Nitrogen-fixing ability of perennial leguminous grasses in various environmental conditions of the Western Siberia

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Abstract. The goal of the research was to determine the nitrogen-fixing ability of perennial leguminous grasses and their use of biological nitrogen to form the crop of fodder in different environmental conditions of Western Siberia. The nitrogen-fixation coefficient ($C_f$) was determined by the method of comparison with a non-leguminous plant. It has been established that in the southern forest-steppe on meadow-chernozem soil biological nitrogen is used more efficiently using Medicago varia, Galega orientalis, Onobrychis arenaria and Astragalus galegiformis. Plants of these species fix up to 233–379 kg/ha of air nitrogen during the growing season, $C_f$ is equal to 0.56–0.78. The accumulation of symbiotic nitrogen by leguminous grasses is 1.1–4.6 times less in the northern forest-steppe in solonetz fine than in meadow-chernozem soil. Medicago varia at solonetz forms due to biological nitrogen to 58% of yield and Astragalus galegiformis only 10–22%.

1. Introduction

The population of Earth is projected to reach more than 8 billion people for the first quarter of the 21st century. To provide it with food by 2025, it will be necessary to produce about 4 billion tons of grain [1]. The solution of this important problem largely depends on increasing crop yields and growing action in the collection of agricultural products. The productivity of any phytocenosis is determined primarily by the amount of nitrogen available to the plant, which plays a crucial role in their life.

According to calculations Pryanishnikov D.N. (1945), to cover the needs of agriculture in nutrient nitrogen, it is necessary to have about 25% of legumes in crop rotation. This, together with mineral and organic fertilizers, would guarantee an optimal level of nitrogen nutrition of plants [2].

It is known that the main supplier of biological nitrogen is legumes grass and leguminous crops. However, ignorance of the real scale of symbiotic nitrogen fixation, underestimation of the role of this extremely important source of energy conservation and human health leads to the fact that in Russia a very modest place in the structure of sown areas is given to the legume component (about 10%). In Western European and the United States, its share is 26% or more [3-4].

According to Food and Agriculture Organization of the United Nations (FAO) in world protein production, the share of leguminous crops accounts for about 20%. Legumes offer great potential for coping with such requests. They have numerous features that can act together at different stages in the
soil-plant-animal-atmosphere system and these are most effective in mixed swards with a legume abundance of 30-50% [5-6].

The annual reserves of nitrogen in the world due to biological fixation are estimated at 160–240 million tons, which is about a tenth of the amount of this element, which is included in the entire biomass of the Earth annually. Leguminous grasses record about 140 million tons of nitrogen from the atmosphere [7-8]. *Trifolium*, with a yield of about 11 t/ha and a nitrogen content of about 2.5–3.0%, can consume 110–113 kg of nitrogen from the atmosphere annually. *Medicago*, with a yield of 4 t/ha and a total nitrogen content of 3.0%, can fix already 14 kg. The nitrogen fixation coefficient in these plants is 0.7. At the same time, perennial legumes leave more than 10 tons of dry organic matter containing up to 250 kg of nitrogen in the soil on an area of one hectare. This amount is enough to increase the wheat yield of 1.0–1.5 t/ha of grain. Soil enrichment with nitrogen after legumes makes it possible to reduce the dose of nitrogen fertilizer application for subsequent crops by 1.5–2 times [9].

The role of legumes as producers of feed protein is great. They convert the nitrogen of the atmosphere into biologically valuable proteins, which are richer in amino acids than grain proteins, and also better absorbed by animals. Biological nitrogen is completely environmentally friendly, while technical nitrogen is easily washed out, pollutes groundwater and open water with nitrates, can accumulate in plant products in excessive amounts and cause serious diseases in humans and animals. That is why researchers pay such important attention to the problem of biological nitrogen fixation. The high efficiency of the use of biological nitrogen determines the great practical importance of research aimed at enhancing its role in the nitrogen balance of agriculture.

Ecologization of agricultural production in the modern world is becoming increasingly important due to global disruption of the processes of circulation of major nutrients in artificial agrocenoses. The tasks of our long-term studies were to determine the volumes of nitrogen fixed from the atmosphere by various types of perennial legumes on the zonal soils of the natural zones of Western Siberia. We tried to identify the share of biological nitrogen in the formation of the crop of these herbs in various environmental conditions of the region.

2. Materials and methods
Long-term studies were carried out in the southern and northern forest-steppe zone of Western Siberia (Omsk region) of the Russian Federation. Western Siberia is characterized by a large variety of soil and climatic conditions. There are sharp fluctuations in temperature during the year, month, and even during the day. In the southern forest-steppe, July temperature varies from 14.3 to 41.0 °C, with an average of 18.3 °C. This is a zone of unstable humidification, the amount of precipitation per year is 300–400 mm, the hydrothermal coefficient is 1.0–1.1, in some years - 0.5. The frost-free period here is 114 days, the sum of positive temperatures over the summer period is about 2000 °C.

