stem elongation (GS30), leaf flag emergence (GS37), flowering (GS60), harvest, post-harvest, and pre-sowing.

Soil	Sowing		GS30		GS37		GS60		Harvest		Post-Harvest		Pre-Sowing	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
1	16.6 AB	4.0	10.2 ABC	3.2	2.6 A	0.5	7.5 ABC	0.7	4.7 BC	0.9	7.2 AB	1.2	3.3 ABC	1.0
2	8.5 EFG	2.1	3.7 C	0.9	0.4 B	0.1	5.2 C	1.0	3.8 CD	0.6	3.0 C	0.7	2.5 C	0.9
3	10.3 ABC	1.8	12.3 AB	1.7	1.7 AB	0.5	6.2 BC	2.2	3.7 CD	0.8	5.6 BC	0.3	3.2 ABC	0.6
4	12.6 ABC	6.1	6.1 BC	1.9	2.2 AB	0.7	7.1 ABC	2.0	4.9 B	0.9	8.0 AB	1.2	3.2 ABC	0.6
5	11.3 ABC	2.3	9.0 ABC	1.9	1.7 AB	0.9	8.2 ABC	2.2	4.6 BC	0.8	6.5 BC	0.9	3.3 ABC	0.6
6	11.4 ABC	5.9	5.3 BC	1.7	2.9 A	0.3	8.0 ABC	0.8	5.1 B	0.5	6.8 BC	0.8	3.6 ABC	0.5
7	6.5 FG	0.4	6.6 BC	0.9	3.0 A	0.7	8.1 A	0.9	6.6 A	0.9	8.9 A	0.6	3.8 ABC	1.0
8	7.3 EFG	2.6	3.3 C	0.9	2.9 A	0.6	7.5 A	1.8	3.1 D	0.7	4.3 BC	1.0	2.7 C	0.2
9	9.7 CDE	3.5	16.4 A	1.2	0.9 AB	0.1	5.9 AB	1.0	4.4 BC	0.6	4.4 BC	0.8	3.2 ABC	0.2
10	12.7 ABC	2.9	4.2 C	0.7	2.2 AB	0.4	8.3 ABC	0.7	4.9 BC	0.9	5.5 ABC	1.0	3.9 A	0.5
11	11.6 ABC	3.2	4.9 BC	1.1	1.6 AB	0.7	7.4 ABC	1.4	5.3 BC	1.7	7.0 AB	1.3	3.6 ABC	0.7
12	5.7 G	2.0	4.4 C	0.7	2.7 A	0.7	8.2 ABC	1.9	5.1 BC	1.2	5.2 ABC	0.7	3.3 ABC	0.8
13	9.4 CDE	1.8	5.0 BC	0.9	1.3 BC	0.6	9.6 AB	1.4	4.7 BC	1.2	5.7 ABC	0.8	3.5 ABC	1.7
14	10.3 CDE	6.0	3.7 C	0.8	2.8 A	0.3	10.2 A	1.2	5.2 BC	1.3	7.1 AB	0.7	4.0 AB	0.9
15	13.7 A	3.5	5.0 BC	1.3	1.5 AB	0.4	8.1 ABC	2.0	4.3 BC	1.0	5.8 ABC	1.7	3.5 ABC	0.2
16	7.9 EFG	1.3	2.8 C	0.8	1.2 AB	0.5	4.9 C	2.3	4.5 BC	1.4	5.4 ABC	0.7	3.0 ABC	0.8

3.3. Wheat N Uptake, Yield, and GPC

There were significant differences in wheat N uptake at harvest, yield, and GPC depending on the soil (Figure 1). The soil with the highest N uptake (mg pot^{-1}) was S-1. The lowest N uptake was in S-2 and S-8. In the case of wheat yield (g pot^{-1}), the highest values were achieved in S-3, S-9, and S-15 and the lowest in S-2, S-8, and S-16. Regarding GPC (%), the highest values were achieved in S-14, and the lowest were in S-2.

