**Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development**
**Vol. 24, Issue 2, 2024**
**PRINT ISSN 2284-7995, E-ISSN 2285-3952**

# STATISTICAL ANALYSIS OF ECONOMIC INDICATORS IN NITROGEN FIXATION OF BIRD'S FOOT TREFOIL CULTIVARS AND ITS INFLUENCE ON THE ECOLOGICAL EFFECT

**Boryana CHURKOVA, Katerina CHURKOVA**

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture – Troyan, 281 Vasil Levski Str., 5600 Troyan, Bulgaria, E-mails: bchurkova@abv.bg, katerina.churkova@abv.bg

***Corresponding author***: bchurkova@abv.bg

***Abstract***

*The amount of fixed nitrogen per decare was studied in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture-Troyan of bird'sfoottrefoil cultivars, such as Targovishte 1, Alvena, Lotanova and Frilo (Italy), Polom (Slovakia), Bonnie (France), Bull (Canada). The Lotanova and Polom varieties have increased nitrogen-fixing capacity and level of tuber formation, as well as fixed nitrogen per decare (17.40 and 17.17 N fix, kg/da), which is a prerequisite for increasing soil fertility and the ecological effect. These two varieties could be recommended as suitable for implementation in production from an economic and ecological point of view. The correlation analysis proved a high positive correlation dependence of gross revenue with yield (r=0.9999), production costs (r=0.9982), and the amount of fixed nitrogen (r=0.9998). Plant nitrogen storage through symbiotic nitrogen fixation of bird'sfoottrefoil, as a suitable meadow legume species for mountain conditions, determines its ecological effect.*

***Key words*****:** *bird's foottrefoil, nitrogen fixation, ecological effect*

## INTRODUCTION

Agriculture in the 21st century is faced with the challenge of overcoming the effects of climate change and at the same time preserving the environment. To achieve this balance, the application of appropriate agricultural practices related to improving soil fertility and biological diversity is essential [25].

For the conditions of sustainable agriculture, legumes are becoming increasingly popular. With their unique ability to establish symbiotic relationships with *Rhizobium* bacteria and atmospheric nitrogen fixation, they are an essential component for ecological forage systems and allow to obtain high yields using less /or no/ mineral nitrogen fertilizers [15, 31].

Legume-rhizobium symbiosis is a significant source of nitrogen and has a huge ecological and agronomic impact on the structure of sustainable agriculture [28, 42, 36]. Nitrogen from biological nitrogen fixation is used directly by plants and is less sensitive to denitrification and leaching [32, 14, 17]. Most legumes through biological nitrogen fixation can meet between 50 and 80% of their nitrogen requirements [20]. Increased symbiotic nitrogen fixation leads to increased nitrogen use efficiency. According to [2], this could be achieved by including more legumes in crop rotations.

Thanks to their nitrogen-fixing capacity, they are an alternative to industrial nitrogen fertilizers and a way to protect the environment [27]. The potential for nitrogen fixation through symbiosis is estimated to average 450 kg N/ha per year. According to [37], legumes fix between 15 and 25 kg of nitrogen per ton of dry matter. About 50 million tons of nitrogen are fixed annually in agroecosystems worldwide [18]. Crops such as alfalfa, red clover, peas, soybeans, and vetch fix about 65 to 335 kg N/ha [35, 22, 34]. Bird's foottrefoil is a valuable legume nitrogen-fixing species [41, 24]. In a study by [39] on the level of tuber formation of different forage legumes, the highest level of tuber formation was found for bird'sfoottrefoil. Likewise, bird'sfoottrefoil has the best phosphorus utilization efficiency (one of the factors that affect the process of

tuber formation and nitrogen fixation) for tuber formation, both as a monoculture and in mixtures.

To increase biological nitrogen fixation and plant productivity, selecting highly productive genotypes with increased nitrogen-fixing potential is important, and in the case of already created varieties to study their capacity for tuber formation [29]. This phenomenon acquires special relevance under the conditions of mountain and foot-hill regions, both in agroclimatic terms and in terms of the reaction and fertility of the soil. Therefore, current farming systems need sustainable intensification by incorporating legumes.

