



# *Article* **Comparison of Organic and Inorganic Fertilization in Fenugreek Cultivation Using Nitrogen Indicators**

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**Abstract:** Nitrogen indices could be used to evaluate organic and inorganic fertilization because they provide quantitative measures of nitrogen availability in the soil, allowing for a more accurate assessment of nutrient-management practices and optimization of crop yields. This study investigates the impact of different fertilization types and salinity on various soil parameters in fenugreek (*Trigonella foenum-graecum* L.) cultivation and nitrogen indices. A field experiment was established at the Agricultural University of Athens during the cropping period of 2018–2019 (CP I), 2019–2020 (CP II), and 2020–2021 (CP III) in a split-plot design with two main salinity treatments (high salinity, HS, and conventional salinity, CS) and five fertilization treatments (biocyclic–vegan humus soil (BHS), manure (FYM), compost (COMP), inorganic fertilization (11–15–15), and the control (C). The Nitrogen Balance Intensity (NBI) was statistically significantly affected by the factors of fertilization  $(p \le 0.01)$  and salinity ( $p \le 0.001$ ) for CP I. The maximum NUEcrop value was recorded in the FYM treatment (0.83  $\pm$  0.04) and the minimum in the COMP treatment (0.64  $\pm$  0.04). Physiological efficiency (PE) was not significantly affected by any treatment for CP III. The fertilization factor significantly affected the NUEsoil index ( $p \leq 0.001$ ) for all three CPs. For CP I, the highest Nitrogen Uptake Efficiency (NUpE) value was recorded in the BHS treatment (27.08  $\pm$  7.31) and the lowest in the C treatment (13.22  $\pm$  7.31). There were no significant differences in CP I and CP II NUEbalance values among the NPK, BHS, and FYM treatments. These findings underscore the potential of organic fertilizers in addressing the global nitrogen challenge and promoting environmentally sustainable farming practices.

**Keywords:** biocyclic-vegan agriculture; fenugreek; salinity; nitrogen indices; NUE

# **1. Introduction**

Fenugreek (*Trigonella foenum-graecum* L.) is an aromatic, medicinal plant rich in phytochemicals [\[1,](#page-15-0)[2\]](#page-15-1). Historically, it has been a key herb in Indian Ayurveda and traditional Chinese and Tibetan medicine for centuries [\[3](#page-15-2)[,4\]](#page-15-3). It was also recognized in ancient Eurasian civilizations, including China and the Indus Valley [\[5\]](#page-15-4). The species name 'foenum-graecum' means 'Greek hay', indicating its ancient use as a forage crop [\[6](#page-16-0)[–8\]](#page-16-1). As an annual legume in the Fabaceae family, it includes alfalfa, known as the 'Queen of Forages'. Fenugreek forage is similar to alfalfa in proteins, fibers, vitamins, and minerals but does not cause bloating [\[9\]](#page-16-2). It supports muscle growth and promotes carcass weight [\[10\]](#page-16-3). Fenugreek seeds and leaves contain phytosterols, phytoestrogens, flavonoids, proteins, amino acids, and other beneficial phytochemicals [\[11\]](#page-16-4).

In recent years, manure has been implicated in nitrate leaching, leading to environmental concerns such as water pollution and ecosystem damage [\[12\]](#page-16-5). As a result, agriculture is increasingly exploring new methods of organic farming that do not rely on animal-based inputs. Biocyclic vegan agriculture is emerging as a promising alternative, emphasizing plant-based practices that minimize the risk of nitrate leaching [\[13\]](#page-16-6).



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Biocyclic vegan agriculture stands on the soil tradition of famous researchers from the 18th, 19th, and 20th centuries (Albrecht Thaer, 1752–1828; Justus von Liebig, 1803–1873; Sir Albert Howard, 1873–1947; and Dr. Hans-Peter Rusch, 1906–1977) and combines it with contemporary organic farming and composting practices as an essential element for enhancing soil fertility [\[14\]](#page-16-7). The Biocyclic Vegan Standards approach in crop cultivation has created a framework that integrates the principles of vegetarianism and animal protection [\[15\]](#page-16-8). This has led to the creation of the Biocyclic Vegan Standard, which has been officially included in the IFOAM Family of Standards since 2017. The use of the Bio-cyclic Vegan Quality Label is based on an accredited certification system and ensures full transparency to consumers at all levels of the supply chain 'from farm to shelf' and the certainty that a Bio-cyclic Vegan product is not 'only' organic and plant-based but has also been produced according to vegan criteria. The key elements of this standard refer to a series of principles that aim to promote organic farming and sustainability in agriculture [\[15\]](#page-16-8). One of the core principles of the Biocyclic Vegan Standard is sustainability. It promotes a sustainable way of farming that minimizes environmental impact. By eliminating animal products and focusing on plant-based agriculture, it reduces greenhouse gas emissions, water usage, and land degradation associated with conventional animal farming [\[16\]](#page-16-9). Industrial animal farming is often linked to high methane production (one of the greenhouse gases) due to the production and storage of animal manure [\[17\]](#page-16-10). Additionally, the extensive use of this standard can protect water resources. Replacing conventional fertilizers with fully mature plant-based compost or Biocyclic Humus Soil helps maintain organic matter in the soil and reduces the need for chemical fertilizers. This reduces the likelihood of nutrient leaching, such as nitrogen, into water systems. Furthermore, this standard respects animals since all forms of animal exploitation have been abolished in biocyclic vegan agriculture, and fertilization with animal-based materials such as manure, meat meal, blood meal, or feather meal has been replaced with purely plant-based means of plant nutrition [\[18\]](#page-16-11).

Last but foremost, it promotes health due to the absence of the risk of soil contamination from antibiotics from animal farming or other pathogens from animal excrement. A healthy, resilient, and living soil is the basis for producing robust plants of high nutritional value for humans [\[19\]](#page-16-12).