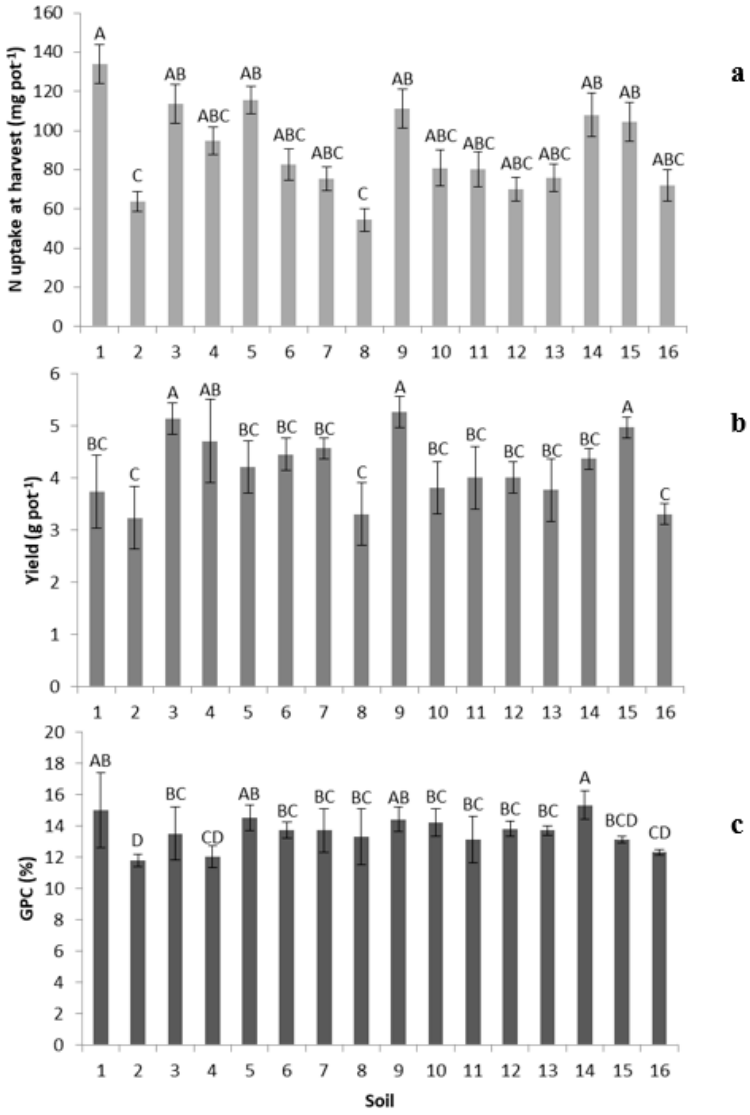

Figure 1. (**a**) Wheat N uptake at harvest (mg pot^{-1}), (**b**) yield (g pot^{-1}), and (**c**) GPC (%) in 16 soils from Araba. Different letters represent significant differences ($p \leq 0.05$) among soils. Values are the mean of three replicates ± SD.

3.4. Relationships between Initial Soil Characteristics with N_{min} throughout the Growing Cycle

Regarding the soil physicochemical properties (Table 3), sand had a negative correlation with N_{min} values at harvest, silt had a positive correlation with N_{min} values at harvest, and clay had a positive correlation with N_{min} values at GS37 and GS60. Ntot was positively correlated with N_{min} at GS37, harvest, and post-harvest. SOM had a positive correlation with N_{min} values at GS37, GS60, harvest, and post-harvest. In the case of aerobic incubations, N2wk had a positive correlation with the sowing values, and N30wk had a positive and significant correlation with the GS60, harvest, post-harvest, and pre-sowing N_{min}. N_o was positively correlated with the GS60 and pre-sowing N_{min} values. Regarding the chemical extractants used to estimate N_o, MI $CaCl_2$ I was correlated with the N_{min} values at GS60. MI-hotKCl was positively correlated with N_{min} values from GS37 to pre-sowing. For $NaHCO_3$, only 205 ABS was correlated with the sowing and pre-sowing N_{min} values. Remarkably, SOM, N30wk, and hotKCl were correlated with the N_{min} values after harvest.

