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Nitrogen Uptake, Use Efficiency, and Productivity of *Nigella sativa* L. in Response to Fertilization and Plant Density

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Abstract: *Nigella sativa* L. has been recognized as one of the most important medicinal plants in many parts of the world for centuries. The purpose of the current study was to evaluate the effects of fertilization and plant density on nitrogen uptake, utilization efficiency, and productivity of *N. sativa* under Mediterranean conditions. The three-year experiment was set up in a split-plot design with three replications. There were 2 plant densities; 200 and 300 plants m^{-2} with 4 fertilization levels: control, seaweed compost, farmyard manure and inorganic fertilizer. The highest seed yield (749–840 kg ha^{-1}) was found in plants subjected to low-density and inorganic fertilization. The seed nitrogen (N) uptake as well as the nitrogen harvest index (NHI) were positively affected by the increase of available nitrogen and negatively by the increase of plant density, with their highest values recorded in the low-density and inorganic fertilization. In conclusion, plant densities greater than 200 plants m^{-2} result in higher crop growth but lower seed yield and decreased nitrogen uptake and use efficiency in *N. sativa* seeds, whereas the application of inorganic fertilizers increases crop yield, nitrogen uptake, and utilization efficiency because these fertilizers present higher nitrogen levels with higher solubility and thus faster availability for the crop in comparison with organic fertilizers.

Keywords: compost; inorganic fertilizer; nitrogen agronomic efficiency (NAE); nitrogen harvest index (NHI); nitrogen utilization efficiency (NUtE); seed yield

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1. Introduction

Nigella sativa L., a diploid plant belonging to the Ranunculaceae family with chromosome number 2n = 12 [1], is mainly cultivated in semi-arid regions, including the Mediterranean, southern Europe, Egypt, Iran, India, Saudi Arabia, Syria, Pakistan, and Turkey [2,3]. *N. sativa* constitutes a short-lived annual plant and has been traditionally used as a medicinal plant that may aid for improving and maintaining human health [4,5]. *N. sativa* seeds are composed of 30–35% fixed and 0.5–1.5% volatile oil considered as alternative sources of oils for nutraceuticals and functional foods [6,7]. One of the most important pharmacologically active constituents of volatile oil is thymoquinone, which imports the plant under research as a medication of a variety of disorders in the respiratory system, digestive tract, cardiovascular system, liver, kidney, immune system [8,9] or metabolic syndrome [10]. The seeds of *N. sativa* have been accepted as a source of aroma in the "blue book" of the European Council since 1981 and have been granted a claim as a novel food ingredient in the context of European Council Regulation No. 258/97 [11,12]. Moreover, a number of studies have shown that the incorporation of *N. sativa* seeds in animal rations improves feed intake, digestibility coefficients, and nutritive values in agricultural livestock [13–18]. Finally, according to Roussis et al. [19], *N. sativa* biomass can also be used as a forage supplement for lactating animals.

As nutrient uptake and crop yields are the primary factors that determine optimal fertilization practices [20], a higher and more balanced nutrient supply is expected to result in higher crop production while maintaining soil health. This is possible when fertilizers are applied in an efficient manner, thereby minimizing the loss of nutrients and improving its efficiency [21,22]. Nitrogen is a critical nutrient for plant growth and development, as well as the most complicated, due to the numerous forms and activities that may occur throughout its cycle [23]. It is heavily involved in all plant metabolic activities, and its rate of uptake and partition is primarily governed by supply and demand throughout the plant's life cycle [24]. Nitrogen availability and supply vary according to crop species and are determined by their needs [25].

However, when unreasonably applied nitrogen fertilizer is neither completely assimilated by plants nor sequestered as soil organic nitrogen, it will result in nitrogen losses and cause environmental problems through nitrate (NO_3^-) leaching, ammonia (NH_3) volatilization, and nitrous oxide (N_2O) emissions, such as greenhouse gases, groundwater contamination, atmosphere pollution, water eutrophication, and biodiversity decline [26,27]. Uncontrolled and unreasonable nitrogen fertilizer use results in massive losses of 40 to 60% of applied nitrogen, which can have a negative impact on crop yields [28,29]. Increasing soil nitrogen use efficiency could reduce fertilizer use and farmer costs while also protecting the environment from the negative effects of nitrogen loss [30,31]. Mineral fertilizers, organic manure, composts, symbiotic N_2 fixation, and atmospheric wet and dry deposition are the main sources of nitrogen in agricultural fields [32]. Organic fertilizers, such as animal manure and composted organic materials, have been considered an excellent soil amendment that can provide nitrogen and enhance nitrogen availability to improve crop yields [33,34].

Nitrogen use efficiency (NUE) can be defined as the maximum economic yield produced per unit of nitrogen applied, absorbed, or utilized by the plant to produce seed and biomass, and constitutes an important approach for estimating the nitrogen losses, as well as the amount of nitrogen absorbed by the crop, and, thus, the efficiency of the applied fertilization [35]. In agronomic research, various indices are commonly used to evaluate the efficiency of applied nitrogen, primarily for purposes that emphasize crop response to nitrogen [36]. Fageria and Baligar [35] defined five different indices for determining NUE in crops: agronomic efficiency (AE), physiological efficiency (PE), agro-physiological efficiency (APE), apparent recovery efficiency (ARE), as well as utilization efficiency (UE). Increased NUE and seed yield are primarily determined by timely planting, proper tillage, optimum plant density, and optimal nitrogen rate and management [37,38]. The primary goal of improving nitrogen utilization, optimizing fertilization, and lowering the risk of contamination of surface water and groundwater resources is to have a better understanding of the plant nitrogen reaction [39].

The nitrogen harvest index (NHI) is defined as the proportion of total plant nitrogen incorporated into the seed. Since nitrogen in the roots has little influence on the efficiency of nitrogen partitioning, the NHI only refers to nitrogen in the above-ground plant parts [40]. It consists of an important index that indicates how efficiently the plant utilized acquired nitrogen for seed production and varies between different crop species as well as among different genotypes of the same species [35].

To our knowledge, there was no evidence available concerning the effects of fertilization and plant density on nitrogen uptake and nitrogen use efficiency of *N. sativa* crop production under Mediterranean semi-arid environments. As a result, the purpose of the current study aimed to investigate the influence of inorganic and organic fertilization, as well as plant density, on crop performance, nitrogen absorption, and assimilation from the soil to the vegetative parts and seeds of *N. sativa*.

2. Materials and Methods

2.1. Site Description and Experimental Design

A field experiment was conducted in 2017 (1st year), and then repeated in the exact location, and under the same experimental design, during 2018 (2nd year) and 2019 (3rd year), in the organic experimental field of the Agricultural University of Athens (Latitude: 37°59′1.70″ N, Longitude: 23°42′7.04″ E, Altitude: 29 m above sea level). The soil properties in the experimental site are presented in Table 1. The site was managed according to European Union regulations on organic agriculture (EC 834/2007). The meteorological data (mean temperature and precipitations) throughout the growing seasons were obtained from the automatic weather station (Davis Vantage Pro2 Weather Station; Davis Instruments Corporation, Hayward, CA, USA) of the Agricultural University of Athens and are shown in Figure 1. Total precipitation in 2017, 2018, and 2019 (from February to June) was 229.4, 218, and 205.8 mm, respectively. The mean temperature during the experimental periods was 18.2 °C for 2017, 19.5 °C for 2018, and 17.2 °C for 2019.

Table 1. Soil properties in the experimental site.

Soil Type	Clay Loam
Clay	29.1%
Silt	35.3%
Sand	35.6%
pH (1:1 H_2O)	7.43
Organic matter	1.82%
$CaCO_3$	15.93%
Total Nitrogen	0.121%
Phosphorus–Olsen P	13.4 mg kg^{-1} soil
Potassium	206 mg kg^{-1} soil

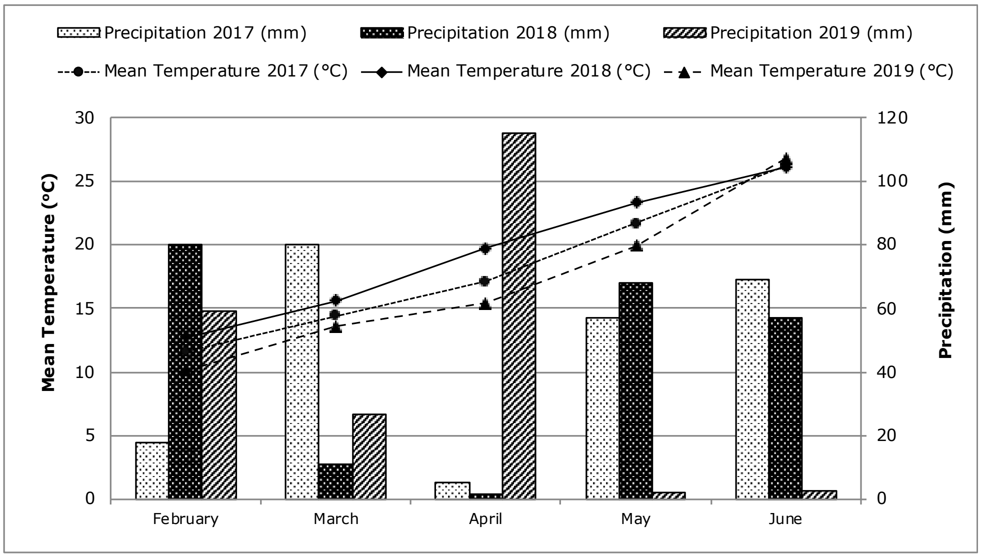


Figure 1. Weather data (mean monthly temperature and precipitation) for experimental site throughout the duration of the 3-year experiment (February–June 2017, 2018, and 2019).