Nitrogen is a key nutrient required for high yields of most agricultural crops [\[20\]](#page-16-13). Nitrogen in the form of  $NO<sub>3</sub>$  is also mobile and susceptible to leaching into groundwater, causing degradation of groundwater quality [\[21\]](#page-16-14). Before the availability of commercial nitrogen fertilizers, agricultural systems generally included crop rotation that involved a nitrogen-fixing crop and animal waste, with manure produced by the animals being returned to the cultivated lands for fertilization purposes. Nitrate concentrations in groundwater have generally increased over the past few decades, and the widespread use of commercial nitrogen fertilizers has been implicated as a contributing factor [\[22\]](#page-16-15). Organic farming is promoted as an environmentally friendly and more sustainable farming practice [\[23\]](#page-16-16).

Organic plots had higher organic matter content, higher nitrogen mineralization potential, and higher levels of microbial biomass than plots receiving commercial fertilizers [\[24](#page-16-17)[–26\]](#page-16-18). In [\[24\]](#page-16-17), the authors reviewed the literature on the use and organic farming. Yields during the conversion period were often lower than those achieved later [\[27\]](#page-16-19).

Soil is considered the main source of nitrogen for most field crops, and most crops receive 50–80% of their nitrogen requirement from the soil, even in cases where nitrogen fertilizer is applied at higher rates [\[28\]](#page-16-20). However, chemical fertilizers are important supplementary sources for maximum economic yield. The main reasons for nitrogen deficiency in crops are the loss of nitrogen through leaching, volatilization, surface runoff, denitrification, and canopy. Under these conditions, increasing nitrogen use efficiency (NUE) and reducing nitrogen fertilizer rates can significantly contribute to maintaining air and water quality [\[29\]](#page-16-21).

Nitrogen fertilizer management can be defined as the management of nitrogen fertilizer so that crops use as much of the applied nitrogen as possible each year [\[30\]](#page-16-22). NUE is an established measure used to compare nitrogen management. There are many approaches to calculating NUE. To date, there have been many scientific contributions on the subject of NUE, mainly focusing on ways to improve crop NUE through agricultural management or breeding innovations [\[31–](#page-16-23)[35\]](#page-17-0).

The body of literature includes a wide range of NUE calculations and recognizes that different NUE indicators have distinct functions [\[36](#page-17-1)[–40\]](#page-17-2). Key reviews have compiled several common measurements of NUE and their proposed applications [\[38](#page-17-3)[,41](#page-17-4)[,42\]](#page-17-5).

At first glance, NUE may seem like a simple term and concept, but its complexity lies in the various nitrogen sources that contribute to plant production (inorganic and organic fertilizers, soil organic matter (SOM), biological nitrogen fixation, and atmospheric deposition); the interaction between soil nitrogen availability, transformation, storage, movement, and loss due to soil conditions; crop genotypes; and the impact of management, weather, and climate [\[35](#page-17-0)[,43\]](#page-17-6). Interpreting NUE results requires a thorough understanding of factors/interventions, spatial and temporal limits, and the intended end use [\[42\]](#page-17-5).

In this three-year study, we utilized indicators based on lubrication, plant, soil, and system ecology. Plant production has a significant impact on the nitrogen cycle, with significant consequences for the climate, the environment, and public health. Designing better nitrogen management will require indicators that accurately reflect the complexity of nitrogen recycling and provide biological significance. Modern agricultural production demands effective, sustainable, and environmentally sound management practices. Under these circumstances, increasing crop yields per unit area through the use of appropriate N-management practices has become a key component of modern plant production technology. Adopting appropriate fertilizer N management strategies can balance the supply of N required for optimal crop production while minimizing potential losses to the environment. The purpose of this study was to compare inorganic with organic fertilization using nitrogen indices to exploit and evaluate all factors influencing nitrogen use or leaching in crop-production systems. Additionally, organic farming has been criticized for reduced yields compared to conventional farming. It is important to evaluate the quality of the produced products and the condition in which the soil is after harvesting.

## **2. Materials and Methods**

# *2.1. Location and Experimental Setup*

The fenugreek cultivation experiments were carried out in the experimental field at the Agronomy Laboratory of the Agricultural University of Athens, specifically the arable crops section (coordinates  $37°59'02.1''$  N  $23°42'08.4''$  E, altitude 28.04 m), consistently over three consecutive growing seasons (Figure [1\)](#page-3-0). The experiment began in the 2018–2019 cropping period (CP I), 2019–2020 (CP II), and continued through 2020–2021 (CP III). Throughout the experiment, only one variety of fenugreek (*Trigonella foenum graecum*) was cultivated. The preceding crop was organic tobacco, with vetch used for green manure. Precipitation during the 1st GS was 217.89 mm, and during the 2nd GS, it was 309.00 mm. The soil at the site is classified as clay loam (CL), slightly alkaline, with a satisfactory SOM content (2.37%). Calcium carbonate (CaCO<sub>3</sub>) content was measured at 29.9%, while nitrogen (N), phosphorus (P), and potassium (K) levels were  $101.3 \text{ mg/L}$ ,  $20.3 \text{ mg/L}$ , and 235 mg/L, respectively.

The experimental design employed a split-plot design, comprising a total of 15 large experimental units. The main factors were two salinity treatments (high salinity, HS, and conventional salinity, CS), and there were 30 smaller plots with five fertilization treatments (biocyclic–vegan humus soil (BHS), manure (FYM), compost (COMP), inorganic fertilization  $(11–15–15)$ , and the control  $(C)$ , which is the baseline condition without any treatment), distributed across three blocks. Sowing was performed manually with a row spacing of 30 cm at a seeding rate of 30 kg ha<sup>-1</sup>. Salinity treatment commenced one week after sowing, with 200 kg ha<sup>-1</sup> of NaCl applied to the surface of the large experimental units, while CS plots received no NaCl. High-salinity treatments are important because they simulate conditions that many crops face in arid and semi-arid regions, where soil salinization is

<span id="page-3-0"></span>

a growing concern. Understanding how crops respond to high salinity can help develop strategies to improve crop resilience and productivity under such stressful conditions.