In modern agricultural conditions, the requirements for obtaining ecologically clean plant production are increasing. Biological nitrogen fixation is a process related to the complete or partial replacement of nitrogen fertilizers in several crops and to protect the environment and production from pollution. Symbiotic nitrogen fixation is characteristic of forage legumes and is carried out by tuber-formation bacteria bacteria. It has been established that the amount of fixed nitrogen from symbiotic nitrogen fixation is 3 million tons per year. This directs the attention of researchers to search for ways to solve the nitrogen problem, so that the obtained forage production is ecologically clean, and the costs incurred in its production are minimal. The findings of [11] that depending on the legume species [30] and its symbiosis with bacteria from the genera *Rhizobium* and *Bradyrhizobium*, they fix atmospheric nitrogen in amounts up to 30 kg/da, determine further work in this direction.

Symbiotic nitrogen fixation determines the possibility of supplying the grass component with nitrogen in the mixed cultivation of forage grasslands [14]. The presence of a legume component in mixed crops regulates the processes of tuber formation and nitrogen fixation [26] and lowers the requirements for mineral nitrogen [5], which determines the positive impact on the nutritional value of the forage. Alfalfa, clover, and bird'sfoottrefoil accumulate 180, 170, and 92 kg/ha$^{-1}$ N per year in the soil, respectively, and exhibit good adaptability to soil-climate conditions, altitudes, and other environmental conditions [12].

Scientific experiments defining the role of grass forages as one of the keys to increasing soil fertility, reducing the application of chemical fertilizers, and preserving agrobiodiversity are scarce [7, 38]. At the present stage, there are no comprehensive studies of the production of biological forage production from artificial grasslands in terms of its ecological effect [19].

The present research work aims to study the ecological effect of nitrogen fixation in different varieties of bird's foottrefoil varieties and to make a statistical analysis of the economic indicators in forage production.

## MATERIALS AND METHODS

The study includes four-year data (2016-2019) from an experiment in the field of Research Institute of Mountain Stockbreeding and Agriculture – Troyan. The experimental design was a complete block with 4 replications with a plot size of 5 m$^2$. The following bird'sfoottrefoil cultivars were tested and compared with the Bulgarian variety Targovishte 1: Alvena, Lotanova and Frilo (Italy), Polom (Slovakia), Bonnie (France) and Bull (Canada). The data, on which basis the ecological effect was determined, were in the annual report P 163 on the scientific research activity of the Agricultural Academy of Bulgaria.

The indicators that determine the ecological effect are related to the study of the impact of the nitrogen-fixing capacity of different bird'sfoottrefoil varieties on the amount of bird'sfoottrefoil, to obtain quality forage production.

The ecological effect [33] of nitrogen fixation was interpreted by analyzing the relative share of bird'sfoottrefoil in the grassland and the impact of nitrogen fixation [40] on the share of the economically significant sown grass species in different bird'sfoottrefoil varieties.

Soil fertility was analyzed based on the amount of fixed nitrogen in the soil [16]. For this purpose, tuber formation was registered as the number of tubers/plant at the beginning

of blossoming of the bird'sfoottrefoil (second regrowth) by taking soil monoliths [1]. According to the formula of [6] for a rough determination of the amount of fixed nitrogen in field conditions, it was calculated by years and averaged over the period. Experimental data were processed statistically using SPSS (2020) software.

The main methods used are index, graphic, tabular, comparative method, method of analysis and synthesis, descriptive statistical analysis, method of economic and index factorial analysis, method of establishing the complex influence of several factors, and correlation analysis.

The statistical processing of the data includes the relative level of the amount of fixed nitrogen, expressed as mean value, standard deviation, minimum and maximum values. Brave and Pearson's correlation coefficients were calculated to prove the relationships between yield and the main economic indicators [21].

Data were processed using Microsoft Excel.