The experimental area was, in total, 302 m^2. The experiment was set up in a split-plot design with three replications, two main plots (plant densities: 200 and 300 plants m^{-2}) and four sub-plots (fertilization treatments: control (untreated), seaweed compost (2000 kg ha^{-1} Posidonia 1.98%N, Compost Hellas S.A., Piraeus, Greece), farmyard manure

(2000 kg ha^{-1}, solid, 1.52%N), and inorganic fertilizer (300 kg ha^{-1} Enpeka 15-15-15+5 S by Compo Expert GmbH, Münster, Germany)) (Table 2). The amount of each type of fertilizer used in the current experiment is the general recommended dose of the corresponding type of fertilizer for *N. sativa* production in clay-loam soils [3,12]. The main plot and sub-plot sizes were 42.25 m^2 (6.5 m × 6.5 m) and 9 m^2 (3 m × 3 m), respectively. Each year, two days prior to the sowing, the soil was prepared by mouldboard ploughing at a depth 0.25 m. Fertilizers were applied as basal dressing through broadcasting by hand and incorporated with the soil by harrowing. *N. sativa* seeds were broadcasted by hand in rows 30 cm apart at a depth of 0.5–1 cm. The sowing rate was 50 kg ha^{-1}, and seed sowing was performed on February 1st in all experimental years (2017, 2018, and 2019). Emergence was on the 25th, 19th, and 27th of February in 2017, 2018, and 2019, respectively. Seedlings were thinned at the 4-true leaf stage to the examined plant densities, which were 200 and 300 plants m^{-2}. Throughout the experimental periods, there was no incidence of pest or disease on *N. sativa* crop. Weeds were controlled by hand-hoeing when needed and before canopy closure.

Table 2. Plant densities and fertilization methods used in the study.

Plant Density	Fertilization Treatment	Fertilization Amount	N Content	N Application Rate
200 plants m^{-2}	Control	No fertilizer	-	-
	Seaweed Compost	2000 kg ha^{-1}	1.98%	40 kg N ha^{-1}
300 plants m^{-2}	Farmyard Manure	2000 kg ha^{-1}	1.52%	30.5 kg N ha^{-1}
	Inorganic Fertilizer	300 kg ha^{-1}	15%	45 kg N ha^{-1}

2.2. Sampling, Measurements, and Methods

As for soil measurements, two topsoil samples (0–30 cm) from each sub-plot were collected at 100 Days After Sowing (DAS). The soil samples were air-dried at room temperature (25 °C), after removing debris, roots, and stones through a square-hole 2-mm sieve and then saved for evaluating soil organic matter (SOM) and soil total nitrogen (STN). The SOM was measured using the wet oxidation method of Walkley and Black [41] and the STN was determined by the Kjeldahl method [42] using a Büchi B-316 (Buchi Labortechnik AG, Flawil, Switzerland) device in order to combust and extract the soil sample.

Plant height and Leaf Area Index (LAI) were determined 85 DAS on ten randomly selected plants from each sub-plot. Leaf area was measured using an automatic leaf area meter (Delta-T Devices Ltd., Burwell, Cambridge, UK). As a result, the plant-based measurements were converted into a LAI by dividing the readings by the plant density of each plot. Moreover, 10 plant samples were randomly collected from each sub-plot at 45, 60, 75, 85, 100, and 115 DAS. The plants were separated into stems, flowers, fruits, seeds, green and yellow leaves, and weighted before being oven-dried for 48 h at 64 °C. The total nitrogen content of all plant samples was determined by grinding them to a fine powder. In addition, the total nitrogen content of the aerial biomass and the seeds was measured by applying the Kjeldahl procedure using a Kjeltec 8400 auto-analyzer (Foss Tecator AB, Höganäs, Sweden). Total plant nitrogen uptake was calculated as nitrogen absorption in the total above-ground (aerial biomass + seeds) dry matter at the time of maturity (115 DAS). For the assessment of the total nitrogen content and nitrogen uptake, the following nitrogen indices were utilized:

Nitrogen Harvest Index (NHI) was defined as given by Ye et al. [43]:

$$\mathrm{NHI} = \frac{\textit{seed N uptake}\ \left(\mathrm{kg\ N\ ha^{-1}}\right)}{\textit{total plant N uptake}\ \left(\mathrm{kg\ N\ ha^{-1}}\right)} \quad (1)$$

Nitrogen Use Efficiency (NUE) was assessed using the indices, Apparent Nitrogen Recovery Efficiency (ANRE), Nitrogen Utilization Efficiency (NUtE), and Nitrogen Agronomic Efficiency (NAE), which were calculated according to Fageria and Baligar [35] and Ye et al. [43], as follows:

$$\text{ANRE }(\%) = \frac{total\ N\ uptake\ of\ the\ fertilized\ plot\ \left(\text{kg N ha}^{-1}\right) - total\ N\ uptake\ of\ the\ unfertilized\ plot\ \left(\text{kg N ha}^{-1}\right)}{quantity\ of\ N\ applied\ \left(\text{kg N ha}^{-1}\right)} \times 100 \quad (2)$$

$$\text{NUtE} = \frac{seed\ yield\ \left(\text{kg ha}^{-1}\right)}{total\ plant\ N\ uptake\ \left(\text{kg N ha}^{-1}\right)} \quad (3)$$

$$\text{NAE} = \frac{seed\ yield\ of\ the\ fertilized\ plot\ \left(\text{kg ha}^{-1}\right) - seed\ yield\ of\ the\ unfertilized\ plot\ \left(\text{kg ha}^{-1}\right)}{quantity\ of\ N\ applied\ \left(\text{kg N ha}^{-1}\right)} \quad (4)$$

Finally, the plants were harvested at full seed maturity (seed moisture 12%) on 3 June 2017 (122 DAS), on 6 June 2018 (125 DAS), and on 8 June 2019 (127 DAS). The seed yield and the weight of 1000 seeds were determined by plants derived from the middle sub-plot area (1 m^2). Harvest index (HI) was calculated by divining seed yield by the biological yield (whole weight of plants derived for seed yield).

2.3. Statistical Analysis

Statistical analysis was carried out using the SigmaPlot 12 statistical software (Systat Software Inc., San Jose, CA, USA). The trait data generated by plant density and fertilization treatments over the 3-year experiment were assessed using a 3 × 2 × 4 factorial design (three years; two plant density treatments and four fertilization treatments) set up in a split-plot design with three replications. A mixed model was used for the analysis of variance (ANOVA), with years and replications as random effects and plant density and fertilization as fixed effects. Tukey's honestly significant difference test (Tukey's HSD) was used to separate mean differences. In order to estimate the levels of correlation between the variables studied, a simple regression analysis was performed. All comparisons were performed at the 5% level of significance.

3. Results

The results of the three-year data analysis (Table 3) indicated that plant density × fertilization interaction was significant on biomass nitrogen (N) content, seed N uptake, and N utilization efficiency. Moreover, the interaction of year × plant density was significant for seed yield, seed N uptake, and N utilization efficiency. The main effects of plant density and fertilization application were significant on productivity and nitrogen uptake and utilization efficiency of *N. sativa* crop. The fertilization regimes had a significant impact on soil properties. In addition, the main effect of the year was statistically significant on soil total nitrogen (STN), plant height, seed N content, and N utilization efficiency (NUtE) (Table 3).

Table 3. Combined analysis of variance (*F* values) for the effects of plant density and fertilization on soil properties and measured traits of *N. sativa* in three experimental years.

Source of Variance	Df	Soil Organic Matter (SOM)	Soil Total Nitrogen (STN)	Plant Height	Leaf Area Index (LAI)	Seed Yield	Harvest Index (HI)
Year (Y)	2	0.0351 ^{ns}	13.961 ***	4.0073 *	0.7294 ^{ns}	1.0164 ^{ns}	1.4479 ^{ns}
Plant Density (PD)	1	0.8345 ^{ns}	1.3599 ^{ns}	21.074 ***	15.560 ***	192.62 ***	141.19 ***
Fertilization (F)	3	41.816 ***	24.680 ***	14.559 ***	49.391 ***	86.882 ***	3.8504 *
Y × PD	2	0.0121 ^{ns}	0.0186 ^{ns}	0.2291 ^{ns}	0.0003 ^{ns}	11.977 ***	1.9516 ^{ns}
Y × F	6	1.3296 ^{ns}	0.4647 ^{ns}	0.1068 ^{ns}	0.0284 ^{ns}	0.4720 ^{ns}	0.2259 ^{ns}
PD × F	3	0.6242 ^{ns}	0.7007 ^{ns}	1.2897 ^{ns}	1.4720 ^{ns}	3.4119 ^{ns}	0.0958 ^{ns}
Y × PD × F	6	0.0860 ^{ns}	0.1276 ^{ns}	0.0808 ^{ns}	0.0410 ^{ns}	0.5430 ^{ns}	0.1046 ^{ns}

Source of Variance	Df	1000 Seed Weight	Biomass N Content 75 DAS	Biomass N Uptake	Total Plant N Uptake	Seed N Content
Year (Y)	2	0.0583 ^{ns}	2.0454 ^{ns}	2.0973 ^{ns}	2.1456 ^{ns}	3.3859 *
Plant Density (PD)	1	0.3819 ^{ns}	35.589 ***	32.151 ***	5.4115 *	14.410 ***
Fertilization (F)	3	2.3924 ^{ns}	82.643 ***	17.876 ***	39.993 ***	57.611 ***
Y × PD	2	0.0125 ^{ns}	0.0944 ^{ns}	0.5695 ^{ns}	0.1464 ^{ns}	1.6664 ^{ns}
Y × F	6	0.0861 ^{ns}	0.3605 ^{ns}	0.3183 ^{ns}	0.1639 ^{ns}	1.7254 ^{ns}
PD × F	3	0.2194 ^{ns}	3.4300 *	1.3991 ^{ns}	1.3836 ^{ns}	1.5031 ^{ns}
Y × PD × F	6	0.0013 ^{ns}	0.4569 ^{ns}	0.3112 ^{ns}	0.4115 ^{ns}	0.6907 ^{ns}

Source of Variance	Df	Seed N Uptake	N Harvest Index (NHI)	Apparent N Recovery Efficiency (ANRE)	N Utilization Efficiency (NUtE)	N Agronomic Efficiency (NAE)
Year (Y)	2	0.5149 ^{ns}	0.5615 ^{ns}	0.4057 ^{ns}	6.0298 **	1.2989 ^{ns}
Plant Density (PD)	1	161.89 ***	184.12 ***	3.1052 ^{ns}	171.58 ***	16.651 ***
Fertilization (F)	3	135.41 ***	10.162 ***	5.2455 *	7.2316 ***	9.6107 ***
Y × PD	2	3.9989 *	2.3714 ^{ns}	0.0808 ^{ns}	6.7993 **	0.2620 ^{ns}
Y × F	6	0.7091 ^{ns}	1.0662 ^{ns}	0.1224 ^{ns}	1.0731 ^{ns}	0.1332 ^{ns}
PD × F	3	5.3673 **	0.4542 ^{ns}	1.9750 ^{ns}	3.2337 *	0.3123 ^{ns}
Y × PD × F	6	0.9550 ^{ns}	0.1338 ^{ns}	0.3889 ^{ns}	0.5864 ^{ns}	0.3835 ^{ns}

F-test ratios are from ANOVA. ns, *, ** and ***: Not-significant and significant at 5%, 1% and 0.1% probability levels, respectively. Df: Degrees of freedom.