Table 3. Pearson correlation coefficients (r) between soil properties, N mineralization indices calculated from the aerobic incubations and chemical extractions with Nmin values at sowing, stem elongation (GS30), leaf flag emergence (GS37), flowering (GS60), harvest, post-harvest, and pre-sowing.

Soil Characteristics		Sowing		GS30		GS37		GS60		Harvest		Post-Harvest		Pre-Sowing	
		r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Soil properties	Sand	−0.11	*ns*	−0.25	*ns*	0.00	*ns*	−0.01	*ns*	−0.63	**	−0.48	*ns*	−0.47	*ns*
	Silt	0.09	*ns*	0.27	*ns*	−0.12	*ns*	−0.11	*ns*	0.50	*	0.36	*ns*	0.33	*ns*
	Clay	0.05	*ns*	−0.11	*ns*	0.55	*	0.56	*	0.38	*ns*	0.40	*ns*	0.47	*ns*
	SOM	−0.16	*ns*	−0.24	*ns*	0.70	**	0.50	*	0.60	**	0.58	*	0.49	*ns*
	Ntot	−0.16	*ns*	−0.12	*ns*	0.57	*	0.43	*ns*	0.54	*	0.59	**	0.44	*ns*
	pH	0.04	*ns*	0.22	*ns*	−0.47	*ns*	−0.38	*ns*	0.06	*ns*	−0.03	*ns*	−0.07	*ns*
	CaCO3	0.33	*ns*	0.40	*ns*	−0.29	*ns*	−0.25	*ns*	0.09	*ns*	0.10	*ns*	0.22	*ns*
Aerobic incubations	N2wk	0.54	*	0.02	*ns*	0.25	*ns*	0.30	*ns*	0.05	*ns*	0.14	*ns*	0.46	*ns*
	N30wk	0.37	*ns*	0.15	*ns*	0.43	*ns*	0.57	**	0.51	*	0.61	**	0.83	***
	No	0.29	*ns*	0.16	*ns*	0.43	*ns*	0.65	**	0.38	*ns*	0.49	*ns*	0.73	**
Chemical extractions	MI $CaCl_2$ I	0.19	*ns*	−0.44	*ns*	0.35	*ns*	0.55	*	0.16	*ns*	0.37	*ns*	0.43	*ns*
	MI $CaCl_2$II	0.34	*ns*	−0.41	*ns*	0.31	*ns*	0.52	*	−0.03	*ns*	0.29	*ns*	0.38	*ns*
	MI-HotKCl	0.36	*ns*	−0.29	*ns*	0.62	**	0.70	*	0.50	*	0.69	**	0.64	**
	205ABS	0.59	**	0.07	*ns*	−0.16	*ns*	0.23	*ns*	0.18	*ns*	0.30	*ns*	0.59	**
	260ABS	0.31	*ns*	0.08	*ns*	−0.15	*ns*	0.15	*ns*	0.26	*ns*	0.32	*ns*	0.33	*ns*

ns, not significant ($p > 0.05$). *, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

3.5. Relationships between Soil Characteristics and Plant N Uptake, Yield, and GPC

Regarding the soil initial properties (Table 4), clay had a positive correlation with GPC. Ntot had a positive correlation with the N uptake between sowing and GS30 and with the N uptake between GS30 and GS37. In the aerobic incubations, N30wk and N_0 showed a positive and significant correlation with the N uptake between GS37 and harvest and GPC. Concerning the chemical extractants, only 205ABS had a positive correlation with yield.

Table 4. Pearson correlation coefficients (r) among soil properties, N mineralization indices calculated from the aerobic incubations and chemical extractions with N uptake values between sowing and stem elongation (GS30), GS30 and leaf flag emergence (GS37), GS37 and flowering (GS60), and yield and GPC (grain protein content).

