Fertilization expression via nitrogen indices in soybean crop under two system tillage

Ioanna KAKABOUKI*, Antigolena FOLINA, Charikleia ZISI, Stella KARYDOGIANNI

Abstract

Soybean (Glycine max L.) constitutes a crop that is currently of interest both for its nutritional properties in humans and animals and for its contribution to soil nitrogen. It belongs to legumes, that means that it can take N₂ and channel it to the soil, to be assimilable from plants. In addition, its high oil and protein content makes it important because of its nutritional properties. Moreover, soybean is a crop that has a major impact on nitrogen indicators. In this study, set up two same experiments in 2018-2019, in Western Greece. There were identified the effects of different fertilizer application (Control, N80, N100, N120), and different tillage (conventional tillage (CT), no tillage (NT)), on soil (organic matter, root density, no nodules/soil) and in agronomic (LAI, height, N% in upper parts, Yield, N% in seeds, N uptake in upper parts, N uptake in seed, N total uptake) characteristics. As well as in nitrogen indicators (nitrogen use efficiency, nitrogen harvest index, nitrogen agronomic efficiency, effects of absorption, effects of uptake). Soil properties were affected mainly by the tillage. However agronomic characteristics presented more differences between the different fertilizer application and finally the indicators were affected on both the parameters.

Keywords: fertilization; nitrogen indicators; nitrogen uptake; soil tillage; soybean

Introduction

Soybean (Glycine max (L.) Merr.) cultivation is gaining ground over the years because it is a versatile crop that is mostly used as animal feed and secondarily in human nutrition (Souza, 2016). This is mainly due to its high adaptation to various conditions, for example on low-N soils (Keyser, 1992) and the high concentration of protein in seed and stems, over 40% (Mere, 2013). The oil and protein content are high so soya bean plants constitute high-quality feed (fish and animals), food and good raw material for biofuels (Hellal et al., 2013). Soybean cultivation in Greece began in the 80’s and a high percentage of oil were recorded. To date, it is mainly used for animal feed (Skoulou et al., 2011). The utilization of applied nitrogen in the crop is related to the N₂ fixation of soybean inasmuch the already available nitrogen in the soil is a factor that affects the amount of fixed-N (Keyser, 1992). The ability of soybean to biological nitrogen fixation is a key factor in the utilization of inorganic fertilization. Soybean plant can
biologically fix 44 to 130 kg N ha⁻¹ per year (Sanginga et al., 2003) and for the high accumulation of N in the seed. requires a large amount of nitrogen (Hao et al., 2011). However, many researchers that N₂ fixation is not enough for the needs of crop and fertilization take place (Wesley et al., 1998; Salvagiotti et al., 2008). On the other hand, adding N fertilizer is not clear if increase yields (Osborne et al., 2008). According to studies, applied N range from 50 kg N ha⁻¹ to 200 kg N ha⁻¹ (Salvagiotti et al., 2008). Salvagiotti et al. (2008) note the rising of the yields with the addition of a large amount of fertilizer (> 4.5 mg ha⁻¹) and a negative relationship with N₂ fixation when the application is done very superficially. Another cost-effective approach to applied N is its split application where a rate provided during the growing season (Mirshekari, 2013).

Existing nitrogen in soil comes from either inorganic or organic fertilization. These two types of fertilizers are widely used to increase yields and protein in seed. Regarding inorganic fertilization, it has been noted that it caused a decrease in the nutritional quality of legumes (Bairwa et al., 2009). However, adding organic fertilizers can improve the efficiency of inorganic fertilizers (Mere et al., 2013). Another factor that offers better utilization of applied nitrogen is the cultivation systems based on legume crops that have the ability to regulate the nitrogen cycle (Crookston et al., 1991; Schipanski et al., 2010). In these systems, the availability of inorganic fertilizers may be limited due to the enrichment of the soil by N₂ fixation (Vitousek et al., 2002). The combination of the above two factors can promote the sustainable use and utilization of mineral nitrogen. As data on Greek conditions is limited, it is necessary to investigate the balance of N from mineral and biological fixation to conserve natural resources and reduce nitration.

However, the biological nitrogen fixation of soybeans starts from the degrees of colonization by soil rhizobia which are affected by the soil and the weather. (Goss et al., 2002) consequently, from agricultural practices applied to the soil such as tillage. Reducing soil disturbance, for example, improves the physical parameters of the soil, such as structure, and at the same time the biological parameters that affect the stabilization of nitrogen (Kihara et al., 2011). Soil biological parameters including the activity of microorganisms such as those of the root nodule bacteria due to the biological nitrogen fixation. The practice of no tillage contributes to the growth of root bacteria in soybean cultivation (Ferreira et al., 2000). Moreover, the applied nitrogen increased the formation of nodules (Seneviratne et al., 2000; Wang et al., 2009). In addition, nodules and applied N can work together if N application is done before planting where nodules have not yet formed. (Bergersen, 1958). The effect of applied N on the biological nitrogen fixation of soybean follows the soil structure as a priority (Schipanski et al., 2009).