3.1. Soil Properties

Soil properties as affected by different plant densities and fertilization regimes during the 3-year experiment are presented in Table 4.

Table 4. Soil organic matter (SOM) and soil total nitrogen (STN) as affected by the plant density and fertilization.

Fertilization	Plant Density (Plants m^{-2})					
	200	300		200	300	
2017	Soil Organic Matter (SOM) (%)		*Mean*	Soil Total Nitrogen (STN) (%N)		*Mean*
Control	1.644	1.686	1.665 b	0.124	0.126	0.125 b
Manure	2.033	1.941	1.987 a	0.156	0.150	0.153 a
Compost	1.903	1.972	1.938 a	0.150	0.149	0.150 a
Inorganic	1.786	1.692	1.739 b	0.148	0.142	0.145 a

Table 4. *Cont.*

Mean	1.842 A	1.823 A		0.145 A	0.142 A	
$F_{Plant\ Density}$	0.3748 ns			0.3907 ns		
$F_{Fertilization}$	7.0649 ** (Tukey = 0.1274)			6.4801 ** (Tukey = 0.0132)		
$F_{Plant\ Density \times Fertilization}$	0.0940 ns			0.0932 ns		
2018						
Control	1.594	1.608	1.601 b	0.138	0.137	0.138 c
Manure	2.104	2.033	2.069 a	0.178	0.169	0.174 a
Compost	2.030	2.013	2.022 a	0.163	0.170	0.167 ab
Inorganic	1.739	1.627	1.683 b	0.160	0.146	0.153 bc
Mean	1.867 A	1.820 A		0.160 A	0.156 A	
$F_{Plant\ Density}$	0.1974 ns			0.6661 ns		
$F_{Fertilization}$	6.8596 ** (Tukey = 0.1557)			8.2321 ** (Tukey = 0.0083)		
$F_{Plant\ Density \times Fertilization}$	0.6888 ns			0.7344 ns		
2019						
Control	1.577	1.549	1.563 b	0.139	0.137	0.138 c
Manure	2.154	2.075	2.115 a	0.181	0.175	0.178 a
Compost	2.071	2.055	2.064 a	0.176	0.173	0.175 ab
Inorganic	1.664	1.564	1.614 b	0.158	0.155	0.157 b
Mean	1.867 A	1.811 A		0.164 A	0.160 A	
$F_{Plant\ Density}$	0.2963 ns			0.3367 ns		
$F_{Fertilization}$	10.8148 *** (Tukey = 0.1276)			10.4148 *** (Tukey = 0.0112)		
$F_{Plant\ Density \times Fertilization}$	0.0999 ns			0.1053 ns		

F-test ratios are from ANOVA. ns, ** and ***: Not-significant and significant at 1%, and 0.1% probability levels, respectively. The capital letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different plant density, and lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different fertilization.

Soil organic matter (SOM) is a chemical and biological soil characteristic that serves as a major organic nitrogen nutrient pool and a substrate for microbial activity. As presented in Table 4, during the experiment, the SOM was significantly affected by fertilization and raised after the application of organic fertilizers. In particular, the fertilizations with manure and compost gradually increased the levels of SOM during the experimental periods, with the highest values (2.115% and 2.064% for manure and compost, respectively) obtained in the third year (2019) of the experiment. In contrast, the application of inorganic fertilizer tended to decrease the SOM content in the course of time, with the lowest value (1.614%) observed in the last year (2019) of this study.

Soil total nitrogen (STN) is identified as an organic matter component, and its levels are enhanced by the application of organic fertilizers. According to Table 4, fertilization only had a significant effect on STN. The STN was significantly higher in the treatments with manure and compost. The highest values were noticed in manure (0.153%, 0.174% and 0.178%, for 2017, 2018, and 2019, respectively) which had no statistically significant differences with compost (0.150%, 0.167%, and 0.175%, for 2017, 2018, and 2019, respectively). Because of the continuous fertilization with organic fertilizers, the final rates of STN were increased by 16.4% and 16.0% in manure and compost, respectively, compared to the first year (2017) of the study.

3.2. Growth, Seed Yield and Yield Components of N. sativa

The results of the present study indicated that the plant height of *N. sativa* was affected by both plant density and fertilization (Table 5). Plant height was higher in the low-density plants (200 plants m^{-2}) than in high-density plots (300 plants m^{-2}) during the experimental periods, with the values of low-density plants being 58.8, 51.6, and 59.6 cm in 2017, 2018, and 2019, respectively. In response to fertilizers, the plant height increased up to the inorganic fertilization with the averaged values being 61.7, 55.2, and 63.5 cm in the first, second, and third year of the experiment, respectively.

Table 5. Plant height and leaf area index (LAI) as affected by plant density and fertilization.

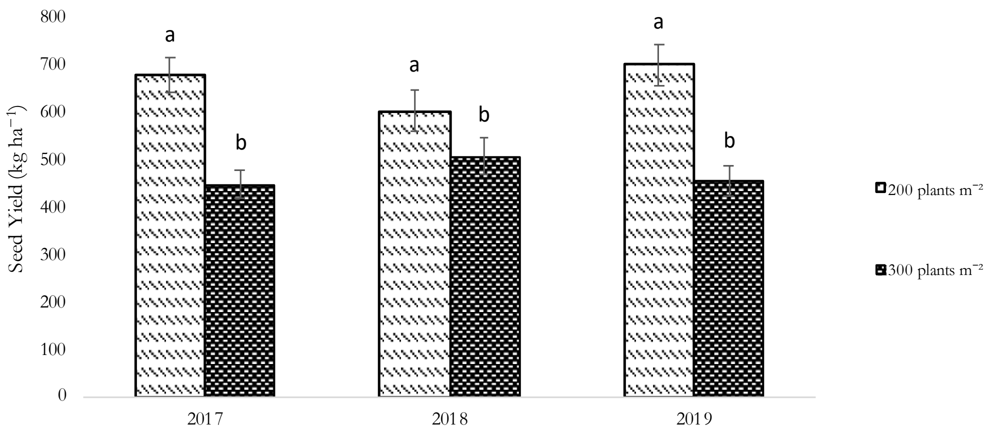
Fertilization	Plant Density (Plants m^{-2})					
	200	300		200	300	
2017	Plant Height (cm)		*Mean*	Leaf Area Index (LAI) (m^2 m^{-2})		*Mean*
Control	45.33	41.98	43.66 b	1.146	1.527	1.337 b
Manure	55.89	45.99	50.94 ab	1.702	1.757	1.730 b
Compost	66.56	53.90	60.23 a	2.173	2.532	2.353 a
Inorganic	67.29	56.09	61.69 a	2.378	2.964	2.671 a
Mean	58.77 A	49.49 B		1.850 B	2.195 A	
$F_{Plant\ Density}$	4.9897 * (Tukey = 9.253)			5.8572 * (Tukey = 0.1866)		
$F_{Fertilization}$	4.1390 * (Tukey = 12.773)			17.7728 *** (Tukey = 0.4575)		
$F_{Plant\ Density\ \times\ Fertilization}$	0.2445 ^{ns}			0.5864 ^{ns}		
2018						
Control	38.02	34.92	36.47 b	1.232	1.617	1.425 b
Manure	49.54	39.96	44.75 ab	1.834	1.860	1.847 b
Compost	56.93	44.77	50.85 a	2.169	2.682	2.426 a
Inorganic	62.04	48.36	55.20 a	2.606	3.081	2.844 a
Mean	51.63 A	42.00 B		1.961 A	2.310 A	
$F_{Plant\ Density}$	6.9992 * (Tukey = 9.024)			4.2587 ^{ns}		
$F_{Fertilization}$	4.9831 * (Tukey = 11.812)			13.6067 *** (Tukey = 0.5193)		
$F_{Plant\ Density\ \times\ Fertilization}$	0.4121 ^{ns}			0.4295 ^{ns}		
2019						
Control	42.68	38.59	40.64 c	1.225	1.630	1.428 b
Manure	55.91	46.08	51.00 bc	1.795	1.842	1.818 b
Compost	68.11	47.41	57.76 ab	2.290	2.656	2.473 a
Inorganic	71.67	55.34	63.51 a	2.537	3.102	2.820 a
Mean	59.59 A	46.86 B		1.962 B	2.308 A	
$F_{Plant\ Density}$	9.5346 ** (Tukey = 10.847)			5.8230 * (Tukey = 0.2043)		
$F_{Fertilization}$	5.6748 ** (Tukey = 14.368)			19.2348 *** (Tukey = 0.4584)		
$F_{Plant\ Density\ \times\ Fertilization}$	0.7822 ^{ns}			0.5742 ^{ns}		

F-test ratios are from ANOVA. ns, *, ** and ***: Not-significant and significant at 5%, 1%, and 0.1% probability levels, respectively. The capital letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different plant density, and lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different fertilization.