Soil Characteristics		N Uptake						Yield		GPC	
		Sowing-GS30		GS30-GS37		GS37-Harvest					
		r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Soil properties	Sand	−0.06	*ns*	−0.04	*ns*	0.01	*ns*	−0.22	*ns*	−0.06	*ns*
	Silt	−0.02	*ns*	0.09	*ns*	−0.10	*ns*	0.22	*ns*	−0.10	*ns*
	Clay	0.39	*ns*	−0.26	*ns*	0.45	*ns*	−0.07	*ns*	0.73	***
	SOM	0.46	*ns*	−0.47	*ns*	−0.10	*ns*	0.09	*ns*	0.31	*ns*
	Ntot	0.52	*	0.49	*	0.06	*ns*	0.24	*ns*	0.26	*ns*
	pH	−0.43	*ns*	0.33	*ns*	−0.06	*ns*	−0.16	*ns*	−0.22	*ns*
	$CaCO_3$	−0.24	*ns*	0.33	*ns*	0.08	*ns*	0.32	*ns*	−0.13	*ns*
Aerobic incubations	N2wk	0.30	*ns*	−0.24	*ns*	0.39	*ns*	0.26	*ns*	0.40	*ns*
	N30wk	0.39	*ns*	−0.25	*ns*	0.64	**	0.34	*ns*	0.75	**
	No	0.47	*ns*	−0.38	*ns*	0.64	**	0.43	*ns*	0.74	**
Chemical Extractions	MI $CaCl_2$ I	−0.01	*ns*	−0.21	*ns*	−0.11	*ns*	−0.07	*ns*	0.04	*ns*
	MI $CaCl_2$ II	−0.06	*ns*	−0.14	*ns*	−0.05	*ns*	−0.07	*ns*	0.03	*ns*
	MI-HotKCl	0.43	*ns*	−0.35	*ns*	0.21	*ns*	−0.07	*ns*	0.05	*ns*
	205ABS	0.29	*ns*	−0.24	*ns*	0.24	*ns*	0.57	*	0.00	*ns*
	260ABS	−0.11	*ns*	0.10	*ns*	−0.04	*ns*	0.10	ns	−0.08	*ns*

ns, not significant ($p > 0.05$). *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

The soil N_{min} values (Table 5) at sowing and GS30 were correlated positively with N uptake between GS37 and harvest. The N_{min} values at GS30 and GS60 had positive correlations with yield and GPC, respectively.

Table 5. Pearson correlation coefficients (r) among the soil N_{min} values, N uptake values between sowing and stem elongation (GS30), GS30 and leaf flag emergence (GS37), GS37 and flowering (GS60), and yield and GPC (grain protein content).

Soil N_{min}	N Uptake						Yield		GPC	
	Sowing-GS30		GS30-GS37		GS37-Harvest					
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Sowing	0.01	*ns*	0.18	*ns*	0.53	*	0.22	*ns*	0.09	*ns*
GS30	−0.02	*ns*	0.31	*ns*	0.65	**	0.59	**	0.31	*ns*
GS37	0.27	*ns*	−0.25	*ns*	0.00	*ns*	−0.04	*ns*	0.38	*ns*
GS60	0.22	*ns*	−0.31	*ns*	0.09	*ns*	−0.07	*ns*	0.53	**
Harvest	0.38	*ns*	−0.25	*ns*	0.00	*ns*	0.22	*ns*	0.24	*ns*

ns, not significant ($p > 0.05$). *, ** Significant at the 0.05 and 0.01 probability levels, respectively.

4. Discussion

The tested soils differed in their initial N_{min} values and their physical and chemical properties [13], which significantly influenced the N mineralization patterns. Depending on their characteristics, soils mineralize in different ways, making available different N_{min} values (Table 2). The first step in mineralization is ammonification, which is the conversion of organic N into ammonium by soil microbes. This process is carried out exclusively by heterotrophic microorganisms that utilize C as an energy source. Nitrate production is mediated via two groups of autotrophic bacteria (*Nitrosomonas* and *Nitrobacter*) that convert ammonium into nitrate by the process called nitrification. Nitrogen availability relies on both the initial availability of N_{min} and the rate of mineralization or immobilization [28], as well as the previous N uptake by the crop, influencing the yield and GPC.