The amount of nitrogen from biological nitrogen fixation is affected by a variety of factors that affect production and yield (Keyser, 1992). Whether soil tillage affects soybean yields is ambiguous, many researchers say it affects and increases yields (Elmore, 1990; Okoth et al., 2014) while others that it does not affect (Lueschen et al., 1993). Soil tillage can also affect nitrogen fixation. The effect of reduced tillage and conventional tillage on nitrogen fixation in grain legumes has been investigated in Kenya and it was noted that nitrogen fixation increased with reduced treatment (Kihara et al., 2011).

The most efficient use of nitrogen (NUE) directly determines the yield and protein content of soybeans. Hao et al. (2011) mentioned that the NUE could be improved by genetic improvement, offering higher yields and better quality of soy products. Attia et al. (2015) mention that intercropping of corn and soybean and the interaction with N rates significantly affected NUE. Contrariwise, if NUE is calculated as the removal of N grains, increasing the rate N the NUE decreases (Halvorson et al., 2006).

In recent years, fertilizers have been developed to maximize nitrogen utilization, such as adding inhibitors of urea. In this experiment, we tried to combine some classic practices that are already widely used by producers to maximize nitrogen utilization. Also, while it is widely reported in the literature that soybean plants fix large amounts of N, there are few studies to evaluate this N. That is, to finally see, for example, how many N uptake the ground part of the plant and seeds.
The objective of this study was to compare the response of soybean crop with various N rate fertilizers and conventional tillage system when planted at 2 years. Also, through nitrogen indices the evaluation of nitrogen fertilization in terms of crop development was expressed. The original hypothesis (H0) was if the indicators could effectively express soybean fertilization in different tillage systems, in contrast (H1) was that they could not express effectively.

Materials and Methods

Experimental design

Two same experiments, with soya crop was established in Western Greece, in Agrinio (38°35'18 N, 21°25'40 E, with altitude 53 m), in 2018 and 2019. The soil was a clay loam, with pH (1:1 H₂O) 6.72 and organic matter content 2.78%. The sites were managed according to organic agriculture guidelines (EC 834/2007). The density of plants was 350 plants ha⁻¹. A drip irrigation system was used to irrigate the crop. The meteorological data on the air temperature and the precipitation of the experimental space during the experimental periods are presented in Figure 1.

The experiment was created on an area of 960 m², according the split plot design with three replicates, two main plots (conventional tillage (CT), moldboard plowing at 25 cm, followed by one rotary hoeing at 10-15 cm, and no tillage, direct sowing (NT)). In additions were four sub-plots (fertilization treatments: control, application with 80 kg N ha⁻¹ (N80), application with 100 kg N ha⁻¹ (N100) and application with 120 kg N ha⁻¹ (N120)). Each main plot was 160 m².

![Figure 1. Meteorological data (mean monthly air temperature and precipitation) for experimental site during the experimental periods (April-September, 2018-2019)](image)

Measurements

The total soil porosity was determined by 1-Db / Dp, where Dp is the particle density (2.5 g cm⁻³) and Db is the soil density. The bulk soil density was determined for each plot by taking undisturbed soil cores with 100 cm³ taken from cylinders from a depth of 0-10 cm. Three samples of 100 cm³ per plot were taken, in the flowering period. The undisturbed samples were finally dried in an oven at 100 °C for 24 hours to obtain the...
dry mass of the soil and the soil density was calculated as follows: $D_b = \frac{\text{dry mass (g)}}{100 \text{ cm}^3}$. Penetration resistance was calculated with penetrating DT. Organic matter was measured at the maturation stage, by Wakley black method (Wakley and Black, 1934).

Root specimens were collected in flowering and from the 0-25 cm layer using a cylindrical drill (25 cm long, 10 cm in diameter) in the middle point between successive plants within a row. For each sample, roots were separated from soil after standing for 24 h in water + (NaPO3)6 + Na2CO3. The dry root weight was then determined after drying one of the paired samples for 48 hours at 70 °C (Kokko et al., 1993). To determine the density and diameter of the roots, the root samples were placed in a high-resolution scanner using DT software (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burrwell, Cambridge, UK).

In addition, at the flowering was counted, the number of nodules at 100 cm$^3$ soil. Other measurements at the flowering stage was the LAI, using SunScan (Delta-T Devices Ltd), and the plants’ height cm. Then, at the time of maturity was measured the number of pods per plant.

Moreover, the other measurements were taken on harvested products. There were weighted 1000 seeds from each experimental part. In dry matter upper measurement, leaves and stems (kg ka$^{-1}$) were determined after drying for 72 h at 75 °C. As well there was counted the yield (kg ha$^{-1}$), by manually harvesting the pods of the plants in moisture content 13%. Nitrogen measurements were N% in upper parts and N uptake in upper parts (kg ha$^{-1}$), N% in seeds and N uptake in seeds (kg ha$^{-1}$), and N total uptake (kg ha$^{-1}$). The total nitrogen was determined by the Kjeldahl method (Bremner, 1960) using a Buchi 316 device. The samples were chosen randomly within each plot. All plant samples were ground to fine powder and used for determination of total nitrogen.