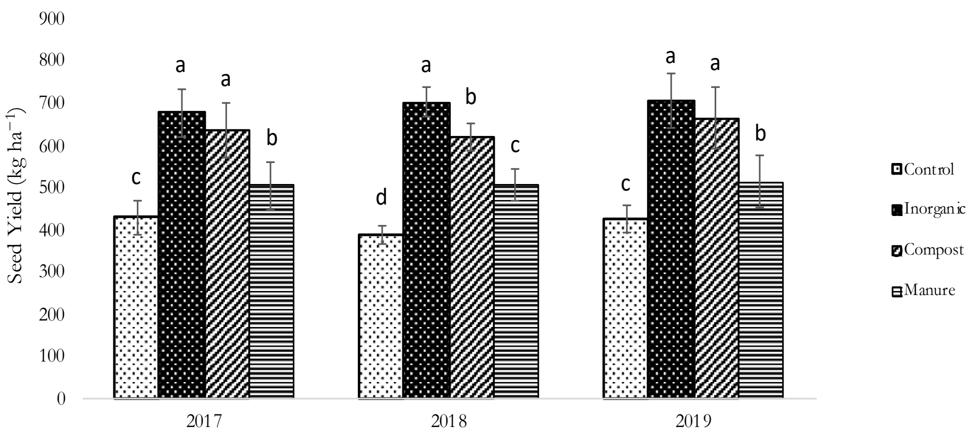
According to the combined analysis of variance (Table 3) and Table 5, leaf area index (LAI) was significantly influenced by plant density and fertilization. With the exception of the second year (2018) of the current study, the LAI was significantly higher in the high-density plots (300 plants m^{-2}), and values were 2.195 and 2.308 m^2 m^{-2} for the years 2017 and 2019, respectively. Concerning the effect of fertilization, averaged over the year and plant densities, the mean values of LAI were higher in the inorganic treatment

(2.778 m^2 m^{-2}) followed by compost (2.417 m^2 m^{-2}), manure (1.798 m^2 m^{-2}), and control (1.396 m^2 m^{-2}).

Seed yield was affected by both examined factors during the experimental periods. Concerning the plant density effect, the seed yields observed in low-density plots (677.3, 602.0, and 699.5 kg ha^{-1} in 2017, 2018, and 2019, respectively) were higher than in high-density plots (446.8, 506.5, and 455.9 kg ha^{-1} in 2017, 2018, and 2019, respectively). As for the fertilization effect, the highest seed yields were found in plots with inorganic fertilization (677.7, 703.4 and 706.3 kg ha^{-1} in 2017, 2018 and 2019, respectively) and compost (636.4, 619.8 and 665.3 kg ha^{-1} in 2017, 2018 and 2019, respectively) (Figure 2).



(**A**)



(**B**)

Figure 2. Seed yields as affected by (**A**) plant density and (**B**) fertilization. Vertical lines represent standard mean errors. Different lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$).

Harvest index (HI) was not affected by fertilization, but it was only influenced by the different plant densities (Table 6). Specifically, the highest HI were found in the case of low-density treatment, with the values being 0.231, 0.229, and 0.233 in 2017, 2018, and 2019, respectively, while the lowest values (0.119, 0.153 and 0.120 in 2017, 2018, and 2019, respectively) were obtained from the high-density plots.

Table 6. Harvest index (HI) and thousand seed weight as affected by the plant density and fertilization.

Fertilization	Plant Density (Plants m^{-2})					
	200	300		200	300	
2017	Harvest Index (HI)		*Mean*	1000 Seed Weight (g)		*Mean*
Control	0.225	0.106	0.166 a	1.496	1.512	1.504 a
Manure	0.217	0.122	0.170 a	1.545	1.487	1.516 a
Compost	0.231	0.119	0.175 a	1.540	1.535	1.538 a
Inorganic	0.251	0.130	0.191 a	1.655	1.596	1.626 a
Mean	0.231 A	0.119 B		1.559 A	1.533 A	
$F_{Plant\ Density}$	47.5421 *** (Tukey = 0.0302)			0.1714 ^{ns}		
$F_{Fertilization}$	0.4673 ^{ns}			0.7616 ^{ns}		
$F_{Plant\ Density \times Fertilization}$	0.1382 ^{ns}			0.0910 ^{ns}		
2018						
Control	0.196	0.129	0.163 a	1.502	1.524	1.513 a
Manure	0.233	0.148	0.191 a	1.532	1.483	1.508 a
Compost	0.232	0.154	0.193 a	1.527	1.529	1.528 a
Inorganic	0.257	0.179	0.218 a	1.621	1.587	1.604 a
Mean	0.229 A	0.153 B		1.546 A	1.531 A	
$F_{Plant\ Density}$	24.5912 *** (Tukey = 0.0325)			0.0591 ^{ns}		
$F_{Fertilization}$	2.1250 ^{ns}			0.5433 ^{ns}		
$F_{Plant\ Density \times Fertilization}$	0.0579 ^{ns}			0.0723 ^{ns}		
2019						
Control	0.213	0.107	0.160 a	1.470	1.481	1.476 a
Manure	0.221	0.113	0.167 a	1.499	1.437	1.468 a
Compost	0.238	0.124	0.181 a	1.553	1.543	1.548 a
Inorganic	0.260	0.137	0.199 a	1.656	1.604	1.630 a
Mean	0.233 A	0.120 B		1.545 A	1.516 A	
$F_{Plant\ Density}$	90.3770 *** (Tukey = 0.0248)			0.1651 ^{ns}		
$F_{Fertilization}$	2.0547 ^{ns}			1.1697 ^{ns}		
$F_{Plant\ Density \times Fertilization}$	0.1083 ^{ns}			0.0615 ^{ns}		

F-test ratios are from ANOVA. ns, ***: Not-significant and significant at 0.1% probability levels, respectively. The capital letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different plant density, and lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different fertilization.

Concerning the thousand seed weight, there were no significant differences between the high- and low-density plots; although, the plants of low-density treatment presented slightly higher values of this trait (1.559, 1.546 and 1.545 g in 2017, 2018 and 2019, respectively) than those of the high-density treatment (1.533, 1.531 and 1.516 g for the respective years). In the same manner, the effect of fertilization was not found to be statistically significant throughout the experimental periods; however, slightly higher values (1.626, 1.604, and 1.630 g in 2017, 2018, and 2019, respectively) were achieved in the plots fertilized with the inorganic fertilizer.

3.3. Nitrogen Content and Uptake in the Aerial Components of *N. sativa*

The effects of the plant density and fertilization on the biomass nitrogen (N) content of *N. sativa* are presented in Table 7. The maximum values were achieved in the timespan between blooming and full flowering (75 DAS) [44]. In the low-density plants, the values of biomass N content were substantially higher (3.08, 3.25, and 3.13%N in 2017, 2018, and 2019, respectively) than the high-density treatment (2.74, 2.86 and 2.81%N for the respective years). In addition, the mean values of biomass N provided good evidence of the effect of fertilization treatments. Averaged over plant densities and years, the highest values were found in inorganic fertilization (3.58%N) followed by compost (3.16%N), manure (2.88%N), and control (2.30%N).

Table 7. Biomass nitrogen content as affected by plant density and fertilization.

Fertilization	Plant Density (Plants m^{-2})								
	200	300		200	300		200	300	
	Biomass N Content (%N)								
2017	45 DAS		*Mean*	60 DAS		*Mean*	75 DAS		*Mean*
Control	1.13	1.01	1.07 b	1.72	1.13	1.43 b	2.39	2.15	2.27 c
Manure	1.53	1.20	1.37 ab	2.34	1.53	1.94 ab	3.12	2.50	2.81 b
Compost	1.67	1.52	1.60 a	2.49	1.67	2.08 a	3.22	2.95	3.09 b
Inorganic	1.68	1.56	1.62 a	2.44	1.68	2.06 a	3.59	3.34	3.47 a
Mean	1.50 A	1.32 A		2.25 A	1.49 B		3.08 A	2.74 B	
$F_{Plant\ Density}$		3.0759 ^{ns}			7.7270 * (Tukey = 0.347)			14.1810 ** (Tukey = 0.424)	
$F_{Fertilization}$		6.3472 ** (Tukey = 0.298)			6.3375 ** (Tukey = 0.432)			30.0160 *** (Tukey = 0.349)	
$F_{Plant\ Density \times Fertilization}$		0.2714 ^{ns}			0.7434 ^{ns}			1.0235 ^{ns}	
2018									
Control	1.21	1.07	1.14 b	1.89	1.71	1.80 b	2.56	2.28	2.42 c
Manure	1.64	1.25	1.45 ab	2.56	1.87	2.22 ab	3.36	2.59	2.98 b
Compost	1.70	1.61	1.66 a	2.59	2.31	2.45 a	3.20	3.21	3.21 b
Inorganic	1.80	1.62	1.71 a	2.74	2.47	2.61 a	3.87	3.36	3.62 a
Mean	1.59 A	1.39 A		2.45 A	2.09 B		3.25 A	2.86 B	
$F_{Plant\ Density}$		3.1729 ^{ns}			5.4360 * (Tukey = 0.392)			11.3631 ** (Tukey = 0.448)	
$F_{Fertilization}$		5.3518 ** (Tukey = 0.333)			4.9055 * (Tukey = 0.494)			18.9643 *** (Tukey = 0.436)	
$F_{Plant\ Density \times Fertilization}$		0.3535 ^{ns}			0.5351 ^{ns}			2.1428 ^{ns}	
2019									
Control	1.09	0.98	1.04 b	1.65	1.50	1.58 b	2.29	2.10	2.20 d
Manure	1.56	1.24	1.40 a	2.37	1.75	2.06 a	3.17	2.57	2.86 c
Compost	1.71	1.58	1.65 a	2.56	2.14	2.35 a	3.31	3.05	3.18 b
Inorganic	1.75	1.64	1.70 a	2.54	2.37	2.46 a	3.75	3.52	3.64 a
Mean	1.53 A	1.36 A		2.28 A	1.94 B		3.13 A	2.81 B	
$F_{Plant\ Density}$		2.1809 ^{ns}			6.2529 * (Tukey = 0.296)			10.7108 ** (Tukey = 0.498)	
$F_{Fertilization}$		7.4514 ** (Tukey = 0.316)			8.2270 ** (Tukey = 0.445)			37.6659 *** (Tukey = 0.351)	
$F_{Plant\ Density \times Fertilization}$		0.2151 ^{ns}			0.6731 ^{ns}			0.8870 ^{ns}	
Fertilization	Plant Density (Plants m^{-2})								
	200	300		200	300		200	300	
	Biomass N Content (%N)								
2017	85 DAS		*Mean*	100 DAS		*Mean*	115 DAS		*Mean*
Control	2.03	1.82	1.93 c	1.81	1.63	1.72 c	1.68	1.59	1.64 b
Manure	2.64	2.17	2.41 b	2.47	1.98	2.23 b	2.29	1.85	2.07 a
Compost	2.73	2.50	2.62 b	2.61	2.23	2.42 ab	2.42	2.08	2.25 a
Inorganic	3.10	2.71	2.91 a	2.65	2.39	2.52 a	2.40	2.21	2.31 a