The moisture content of the northern forest-steppe zone is characterized by a multi-year precipitation rate of 350–420 mm and a hydrothermal coefficient of 1.2–1.3. In some years, there is a lack of moisture. In the southern forest-steppe zone, experiments were laid on the meadow-chernozem soil, in the northern forest-steppe zone - on solonetz fine. The object of research was promising species of perennial legumes. The experiments were laid in two or three repetitions in time and four times in space, the accounting area of plots 25–100 m². Schemes of experiments are given when discussing the results.

The nitrogen fixation coefficient (Cf) was taken as the fraction of nitrogen fixed from the atmosphere for the production of legume biomass. A comparative method was used. The essence of the method lies in the assumption that under the same growing conditions for leguminous and cereal crops, the amount of nitrogen taken by them from the soil is approximately the same. For comparison, we used data on a perennial cereal culture – *Bromopsis inermis*. Nitrogen consumption by legumes and nitrogen takeaway by cereals were attributed to fixed nitrogen by legumes. Surveillance of plants and yield registration we used the generally accepted methodology proposed by the All-Russian Scientific Research Institute of Forage named after V.R. Williams [11], for statistical processing of
experimental data – the method of variance and correlation analysis [12]. Statistics was calculated by database software Excel.

3. Results
The research results are presented in tables (tables 1-3).

| Grass species                             | Completely dry substance, kg/ha | overall Nitrogen kg/ha | symbiotic Nitrogen kg/ha | C_f | Takeaway, kg/ha |
|-------------------------------------------|---------------------------------|------------------------|--------------------------|-----|------------------|
| *Medicago varia*                          | 9.21                            | 295                    | 185                      | 20.1| 0.63             |
| *Melilotus album*                         | 5.21                            | 178                    | 68                       | 13.1| 0.38             |
| *Melilotus officinalis*                   | 4.26                            | 149                    | 39                       | 9.2 | 0.26             |
| *Trifolium pratense*                      | 4.32                            | 126                    | 16                       | 3.7 | 0.13             |
| *Trifolium hybridium*                     | 3.67                            | 127                    | 18                       | 4.9 | 0.14             |
| *Trifolium repens*                        | 2.50                            | 91                     | 12                       | 4.8 | 0.13             |
| *Onobrychis arenaria*                     | 7.64                            | 241                    | 131                      | 17.2| 0.54             |
| *Galega orientalis*                       | 7.91                            | 274                    | 164                      | 20.7| 0.60             |
| *Lotus corniculatus*                      | 6.19                            | 170                    | 60                       | 9.7 | 0.35             |
| *Lupinus perennis*                        | 4.03                            | 146                    | 37                       | 9.2 | 0.25             |
| *Astragalus galegiformis*                 | 7.24                            | 242                    | 132                      | 18.2| 0.55             |
| *Astragalus cicer*                        | 6.40                            | 207                    | 97                       | 15.2| 0.47             |
| Least significant difference (LSD_{0.05}) | 1.05                            |                        |                          |     |                  |

Table 2. The use of overall and symbiotic nitrogen by *Astragalus galegiformis* of different years of life (the southern forest-steppe, meadow-chernozem soil).

| Year of life | Completely dry substance, t/ha | overall Nitrogen kg/ha | symbiotic Nitrogen kg/ha | C_f |
|--------------|---------------------------------|------------------------|--------------------------|-----|
| Second       | 3.10                            | 75                     | 18                       | 5.8 | 0.24             |
| Third        | 8.27                            | 206                    | 105                      | 12.7| 0.51             |
| Fourth       | 9.25                            | 274                    | 186                      | 20.1| 0.68             |

4. Discussion
The assimilation of nitrogen from the atmosphere by leguminous herbs, as shown by our experiments, largely depends on the biological characteristics of the species. High utilization of total and symbiotic nitrogen in the southern forest-steppe zone on the meadow-chernozem soil differed *Medicago varia*, *Galega orientalis*, *Onobrychis arenaria* and *Astragalus galegiformis*. During the growing season, the amount of nitrogen fixed by them averaged 131–185 kg / ha, and the nitrogen fixation coefficient (C_f) reached 0.54–0.63. Due to biological nitrogen, these herbs allowed to get from 3.98 to 5.80 t/ha of dry substance (Table 1). At the same time, these species also showed an increased removal with a yield of phosphorus (38–70 kg / ha) and potassium - 120–166 kg/ha.
Table 3. The use of overall and symbiotic nitrogen by perennial leguminous grasses on various soil types of the forest-steppe zone of Western Siberia.