Nitrogen uptake and nitrogen uptake total were measured by the following equations (eq 1) and (eq 2) respectively.

\[ N\text{-uptake} = N\% \text{ tissue} \times \text{Dry matter yield upper parts} \]  
\[ N\text{-uptake total} = N\text{yield upper parts (kg ha}^{-1}\text{)} + N\text{yield seed (kg ha}^{-1}\text{)} \]  

In addition, was calculated some nitrogen indicators. Nitrogen use efficiency were estimated by the equation 3.

\[ NUE = (N\text{ total uptake (kg ha}^{-1}\text{)} - N\text{ total uptake (kg ha}^{-1}\text{) control}) / kg \text{ N ha}^{-1} \]  

Nitrogen Harvest Index is an indicator which is defined as a ratio of the concentration of N in seeds (seeds N uptake) to the total N in the plant (total plant N uptake) (eq 4). This indicator is positively related to seed yield.

\[ NHI = \text{seed N uptake (kg)/ total plant N uptake (kg)} \]  

Another Nitrogen indicator, is Nitrogen Agronomic Efficiency, which shows the amount of seed produced per kg of N fertilizer (eq 5).

\[ NAE = (\text{seed yield fertilizer-seed yield control}) / N \text{ kg of fertilizer} \]  

In this study, were calculated also the effects of absorption, which are defined the ration of nitrogen total uptake to quantity of N fertilizer (eq 6) and Effects of Uptake which are shown the ration of yield to nitrogen total uptake (eq 7).

\[ \text{Effects of Absorption} = N\text{ total uptake (kg/ha) / N Kg fertilizer (kg ha}^{-1}\text{)} \]  
\[ \text{Effects of Uptake} = \text{Yield (kg/ha) / N total uptake (kg ha}^{-1}\text{)} \]
Statistical analysis

Analysis of variance was carried out on data using the Statistica (Stat Soft, 2011) logistic package as a completely randomized design. The analysis of variance (ANOVA) used a mixed model, with years and replications as random effects and tillage system and fertilization as fixed effects. Differences between means were separated using Tukey’s test. Correlation analyses were used to describe the relationships between growth parameters and yield components using Pearson’s correlation. All comparisons were made at the 5% level of significance (p ≤ 0.05).

Results

In the Table 1, presented the soil characteristics as like as the total soil porosity. The values ranged from 39.6 to 41.3% in the first year in CT. Also, in NT the values were higher than CT ranged from 43 to 45%. Correspondingly, in year B the values ranged from 39 to 41.7% in CT, and from 42.3 to 44.7% in NT. In the first and second year, and in the CT and NT, the control had not statistically significant difference with the N100 and the N80 had not statistically significant difference with the N120. In year 2018 in NT, the N100 had not statistically significant difference with the N120. The highest value was 44.7 % in the N80, NT in second year (Table 1).

Table 1. The soil characteristics as effected by fertilizer treatments (control, inorganic fertilization 80 kg N ha⁻¹ (N80), inorganic fertilization 100 kg N ha⁻¹ (N100) and inorganic fertilization 120 kg N ha⁻¹ (N120)) and different tillage system (conventional and no tillage: CT and NT, respectively)