## RESULTS AND DISCUSSIONS

The soil of the experiment is a light gray pseudopodzolic with an acidic ($pH_{H2O}$ 5.2-5.5; $pH_{KCL}$ 4.3-4.4) reaction, which defines it as suitable for growing forage grasses. The availability of total and digestible phosphorus is 1.2-2.4 mg/100 g soil, which is very low, and absorbable potassium with 5.9-9.9 mg/100 g soil. The humus content (0.96-1.44%) is low. Their mechanical composition is from light to heavy sandy loam.

### Amount of fixed nitrogen of bird'sfoottrefoil cultivars

The amount of fixed nitrogen per decare (Table 1) during the experimental period is influenced by weather conditions and the dry matter yield formed [10], as well as by the level of tuber formation. The growth of bird's foottrefoil and its rates of development largely determine the productivity of the grassland [3, 8], the activity of tuber-formation bacteria [14], and the degree of nitrogen fixation in individual bird's foottrefoil varieties. The least amount of fixed nitrogen per decare was found in the first experimental year, when the variation was from 4.12 to 5.78 kg/da, with an average value of 5.15 kg/da. The low values of fixed nitrogen correspond to low productivity, which is typical of the species during the initial stage of development.

Table 1. Amount of fixed nitrogen per decare of bird's foottrefoil varieties (N fix, kg/da)

| ***Cultivars*** | ***2016*** | | ***2017*** | | ***2018*** | | ***2019*** | | ***Average for the period*** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *N fix, kg/da* | *+, - kg/da* | *N fix, kg/da* | *+, - kg/da* | *N fix, kg/da* | *+, - kg/da* | *N fix, kg/da* | *+, - kg/da* | *N fix, kg/da* | *+, - kg/da* |
| *Targovishte 1* | 5.13 | - | 13.25 | - | 20.40 | - | 20.19 | - | 14.74 | - |
| *Alvena* | 5.55 | +0.42 | 14.69 | +1.44 | 21.63 | 1.23 | 24.47 | 4.28 | 16.58 | +1.84 |
| *Lotanova* | 4.12 | -1.01 | 14.38 | 1.13 | 20.70 | 0.30 | 30.39 | 10.20 | 17.40 | +2.66 |
| *Frilo* | 5.78 | +0.65 | 13.14 | -0.11 | 18.78 | -1.62 | 26.91 | 6.72 | 16.15 | +1.41 |
| *Polom* | 5.10 | -0.03 | 15.65 | 2.40 | 21.16 | 0.76 | 26.78 | 6.59 | 17.17 | +2.43 |
| *Bonnie* | 5.20 | +0.07 | 13.41 | 0.16 | 20.97 | 0.57 | 24.35 | 4.16 | 15.98 | +1.24 |
| *Bull* | 5.16 | +0.03 | 15.40 | 2.15 | 17.16 | -3.24 | 23.49 | 3.30 | 15.30 | +0.56 |
| ***Average*** | 5.15 | | 14.27 | | 20.11 | | 25.23 | | 16.19 | |
| ***Stdev*** | 0.52 | | 1.04 | | 1.58 | | 3.21 | | 0.96 | |
| ***Min*** | 4.12 | | 13.14 | | 17.16 | | 20.19 | | 14.74 | |
| ***Max*** | 5.78 | | 15.65 | | 21.63 | | 30.39 | | 17.40 | |

**Source:** Own calculations.

The maximum value was reported for Frilo, followed by Alvena. Alvena has the highest level of tuber formation and the highest dry matter yield. In the second experimental year, the dry matter yield was twice compared to the first year, and the amount of fixed nitrogen exceeded the values of the first year by almost three times. The variation is from 13.14 to 15.65 N fix,kg/da, as Polom and Bull cultivars had the highest values. The