**Figure 1.** Experimental field of fenugreek.

Fertilization treatments, including BHS, FYM, COMP, and NPK, were applied at a consistent nitrogen rate of 110 kg N ha<sup>-1</sup>. BHS was used as a substitute for manure or other animal-based fertilizers, recommended for producers following the Biocyclic Vegan Standard. The applied amount of BHS was 3.928 tons ha $^{-1}$ , with a composition of 46.3 g of organic matter per 100 g, 2.8 g of nitrogen per 100 g, and a pH of 7.6. The compost, a commercial preparation, was applied at a rate of 9.166 tons ha $^{-1}$ , containing 70% compost, 15% black peat, organic materials, 10% perlite, and 5% soil, with a pH of 5.5–6.8 and 1.2% nitrogen. FYM was sourced from the Agricultural University of Athens stables, applied at 6.875 tons ha−<sup>1</sup> , with a physico-chemical composition of pH of 7.39, 1.60% total N, 8.9 mg/L of P (Olsen), and  $4.4\%$  organic C. NPK (11–15–15) was applied at a rate of 1 ton ha<sup>-1</sup>.

## *2.2. Soil Sampling and Methods*

Soil samples were collected from the 0–25 cm soil layer. SOM was calculated using the Walkley and Black method [\[44\]](#page-17-7). Soil total nitrogen (STN) content was determined according to ISO 11261:1995 protocol [\[45\]](#page-17-8), cation-exchange capacity was determined following ISO 11260:1994 [\[46\]](#page-17-9), and electrical conductivity was determined in a soil water extract according to ISO 11265:1994 standard [\[47\]](#page-17-10).

## *2.3. Indices*

Table [1](#page-4-0) lists various fertilization-based, plant-based, soil-based, and ecology-based NUE indicators. These indices are crucial for understanding the efficiency of nitrogen utilization in agricultural systems. Each indicator is defined by a specific formula and referenced accordingly.

## *2.4. Statistical Analysis*

Experimental data were analyzed using analysis of variance (ANOVA) for the three years, with Tukey's method used to form homogeneous groups of means, as the experiment is factorial. ANOVA analysis was conducted using Sigma Plot (ver. 10; Systat Software Inc., San Jose, CA, USA). The choice of Tukey's Honestly Significant Difference (HSD) test for our split-plot design in the field experiment allowed us to maintain stricter control of

the type I error rate, reducing the likelihood of false positives and ensuring more reliable results. Tukey's HSD is well-suited for the hierarchical structure of split-plot designs, providing comprehensive pairwise comparisons across different levels of the experiment. Additionally, the Pearson correlation coefficient (PCC) was calculated using R software 4.4.0., with all analyses performed at a significance level of  $\alpha$  = 0.05 (5%).

<b>Index Name</b>	<b>Short Name</b>	Formula	Reference	No.
<b>Fertilization-based indicators</b>				
Partial-factor Seed Productivity	PFP <sub>seed</sub>	$\text{PFP}_{\text{seed}} = \frac{\text{Seed yield}_{\text{fert}}}{\text{Fertilizer N}}$	$[38]$	(1)
Partial-factor <b>Biomass Productivity</b>	PFP <sub>biomass</sub>	$\text{PFP}_{\text{biomass}} = \frac{\text{Biomass yield}_{\text{fert}}}{\text{Fertilizer N}}$	[38]	(2)
N Balance Intensity	<b>NBI</b>	$NBI =$ Seed N yield - Fertilizer N	[48]	(3)
	$NUE_{\text{crop}}$		$\left[35\right]$	(4)
Partial N Balance	<b>PNB</b>		[38]	(5)
Agr. Efficiency	AE	$\begin{array}{rcl} \text{NUE}_{\text{crop}} &=& \frac{\text{Seed N Yield}_{\text{fer}}}{\text{Fertilizer N}} \\ \text{PNB} &=& \frac{\text{Plant N}_{\text{fer}}}{\text{Fertilizer N}} \\ \text{AE} &=& \frac{\text{Yield}_{\text{ferri}}}{\text{Fertilizer N}} \end{array}$	[38]	(6)
$Fertilizer-N = Recovery$ Efficiency	REfertN	$\text{REfertN} = \frac{\text{Plant N}_{\text{fert}} - \text{Plant N}_{\text{control}}}{\text{Fertilizer N}} \times 100$	[38]	(7)
Plant-based indices				
Physiol. Efficiency	PE	$\begin{array}{rcl} \mathrm{PE} & = & \frac{\mathrm{Yield_{fert}-Yield_{control}}}{\mathrm{Plant\, N_{fert}-Plant\, N_{control}}} \\ & & \mathrm{NUtE} & = & \frac{\mathrm{Yield}}{\mathrm{Plant\, N}} \\ \end{array}$	[38]	(8)
N Utiliz. Efficiency	<b>NUtE</b>		[31]	(9)
Internal Efficiency	IE		[38]	(10)
N Harvest Index	<b>NHI</b>	$\begin{aligned} \n\text{IE} &= \frac{\text{Seed N Yield}_{\text{fert}}}{\text{Plant N}_{\text{fert}}}\\ \n\text{NH} &= \frac{\text{Seed N yield}}{\text{Plant N}_{\text{bert}}} \times 100\\ \n\text{NHE}_{\text{ref}} &= \frac{\text{Sum N} \times \text{Plant}}{\text{Sum N}_{\text{int}}}\n\end{aligned}$	[31]	(11)
	NUE <sub>soil</sub>	$NUEsoil$ = $\frac{1 \text{ km}}{\text{Fertilizer N} + \text{Soil N}}$	$\left[31\right]$	(12)
Soil-based indices				
N Uptake Efficiency	<b>NUpE</b>	$NUpE = \frac{Plant N}{Fertilizer N + Soil N} \times 100$	[31]	(13)
	NUE <sub>yield</sub>	$NUE_{yield}$ = $NUpE \times NUtE$	[49]	(14)
	NUE <sub>balance</sub>	$\overline{\text{NUE}}_{\text{balance}} = \frac{\text{N}_{\text{outputs}}}{\text{N}_{\text{inputs}}}$	$[35]$	(15)
<b>Ecology-based indices</b>				
Nitrogen Productivity	NP	$NP = \frac{RGR}{Plant N}$	[50]	(16)
	$\text{NUE}_{\text{ecology}}$	$NUE_{ecology}$ = $NP \times MRT$	[51]	(17)

<span id="page-4-0"></span>**Table 1.** Indices and formulas used for calculating various NUE indicators in fenugreek crop.