| Fertilization | Total soil porosity (%) | Penetration resistance 0-30 cm (MPa) | Organic matter | Root density (cm 100cm⁻³) | no nodules (100 cm⁻³ soil) |
|---------------|-------------------------|-------------------------------------|----------------|-------------------------|---------------------------|
|               | 2018 CT                 |                                     |                |                         |                           |
| Control       | 40.3a                   | 2.627a                             | 2.083a         | 12.667a                 | 8.102a                    |
| N80           | 39.6b                   | 2.523a                             | 2.250a         | 16.667b                 | 10.185ab                  |
| N100          | 41a                     | 2.570a                             | 2.210b         | 20.667b                 | 12.037ac                  |
| N120          | 41.3b                   | 2.630a                             | 2.273b         | 23.333b                 | 13.426c                   |
|               | 2018 NT                 |                                     |                |                         |                           |
| Control       | 43a                     | 1.963a                             | 2.700a         | 20a                     | 10.648a                   |
| N80           | 44.3b                   | 1.977a                             | 2.820a         | 24.667c                 | 13.194ab                  |
| N100          | 45a                     | 1.860a                             | 2.897b         | 28.667c                 | 15.278b                   |
| N120          | 45c                     | 2.010a                             | 2.817c         | 32.667c                 | 17.361c                   |
|               | 2019 CT                 |                                     |                |                         |                           |
| Control       | 39a                     | 2.627a                             | 2.083a         | 13a                     | 7.407a                    |
| N80           | 39.7b                   | 2.547a                             | 2.250a         | 17.667c                 | 9.954ab                   |
| N100          | 40.3a                   | 2.550a                             | 2.263b         | 22.667c                 | 11.111b                   |
| N120          | 41.7b                   | 2.550a                             | 2.320c         | 26c                     | 13.194c                   |
|               | 2019 NT                 |                                     |                |                         |                           |
| Control       | 42.3a                   | 1.977a                             | 2.627a         | 21.667a                 | 12.731b                   |
| N80           | 44.7b                   | 2.083a                             | 2.767a         | 29.000a                 | 13.889b                   |
| N100          | 44.3a                   | 2.020a                             | 2.967b         | 30.333c                 | 16.204a                   |
| N120          | 44b                     | 1.927a                             | 3.117c         | 33.667c                 | 20.370c                   |
| F_tillage     | 74.726***               | 301.619***                         | 160.259***     | 83.566***               | 58.724***                  |
| F_fertilization | 5.506                  | 0.339***                           | 5.227**        | 30.945***               | 23.489***                  |
| F_year        | 1.142**                 | 0.188**                            | 0.763**        | 3.888**                 | 1.072**                   |
In the penetration resistance 0-30 cm none value in the both years and in the different tillage had statistically significant difference between them. The highest value was 2.627 MPa in control, in the first and second year in CT and the lowest was 1.860 MPa in N100, in the first year in NT. The organic matter’s values ranged from 2.083 to 2.273 in year 2018 in CT and from 2.083 to 2.320 in year 2019, respectively. In NT the values ranged from 2.817 to 2.820 in first year and from 2.627 to 3.117 in second year. It is emphasized that there were higher values in NT than CT, in both years. The N100 had statistically significant difference with the other treatments and the N80 had not statistically significant difference with the control, in CT and NT in both years. Concerning the root density, the values in CT ranged from 12.667 to 23.333 cm 100 cm⁻³, in first year and from 13 to 26 cm 100 cm⁻³ in second year. The N100 had not statistically significant difference with the other fertilizers in both years in CT and NT. Moreover, in yield the highest value was 4,570 kg ha⁻¹ in N120, NT in first year and from 4,050 to 4,312 kg ha⁻¹ in CT and from 4,140 to 4,570 kg ha⁻¹ in NT in the first year. In the second year, in CT the N80 had not statistically significant difference with the N120 and the N80 had not statistically significant difference with the N100. And the N100 had not statistically significant difference with the N120, in CT and NT. Furthermore, in second year, only the N100 had not statistically significant difference with the N120. The highest value was 7.350 in N100, NT in year B. The lowest value was 6.477 in control, CT in year A (Table 2).

| Fertilization x tillage | Fertilization x year | Soil x year | Year |
|-------------------------|---------------------|-------------|------|
| Fertilization x tillage | 1.066**          | 0.912**     | 0.538** |
| Fertilization x year   | 0.009**          | 0.975**     | 0.133** |
| Soil x year            | 0.336**          | 1.04**      | 0.985** |
| Year                    | 0.198**          | 0.129**     | 0.3**  |

In the penetration resistance 0-30 cm none value in the both years and in the different tillage had statistically significant difference between them. The highest value was 2.627 MPa in control, in the first and second year in CT and the lowest was 1.860 MPa in N100, in the first year in NT. The organic matter’s values ranged from 2.083 to 2.273 in year 2018 in CT and from 2.083 to 2.320 in year 2019, respectively. In NT the values ranged from 2.817 to 2.820 in first year and from 2.627 to 3.117 in second year. It is emphasized that there were higher values in NT than CT, in both years. The N100 had statistically significant difference with the other treatments and the N80 had not statistically significant difference with the control, in CT and NT in both years. Concerning the root density, the values in CT ranged from 12.667 to 23.333 cm 100 cm⁻³, in first year and from 13 to 26 cm 100 cm⁻³ in second year. The N100 had not statistically significant difference with the other fertilizers in both years in CT and NT. Moreover, in yield the highest value was 4,570 kg ha⁻¹ in N120, NT in first year and from 4,050 to 4,312 kg ha⁻¹ in CT and from 4,140 to 4,570 kg ha⁻¹ in NT in the first year. In the second year, in CT the N80 had not statistically significant difference with the N120 and the N80 had not statistically significant difference with the N100. And the N100 had not statistically significant difference with the N120, in CT and NT. Furthermore, in second year, only the N100 had not statistically significant difference with the N120. The highest value was 7.350 in N100, NT in year B. The lowest value was 6.477 in control, CT in year A (Table 2).
Table 2. The agronomic characteristics as affected by fertilizer treatments (control, inorganic fertilization 80 kg N ha⁻¹ (N80), inorganic fertilization 100 kg N ha⁻¹ (N100) and inorganic fertilization 120 kg N ha⁻¹ (N120)) and different tillage system (conventional and no tillage; CT, and NT, respectively).