Table 7. *Cont.*

Mean	2.63 A	2.30 B		2.39 A	2.06 B		2.20 A	1.93 B	
$F_{Plant\ Density}$	14.3382 ** (Tukey = 0.354)			12.0698 ** (Tukey = 0.324)			5.6917 * (Tukey = 0.308)		
$F_{Fertilization}$	22.8282 *** (Tukey= 0.321)			13.9822 *** (Tukey = 0.316)			7.2337 ** (Tukey = 0.354)		
$F_{Plant\ Density \times Fertilization}$	0.5520 ^{ns}			0.4812 ^{ns}			0.4797 ^{ns}		
2018									
Control	2.17	1.90	2.04 c	1.86	1.74	1.80 b	1.90	1.70	1.80 b
Manure	2.82	2.29	2.56 b	2.63	2.08	2.36 a	2.44	1.91	2.18 a
Compost	2.74	2.68	2.71 b	2.62	2.41	2.52 a	2.39	2.27	2.33 a
Inorganic	3.30	2.82	3.06 a	2.81	2.47	2.64 a	2.64	2.29	2.47 a
Mean	2.76 A	2.42 B		2.48 A	2.18 B		2.34 A	2.04 B	
$F_{Plant\ Density}$	8.2015 * (Tukey = 0.393)			7.5441 * (Tukey = 0.301)			6.2373 * (Tukey = 0.271)		
$F_{Fertilization}$	13.2084 *** (Tukey = 0.398)			11.0254 *** (Tukey = 0.370)			5.8531 ** (Tukey = 0.383)		
$F_{Plant\ Density \times Fertilization}$	0.8515 ^{ns}			0.6719 ^{ns}			0.5636 ^{ns}		
2019									
Control	1.95	1.78	1.87 c	1.74	1.59	1.67 c	1.43	1.37	1.40 c
Manure	2.69	2.24	2.47 b	2.51	2.04	2.28 b	1.98	1.57	1.78 b
Compost	2.80	2.59	2.70 b	2.69	2.31	2.50 ab	2.15	1.73	1.94 b
Inorganic	3.24	2.84	3.04 a	2.77	2.51	2.64 a	2.29	2.18	2.24 a
Mean	2.67 A	2.36 B		2.43 A	2.11 B		1.96 A	1.71 B	
$F_{Plant\ Density}$	11.6009 ** (Tukey = 0.295)			9.6582 ** (Tukey = 0.237)			6.6947 * (Tukey = 0.229)		
$F_{Fertilization}$	29.9780 *** (Tukey = 0.322)			18.0244 *** (Tukey = 0.346)			12.816 *** (Tukey = 0.265)		
$F_{Plant\ Density \times Fertilization}$	0.5447 ^{ns}			0.4465 ^{ns}			0.9498 ^{ns}		

F-test ratios are from ANOVA. ns, *, ** and ***: Not-significant and significant at 5%, 1%, and 0.1% probability levels, respectively. The capital letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different plant density, and lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different fertilization.

Nitrogen (N) uptake in the aerial biomass was estimated by multiplying the N content of the aerial biomass and the aerial biomass yield at the time of maturity (115 DAS). According to the combined analysis (Table 3), N uptake of the aerial biomass was influenced by the plant density and fertilization. Averaged over fertilization treatments, the highest yields (59.57, 52.14, and 50.18 kg N ha^{-1} in 2017, 2018, and 2019, respectively) were recorded when plants subjected to high-density (Table 8). During the three-year experiment, the mean values of biomass N uptake were greatest in the inorganic treatment (59.05, 53.13, and 56.09 kg N ha^{-1} in 2017, 2018, and 2019, respectively) followed by compost (60.23, 54.84, and 49.75 kg N ha^{-1} in 2017, 2018, and 2019, respectively), while the lowest values (32.96, 32.92, and 29.99 kg N ha^{-1} for the respective years) were found in the untreated (control) plants.

Table 8. Biomass nitrogen (N) uptake, total plant N uptake, seed N content, and seed N uptake as affected by plant density and fertilization.

Fertilization	**Plant Density (Plants m^{-2})**											
	200	**300**		**200**	**300**		**200**	**300**		**200**	**300**	
2017	**Biomass N Uptake (kg N ha^{-1})**		*Mean*	**Total Plant N Uptake (kg N ha^{-1})**		*Mean*	**Seed N Content (%N)**		*Mean*	**Seed N Uptake (kg N ha^{-1})**		*Mean*
Control	23.88	42.03	32.96 b	38.71	51.71	45.21 c	2.88	2.80	2.84 c	14.83	9.67	12.25 d
Manure	41.89	52.77	47.33 ab	64.96	64.24	64.60 b	3.76	3.00	3.38 b	23.06	11.47	17.27 c
Compost	51.72	68.74	60.23 a	81.88	86.27	84.08 a	3.87	3.54	3.71 b	30.16	17.54	23.85 b
Inorganic	43.35	74.75	59.05 a	77.95	97.51	87.73 a	4.31	4.09	4.20 a	34.60	22.76	28.68 a
Mean	40.21 B	59.57 A		65.88 A	74.93 A		3.71 A	3.36 B		25.66 A	15.36 B	
$F_{Plant\ Density}$	12.2542 ** (Tukey = 14.188)			2.2639 ^{ns}			7.9482 * (Tukey = 0.499)			88.9179 *** (Tukey = 6.009)		
$F_{Fertilization}$	5.2763 * (Tukey = 20.011)			10.6237 *** (Tukey = 17.738)			21.7320 *** (Tukey = 0.427)			43.7798 *** (Tukey = 3.639)		
$F_{Plant\ Density \times Fertilization}$	0.6103 ^{ns}			0.5589 ^{ns}			1.4044 ^{ns}			2.5077 ^{ns}		
2018												
Control	26.61	39.22	32.92 b	38.87	48.84	43.86 c	3.05	2.59	2.82 c	12.27	9.62	10.95 d
Manure	39.38	45.40	42.39 ab	61.54	58.03	56.79 b	3.82	2.94	3.38 bc	22.16	12.63	17.40 c
Compost	45.16	64.52	54.84 a	69.30	85.20	77.25 a	3.63	3.65	3.64 ab	24.14	20.68	22.41 b
Inorganic	46.84	59.41	53.13 a	79.89	84.65	82.27 a	4.93	3.81	4.37 a	33.05	25.25	29.15 a
Mean	39.50 B	52.14 A		62.40 A	69.18 A		3.86 A	3.25 B		22.91 A	17.05 B	
$F_{Plant\ Density}$	9.1053 ** (Tukey = 10.975)			1.7885 ^{ns}			6.0353 * (Tukey = 0.551)			16.4998 *** (Tukey = 6.556)		
$F_{Fertilization}$	5.9493 ** (Tukey = 14.189)			11.9203 *** (Tukey= 16.442)			7.6640 ** (Tukey = 0.634)			28.5723 *** (Tukey = 3.748)		
$F_{Plant\ Density \times Fertilization}$	0.4223 ^{ns}			0.6585 ^{ns}			0.9271 ^{ns}			1.3379 ^{ns}		
2019												
Control	22.68	37.31	30.00 c	33.72	44.70	39.21 c	2.20	2.12	2.16 c	11.04	7.39	9.22 d
Manure	33.87	39.62	36.74 b	56.27	52.87	54.57 b	3.55	3.36	3.46 b	22.40	13.26	17.83 c
Compost	44.65	54.84	49.75 a	74.81	72.31	73.56 a	3.65	3.46	3.56 b	30.16	17.48	23.82 b
Inorganic	43.22	68.96	56.09 a	76.41	91.72	84.07 a	3.97	3.98	3.98 a	33.19	22.76	27.98 a
Mean	36.11 B	50.18 A		60.30 A	65.40 A		3.34 A	3.23 A		24.20 A	15.22 B	

Table 8. *Cont.*

$F_{Plant\ Density}$	11.0706 ** (Tukey = 12.232)	1.3639 ns	1.5880 ns	128.0487 *** (Tukey = 6.559)
$F_{Fertilization}$	7.9176 ** (Tukey = 16.335)	20.8526 *** (Tukey = 13.162)	76.5050 *** (Tukey = 0.254)	105.3903 *** (Tukey = 3.652)
$F_{Plant\ Density \times Fertilization}$	1.0293 ns	1.1758 ns	0.3006 ns	5.8659 ns

F-test ratios are from ANOVA. ns, *, ** and ***: Not-significant and significant at 5%, 1%, and 0.1% probability levels, respectively. The capital letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different plant density, and lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different fertilization.