Seed yield<sub>fert</sub>: represents the seed yield in treatments that have received fertilization, measured in kg ha<sup>-1</sup>. Seed yield<sub>control</sub> (kg ha<sup>-1</sup>): represents the seed yield in treatments that have not received fertilization, measured in kg ha<sup>-1</sup>. Seed N yield<sub>fert/control</sub>: calculated by seed yield (kg ha<sup>-1</sup>) × seed N content (%) in kg N ha<sup>-1</sup>. PlantN<sub>fert</sub><br>(kg N ha<sup>-1</sup>): plant (leaves and stems) dry matter in Kg ha<sup>-1</sup> × Plant N content (%); treatmen PlantN<sub>control</sub> (kg N ha<sup>-1</sup>): plant (leaves and stems) dry matter in Kg ha<sup>-1</sup> × Plant N content (%); treatments have not been fertilized. N<sub>inputs</sub>: the units of nitrogen applied through the fertilizer; the units were the same for all treatments. N<sub>outputs</sub>: upper parts N uptake + seed N uptake (kg ha<sup>-1</sup>). RGR (Relative Growth Rate): (ln W2 − ln W1)/(t2 − t1), where W1 and W2 are plant dry weights at times t1 and t2. MRT: the Mean Residency Time of cultivation Fertilizer N: the units of nitrogen applied through the fertilizer; the units were the same for all treatments.

## **3. Results**

#### *3.1. Soil Measurements*

In CP I, STN was significantly affected by the fertilization factor  $(p < 0.001)$ . The highest STN value was recorded in the BHS and the lowest in the C treatment. The FYM treatment produced 13.15% less soil nitrogen compared to BHS, while the NPK and COMP treatments produced 22.96% and 33.15% less, respectively. In addition, TSN was significantly affected by the fertilization factor ( $p \le 0.001$ ) and the interaction of fertilization and salinity ( $p \leq 0.05$ ) in CP III. The FYM produced 8.51% less TSN compared to BHS, while the NPK and COMP treatments produced 17.51% and 21.43% less, respectively (Table [2\)](#page-5-0).



<span id="page-5-0"></span>Table 2. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on soil total nitrogen (STN), cation-exchange capacity (CEC), and soil organic matter (SOM).

The F-test indicators are from the ANOVA. Different letters (a, b, c, and d) within a column indicate significant differences according to the Tukey test. Significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns: not significant  $(p > 0.05)$ .

CEC was significantly affected only by the fertilization factor ( $p < 0.001$ ) for all three experimental periods (Table 2). In CP I, the highest CEC value was recorded in the BHS treatment and the lowest in the C treatment. The FYM produced 9.20% less CEC compared to BHS, while the NPK and COMP treatments produced 13.31% and 17.83% less, respectively. In CP III, the highest CEC value was recorded in the BHS treatment and the lowest in the C treatment. The COMP and FYM treatments produced 8.18% less CEC compared to BHS, while the NPK treatment produced 14.43% less.

The SOM was significantly affected by the fertilization factor ( $p \leq 0.001$ ) for all three cropping periods. For CP I, the SOM reached its maximum value in the BHS treatment and its minimum in the NPK treatment. FYM and COMP provided  $3.5 \pm 0.15\%$  and  $3.4 \pm 0.15\%$ SOM, respectively, while C provided 2.74  $\pm$  0.15%. For CP II, the FYM treatment recorded 3.51  $\pm$  0.07% SOM, while COMP and NPK recorded 3.41  $\pm$  0.07% and 2.4  $\pm$  0.07%, respectively. In the FYM and COMP treatments, SOM was recorded at  $3.63 \pm 0.08\%$  and  $3.53 \pm 0.08$ %, respectively, while with the NPK, it was  $2.40 \pm 0.08$ % during CP III (Table 2).

## *3.2. Fertilization-Based Indicators*

For CP I, the factor that statistically significantly affected the PFPseed is fertilization  $(p \leq 0.01)$ . The maximum PFPseed value was observed in the FYM treatment and the minimum in the BHS treatment. The NPK treatment resulted in a PFPseed of  $144.71 \pm 6.77$ , and the COMP treatment resulted in 127.64  $\pm$  6.77. For CP II, PFPseed was statistically affected by salinity. For CP III, PFPseed was significantly affected by fertilization ( $p \leq 0.001$ ), with the highest value in the BHS treatment and the lowest in the COMP treatment (Table [3\)](#page-6-0).

<span id="page-6-0"></span>**Table 3.** Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11–15–15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on fertilization-based indicators; Partial-factor Seed Productivity (PFPseed), Partial-factor Biomass Productivity (PFPbiomass), N Balance Intensity (NBI), NUEcrop, Partial N Balance (PNB), Agronomic Efficiency (AE), and Fertilizer-N Recovery Efficiency (REfertN).



The F-test indicators are from the ANOVA. Different letters (a, b, c, and d) within a column indicate significant differences according to the Tukey test. Significance levels: \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001, ns: not significant  $(p > 0.05)$ .

PFPbiomass was significantly affected by fertilization ( $p \leq 0.001$ ) for all three CPs (Table [3\)](#page-6-0). For CP I, the maximum PFPbiomass was recorded in the NPK treatment  $(42.75 \pm 0.72)$  and the minimum in the COMP treatment. The FYM treatment resulted in a PFPbiomass of 42.62  $\pm$  0.72, and the BHS treatment resulted in 42.26  $\pm$  0.72. For CP II, FYM resulted in  $40.85 \pm 0.45$ , and NPK resulted in 39.49  $\pm$  0.45, and they were not statistically different from each other. For CP III, the maximum partial productivity factor for biomass was recorded in the BHS treatment and the minimum in the COMP treatment. All fertilization treatments differed significantly from each other.

NBI was statistically significantly affected by the factors of fertilization ( $p \leq 0.01$ ) and salinity ( $p \le 0.001$ ) for CP I. For CP I, the maximum NBI value was recorded in the FYM treatment and the minimum in the C treatment. For CP II, no treatment was statistically significant (Table [3\)](#page-6-0).