| Fertilization | LAI  | Plant height (cm) | No pods plant⁻¹ | 1000 seeds weight (g) | DM yield upper parts STOVER kg ha⁻¹ | N in upper parts (%) | Yield Kg ha⁻¹ Gw | N in seeds (%) |
|---------------|------|-------------------|------------------|-----------------------|--------------------------------------|---------------------|-----------------|----------------|
| **2018**      |      |                   |                  |                       |                                      |                     |                 |                |
| Control       | 4.62³ | 42     | 37    | 28     | 3.747³ | 1.700³ | 4.050³ | 6.477³ |
| N80           | 4.92³ | 45     | 42    | 29     | 3.923³ | 1.777³ | 4.156³ | 6.840³ |
| N100          | 5.13³ | 47     | 42    | 30     | 4.045³ | 1.827³ | 4.243³ | 6.923³ |
| N120          | 5.34⁴ | 52     | 44    | 31     | 4.195⁴ | 1.860⁴ | 4.312⁴ | 7.040⁴ |
| **2018**      |      |                   |                  |                       |                                      |                     |                 |                |
| Control       | 4.92⁰ | 44     | 40    | 29     | 3.978⁰ | 1.793⁰ | 4.140⁰ | 6.877⁰ |
| N80           | 5.22⁰ | 47     | 44    | 31     | 4.118⁰ | 1.873⁰ | 4.300⁰ | 7.7⁰ |
| N100          | 5.39⁰ | 52     | 47    | 32     | 4.237⁰ | 1.987⁰ | 4.410⁰ | 7.127⁰ |
| N120          | 5.90⁴ | 54     | 51    | 33     | 4.348⁴ | 2.117⁴ | 4.570⁴ | 7.246⁴ |
| **2019**      |      |                   |                  |                       |                                      |                     |                 |                |
| Control       | 4.65⁰ | 44     | 36    | 29     | 3.700⁰ | 1.753⁰ | 4.015⁰ | 6.857⁰ |
| N80           | 4.81³ | 47     | 39    | 30     | 3.860³ | 1.740³ | 4.145³ | 6.950³ |
| N100          | 5.14³ | 51     | 41    | 32     | 4.042³ | 1.783³ | 4.297³ | 7.150³ |
| N120          | 5.20⁴ | 53     | 42    | 31     | 4.148³ | 1.857³ | 4.373³ | 7.230³ |

Concerning the N uptake in upper parts, in the first year, the values ranged from 64 to 78 kg ha⁻¹ in CT and from 71 to 92 kg ha⁻¹ in NT. In the second year, in CT ranged from 65 to 77 kg ha⁻¹ and from 69 to 86 kg ha⁻¹ in NT (Table 3). The N80 had not statistically significant difference with the control, and the N100 had not statistically significant difference with the N80 and with the N120, in both years and in the CT, NT. In N uptake in seed, all treatments had statistically significant difference between them in year A and B and in the CT, NT. The highest value was 336 kg ha⁻¹ in N120, NT in second year. Also, the lowest value was 262 kg ha⁻¹ in control, CT in the first year.
Conventional tillage. In the present study, it was shown that the no tillage treatments increased the soil porosity. A penetration register was 27% lower compared with conventional tillage. As well, Alvarez and Steinbach (2009) lower its penetration, because of the soil pores size. Our results shown that in no tillage treatment the nodules almost 48% and 30% respectively. Soil leverage promotes the oxidation of organic matter that has risen roots growth is correlative with the register, so if there are more roots on 100 cm soil, there are more nodules.

Table 3. The nitrogen indicators as effected by fertilizer treatments (control, inorganic fertilization 80 kg N ha⁻¹ (N80), inorganic fertilization 100 kg N ha⁻¹ (N100) and inorganic fertilization 120 kg N ha⁻¹ (N120)) and different tillage system (conventional and no tillage: CT and NT, respectively)

| Fertilization | N uptake in upper parts kg ha⁻¹ | N uptake in SEED kg ha⁻¹ | N total uptake kg ha⁻¹ | NUE | NHI | NAE | Eff of absorption Nt Nv⁻¹ | Eff of uptake Gw Nt⁻¹ |
|---------------|---------------------------------|--------------------------|-----------------------|-----|-----|-----|--------------------------|-----------------------|
| 2018          |                                 |                          |                       |     |     |     |                          |                       |
| Control       | 64ᵃ 262ᵇ 326ᵃ                  | -                        | 0.805ᵃ                |     |     | (-) | 12.429ᵇ                 |                       |
| N80           | 70ᵇ 284ᵇ 354ᵇ                  | 0.269ᵇ 0.803ᵇ 1.25ᵇ     | 4.420ᵇ                | 11.739ᵇ |
| N100          | 74ᵇ 294ᵇ 368ᵇ                  | 0.181ᵇ 0.799ᵇ 1.933ᵇ    | 3.677ᵇ                | 11.543ᵇ |
| N120          | 78ᵇ 304ᵈ 382ᵈ                  | 0.177ᵇ 0.796ᵇ 2.181ᵇ    | 3.180ᶜ                | 11.306ᶜ |
| 2018          |                                 |                          |                       |     |     |     |                          |                       |
| Control       | 71ᵃ 285ᵃ 356ᵃ                  | -                        | 0.80ᵃ                 |     |     | (-) | 11.631ᵃ                 |                       |
| N80           | 77ᵇ 301ᵇ 378ᵇ                  | 0.203ᵇ 0.796ᵇ 2ᵇ        | 4.727ᵇ                | 11.376ᵇ |
| N100          | 84ᵇ 314ᵇ 399ᵇ                  | 0.133ᵇ 0.789ᵇ 2.7ᵇ      | 3.985ᵇ                | 11.077ᵇ |
| N120          | 92ᶜ 331ᵈ 423ᵈ                  | 0.140ᶜ 0.783ᵇ 3.583ᶜ    | 3.527ᶜ                | 10.807ᶜ |
| 2019          |                                 |                          |                       |     |     |     |                          |                       |
| Control       | 65ᵃ 275ᵃ 340ᵃ                  | -                        | 0.80ᵃ                 |     |     | (-) | 11.803ᵃ                 |                       |
| N80           | 67ᵇ 288ᵇ 355ᵇ                  | 0.159ᵇ 0.811ᵇ 1.625ᵇ    | 4.441ᵇ                | 11.670ᵇ |
| N100          | 72ᵇ 307ᶜ 379ᵇ                  | 0.191ᵇ 0.810ᵇ 2.817ᵇ    | 3.793ᵇ                | 11.329ᵇ |
| N120          | 77ᶜ 316ᵈ 393ᵈ                  | 0.074ᵈ 0.804ᵈ 2.986ᵈ     | 3.277ᶜ                | 11.123ᶜ |