combination of favorable soil-climatic conditions, a high level of tuber formation, and high productivity determine the high nitrogen-fixing capacity in the third experimental year. Fixed nitrogen in Alvena and Polom cultivars per a decare registered almost similar values of the amount of fixed nitrogen per decare, 21.63 and 21.16 N fix, kg/da, respectively. The lowest amount of fixed nitrogen per decare was found in Bull (17.16 N fix, kg/da). In the fourth experimental year, the cultivars reached the highest amount of fixed nitrogen, from 20.19 to 30.39 N fix, kg/da, respectively, as Lotanova variety appeared as the most suitable of the studied varieties in terms of this indicator. On average over the study period, Lotanova and Polom had the highest yield of fixed nitrogen per decare (17.40 and 17.17 N fix, kg/da), exceeding the standard variety by 2.66 and 2.43 N fix, kg/da. These two varieties could be recommended as suitable for implementation in production from an economic and ecological point of view. The relatively high content of nitrogen fixed by them predetermines low costs in terms of fertilizing and defines the species as a suitable component for growing subsequent crops. The lack of mineral nitrogen fertilizer also proves the ecological effect of growing these varieties, due to the lack of nitrates in the soil.

The statistical processing of the nitrogen fixation data of bird's foottrefoil varieties shows the highest average value x=25.23 fix, kg/da of the fixed nitrogen in the fourth year of the experimental period, and the lowest (x=5.15 fix, kg/da) in the first experimental period year when the plants are weak and not strong. The standard deviation has a maximum value (Stdev = 3.21) in the fourth year when the average value of the amount of fixed nitrogen is the highest. The maximum amount of fixed nitrogen in the fourth experimental year for the Lotanova variety is 30.39 fix,kg/da.

**Ecological effect of nitrogen fixation**

The capacity of bird'sfoottrefoil to accumulate a certain amount of atmospheric nitrogen from the air contributes to its assimilation by plants. In this way, plants naturally acquire the necessary amount of nutrients for their growth and development. The increased nitrogen-fixing capacity and level of tuber formation, as well as the amount of fixed nitrogen per decare, are a prerequisite for increasing soil fertility of Lotanova and Polom bird's foottrefoil varieties. Plant nitrogen storage through symbiotic nitrogen fixation of bird's foottrefoil, as a suitable meadow legume crop for mountain conditions, determines the ecological effect of forage legumes. Nitrogen fixation is also important for increasing the quality of forage because nitrogen is the main element in the composition of all amino acids. The lack of chemical substances, which are introduced in the form of mineral fertilizers, complements the role of legumes in their cultivation in an ecological aspect related to improving soil fertility.

Figure 1 shows the impact of nitrogen-fixing capacity on the share of bird'sfoottrefoil (%) in the grassland over the years. In the first experimental year, the Alvena variety had the highest share of bird's foottrefoil in the grassland (54.5%), followed by the Frilo variety (50.0%), whereas the lowest share was registered in the Bull variety (18.8%) and Bonnie (19.5%).

There is a correlation between the high share of bird's foottrefoil in the grassland and the amount of fixed nitrogen by the legume species [13], which proves that bird’s foottrefoil forms root tubers that accumulate nitrogen and stimulate the development of the sown crop [23].

In the second year, the combination of favorable climatic conditions with maximum growth and development of the plants justified the reported high values regarding the share of the bird's foottrefoil in the grassland [4].

Polom (97.0%) and Alvena (93.8%) had the highest relative share of bird's foottrefoil, registering the highest amount of fixed nitrogen per decare, respectively 15.65 and 14.69 N fix, kg/da.