Seed nitrogen (N) content was significantly influenced by both plant density and fertilization during the three-year experiment. With the exception of the third year (2019), the seed N content was substantially higher in the plants of low-density plots, and the values were 3.70 and 3.72% N for the years 2017 and 2018, respectively (Table 8). In response to fertilization, the highest seed N content was achieved in inorganic fertilization (4.19, 4.10, and 3.98% N in 2017, 2018, and 2019, respectively) followed by compost (3.71, 3.64, and 3.55% N in 2017, 2018, and 2019, respectively) and manure (3.38, 3.38, and 3.46% N for the respective years) treatments.

Nitrogen (N) uptake in seeds was defined by multiplying the N content of the seeds with the seed yield. The results of the experiment indicated that seed N uptake were affected by the plant density and fertilization during the experimental periods. In regard to plant density, the highest values (25.66, 22.91, and 24.20 kg N ha^{-1} in 2017, 2018, and 2019, respectively) were obtained when plants were subjected to low density (200 plants m^{-2}). The highest seed N uptake value was achieved in inorganic fertilization treatment with the values being 28.68 (134% higher than control), 29.15 (166% higher than control), and 27.98 kg N ha^{-1} (204% higher than control) in 2017, 2018, and 2019, respectively (Table 8).

Total plant nitrogen (N) uptake was determined by multiplying the N content of total above-ground (aerial biomass + seeds) dry matter and the total above-ground dry matter yield at the time of maturity (115 DAS). Total plant N uptake was significantly affected by the different plant density and fertilization treatments. Averaged over years and fertilization treatments, the highest value (69.84 kg N ha^{-1}) was recorded when plants were subjected to high density (Table 8). Concerning the effect of fertilization, the highest total plant N uptake values, averaged over years and plant density treatments, were achieved in inorganic fertilization (84.69 kg N ha^{-1}) and compost (78.30 kg N ha^{-1}), while the lowest value (42.76 kg N ha^{-1}) was obtained in the untreated (control) plot.

3.4. Nitrogen Use Efficiency of *N. sativa*

The nitrogen use efficiency of *N. sativa* crop as affected by different plant densities and fertilization regimes are shown in Table 9.

Table 9. Nitrogen harvest index (NHI), apparent nitrogen recovery efficiency (ANRE), nitrogen utilization efficiency (NUtE), and nitrogen agronomic efficiency (NAE) as affected by the plant density and fertilization.

Fertilization	Plant Density (Plants m^{-2})											
	200	300		200	300		200	300		200	300	
2017	N Harvest Index (NHI)		*Mean*	Apparent N Recovery Efficiency (ANRE) (%)		*Mean*	N Utilization Efficiency (NUtE) (kg kg^{-1})		*Mean*	N Agronomic Efficiency (NAE) (kg kg^{-1})		*Mean*
Control	0.383	0.188	0.286 b	-	-	-	13.29	6.68	9.99 a	-	-	-

Table 9. *Cont.*

Manure	0.357	0.184	0.271 b	86.35	41.23	63.79 a	9.49	6.39	7.94 b	3.27	1.65	2.46 b
Compost	0.369	0.204	0.287 b	107.94	86.42	97.18 a	9.52	5.76	7.64 b	6.61	3.72	5.17 a
Inorganic	0.452	0.243	0.348 a	87.20	101.79	94.50 a	10.56	5.97	8.27 b	6.34	4.68	5.51 a
Mean	0.390 A	0.205 B		93.83 A	76.48 A		10.72 A	6.20 B		5.41 A	3.35 A	
$F_{Plant\ Density}$		97.9952 *** (Tukey = 0.0429)			0.7851 ^{ns}			63.8975 *** (Tukey = 1.409)			4.6058 ^{ns}	
$F_{Fertilization}$		3.3397 * (Tukey = 0.0323)			1.1973 ^{ns}			3.4509 * (Tukey = 1.621)			4.0459 * (Tukey = 2.318)	
$F_{Plant\ Density} \times$ $_{Fertilization}$		0.2970 ^{ns}			0.7864 ^{ns}			1.8166 ^{ns}			0.1899 ^{ns}	
2018												
Control	0.317	0.197	0.257 b	-	-	-	10.42	7.59	9.01 a	-	-	
Manure	0.363	0.223	0.293 b	74.57	30.21	52.39 a	9.52	7.65	8.58 a	6.06	1.85	3.96 a
Compost	0.352	0.242	0.297 b	76.08	90.88	83.48 a	9.70	6.78	8.24 a	6.70	4.94	5.82 a
Inorganic	0.425	0.296	0.361 a	91.15	79.59	85.37 a	9.79	7.78	8.79 a	7.71	6.37	7.04 a
Mean	0.364 A	0.240 B		80.60 A	66.89 A		9.86 A	7.45 B		6.82 A	4.39 A	
$F_{Plant\ Density}$		43.4772 *** (Tukey = 0.0472)			0.5093 ^{ns}			24.2701 *** (Tukey = 0.923)			3.7986 ^{ns}	
$F_{Fertilization}$		5.2374 * (Tukey = 0.0563)			1.2400 ^{ns}			0.4454 ^{ns}			2.0503 ^{ns}	
$F_{Plant\ Density} \times$ $_{Fertilization}$		0.1118 ^{ns}			0.7942 ^{ns}			0.3020 ^{ns}			0.5102 ^{ns}	
2019												
Control	0.328	0.167	0.248 b	-	-	-	14.90	7.88	11.39 a	-	-	
Manure	0.399	0.247	0.323 a	74.18	26.88	50.53 b	11.23	7.37	9.30 b	4.22	1.56	2.89 b
Compost	0.403	0.248	0.326 a	102.73	69.03	85.88 ab	11.04	7.18	9.11 b	8.10	3.88	5.99 a
Inorganic	0.456	0.250	0.353 a	94.86	104.48	99.67 a	11.39	6.27	8.83 b	7.51	4.95	6.23 a
Mean	0.397 A	0.228 B		90.59 A	66.80 A		12.14 A	7.18 B		6.61 A	3.46 B	
$F_{Plant\ Density}$		52.9955 *** (Tukey = 0.0544)			2.6135 ^{ns}			92.7315 *** (Tukey = 1.401)			9.6704 ** (Tukey = 2.493)	
$F_{Fertilization}$		3.8349 * (Tukey = 0.0975)			3.9543 * (Tukey = 41.301)			5.1415 * (Tukey = 1.343)			4.5135 * (Tukey = 2.975)	
$F_{Plant\ Density} \times$ $_{Fertilization}$		0.2896 ^{ns}			1.3600 ^{ns}			2.0984 ^{ns}			0.2822 ^{ns}	

F-test ratios are from ANOVA. ns, *, ** and ***: Not-significant and significant at 5%, 1%, and 0.1% probability levels, respectively. The capital letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different plant density, and lowercase letters denote statistically significant differences according to the Tukey's HSD test ($p \leq 0.05$) under different fertilization.

The nitrogen harvest index (NHI) was affected by both plant density and fertilization during the experimental periods. Concerning the plant density effect, the NHI recorded in low-density plots (0.390, 0.364, and 0.397 in 2017, 2018, and 2019, respectively) were higher than in high-density treatments (0.205, 0.240, and 0.228 in 2017, 2018, and 2019, respectively) (Table 9). Regarding the fertilization treatments, the highest NHI ratios were found in inorganic fertilization (0.348, 0.361, and 0.353 in 2017, 2018, and 2019, respectively).

The results of the experiment indicated that apparent nitrogen recovery efficiency (ANRE) was not affected by plant density during the experimental periods; although, the plants of low-density treatment presented slightly higher values of this index (93.83, 80.60, and 90.59% in 2017, 2018, and 2019, respectively) than those of the high-density treatment (76.49, 66.89 and 66.80% for the respective years). Concerning the effect of fertilization, this had a great impact on ANRE during the third year (2019) of the experiment, with the mean values being 99.67, 85.88 and 50.33% for application of inorganic fertilizer, compost, and manure, respectively (Table 7).

Nitrogen utilization efficiency (NUtE) was significantly higher in the low-density plants (200 plants m^{-2}) than in high-density plants (300 plants m^{-2}) during the experimental periods, with the values in low-density plants being 10.72, 9.86, and 12.14 kg kg^{-1} in 2017, 2018, and 2019, respectively (Table 9). In response to fertilization, this has a significant effect in the first (2017) and third year (2019) of the experiment, and the highest value was found in the control (9.99 and 11.39 kg kg^{-1} in 2017 and 2019, respectively), followed by inorganic fertilization (8.27 and 8.83 kg kg^{-1} in 2017 and 2019, respectively) manure (7.94 and 9.30 kg kg^{-1} in 2017 and 2019, respectively), and compost (7.64 and 9.11 kg kg^{-1} in 2017 and 2019, respectively).

Nitrogen agronomic efficiency (NAE) did not differ among plant densities in 2017 and 2018; however, significant differences were found in the third year (2019) of the experiment, where the highest value (6.61 kg kg^{-1}) was obtained in low-density plants (Table 9). With the exception of the second year (2018) of the present study, the NAE ratio was significantly higher in inorganic fertilization (5.51 and 6.24 kg kg^{-1} in 2017 and 2019, respectively) and compost treatments (5.16 and 5.99 kg kg^{-1} in 2017 and 2019, respectively).

4. Discussion

Organic fertilizers typically increase soil microbial mass by providing carbon-rich organic compounds to the generally low-carbon microbial communities present in arable soils [45]. The incorporation of organic fertilizers can increase soil microbial activity by between 16% and 20% compared to inorganic fertilizers [46,47]. In addition, several studies have found that the application of organic fertilizers leads to an increase in enzymatic activities involved in the release of major macronutrients for plants [46,47]. Consequently, organic fertilizers can stimulate soil microbial processes and increase crop yields compared to inorganic fertilization. This property has been associated with increased organic matter and soil fertility after the continuous application of organic fertilizers [45]. Indeed, in the present study, organic fertilizers and their application resulted in a higher content of organic matter than inorganic fertilizer (Table 4). The concentration of soil organic matter (SOM) during the three-year experiment increased compared to the initial concentration by 6% and 6.2% in the manure and compost plots, respectively, while it decreased by 7.5% in the inorganic fertilization plots. In general, the continuous use of inorganic fertilizers can reduce organic matter reserves as it enhances its mineralization [48] with the consequent reduction of cultivated soil quality and even the increase of soil acidification and environmental pollution [49].