NUEcrop was statistically significantly affected both by fertilization ( $p \leq 0.01$ ) and salinity ( $p \leq 0.001$ ) for CP I. During the same period, the maximum NUE crop value was recorded in the FYM treatment and the minimum in the COMP treatment. The NPK treatment resulted in an NUEcrop of  $0.77 \pm 0.41$ , while the BHS and COMP treatments resulted in 0.71  $\pm$  0.41 and 0.64  $\pm$  0.41, respectively. For CP II, salinity statistically significantly affected NUEcrop, while for CP III, no treatment had a statistically significant effect.

For CP I and CP III, the PNB was statistically significantly judged by fertilization  $(p < 0.05$  and  $p < 0.001$ , respectively), and FYM and NPK were not statistically significantly different from each other, with a higher value in the BHS for the second and CP III, respectively (Table [3\)](#page-6-0).

AE was statistically affected by fertilization at  $p \leq 0.01$  for CP I and  $p \leq 0.001$  for CP III. In CP I, fertilization treatments showed statistically significant differences between them. The maximum value of AE was recorded in FYM and the minimum in BHS. FYM and NPK were not statistically significantly different from each other. For CP III, BHS and FYM did not differ statistically significantly from each other.

REfertN was statistically significantly influenced by fertilization ( $p \leq 0.001$ ) and salinity ( $p \leq 0.01$ ) (Table [3\)](#page-6-0). For CP I, the maximum value for REfertN was recorded with BHS and the minimum with COMP. FYM and NPK gave REfertN values of 0.13 and 0.12, respectively. For CP II, FYM and NPK achieved REfertN values of 0.14 and 0.11, respectively. For CP III, CS significantly differed from HS.

## *3.3. Plant-Based Indices*

The index PE was statistically significantly affected by fertilization  $p \leq 0.05$  and  $p \geq 0.001$  for CPI and CP II, respectively. For CP I, the highest PE value was recorded in the COMP treatment and the lowest in the BHS treatment, which differed significantly from each other. The NPK and FYM treatments resulted in PE values of 61.11  $\pm$  9.67 and  $58.93 \pm 9.67$ , respectively, in CP II. PE was not significantly affected by any treatment for CP III (Table [4\)](#page-8-0).

The NUtE index was not significantly affected by any factor.

Fertilization significantly affected the IE index. For CP I, the highest IE value was recorded in the FYM treatment and the lowest in the C treatment. For CP II, NPK resulted in an IE value of 4.05  $\pm$  0.26, while FYM and BHS resulted in 3.94  $\pm$  0.26 and 3.30  $\pm$  0.26, respectively. For CP III, the highest IE value was recorded in the NPK treatment and the lowest in the C treatment. The COMP and FYM treatments resulted in IE values of  $4.08 \pm 0.19$  and  $3.99 \pm 0.19$ , respectively, while BHS resulted in  $3.92 \pm 0.19$ .

The fertilization factor significantly affected the NHI. For CP I, the highest NHI value was recorded in the FYM treatment and the lowest in the C treatment. The NPK and COMP treatments resulted in NHI values of 304.96  $\pm$  21.43 and 302.38  $\pm$  21.43, respectively, while BHS resulted in  $259.23 \pm 21.43$  (Table [4\)](#page-8-0). For CP II, the NPK and FYM treatments resulted in NHI values of  $405.86 \pm 26.99$  and  $394.29 \pm 26.99$ , respectively, while BHS resulted in 330.21  $\pm$  26.99. For CP III, NHI was significantly affected by fertilization ( $p \le 0.05$ ), with the highest value recorded in the NPK treatment and the lowest in the C treatment.

<span id="page-8-0"></span>Table 4. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on Fertilization-based indicators; Partial-factor Seed Productivity (PFPseed), Partial-factor Biomass Productivity (PFPbiomass), N Balance Intensity (NBI), NUEcrop, Partial N Balance (PNB), Agronomic Efficiency (AE), and Fertilizer-N Recovery Efficiency (REfertN).



The F-test indicators are from the ANOVA. Different letters  $(a, b, c)$  within a column indicate significant differences according to the Tukey test. Significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns: not significant ( $p > 0.05$ ).

The fertilization factor significantly affected the NUEsoil index ( $p \le 0.001$ ) for all three CPs. For CP I, the highest NUEsoil value was recorded in the NPK treatment and the lowest in the C treatment. The FYM and BHS treatments resulted in NUEsoil values of  $10.19 \pm 0.27$ and  $10.08 \pm 0.27$ , respectively. FYM and NPK did not differ significantly. For CP II, FYM, NPK, and BHS did not differ significantly from each other. For CP III, the highest NUEsoil value was recorded in the BHS treatment and the lowest in the C treatment (Table 4).

## 3.4. Soil-Based Indices

The NUpE index was significantly influenced by the fertilization factor ( $p \leq 0.001$ ) for all three CPs (Table 5). For CP I, the highest NUpE value was recorded in the BHS treatment and the lowest in the C treatment. The FYM and NPK treatments did not differ significantly from each other. For CP II, the FYM and NPK treatments also did not differ significantly, while the highest value was given by BHS. For CP III, the highest NUpE value was recorded in the BHS treatment and the lowest in the C treatment. The FYM and NPK treatments resulted in NUpE values of  $25.51 \pm 8.29$  and  $22.58 \pm 8.29$ , respectively.

<span id="page-9-0"></span>Table 5. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on ecology-based indices, nitrogen productivity (NP), and NUE ecology.



The F-test indicators are from the ANOVA. Different letters  $(a, b, c,$  and d) within a column indicate significant differences according to the Tukey test. Significance levels: \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , ns: not significant ( $p > 0.05$ ).

The NUE yield index was not significantly affected by any treatment.

The NUE balance index was significantly influenced by both fertilization ( $p \le 0.001$ ) and salinity ( $p \leq 0.05$ ) factors for all three CPs. For CP I, the HS treatment resulted in NUEbalance values of 0.84  $\pm$  0.19, while the CS treatments had values of 1.00  $\pm$  0.19. This indicates that under high-salinity conditions, 84% of the nitrogen applied to the field was removed with the crop harvest, while the remaining 16% might have been lost or remained in the soil. The NPK, BHS, and FYM treatments did not differ significantly for CP I and CP II. For CP III, the HS treatment resulted in NUEbalance values of  $1.02 \pm 0.51$ , while the CS treatments had values of  $0.98 \pm 0.51$ . The highest NUEbalance value was recorded in the BHS treatment and the lowest in the COMP treatment (Table 5).