There were no correlations between the soil properties and N_{min} values at sowing or GS30. It should be mentioned that the soil preparation (drying, sieving, and rewetting) prior to the experiment could have affected soil structure and functioning. This could explain the lack of correlation in the early stages. It should be mentioned that soil rewetting often causes abundant mineralization because microorganisms recover their activity [29]. However, Mikha et al. [30] suggested that N immobilization occurred in response to the easily accessible C due to the rapid increase in microbial activity. Later, in the growing cycle, from GS37 onwards, SOM, Ntot, sand, silt, and clay were relatively effective predictors of soil N_{min} dynamics.

Soil organic matter (SOM) is a heterogeneous mixture of organic compounds that vary in their nutrient composition, molecular characteristics, age, and biological stability. Increasing SOM by adding carbon is a beneficial agronomic practice that stimulates microbial communities and enhances soil N and C pools [31]. The youngest compounds are the most biologically active compounds, and the materials with intermediate ages contribute to soil physical characteristics [28]. We found positive correlations from GS37 to post-harvest with the N_{min} values (Table 3). Ros et al. [6] found that SOM explained 78% of the variation in mineralizable N, whereas other soil properties only explained 8%. In some studies, SOM fractions have been preferred to total SOM for predicting N_o due to the easy release of labile compounds during the extractions [32]. However, other studies suggested that none of those SOM fractions is an a priori preferable indicator of N_o [6,33].

Debosz and Kristensen [34] found that Ntot content had a positive relationship with N mineralization. Similarly, Dessureault-Rompré et al. [9] showed that Ntot was one of the best predictors of soil mineralizable N pools. However, in our case, Ntot only had positive correlations with the N_{min} values at harvest and post-harvest (Table 3). It is remarkable that only approximately 1–4% of the Ntot is mineralized as plant-available N (NH_4-N and NO_3-N) each year [34]. Many authors have found that soil Ntot and SOM were the best predictors of N_o [9,11].

The mineralization of N is often affected by the clay content, likely due to SOM binding to mineral particles. Clay was correlated with N_{min} at the end of the growth cycle and with the GPC. In this experiment, soils with the highest GPC were S-1, S-5, S-9, and S-14 (15–16%), where the clay

values were 32.4, 28.3, 26.4, and 34.8%, respectively. Clay has indirect affects through the formation of aggregates that protect SOM, and therefore microbial biomass, and direct effects with the stabilization of organic N [35]. Hassink [36] found that the mineralization of organic N was negatively affected by a high clay content due to SOM binding to mineral particles. Similarly, Ros et al. [6] found that clay had a negative influence on mineralizable N because mineralization in clayey soils was lower than that in sandy soils. In contrast, in this experiment, clay was positively correlated with N_{min} at GS37 and GS60, and sand was negatively correlated with N_{min} at GS60 (Table 3). Some clayey soils are able to fix and release ammonium, but the regulation of N availability is not fully understood [37]. Chantigny et al. [38] showed that in clay soils, the fixation of the recently added ammonium was higher than that in sandy soil (34% and 11%, respectively). The recently fixed ammonium that can be derived from added fertilizer or from soil organic matter [39] is quickly fixed by clay minerals and later released slowly during the crop growth season due to the increased crop demand. In a greenhouse experiment, Dou and Steffens [40] found that 90–95% of the recently fixed ammonium was released during a 14-week period. Under field conditions, 66% of the recently fixed ammonium was released 86 days after fixation [41]. Provision of root exudates by plants improved the activity of heterotrophic microorganisms, which foster the release of fixed ammonium [37], retarding nitrification. The silt fraction has also been reported to bind NH_4^+ in a non-exchangeable form [37]; in our study, it was correlated with N_{min} at harvest. In S-9, where high values of GPC were achieved (15%), the silt content was 54.8.

The pH and $CaCO_3$ did not present any effect on N mineralization. Dessureault-Rompré et al. [11] found that the effect of pH on soil mineralization was very low. In other studies, soil pH, moisture and temperature were often non-linearly related to the dynamics of N [42,43]. The pH range among our soils was very low.