Discussion

It is well known, that tillage is a method that causes fine-grain soil. In addition, the smaller the soil grains, the lower its porosity. So, on the treatment of no tillage the porosity will be great unlike the soil after conventional tillage. In the present study, it was shown that the no tillage treatments increased the soil porosity rate by about 10%. Porosity is negative correlated in the penetration register. Thus, the greater the porosity, the lower its penetration, because of the soil pores size. Our results shown that in no tillage treatment the penetration register was 27% lower compared with conventional tillage. As well Alvarez and Steinbach (2009) reported that under no tillage the cone penetration increased by 50%. This fact affects the roots density, due to roots growth is correlative with the register, so if there are more roots on 100 cm soil, there are more nodules in the same soil area. Thus, the present experiments had an increase in the root density and the number of nodules almost 48% and 30% respectively. Soil leverage promotes the oxidation of organic matter that has risen.
to the surface of the soil due to its contact with O₂, high atmospheric temperature and solar radiation this is how the organic matter in our study increased by about 27%. There are references in the literature which showed that lower organic matter was found in conventional treatment (Bilalis et al., 2010).

In terms of fertilization applications, total soil porosity and penetration register, are not significantly affected by them. On the other hand, organic matter is positively associated with nitrogen application. This is due to the replacement of nitrogen in the soil. It is known that legumes for their development mainly use nitrogen that is injected into the soil by the process of nitrogen fixation and organic matter. Thus, by nitrogen application, soil exhaustion is reduced. In addition, the application of nitrogen is positively related to root density, and therefore to the number of nodules. Sidiras et al. (1999) reported that in vetch crop no tillage treatment have a positive effect on root weight. Furthermore, if there is not enough N available in the soil during the nodule growth, N levels in legumes are insufficient (Hardy and Havelka, 1975; Sprent, 1985; Streeter, 1988). This is why, unlike other studies (Ntambo et al., 2017), in this study was observed an increase in the number of nodules per 100 cm³.

Nitrogen (N), is an important nutrient for soybean growth and development. In soybean cultivation the application of nitrogen helps the vegetative growth of the plant. The agronomic characteristics such as plant height, dry weight and crop yield are improving by the application but also by the amount of nitrogen in the crop. In general, the application of nitrogen in all crops results an increase in yields. According to a study that has been carried out, there is a need to supply inorganic nitrogen to soybeans for higher yields due to the fact that it cannot meet the high nitrogen needs through nitrogen fixation (Kumawat et al., 2000).

A study conducted by Saxena and Chandal (1992), showed that the maximum plant height was presented in 40 N kg ha⁻¹. Naik and Rao (2004), conducted an experiment in which they recorded the same result in terms of height increase. In our results the highest value in plant height was in N100 and in N120. It was observed that the plant height increased from control to N120. This is due to the fact, that nitrogen promotes plant growth in general, with the result that as the levels of applied nitrogen increase, so does the plant height, the same observed by Pradhan et al. (1995), in nitrogen levels from 0 to 80 kg ha⁻¹.

It was observed, that as the amount of nitrogen applied increased, so increased the LAI, where in NT values were higher than in CT in both years. Warner and Newton (2005), reported similar results in LAI, that is, an increase in the amount of nitrogen applied resulted an increase in LAI. The increase in nitrogen resulted in higher values for pods per plant as well as in yield. According to our results, the treatments with nitrogen fertilizers were had statistically significant difference with the control, in number for pods plant⁻¹ and in the yield. As reported by, Jadhav et al. (2009) the number of pods plant⁻¹ and 1000 seed weight of soybean were increased with a application of nitrogen fertilizer. Also, and the Gai et al. (2017), had recorded higher seed yields after the nitrogen application.
Yield Kg/ha 13% MC = 2176,7755+6,9027*x+5,2352*y

Yield =2176+6.90Nupt upper +5.24Nupt seed

$R^2_{adjusted}=0.923^{***}$

Figure 2. 3D-graph and linear equation between Yield and Nuptake in seeds & N total uptake

The percentage of nitrogen in seed was higher with nitrogen fertilizers and more specifically the highest amounts of nitrogen had the highest percentages of nitrogen content. The control had the lowest value in the both years and in the both tillage systems. Kumawat et al. (2000), had reported that the protein content increased with an increase in the nitrogen levels. The results of our experiment regarding the content of nitrogen in the seed can be followed by this report due to the fact that the synthesis of proteins has as a necessary component nitrogen, so the high amounts of nitrogen therefore have a high protein content.