## *3.5. Ecology-Based Indices*

NP was significantly affected by the fertilization factor ( $p \le 0.001$ ) for all three cropping periods. The fertilizations did not differ significantly from each other but did differ significantly from the C. For CP I, the highest NP was recorded in C and the lowest in the BHS. The COMP and NPK treatments resulted in nitrogen productivity of  $3.32 \pm 0.2$  and  $3.17 \pm 0.2$ , respectively, while the FYM treatment resulted in  $0.03 \pm 0.002$ . For CP II, the NP was maximized in C and minimized in BHS. The COMP and NPK treatments led to NP values of  $3.30 \pm 0.1$  and  $3.22 \pm 0.001$ , respectively, while the FYM treatment resulted in 2.97  $\pm$  0.1. The COMP and NPK treatments resulted in NP values of 3.32  $\pm$  0.1 and  $3.21 \pm 0.1$ , respectively, while the FYM treatment resulted in 2.97  $\pm$  0.1 in CP III.

The NUEecology index was significantly influenced by the fertilization factor. For CP I, the highest NUEecology value was observed in C and the lowest in the BHS treatment. For CP III, the highest NUEecology value was recorded in C and the lowest in the BHS treatment. The COMP and NPK treatments resulted in NUE ecology values of  $597.90 \pm 27.00$  and 578.48  $\pm$  27.00, respectively (Table [6\)](#page-10-0).

<span id="page-10-0"></span>**Table 6.** Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11–15–15) and salinity level (CS: conventional salinity, HS: high salinity) effect on ecology-based indices, nitrogen productivity (NP), and NUEecology.



The F-test indicators are from the ANOVA. Different letters (a and b) within a column indicate significant differences according to the Tukey test. Significance levels: \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , ns: not significant ( $p > 0.05$ ).

# **4. Discussion**

<span id="page-11-0"></span>Pearson's correlation with r and *p*-values between nitrogen indices and soil characteristics is presented in Figure [2.](#page-11-0)



**Figure 2.** Pearson's correlation matrix with r and *p*-values between nitrogen indices and soil characteristics.

The behavior of nitrogen in soil systems is complex and heavily influenced by the type of fertilizer used, whether organic or inorganic. The efficiency of nitrogen absorption and utilization by crops is affected by several factors, including soil texture, mineral composition, and the presence of organomineral complexes. The soil texture, defined by the proportions of sand, silt, and clay, significantly impacts nitrogen dynamics. The mineral content of the soil, especially clay minerals like montmorillonite and kaolinite, plays a crucial role in nitrogen retention. These minerals have high surface areas and specific charge properties that allow them to effectively adsorb nitrogen in the forms of ammonium  $(NH<sub>4</sub><sup>+</sup>)$  and nitrate  $(NO<sub>3</sub><sup>-</sup>)$  ions [\[52\]](#page-17-15).

Clays are renowned for their ability to retain nitrogen. Their large surface area and negative charge enhance their capacity to hold onto cations like ammonium, reducing nitrogen leaching losses, which is a major pathway for nitrogen loss from the soil system [\[53\]](#page-17-16).

The soil total nitrogen (STN) was significantly affected by the type of fertilization in the fenugreek crop. The highest STN was recorded with BHS. Due to the stability of BHS and its resistance to nutrient leaching, the risk of over-fertilization is essentially eliminated, even with the application of large quantities. Consequently, BHS could play an important role in addressing the current global nitrogen challenge [\[14\]](#page-16-7). Additionally, the authors in [\[54\]](#page-17-17) and in [\[55\]](#page-17-18) demonstrated that the presence and decomposition of legume roots have a small positive effect on increasing soil nitrogen. Therefore, the presence of fenugreek roots in the soil after seed harvest may positively impact the increase of soil nitrogen. Additionally, STN shows a positive correlation with the Leaf Area Index (LAI) ( $r = 0.58$ ,  $p \le 0.001$ ) and SOM (r = 0.62,  $p \le 0.001$ ).

The CEC was significantly affected by fertilization in fenugreek cultivation. This result is consistent with Brar et al. in wheat cultivation [\[56\]](#page-17-19). However, unlike Brar et al. [\[54\]](#page-17-17), in our experiment, higher CEC values were observed with organic fertilizers. The increase in CEC due to organic fertilizers is also confirmed by Schulz and Glaser [\[57\]](#page-17-20). Also, the BHS fertilization provided the highest CEC values and showed a positive correlation with STN  $(r = 0.77, p \le 0.001).$ 

Agriculture is considered a factor in the degradation of natural resource quality. One of the reasons leading to this situation was the low input of organic matter into the soil [\[58\]](#page-17-21). Therefore, the use of organic fertilizers is deemed important. SOM was significantly affected by fertilization in fenugreek cultivation. This has also been confirmed in a plethora of crops [\[59](#page-17-22)[–62\]](#page-18-0). Organic fertilizers, specifically BHS fertilization, provided the highest values of organic matter. This is supported by numerous studies that have argued that organic additions promote better plant growth, which can be linked to improved root development and the more efficient use of water and nutrients [\[63–](#page-18-1)[65\]](#page-18-2). The soil matrix, including its physical and chemical properties, also affects nitrogen mobility and distribution. Soil texture, structure, and porosity determine the movement of water and nutrients through the soil profile [\[66\]](#page-18-3).