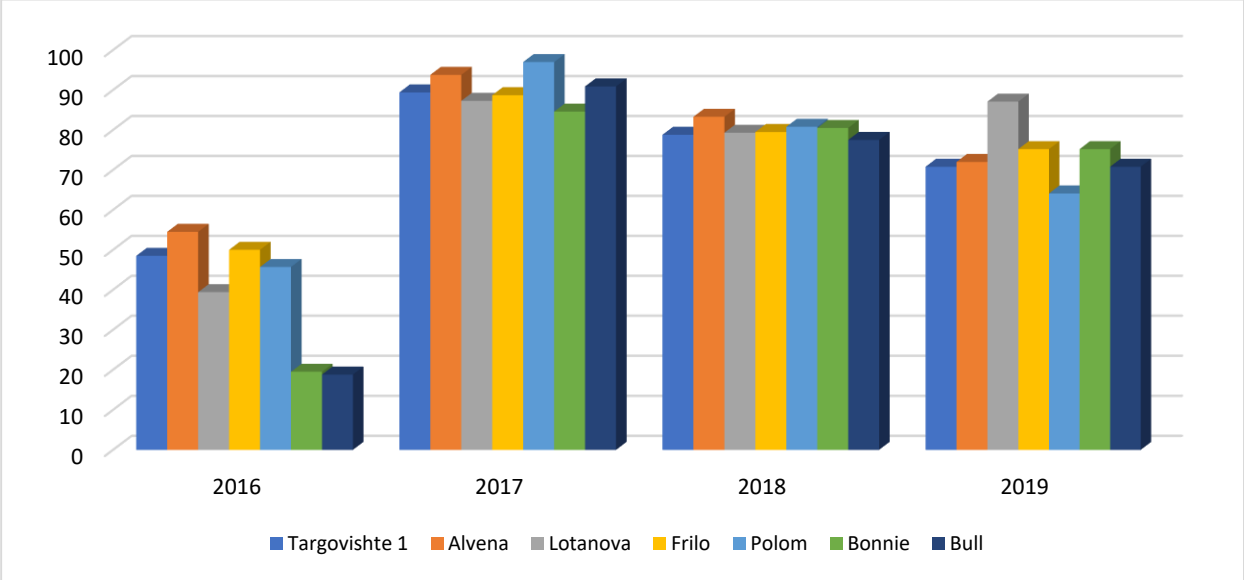


Fig. 1. The impact of nitrogen-fixing capacity on the share of bird's foottrefoil (%) in the grassland over the years.
Source: Own design and results.

This again proves the stimulating effect of the amount of fixed nitrogen of these varieties on the growth and development of the bird'sfoottrefoil [4].

In the third experimental year, all tested varieties registered a high share of bird'sfoottrefoil in the grassland at both mowings. Alvena (83.3%) and Polom (80.8%) N fix kg/da had the highest relative share of bird'sfoottrefoil in the grassland.

Bird'sfoottrefoil maintains a high percentage share in a large part of the tested varieties in the fourth year of the experimental period. Its share was maximum in the grassland with Lotanova variety (87.1%) with the highest amount of fixed nitrogen per decare, respectively 30.39 N fix, kg/da.

**Correlation dependences**

Table 2 presents the correlation dependences between the main economic indicators with the dry matter yield and the amount of fixed nitrogen per decare [9].

Table 2. Correlation dependences among the average yield, the amount of fixed nitrogen, and the main economic indicators in forage production from bird's foottrefoil varieties

| | ***Average yield*** | ***N fix*** | ***Production costs*** | ***Cost price*** | ***Gross income*** | ***Gross profit*** | ***Profitability*** | ***Efficiency coefficient*** |
|---|---|---|---|---|---|---|---|---|
| *Average yield* | 1 | | | | | | | |
| *N fix* | 1.0000 | 1 | | | | | | |
| *Production costs* | 0.9990 | 0.9991 | 1 | | | | | |
| *Cost price* | -0.9992 | -0.9991 | -0.9984 | 1 | | | | |
| *Gross income* | 0.9999 | 0.9998 | 0.9982 | -0.9990 | 1 | | | |
| *Gross profit* | 0.9998 | 0.9998 | 0.9982 | -0.9990 | 1 | 1 | | |
| *Profitability* | 0.9998 | 0.9998 | 0.9981 | -0.9990 | 1.0000 | 1.0000 | 1 | |
| *Efficiency coeficient* | 0.9995 | 0.9995 | 0.9978 | -0.9986 | 0,9998 | 0.9998 | 0.9998 | 1 |

Source: Own calculations.

A high degree of correlation was observed between forage yield and the amount of fixed nitrogen per decare (r=1.0000). This dependence proves the role of legumes as a nitrogen source that affects plant growth and development and determines the high dependence between production costs and yield (r=0.9990). This dependence

predetermines and explains the reduced costs of dry matter production with reduced fertilizer inputs.