N uptake in upper parts kg ha$^{-1}$ was affected by tillage and fertilization. The N uptake in upper parts was increased with the rise of N and the undisturbed soil. It is noted that treatment of tillage gave lower values to N uptake in upper parts. This may be because undisturbed soil does not affect AM and soil microorganisms (Bilalis et al., 2012), so they act better and absorb more N. This is also confirmed by the positive correlation between Total soil porosity (%) and N uptake in upper parts ($r = 0.5815$, $p = 0.001$). This positive relationship is also noted with N uptake in seed ($r = 0.58^{***}$) and with N total uptake ($r = 0.60^{***}$).

N uptake in seed kg ha$^{-1}$ was affected by tillage, fertilization and year. In both years, in conventional tillage we had a lower difference between the maximum value (N120) and the control. So, we noticed that keeping the soil undisturbed helps the soybean to absorb more N in the seeds, therefore better seed quality. Additionally, keeping the soil undisturbed during these 2 years, it is noted that in the 2nd year the N uptake in seed was higher. Also, the direct effect of N fertilizers on the increase of seed storage proteins mentioned by Martre et al. (2003).

N total uptake kg ha$^{-1}$ was affected by tillage ($p=0.001$), fertilization ($p=0.001$) and year ($p=0.01$). The total N uptake (above-ground and seed) was consistently above 350 kg N ha$^{-1}$ for the three rate of N fertilizer while the controller was consistently 10 kg N ha$^{-1}$ lower. These high prices are due to the N content, as well as the higher plant mass in N120 fertilization and the higher seed yields in the same fertilization. This can also be explained by the high linear correlations between total N uptake and crop height ($r=0.67^{**}$, Table 4.).

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NUE was affected by fertilization. According to our results, NUE decreased with the increase of nitrogen units. Expressly, the yield of soybeans in seed decreased with increasing N units. The results agree with Halvorson et al. (2006).

NHI was affected by tillage fertilization and year. The nitrogen harvest index of the crop was lower than the N120 fertilizer compared to the control and generally followed the opposite course. This is verified by the negative relationship with N uptake in upper parts kg ha\(^{-1}\) (\(r = -0.907\)*** \(p < 0.001\)). The nitrogen harvesting index is 0.8 for all operations. It is therefore noted that when the direction of production of the crop is the seed, the nitrate fertilization yields.

NAE was affected by tillage and fertilization. The factor of fertilization had a positive effect on the price of the NAE index with the price range ranging from 1.25 (N80, Year A) to 3.61 (N120, Year B). So, the higher prices of index, the higher soybean seed yields. If the cultivation of soy focusses on seed production, it will exploit as much of applied N. While NAE is only determined by seed yield, we see that there is a significant correlation with N uptake in seed kg ha\(^{-1}\) (\(r = 0.84\)***).

Eff of absorption (Nt/Ns) was by tillage, fertilization and tillage* fertilization. With the rising of N rates, the absorption of N from the whole plant decreased. For the first year, the difference between N80 and N120 in CT was 39% while in NT it was 34%. Therefore, the increase of nitrogen units was greater than the increase in N total uptake. There is also a small negative correlation with N uptake in seed (\(r = -0.3599\)).

Tillage, fertilization and year statistically affect Eff of uptake Gw/NT. The effects of uptake were decreased while N rates rise in both years. Regarding the tillage, in the first year the difference between witness and N120 in CT was 9.9% while for NT it was 7.8%. In contrast to the 2nd year, the difference between witness and N120 was higher in NT. The correlation between effects of uptake with N% in seeds and N uptake in seed kg ha\(^{-1}\) is negative (\(r = -0.89\) and \(r = -0.95\) respectively) (Table 5).

The given Figure (Figure 2) illustrate that there is a linear correlation between yield (kg ha\(^{-1}\)), N uptake in seed (kg N ha\(^{-1}\)) and N uptake upper parts (kg N ha\(^{-1}\)). As the yield increases, the nitrogen absorption from the seeds and the aboveground part of the plant also increases (\(R^2 = 0.923\)). This result is important if we consider that soybean cultivation is aimed at animal feed and seeds.