PFPseed and PFPbiomass were significantly affected by fertilization. PFP is a useful measure of nutrient use efficiency as it provides an integrated index that quantifies the total economic output in relation to the use of all nutrient resources in the system [\[67\]](#page-18-4). PFP was shown to decrease with the application of inorganic fertilizers. In [\[68\]](#page-18-5), it also showed that the excessive use of N fertilizers is responsible for lower PFP values. Therefore, we conclude that the application of inorganic fertilizers in fenugreek cultivation may be excessive. A decrease in PFP can be attributed to nutrient imbalances, a reduced supply of indigenous soil N, subsoil compaction, reduced root volume, and increased incidence of pests and diseases [\[69\]](#page-18-6). PFP for both seed yield and biomass showed an increase with the addition of organic fertilizers. It is also noted that PFPbiomass decreased in the third experimental year due to high soil salinity. PFPbiomass shows a positive correlation with STN ( $r = 0.58$ ,  $p \le 0.001$ ).

The NBI index in CP I showed negative values for all fertilizations, which means that not all the fertilizer was used and remained in the soil. NBI is a ratio that reflects the balance between the nitrogen available to the plant and the actual nitrogen needs of the plant. It can be an important indicator for agriculture as its use can ensure that crops receive the optimal amount of nitrogen—enough to maximize growth and yield without causing excessive nitrogen leaching or runoff, which can be harmful to the environment. The closer the NBI is to zero, the lower the nitrogen accumulation in the system, thus reducing leaching. In our experiment, we confirmed that NPK gave the largest negative values, thus creating an excess of nitrogen. Negative values indicate that not all the fertilizer was used and remained in the soil. This is good when using organic fertilizers but negative when using inorganic ones [\[48\]](#page-17-11). When the values are positive, it is necessary to utilize other forms of nitrogen, such as soil nitrogen or nitrogen from nitrogen fixation. In CP I,

salinity significantly affected the NBI. NBI showed a positive correlation with STN  $(r = 0.72)$ ,  $p \leq 0.001$ ). This conclusion is in agreement with [\[70\]](#page-18-7) in wheat cultivation. Fenugreek is considered to have a relatively moderate to low nitrogen absorption rate, in contrast to maize, for example, which is considered to have a high absorption rate [\[71\]](#page-18-8). Therefore, it would be manageable to fertilize only with organic nitrogen to meet the maximum requirements without applying excessive nitrogen to the soil.

NUEcrop was statistically significantly affected by the type of fertilization. Higher NUEcrop values were recorded with organic fertilizations compared to inorganic ones. In CP I, all values are less than one, indicating a net removal of nitrogen [\[35\]](#page-17-0). Additionally, with organic fertilizations, the values were closer to one, and in CPII and CP III, the BHS and FYM treatments even gave values higher than one, meaning the plant better utilized the added nitrogen units. This is also confirmed by [\[72\]](#page-18-9), who noted that organic fertilizers, since they release nitrogen more slowly compared to inorganic fertilizers, improve NUE in the long term by reducing the risk of nitrogen loss and improving soil health. Another explanation for this could be that organic fertilization enhances soil microbial activity, organic matter content, and overall soil fertility, which can contribute to the continuous improvement in NUE [\[73\]](#page-18-10). This is also confirmed by the present experiment, where NUEcrop showed a positive correlation with SOM ( $r = 0.44$ ,  $p \le 0.001$ ) and CEC ( $r = 0.63$ ,  $p \leq 0.001$ ).

PNB is used to understand the role of legumes in agricultural systems [\[74\]](#page-18-11). Both inorganic and organic fertilization significantly affected PNB in fenugreek cultivation. Inorganic fertilizers offer immediate nitrogen availability, potentially leading to higher PNB if managed correctly. However, improper management can lead to nitrogen losses and a reduction in PNB [\[75\]](#page-18-12). Organic fertilizers provide slow nitrogen release and improve soil health, contributing to continuous nitrogen uptake and potentially higher PNB in the long term [\[76](#page-18-13)[,77\]](#page-18-14). Indeed, PNB showed a positive correlation with STN ( $r = 0.80$ ,  $p \le 0.001$ ). Additionally, in [\[74\]](#page-18-11), the authors demonstrated significant regressions between partial nitrogen balance and NHI. This is not confirmed in our experiment.

The AE was significantly affected by fertilization, with higher values observed in organic fertilization. This result is also confirmed by [\[78\]](#page-18-15). In [\[79\]](#page-18-16), the results showed that the combination of organic and inorganic fertilization resulted in higher AE values compared to the application of only inorganic fertilization. AE indicates that organic fertilizations were more effectively utilized by the fenugreek crop. AE showed no correlation with soil characteristics. This is also confirmed in soybean cultivation [\[80\]](#page-18-17). Fine-textured soils with higher clay content typically retain more water and nutrients, reducing the risk of nitrogen leaching. Conversely, sandy soils with larger pore spaces may require more frequent applications of nitrogen fertilizers to maintain adequate nutrient levels for crops [\[81,](#page-18-18)[82\]](#page-18-19).

Salinity and fertilization factors affected REfertN in fenugreek cultivation. The REfertN equation represents the recovery efficiency of the applied nitrogen fertilizer in plants. REfertN indicates the percentage of applied nitrogen that was absorbed by the plants. Higher REfertN values were recorded in organic fertilizations, indicating that fenugreek plants absorbed nitrogen more effectively in these forms compared to the control. Additionally, high salinity hindered the more effective absorption of nitrogen by fenugreek plants, resulting in lower REfertN values. This is also confirmed by the negative correlation of REfertN with soil electrical conductivity ( $r = -0.44$ ,  $p \le 0.001$ ). This is also confirmed in bean cultivation [\[83\]](#page-18-20). In addition, high-salinity treatments are particularly significant in the context of agricultural sustainability and fenugreek crop productivity [\[84\]](#page-18-21).

High salinity can lead to soil compaction and reduced soil aeration, which negatively impacts root growth and microbial activity [\[73\]](#page-18-10). In this study, high-salinity treatments significantly influenced some NUE indices, indicating that salinity management is crucial for optimizing fertilization practices.

The PE was significantly affected by fertilization in fenugreek cultivation. This is also confirmed by [\[85\]](#page-18-22). PE indicates a plant's ability to convert nitrogen obtained from fertilizer beyond what is available in the soil into economic yield [\[38\]](#page-17-3). In fenugreek cultivation, it

was noted that COMP fertilization effectively converts the applied nitrogen into economic yield. In [\[86\]](#page-18-23), the results showed that a combination of organic and inorganic fertilization resulted in higher PE values compared to the application of only inorganic fertilization in pea cultivation. PE showed a negative relationship with STN ( $r = -0.36$ ,  $p \le 0.01$ ).