Soil total nitrogen (STN) is recognized as a factor that is important for soil fertility in both managed and natural ecosystems [50] and may reflect the nitrogen status of the soil. In the present study, the average STN was affected by the type of fertilizer. All fertilization systems had a very significant influence on increasing the STN (Table 4). In particular, manure and compost were found to have the greatest effect on increasing the STN, while, with the exception of the control, the least positive effect was observed in the case of inorganic fertilizer. The beneficial effect of the continuous application of manure on the increase of STN has also been reported by Sadej and Przekwas [51] and Zhengchao et al. [52]. Specifically, continuous application of organic fertilizer can accelerate the activation of soil nutrients, improve soil nutrient content, maintain available nutrient balance, and then improve soil fertility [53].

In the surface layer of most soils, about 90–98% of the STN occurs in organic forms [54]. STN, according to its origin, can be divided into two broad categories: (a) nitrogen from organic residues, consisting of residues of plant or animal origin that have not been treated and partially decomposed products, and (b) nitrogen from soil organic matter or humus [54]. Therefore, the amount of total nitrogen in the soil is directly affected by the soil organic matter (SOM) and indirectly by the factors that affect the organic matter, such as fertilization. This is also proved by the significant linear correlation between the SOM and STN (r = 0.5515, $p \leq 0.001$; Table 10).

Plant height received the highest values at a plant density of 200 plants m^{-2} with a 3-year average value being 23% higher compared to the plant density of 300 plants m^{-2}. A similar response of plant height to plant density has been reported by Mollafilabi et al. [55], who found that height increased with increasing plant density to 180 plants m^{-2}, and then decreased with further density increase. The increase in height at high plant densities is probably caused by the elongation of the shoots and the increase in the number of nodes per plant due to mutual shading [55–57]. Specifically, mutual shading results in the accumulation of auxin, which, as a bioactive hormone, stimulates cell division and elongation [58]. The decrease in height above the optimal plant density is caused by competition between plants for factors that contribute to their growth, such as soil moisture, light, and nutrients [56].

Regarding the effect of fertilization on plant height, statistically significant differences were observed between the fertilization systems with the highest values being found in inorganic fertilization and compost, followed by manure, while the lowest value was found in the control (Table 5). The increase in height of plants with different sources of nitrogen can be attributed to the fact that nitrogen, which promotes plant growth, increases the number and length of internodes resulting in a gradual increase in height [59]. The addition of nitrogen (up to the optimal level) increases the production of cytokines, which in turn affects the elasticity of cell walls [60], the number of meristematic cells and cell growth [61]. In the present study, the significant increase in plant height achieved with the application of inorganic fertilizer and compost was due to the fact that they provided greater and similar amounts of nitrogen available to the plants than manure, but also control, where observed lower values due to insufficient supply of nutrients [62].

Compost generally improves soil fertility by playing an essential role in improving the physicochemical and biological properties of the soil. In addition to preserving and improving soils, it also acts as slow-release fertilizer during mineralization compared to inorganic fertilizers, most of which are very soluble when applied to the soil. In particular, the application of compost can increase the organic matter of the soil. The organic matter of the soil improves its structure and at the same time increases the availability of nutrients. The availability of organic matter also contributes to crop growth and yield by directly providing nutrients and indirectly modifying soil physical properties, such as soil aggregate stability and porosity, which can improve root growth, rhizosphere, and promote plant growth [63]. Moreover, compared to manure, composts contain a higher amount of humic substances [64]. Humic substances are heterogeneous organic macromolecules consisting of humic acids (HAs), fulvic acids (FAs) and humine. Humic substances improve soil fertility by improving the physicochemical properties of the soil and, in particular, by improving the structure of the soil as a source of nutrients and trace elements for the intake of plants with induced activities of microflora and fauna, which are important in the life cycle on Earth. In addition, they affect the physiological, metabolic, and growth processes of plants. Finally, humic substances activate the plasma membrane H-ATPase, respiration, and activation of genes involved in nitrate (NO_3^-) uptake in plants [65,66]. Thus, compost could constitute a valuable alternative fertilization source to increase crop production.

Table 10. Correlation coefficients between evaluated traits.

Trait	Coefficient of Correlation (*r*)														
	Soil Organic Matter (SOM)	**Soil Total Nitrogen (STN)**	**Plant Height**	**Leaf Area Index (LAI)**	**Seed Yield**	**Harvest Index (HI)**	**1000 Seed Weight**	**Biomass N Content 75 DAS**	**Biomass N Uptake**	**Total Plant N Uptake**	**Seed N Content**	**Seed N Uptake**	**N Harvest Index (NHI)**	**Apparent N Recovery Efficiency (ANRE)**	**N Utilization Efficiency (NUtE)**
Soil Total Nitrogen (STN)	0.5515 ***														
Plant Height	0.2150 ns	0.2958 *													
Leaf Area Index (LAI)	0.0893 ns	0.2836 *	0.6263 ***												
Seed Yield	0.1639 ns	0.3130 **	0.7711 ***	0.4676 ***											
Harvest Index (HI)	0.0162 ns	0.1017 ns	0.1987 ns	−0.2077 ns	0.6806 ***										
1000 Seed Weight	−0.3074 ns	−0.0077 ns	0.1965 ns	0.2333 *	0.2360 *	0.1363 ns									
Biomass N Content 75 DAS	0.1686 ns	0.4463 ***	0.6613 ***	0.6839 ***	0.7717 ***	0.4066 ***	0.2554 *								
Biomass N Uptake	0.1430 ns	0.1567 ns	0.3949 ***	0.7980 ***	0.1791 ns	−0.4090 ***	0.1317 ns	0.4134 ***							
Total Plant N Uptake	0.1819 ns	0.2633 *	0.6469 ***	0.8865 ***	0.5307 ***	−0.0969 ns	0.2121 ns	0.7002 ***	0.9189 ***						
Seed N Content	0.2215 ns	0.3467 **	0.6493 ***	0.6730 ***	0.6319 ***	0.2659 *	0.2214 ns	0.8910 ***	0.4115 ***	0.6775 ***					
Seed N Uptake	0.1631 ns	0.3344 **	0.8019 ***	0.5969 ***	0.9413 ***	0.5637 ***	0.2587 *	0.8956 ***	0.2808 *	0.6366 ***	0.8438 ***				
N Harvest Index (NHI)	0.0582 ns	0.1990 ns	0.4549 ***	−0.0113 ns	0.7344 ***	0.8391 ***	0.1481 ns	0.5441 ***	−0.4296 ***	−0.0515 ns	0.5086 ***	0.7146 ***			
Apparent N Recovery Efficiency (ANRE)	−0.1835 ns	−0.0217 ns	0.6979 ***	0.6321 ***	0.5394 ***	0.0088 ns	0.1447 ns	0.5964 ***	0.6667 ***	0.8550 ***	0.4750 ***	0.5731 ***	0.0338 ns		
N Utilization Efficiency (NUtE)	−0.1343 ns	−0.0616 ns	0.0492 ns	−0.4813 ***	0.3714 **	0.7525 ***	−0.0026 ns	−0.0257 ns	−0.7494 ***	−0.5257 ***	−0.1526 ns	0.1860 ns	0.7516 ***	-0.1671 ns	
N Agronomic Efficiency (NAE)	−0.2682 ns	0.1042 ns	0.5535 ***	0.3699 **	0.8375 ***	0.4921 ***	0.2129 ns	0.6015 ***	0.1539 ns	0.4541 ***	0.2810 *	0.7316 ***	0.4428 ***	0.6105 ***	0.4081 **

ns, *, ** and ***: Not-significant and significant at 5%, 1%, and 0.1% probability levels, respectively.

The leaf area index (LAI) was significantly affected by both sowing density and fertilization. Regarding the effect of plant density, it was observed that with increasing density, the LAI increased. The increase in LAI with the increase in plant density is related to the effective inhibition of light [67] and can therefore enable higher plant densities to achieve higher photosynthetic production per unit area and higher biomass production [68]. This result is also supported by the significant and positive correlations of LAI with total above-ground dry matter (r = 0.8371, $p \leq 0.001$; data not shown). In terms of fertilization, this had a positive effect on the LAI ratio, with the highest values being found in plants that received inorganic fertilizer. These findings are consistent with the findings of Özgüven and Serekoglu [69] and Tuncturk et al. [3], which shows the positive effect of increasing nitrogen levels on the leaf area of *N. sativa* plants.

Plant density had a significant effect on seed yield of *N. sativa* crop throughout the three-year experiment. Seed yield was higher at the plant density of 200 plants m^{-2} with the three-year average value being 40.4% higher than the density of 300 plants m^{-2}. According to the study of Mollafilabi et al. [55], it was found that the highest seed yield in *N. sativa* was achieved at a sowing density of 180 m^{-2} plants (809 kg ha^{-1}) and an increase in density to 240 plants m^{-2} reduced the yield by 38%. In terms of fertilization, there was a significant effect of different fertilizations on the seed yield of *N. sativa*. In particular, the three-year average value of seed yield was statistically significantly higher in the plots that had received the inorganic fertilizer (696 kg ha^{-1}), with the compost (641 kg ha^{-1}) following, while the lowest yield presented by the control (414 kg ha^{-1}). According to several authors, higher yields have been observed in various crop fertilizers with inorganic fertilizers, as these fertilizers, in relation to the organic ones, contained soluble inorganic nitrogen with rapid availability to the cultivated plant species resulting in greater growth and higher yields [29,70–72]. This result is also supported by the significant and positive correlations of seed yield with plant height (r = 0.7711, $p \leq 0.001$; Table 10), total above-ground dry matter (r = 0.5467, $p \leq 0.001$; data not shown), and LAI (r = 0.4676, $p \leq 0.001$; Table 10).