Gross revenues are highly positively correlated with yield (r=0.9999), production costs (r=0.9982), and the amount of fixed nitrogen (r=0.9998). Profitability is also highly correlated with gross revenue (r=1.0000) and gross profit (r=1.0000). The efficiency coefficient is highly correlated with yield (r=0.9995), amount of fixed nitrogen (r=0.9995), production costs (r=0.9978), gross revenue (r=0.9998), gross profit and profitability (r=0.9998). The correlation dependence of the cost price of production with gross revenues (r=-0.9990), gross profit (r=-0.9990), profitability (r=-0.9990), and respectively the efficiency ratio (r=-0.9986) is negative. The obtained values of the correlation coefficients confirm the obtained data on the economic indicators of the increased gross profit, gross revenue, and profitability, and reduced cost price of the obtained forage production from bird's foottrefoil varieties.

## CONCLUSIONS

In the comparative test of seven bird'sfoottrefoil varieties, it was found that the Lotanova variety had the highest tuber-forming capacity. This predetermines the resulting high amount of fixed nitrogen per decare.

The amount of fixed nitrogen largely determines the average yield, gross revenue, and profitability of the obtained forage production, which is proven by the high values of the correlation coefficients.

The high nitrogen-fixing capacity and accumulated nitrogen per decare enhanced natural soil fertility and increased the ecological effect of meadow legume crops.

Lotanova and Polom with their high productivity, amount of fixed nitrogen per decare, economic efficiency and ecological effect can be recommended as suitable for growing in mountain conditions.

## REFERENCES

[1]Beck, D.P., Materon, L.A., Afandi, F., 1993, Practical *Rhizobium*-Legume Technology Manual, ICARDA, Aleppo, Syria.

[2]Blesh, J., Drinkwater, L.E., 2013, The impact of nitrogen source and crop rotation on nitrogen mass balances in the Mississippi River Basin. Ecological Applications, 23(5), 1017–1035.

[3]Bozhanska, T., 2020, Study on the influence of Lumbrical and Lumbrex biofertilisers on an artificial grassland of bird's-foot-trefoil (*Lotus corniculatus* L.). Forest science, no 1, 45-54.

[4]Bozhanska, T., Churkova, B., 2019, Growth and development of legume and grass components in mixed grasslands grown in the Central Balkan mountain. Trakia Journal of Sciences, No 1,19-27. doi:10.15547/tjs.2019.01.004

[5]Carlier, L., Vlahova, M., Todorov, M., Mihovsky, Ts., 2008, Importance of grasslands. Journal of Mountain Agriculture on the Balkans, 11(3), 534-553.

[6]Carlsson, G., Huss-Danell, K., 2003, Nitrogen fixation in perennial forage legumes in the field. Plant and Soil 253(2), 353-372.

[7]Cavaco, T., Torres, M.O., Faria, J.M.S., 2023, Grassland crops as drivers for the improvement of soil fertility. Biology and life sciences forum, 27(1), 25; https://doi.org/10.3390/IECAG2023-14991.

[8]Churkova, B., 2013, Influence of some bioproducts on the biological and productive characteristics of bird`sfoot trefoil grown for forage. Biotechnology in Animal Husbandry, 29 (1), 123-132.

[9]Churkova, B., 2019, Correlation and regression dependences between quantity and quality indicators depending on fertilizing of bird's-foot-trefoil with humate fertilizers. Journal of BioScience and Biotechnology, 8 (2), 123-127.

[10]Churkova, B., Bozhanska, T., Naydenova, Y., 2016, Feeding value of bird's–foot trefoil (*Lotus corniculatus* L.) cultivar under conditions of the Central Northern part of Bulgaria. Banat's Journal of Biotechnology, VII(14), 38-45. DOI: 10.7904/2068–4738–VII(14)–38.

[11]Donkova, R., Dimitrova, A, Jonova, E., Petkova, G., Nedyalkova, K., Markova, A., Altimirska, R., 2014, Biological methods for increasing the yield and improving the quality of the production when growing legume species on black soils. Soil science, agrochemistry and ecology, XLVIII, No. 3-4, 49-58.