In our experiment, NutE was not affected by the type of fertilization and salinity. This contradicts [\[87\]](#page-18-24). However, the authors in [\[88\]](#page-18-25) noted that different levels of fertilization could affect NutE, something we cannot confirm as the applied nitrogen units in our experiment were constant. They also showed that the highest values were recorded in the control, which is also confirmed in our experiment.

The index of HI was significantly affected by the type of fertilization in fenugreek cultivation. The highest HI values were recorded with inorganic fertilization.

Understanding the impact of fertilization practices on NHI is essential for optimizing nitrogen use efficiency and crop yields [\[42\]](#page-17-5). NHI was significantly affected by the type of fertilization in fenugreek cultivation. Lower NHI values were recorded with organic fertilization compared to inorganic fertilization in fenugreek cultivation. Studies on NHI can reveal how different management practices can improve the allocation of nitrogen to economically important parts of the plant, such as the seed [\[89\]](#page-18-26). Thus, using NHI, it was understood that nitrogen allocation to the harvested product, which is the seed, was better with organic fertilization in fenugreek cultivation. Additionally, NHI in fenugreek cultivation showed no correlation with SOM. This result contradicts those found by Kakabouki et al. [\[80\]](#page-18-17) regarding soybean cultivation.

NUEsoil was significantly affected by fertilization. This aligns with [\[90\]](#page-18-27). While inorganic fertilization did not differ significantly from organic fertilization during the first two experimental periods, higher NUEsoil values were recorded in the organic fertilization during the last experimental period. The higher NUEsoil values under organic fertilization are mainly due to the more gradual and sustained release of nitrogen. These factors collectively contribute to more efficient nitrogen uptake by plants and increased biomass, resulting in higher nitrogen use efficiency compared to inorganic fertilization [\[91–](#page-19-0)[93\]](#page-19-1). This is confirmed in our experiment by the positive correlation of NUEsoil with CEC ( $r = 0.50$ ,  $p \le 0.001$ ) and STN ( $r = 0.61$ ,  $p \le 0.001$ ).

According to the NupE values, during the first two experimental periods, nitrogen accumulation in plant biomass per unit of available soil nitrogen (originating from fertilizer and soil) did not differ between inorganic and organic fertilization. However, higher NupE values were recorded in organic fertilization during the last experimental period. This conclusion is consistent with [\[94\]](#page-19-2), who noted that NupE is influenced by nitrogen fertilizer supply. Additionally, higher NupE values may be due to the sequestration of available nitrogen by plant roots and its subsequent use by the plant [\[95\]](#page-19-3). High organic matter improves soil structure, water-holding capacity, and microbial activity, which enhance nitrogen uptake [\[96\]](#page-19-4). This is also confirmed in our experiment, where NupE showed a positive correlation with SOM ( $r = 0.50$ ,  $p \le 0.001$ ). Moreover, the gradual release of nitrogen from organic fertilizers may have allowed fenugreek plants to better utilize the applied nitrogen units.

NUEbalance, which represents the balance between nitrogen inputs and outputs in fenugreek cultivation, was significantly affected by salinity levels and the type of fertilization. The presence of high salinity reduced NUEbalance. Additionally, NPK, BHS, and FYM did not differ significantly from each other. In the second experimental period, exceptional nitrogen use efficiency was recorded in fenugreek cultivation, raising potential concerns about the long-term sustainability of soil nitrogen levels. According to the literature, this index is used to evaluate existing best agricultural practices and inform policy-making [\[97\]](#page-19-5). NUEbalance showed a positive correlation with LAI ( $r = 0.66$ ,  $p \le 0.001$ ) and CEC ( $r = 0.68$ ,  $p \leq 0.001$ ).

The NP showed a negative relationship with STN ( $r = -0.37$ ,  $p \le 0.001$ ) and LAI  $(r = -0.47, p \le 0.001)$ . This may occur because organic matter decomposes slowly, gradually releasing nitrogen, and this slow release can limit the immediate availability of nitrogen to plants, affecting their growth [\[98](#page-19-6)[,99\]](#page-19-7). NP was influenced by the addition of fertilization, but organic and inorganic fertilization did not differ significantly from each other. Higher NP is associated with rapid growth, a relatively large investment of nitrogen in photosynthetic tissues, the efficient use of nitrogen invested in leaves for the photosynthesis process, and a relatively low use of carbon in respiration, as explained by Lambers and Oliveira [\[51\]](#page-17-14).

Finally, the NUE ecology showed a negative relationship with STN ( $r = -0.59$ ,  $p \le 0.001$ ) and CEC ( $r = -0.51$ ,  $p \le 0.001$ ). Because this index includes the time the crop remains in the field, it is logical that as the crop stays longer in the field, it absorbs and consumes STN, hence the negative correlation between NUEecology and STN. NUEecology was influenced by the addition of fertilization, but organic and inorganic fertilization did not differ significantly from each other.

## **5. Conclusions**

This study demonstrates that the type of fertilization significantly impacts several soil and crop metrics in fenugreek cultivation. Organic fertilization, particularly with BHS, showed superior performance in increasing STN, CEC, and SOM while also reducing the risks associated with nutrient leaching and over-fertilization. This aligns with the global need to improve NUE and manage soil health sustainably.

Organic fertilizers enhanced various nitrogen efficiency indices, such as NUEcrop, PFP, REfertN, and NupE, indicating a more effective and sustained nitrogen utilization by fenugreek plants compared to inorganic fertilizers. This improvement is attributed to the gradual nitrogen release from organic sources, better soil structure, and enhanced microbial activity. Notably, organic fertilization also resulted in higher PNB and AE values. Salinity hinders effective nitrogen absorption, reducing the efficiency of fertilization practices. Thus, managing salinity levels is crucial for optimizing nitrogen uptake and maintaining soil health.

Conversely, inorganic fertilizers, while providing immediate nitrogen availability, often led to nutrient imbalances, lower NUE, and potential nitrogen losses, highlighting the necessity for balanced and well-managed fertilization practices.

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