**Table 4. Linear Correlation (r-values) matrix between soil & plant properties.**

|                      | DM yield upper parts | N in upper parts (%) | Yield (kg ha\(^{-1}\)) | N in seeds (%) | N uptake in upper parts (kg ha\(^{-1}\)) | N uptake in SEED (kg ha\(^{-1}\)) | N total uptake (kg ha\(^{-1}\)) | NUE | NHI | NAE | Effect of absorption (Nt/Ns) | Effect of uptake (Gw/NT) |
|----------------------|---------------------|----------------------|------------------------|----------------|----------------------------------------|---------------------------------|---------------------------------|-----|-----|-----|-----------------------------|---------------------------|
| Total soil porosity (%) | .618*** .492** .591** .486** .582** .585** .608** .127ns | - .463* .493ns .096** .603* |
| Penetration resistance 0-30 cm, (MPa) | -.549*** -.495*** -.561*** -.501*** -.571*** -.577*** -.599*** .023ns | -.446ns -.356ns -.250*** -.601*** |
| Organic matter % | .627*** .552** .634*** .653*** .654*** .652*** .673*** .125ns -.486*** .442ns .148*** .670*** |
| Root density (cm 100 cm\(^{-1}\)) | .751*** .529** .726** .728** .659** .780** .770** -.207ns | -.432ns -.598*** -.235*** -.774** |
| No nodules (cm 100 cm\(^{-1}\)) | .772*** .325** .671** .648** .513** .713** .672** -.229ns | -.261ns .619*** -.344*** .638*** |
| LAI | .826** .484 .754 .661 .652 .771 .761 -.244ns -.426ns -.627*** -.416 -.721** |
| height (cm) | -.795*** -.265** .805** .615** .474* .774* .670** -.387ns -.156ns .719*** -.548*** -.718** |
| No pods (plant\(^{-1}\)) | .818*** .781*** .868*** .616*** .875** .821** .875** -.034ns | .728*** .590*** -.256*** -.818** |
| 1000 seed weight (g) | .771*** .322** .659** .695** .511** .729** .682** -.470** | -.243ns .659*** -.406*** -.668** |

Significance levels: * \(p < 0.05\); ** \(p < 0.01\); *** \(p < 0.001\); ns, not significant (\(p > 0.05\))
Table 5. Linear correlation (r-values) between N indices

|                          | NUE      | NHI     | NAE     | Effective of absorption (Nt Ns⁻¹) | Effective of uptake (Gw Nt⁻¹) |
|--------------------------|----------|---------|---------|-----------------------------------|-----------------------------|
| N in upper parts (%)     | .047ns   | -.932** | .486    | -.125ns                           | -.780**                     |
| N in seeds (%)           | -.192ns  | -.215ns | .681**  | -.261ns                           | -.987**                     |
| N uptake in upper parts (kg ha⁻¹) | -.042ns  | -.908** | .632**  | -.255ns                           | -.873**                     |
| N uptake in SEED (kg ha⁻¹) | -.170ns  | -.513** | .838**  | -.356                           | -.956*                      |
| N total uptake (kg ha⁻¹) | -.132ns  | -.675** | .800**  | -.338                           | -.967**                     |

Significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant (p > 0.05).

Nitrogen indicators are used to help us understand if the nitrogen applied is used by plants or lost. Nitrogen Used Efficiency, express the ability of plants to use nitrogen effectively. More specifically, the higher this index, the better the plant uses nitrogen. In the present experiment in the application of 80 kg n per hectare, it seems to be the best dose of nitrogen. Garrido and López-Bellido (2001), reported that whether the indicator’s value, is higher than 1, then the plants get the excess nitrogen and can be used for crop phytoremediation. Nitrogen harvest index express how much of the applied nitrogen is contained in the seed, compared to the nitrogen content of the plant. This indicator informs us which is the most important part of the plant, in terms of nitrogen accumulation. In this study was observed that the control and immediately after the lower nitrogen dose (N80) had the higher NHI. This means that excess nitrogen was used to grow the plants and not the seeds. This can be useful for crops used to feed animals as a silage.

Nitrogen agronomic efficiency refers to the effects of the amount of nitrogen on seed yield. Thus, this indicator in the present study was positive correlated with the nitrogen amount that is to the 120 kg nitrogen per hectare. So, the higher this indication, the higher the yield of the seeds.

However, the effects of absorption show how much of the nitrogen fertilizer is absorbed by the plant. This indicator is negatively related to the dose of nitrogen. This informs us that the best amount the plant can get is 80 kg ha⁻¹. In addition, the Effects of uptake express how total nitrogen uptake is related to yield. Our results show that the plants did not use the uptake nitrogen to increase the yield but for other reasons, such as to increase their height. Through the indicators, it is therefore understandable that soybean uses the nitrogen derived from the N₂ fixation and the organic matter to increase the yield and not the nitrogen applied. This is why the control had the highest value in effects of nitrogen uptake. Therefore, nitrogen indicators can efficiently express the soybean fertilization in two different tillage systems.

Conclusions

In conclusion, different doses of nitrogen had different effects on plant characteristics, for example at plant height, and on soil properties such as organic matter and the number of nodules. In addition, it has been observed that no tillage has a positive effect on soil properties more than agronomic characteristics. However, it is well known that the condition of the soil indirectly effects on soybean growth. The role of indicators is to show whether each dose of nitrogen has a significant effect on plant growth or whether its effects are not beneficial to plants and soil properties. Due to the factors on which they depend, such as the N% and N uptake in upper parts, they seem to be to be affected by both different doses of nitrogen and tillage systems. However,
at high amounts of nitrogen, while the nitrogen content is increased, the indicators decrease, which means that the excess nitrogen does not have essential effects on plant growth. Hence, nitrogen indicators can express the fertilization in soybean cultivation in two different tillage systems.

**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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