[12]Frame, J., 2005, Forage legumes for temperate grasslands. FAO and Science Publishers, Rome, p. 309.

[13]Fustec, J., Bernard, F., 2008, Comparison of yields and biological nitrogen fixation of two legumes grown in different silt soils. In: 22nd General meeting of the European Grassland Federation, June 9-12, 2008, Uppsala, Sweden. Grassland Science in Europe, Vol.13, in press.

[14]Graham, P.H., 2008, Ecology of the root-nodule bacteria of legumes. In: Dilworth, M.J., James, E.K., Sprent, J.I., Newton, W.E. (eds). Nitrogen-fixing leguminous symbiosis. Springer, Dordecht, The Netherland, 23-58.

[15]Graham, P., Vance, C., 2003, Legumes: Importance and constraints to greater use. Plant Physiology, Volume 131 (3), 872–877. https://doi.org/10.1104/pp.017004.
[16]Grzebisz, W., Diatta, J., Barłóg, P., Biber, M., Potarzycki, J., Łukowiak, R., Przygocka-Cyna, K., Szczepaniak, W., 2022, Soil Fertility Clock—Crop Rotation as a Paradigm in Nitrogen Fertilizer Productivity Control. Plants, 11(21), 2841; https://doi.org/10.3390/plants11212841.
[17]Guo, K., Yang, J., Yu, N., Luo, L., Wang, E., 2023, Plant Commun, 2023. Biological nitrogen fixation in cereal crops: Progress, strategies, and perspectives, 13, 4(2):100499. doi: 10.1016/j.xplc.2022.100499.
[18]Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008, Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311,1–18. https://doi.org/10.1007/s11104-008-9668-3.
[19]Kizeková, M., Feoli, E., Parente, J., Kanianska, R., 2017, Analysis of the effects of mineral fertilization on species diversity and yield of permanent grasslands: revisited data to mediate economic and environmental needs. Community Ecology, 18(3), 295–304. https://doi.org/10.1556/168.2017.18.3.8.
[20]Kretovich, B. J. I., 1983, Plant nitrogen sources and the nitrogen cycle in nature. Molecular mechanisms of nitrogen absorption by plants. M.: Nauka, 1983, p. 5-10.
[21]Lidanski T., 1988, Statistics in agriculture. Zemizdat, Sofia.
[22]Lilley, J.M., Bolger, T.P., Peoples, M.B., Gifford, R.M., 2001, Nutritive value and the nitrogen dynamics of *Trifolium subterraneum* and *Phalaris aquatica* under warmer, high $CO_2$ conditions. New Phytologist, 150, 385-395.
[23]Liu, Y., Wu, L., Baddeley, J.A., Watson, C.A., 2011, Models of biological nitrogen fixation of legumes. A review. Agron. Sustain. Dev., 31, 155-172.
[24]MacAdam, J.W., Griggs, T.C., 2013, Irrigated birds foot trefoil variety trial: Forage nutritive value. Utah State Univ. Ext. Fact Sheet AG/Forages/2013-02pr, Logan, UT. http://extension.usu.edu/files/ publications/publication/AG_Forages__2013-01pr.pdf., Accessed on January 5, 2024.
[25]Nikolova, I., 2020, Forage production - essence and challenges. Plant protection.
[26]Nyfeler, D., Huguenin-Elie, O., Frossard, E., Luscher, A., 2006, Regulation of symbiotic nitrogen fixation in grass-clover mixtures. In: Lloveras J., Gonzales-Rodríguez, A., Vazquez-Yañez, O., Piñeiro, J., Santamaría, O., Olea, L., Poblaciones, M.J. (eds). Proc. 21st General Meeting of the European Grassland Federation, Badajoz, Spain, Grassland Science in Europe, 11, 246-248.
[27]Peeters, A., Parente, G., Gall, A., 2006, Temperate legumes: key–species for sustainable temperate mixtures. In: Sustainable Grassland Productivity. Lloveras J., Gonzales-Rodríguez, A., Vazquez-Yañez, O., Piñeiro, J., Santamaría, O., Olea, L. and Poblaciones, M.J. (Eds.). Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, 3-6 April, 2006. Grassland Science in Europe, vol. 11, 205-220.