The harvest index (HI) had a negative response to the increase in sowing density (Table 6). The highest value of the index was recorded at the low plant density (200 plants m^{-2}) with the 3-year average being 76.8% higher compared to the high plant density (300 plants m^{-2}). In general, it has been observed that the harvest index can be increased and maintained as relatively stable over a wide range of sowing densities, and decreases linearly when sowing density is above the optimum for crop yield or when dry weight per plant during maturation is too low or too high [73]. Regarding fertilization, the highest values of the harvest index were found after the application of inorganic fertilizer (Table 6). These results are consistent with the findings of Yimam et al. [74], where they argued that an adequate increase and supply of nitrogen in *N. sativa*, up to 60 kg N ha^{-1}, may be associated with strong vegetative growth and efficient use of available nutrients, which may lead to higher productivity, with higher yields and higher harvest index.

The thousand seed weight was not affected by either the seed density or the different fertilizations. At this point, it is worth noting that the plants of low plant density, as well as the plants of inorganic fertilization, showed slightly higher values (Table 6). Their mean values ranged from 1.476–1.630 g. In various studies, the thousand seed weight of *N. sativa* ranged from 1.77 g [56] to 3.50 g [75]; however, the results of the present study showed that this was lower than that referred to the international literature. In general, the thousand seed weight is affected by a wide range of factors such as variety, cultivation techniques, climatic factors as well as soil properties. According to the study of Talafih et al. [57], increasing the sowing rate of *N. sativa* from 35 to 40 kg of seed per hectare resulted in a reduction in the thousand seed weight by 3%. In contrast, in the study of Toncer and Kizil [56], increasing the sowing rate from 10 to 50 kg of seed per hectare did not significantly change the thousand seed weight. Moreover, Özgüven and Serekoglu [69] observed that the increase in nitrogen levels (from 0 to 90 kg N ha^{-1}) did not affect the weight of the thousand seeds of *N. sativa*, but the increase in phosphorus levels (from 0 to 60 kg P_2O_5 ha^{-1}) had a significant effect on this trait.

The results of the nitrogen (N) content of the above-ground biomass and the seeds of *N. sativa* are presented in Tables 7 and 8, respectively. The seed N content of *N. sativa* was positively affected by the content of total N in the biomass. This is confirmed by the significantly high and positive linear correlation between these two parameters (r = 0.8245, $p \leq 0.001$), as shown in Table 10. Increasing the amount of N available for plant uptake increases the vegetative growth, resulting in a higher percentage of N in the plant [76]. The fact that the administered N is one of the most important elements in increasing the content of total N in the seeds is shown by the fact that the increased amount of available N in the plant caused an increase in the accumulation of this element in the seeds. These results are similar to those of other researchers who reported that N concentrations in plant shoots and then in seeds increased with increasing amount of available N [76–78].

Seed nitrogen (N) uptake was significantly affected by seed density and fertilization during the three-year experiment. N uptake into seeds received the highest values at the sowing density of 200 plants m^{-2} with the 3-year average value being 71% higher than the sowing density of 300 plants m^{-2}. Concerning the effect of fertilization, the highest mean value of the three experimental years was found in inorganic fertilization (28.6 kg N ha^{-1}), while the lowest value was presented in the control (10.8 kg N ha^{-1}).

Regarding the absorption of nitrogen (N) in total above-ground dry matter (Total plant N uptake), the combined analysis of variability showed that both examined factors significantly influenced this characteristic. In particular, the high plant density (300 plants m^{-2}) with a 3-year average value of 69.8 kg N ha^{-1}, was significantly superior to that of the 200 plants m^{-2} with a 3-year average value of 6.29 kg N ha^{-1}. In terms of fertilization, the highest average three-3 values were recorded in inorganic fertilization and compost, with the values being 97.9% and 82.9% higher than the control, respectively (Table 8).

The response trends for N uptake into seeds and total above-ground biomass at different levels of fertilization and seed density are similar to crop yield and total crop dry weight, respectively, as determined and described by Raymond et al. [77] and Johnson et al. [79]. In the present study, this is confirmed by the significant positive correlations of seed N uptake with seed yield (r = 0.9413, $p \leq 0.001$; Table 10) and total plant N uptake with total above-ground biomass of the crop (r = 0.7770, $p \leq 0.001$; data not shown). In general, increased nitrogen uptake with increased nitrogen availability and an increase in sowing density can be attributed to increased dry matter production and also to increased nitrogen concentration in plant tissues [77,80].

The nitrogen harvest index (NHI) is an important indicator for measuring the re-translocation efficiency of absorbed nitrogen from the vegetative parts of the plant to its seeds. This indicator is very useful for measuring the nitrogen distribution in cultivated plants, providing an indication of how efficiently the absorbed nitrogen was used for seed production [35]. High NHI values indicate increased nitrogen distribution in seeds [81]. Indeed, the effect of plant density and fertilization on the NHI index was proportional to that of the harvest index (HI). Specifically, the NHI index received the highest values in the sowing density of 200 plants m^{-2} with the 3-year average value being 71% higher than the sowing density of 300 plants m^{-2}. Regarding the effect of fertilization, statistically significant differences were observed between the fertilization systems with the highest 3-year mean value being found in inorganic fertilization (0.3539), while the lowest value was found in the control (0.2634).

The apparent nitrogen recovery efficiency (ANRE) index depends on the correlation between the demand of the crop for nitrogen and the amount of nitrogen released from the nitrogen applied to the crop [82]. The index showed the highest values in the sub-plots of inorganic fertilization and compost. For the three-year experiment, averaged values were 93.18% for inorganic fertilizer, 88.85% for compost, and 55.58% for manure. These data are consistent with other studies where the higher the available nitrogen levels in the soil, the higher the ANRE index, provided that the amounts of fertilizer applied are not high enough in relation to the optimum for cultivation, since there the specific index can be significantly reduced [83,84]. As noted above, plant density and fertilization had a significant effect on seed yield and harvest index (HI). The NUtE index also represents the ability of a plant to convert uptake of N into seeds [23]. Therefore, significant positive correlations of the NUtE index with seed yield and HI of the crop were expected ($r = 0.3714$, $p \leq 0.001$; $r = 0.7525$, $p \leq 0.001$, respectively; Table 10).

The nitrogen agronomic efficiency (NAE) index describes the ability of the crop to increase its seed yield relative to the amount of applied N. In the present study, the combined analysis of variance showed that plant density and fertilization had an equally significant effect on the NAE index. Specifically, the NAE index received the highest values in the sowing density of 200 plants m^{-2} with the 3-year average value being 68.4% higher than the sowing density of 300 plants m^{-2}. Regarding the effect of fertilization, the highest average 3-year values were recorded in inorganic fertilization and compost, with the values being 101.9% and 82.6% higher than the control, respectively. By definition, the NAE index is significantly positively correlated with seed yield ($r = 0.6894$, $p \leq 0.001$; Table 10). Therefore, considering the above, it is understood that the ideal plant density for seed production is 200 plants m^{-2}, while the ideal types of fertilization are inorganic and compost, since they did not differ statistically significantly between each other. Similar behavior of the NAE index at increasing levels of available N has been reported in other crops, such as wheat [85], cotton [84], and maize [86]. Moreover, Yan et al. [87], studying the effect of sowing density of maize on the NAE index, found that when the sowing density exceeds its ideal density (optimum), the NAE index begins to decrease significantly.

5. Conclusions

According to the results of the present study and their evaluation, soil parameters were affected only by fertilization. In particular, the application of organic fertilizers (manure and compost) for three consecutive years significantly increased the content of organic matter (SOM) and soil total nitrogen (STN). Growth parameters and yield of *N. sativa* were affected by both plant density and fertilization. Plant height showed the highest values in the plants of the plant density of 200 m^{-2} plants, as well as in those that had received inorganic fertilizer or compost. At the level of crop, the leaf area expressed by the leaf area index (LAI) ratio, increased with increasing plant density and the highest values were found in the density of 300 m^{-2} plants. In addition, fertilization had a significant effect on the trait with the highest ratio found after the application of inorganic fertilizer. In the same manner, seed yield was negatively affected by the increase in plant density and positively by the application of fertilizer with the highest values found in plants with low seed density and those that had received inorganic fertilizer. Regarding the harvest index (HI), similar results were followed to those of seed yield. The absorption of total nitrogen in the seeds (Seed N uptake) as well as the nitrogen harvest index (NHI) were positively affected by the increase of available nitrogen and negatively by the increase of sowing density, with their highest values found in the plant density of 200 plants m^{-2} and inorganic fertilization. The absorption of total nitrogen in the total above-ground biomass of the crop (Total Plant N uptake) presented the highest values in high plant density and inorganic fertilizer; however, the application of compost was also equally important. The apparent nitrogen recovery efficiency (ANRE) ratio was only affected by fertilization with values indicating that inorganic fertilizer and compost had no significant difference. In addition, nitrogen utilization efficiency (NUtE) declined by the increase in plant density and available nitrogen. Also, the nitrogen agronomic efficiency (NAE) index showed that sowing densities greater than 200 m^{-2} plants result in a decrease in the index, while an increase of available nitrogen led to an increase in the index with the highest values being found in inorganic fertilizer and compost. As a conclusion, plant densities greater than 200 plants m^{-2} result in higher crop growth but lower seed yield and decreased nitrogen uptake and use efficiency in *N. sativa* seeds, whereas the application of inorganic fertilizers (at a rate of 45 kg N ha^{-1}) increases crop yield, nitrogen uptake, and utilization efficiency because these fertilizers present higher nitrogen levels with higher solubility and thus, faster availability for the crop in comparison with organic fertilizers. Moreover, further research should be conducted, as in recent years the demand for organic medicinal products increased the tendency to medicinal plant cultivation with the use of organic inputs, and according to this study, seaweed compost seems to be a valuable alternative fertilization source to increase *N. sativa* crop production in organic cultivation systems.

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