With respect to wheat N uptake (Table 4), among the soil properties, only soil Ntot was positively correlated with the wheat N uptake at two times: from sowing to GS30 and from GS30 to GS37. In the case of aerobic incubation, N30wk and N_0 were correlated with the N uptake from GS37 to harvest and with GPC. However, only soil N_{min} at sowing and GS30 was correlated with the N uptake from GS37 to harvest (Table 5). Historically, soil N availability has been seen as an inaccurate indicator of plant N availability because plant roots are considered poor competitors for inorganic N against soil microorganisms [44]. This idea could explain the lack of correlation between the soil N availability and the N uptake from the crop. Conversely, it has been determined that a cereal crop was able to accumulate a greater amount of added inorganic N than microorganisms [45]. The results showed that different soils followed different mineralization patterns affecting yield and GPC. The soil N_{min} at GS30 and at GS60 was positively correlated with the yield and GPC, respectively (Table 5). This suggests that the N status at those times is essential for determining the yield [15] or GPC [46,47]. Soils with the lowest N availability at GS30 and GS60 as S-2 and S-16 presented the lowest yields and GPC, respectively. However, when the N availability was high in these growth stages, the yields and GPC values were high. Remarkably, none of the soil properties was correlated with yield, but clay was positively correlated with GPC (Table 4). However, one of the methods of chemical extraction (205 ABS) was correlated with the yield.

The key to optimizing high yields, wheat quality (GPC), and environmental protection is to achieve synchronicity between the N supply and crop demand, while accounting for spatial and temporal variability in soil N. As previously observed, many factors affect soil N mineralization, and therefore wheat N uptake. In Western Europe, the soil N_{min} at the end of winter is used to correct the values of N fertilizer rates calculated from the potential yield. Nevertheless, it implies laborious and expensive sampling and analysis. In Araba (Basque Country, northern Spain), the usual last and greater N dressing application occurred at GS30, but there were no correlations between soil initial properties and N_{min} values at GS30 (Table 3). This is remarkable because a high N availability at GS30 is key for achieving high yields [15]. As the last N dressing is at GS30, it is common to have low N in wheat plants at the end of the growing cycle (GS60–harvest) in Araba [46]. Moreover, the climatic

conditions in this area, humid Mediterranean, can lead to very high yields and therefore low protein concentrations in grain. Fuertes-Mendizabal et al. [47] showed that late N availability (GS37 onwards) in wheat under humid Mediterranean conditions increased GPC, especially when no N was applied in the late stages, as in the area of study. As stated above, clay apparently allowed higher N availability at GS37 and GS60. This could be explained by the positive correlation between N_{min} at GS60 and GPC or the correlation between clay and GPC. However, soils presented a narrow clay range (24.6–34.8%) to confirm that finding. Inside the aerobic incubation, N30wk and N_o were able to estimate the N available for the wheat crop at the end of the growing cycle and thus the GPC. Identifying soils in Araba where it would be possible to have late N availability with the aim of improving GPC would be interesting. In the humid Mediterranean climatic conditions of Araba, a third application at GS37 is possible since rain water usually allows the utilization by wheat of this N applied late [46,47]. However, the chemical methods that avoid the long periods required for aerobic incubation did not correlate properly with the N uptake values in the late growth stages.

In order to make N recommendations that guarantee adequate levels of GPC, methods for the diagnosis of soil available N must be improved, especially in the later stages of the growing cycle. In this sense, it is necessary to explore quick and simple methods because the most effective ones require periods of incubations that are too long. This is even more important in certain circumstances, such as organic farming, where it is difficult to make late applications of N with authorized fertilizers (organic fertilizers).

5. Conclusions

Even in a relatively small cropping area where the variability of soil properties is narrow, the dynamics of soil nitrate and ammonium throughout the cropping season were variable, and therefore, so was the crop N uptake. The soil N_{min} values at early wheat growth stages were well-correlated with yield, and at late stages, they were well-correlated with GPC.

N_0 was correlated with late N uptake and GPC. However, the chemical methods that avoid the long periods required for N_0 determinations were not correlated properly with the N uptake in the late wheat growth stages or GPC. Conversely, clay was positively correlated with the late N_{min} values and GPC, although the clay range was not very wide. Chemical methods were unable to estimate the available soil N in the later stages of the growing cycle. Consequently, as incubation methods are too laborious for their widespread use, further research must be conducted.

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