[28]Peoples, M.B., Herridge, D.F., Ladha, J.K., 1995, Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. Plant and Soil, 174, 3-28.
[29]Peoples, M.B., Brockwell, J., Herridge, D.F., Rochester, J., IAlves, B.J., Urquiaga, S., Boddey, R.M., Dakora, F.D., Bhattarai, S., Maskey, S.L., Sampet, C., Rerkasem, B., Khan, D.F., Hauggaard-Nielsen, H., Jensen, E.S., 2009, The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis, 48, 1-17.
[30]Perfanova, Y., 2011, Application of nitrogen-fixing and other soil microorganisms in chickpea cultivation. Dissertation, Sofia, p. 111.
[31]Pypers, P., Verstraete, S., Thi, C.P., Merckx, R., 2005, Changes in mineral nitrogen, phosphorus availability and salt-extractable aluminium following the application of green manure residues in two weathered soils of South Vietnam. Soil Biology & Biochemistry 37 (1), 163–172.
[32]Rosecrance, R.C., McCarty, G.W., Shelton, D.R., Teasdale, J.R., 2000, Denitrification and N mineralization from hairy vetch (*Vicia villosa* Roth) and rye (*Secale cereale* L.) cover crop monocultures and bicultures. Plant and Soil, 227, 283–290. https://doi.org/10.1023/A:1026582012290.
[33]Schipanski, M.E., & Drinkwater, L.E., 2012, Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. Plant Soil, 357, 147–159. DOI 10.1007/s11104-012-1137-3.
[34]Sidorova, K.K., Levko, G.D., Shumny, V.K., 2013, Investigation of nodulation and nitrogen fixation in annual species and varieties of vetchling, genus Lathyrus. Russian Journal of Genetics: Applied Research volume 3, 197–202.
[35]Sparrow, S.D., Cochran, V.L., Sparrow, E.B., 1995, Dinitrogen fixation by seven legume crops in Alaska. Agronomy Journal, 87 (1), 34-41.
[36]Spiertz, J.H.J., 2010, Nitrogen, sustainable agriculture and food security. A review Agronomy Sustainable. Development, 30, 43–55.
[37]Unkovich, M., Herridge, D., Peoples, M.,Cadisch, G.,Boddey, B., Giller, K., Alves, B.& Chalk, P., 2008, Measuring plant-associated nitrogen fixation in agricultural systems. Australian Centre for International Agricultural Research (ACIAR), p. 45.
[38]Vargová, V., Kanianska, R., Kizeková, M., Šiška, B., Kováčiková, Z., Michalec, M., 2020, Changes and interactions between Grassland Ecosystem Soil and Plant Properties under Long-Term Mineral Fertilization. Agronomy, 10(3), 375; https://doi.org/10.3390/agronomy10030375.
[39]Vasileva, V., 2017, Parameters related to nodulating ability of some legumes. International Journal of Agriculture Food Science & Technology, 3 (1), 005-008.
[40]Vitousek, P.M., Menge, D.N.L., Reed, S.C., Cleveland, C.C., 2013, Biological nitrogen fixation:

rates, patterns and ecological controls in terrestrial ecosystems. Philos Trans R Soc Lond B Biol Sci., 368(1621): 20130119. doi: 10.1098/rstb.2013.0119.
[41]Vuckovic, S., Stojanovic, I., Prodanovic, S.,Cupina, B., Zivanovic, T., Vojin, S.&Jelacic, S., 2007, Morphological and nutritional properties of birdsfoot trefoil (*Lotus corniculatus* L.) autochthonous populations in Serbia and Bosnia and Herzegovina. Genetic Resources and Crop Evolution (2007) 54(2), 421–428. DOI: 10.1007/s10722-006-0004-6
[42]Zahran, H.H., 1999, Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Review 63, 968–989.