| Grass species               | Com-pletely dry substance, t/ha | overall kg/ha | Nitrogen symbiotic kg/ha | kg/t dry substance | C_f |
|-----------------------------|----------------------------------|---------------|--------------------------|-------------------|-----|
| Southern forest-steppe, meadow-chernozem soil (an average of 6 years) |                                  |               |                          |                   |     |
| *Medicago varia*            | 8.92                             | 262           | 153                      | 17,2              | 0.58|
| *Onobrychis arenaria*       | 8.13                             | 256           | 147                      | 18,1              | 0.57|
| *Lotus corniculatus*        | 6.53                             | 179           | 69                       | 10,6              | 0.39|
| *Lupinus perennis*          | 4.32                             | 157           | 47                       | 10,9              | 0.30|
| LSD_{0.05}                  | 1.15                             |               |                          |                   |     |
| Northern forest-steppe, solonetz fine (an average of 4 years) |                                  |               |                          |                   |     |
| *Medicago varia*            | 8.18                             | 242           | 127                      | 15,5              | 0.52|
| *Onobrychis arenaria*       | 4.85                             | 148           | 33                       | 6,8               | 0.22|
| *Astragalus galegiformis*   | 3.44                             | 105           | 11                       | 3,2               | 0.10|
| LSD_{0.05}                  | 1.10                             |               |                          |                   |     |

*Astragalus cicer, Melilotus album, Lotus corniculatus and Lupinus perennis* recorded nitrogen from the atmosphere in smaller sizes – 37–97 kg/ha and formed through it a yield of about 1.11–3.01 t/ha of dry substance or 25–47% of the total crop during vegetation. *Trifolium pratense, Trifolium hybridum* and *Trifolium repens* under the conditions of the southern forest-steppe, symbiotic nitrogen was used very little (C_f = 0.13–0.14), they consumed mainly the mineral nitrogen of the soil. However, when analyzing the symbiotic activity of herbs for years of life, several other patterns are revealed.

In the first year of life, the greatest amount of nitrogen from the atmosphere was used by herbs that grew rapidly and formed nodules on the roots. This is primarily *Melilotus album, Trifolium pratense, Astragalus galegiformis* and *Medicago varia*. They used symbiotic nitrogen from 7 to 24 kg/ha, C_f was 0.11–0.29. Very weak nitrogen fixation in the first year of life was observed in *Astragalus cicer, Onobrychis arenaria, Lotus corniculatus, Galega orientalis* and *Lupinus perennis*, since these species grew very slowly in the first year [13]. We observed nodules on the roots of these plants only at the end of the growing season. In the subsequent years of life, the nitrogen fixation of herbs increased significantly, which is all with the formation of a good symbiotic apparatus. For example, *Astragalus galegiformis* plants increased the intensity from 18 kg/ha in the second year of life to 105 in the third and 186 kg/ha in the fourth year, which is 5.8 and 10.3 times more, respectively (Table 2). The fixation of atmospheric nitrogen was 12.7–20.1 kg per 1 ton of dry substance, C_f – 0.51–0.68 [14].

During the years of maximum productivity of grass stands, the use of symbiotic nitrogen by perennial leguminous herbs reached 379 kg/ha for *Medicago varia* and *Onobrychis arenaria*, 233 kg/ha for *Galega orientalis* and *Lotus corniculatus*, 193 kg/ha for *Astragalus galegiformis* and *Astragalus cicer*, 162 kg/ha for *Melilotus album, Lupinus perennis, Trifolium pratense* and *Trifolium hybridum*. The fixation of atmospheric nitrogen was 19–25 kg per 1 ton of dry substance, C_f – 0.56–0.78.

Ecological conditions in which perennial leguminous plants grew have a great influence on the use of nitrogen from the soil and atmosphere. In the northern forest-steppe in the solonetz fine, the use of symbiotic nitrogen decreased, compared with the meadow-chernozem soil of the southern forest-steppe, especially of *Onobrychis arenaria* - by 4.5 times and *Astragalus galegiformis* – by 4.6 times, *Medicago varia* – by 1.1–1.4 times (table 3).

This is due to the fact that *Onobrychis arenaria* and *Astragalus galegiformis* grow poorly on saline (pH = 8.0–9.2), poorly drained and soils floating from the surface. At the same time, they practically do not form under these conditions on the roots of nodules, they have dry substance collection: *Astragalus galegiformis* – 3.44, *Onobrychis arenaria* – 4.85 t/ha. Of this amount, only 0.34–1.07 t/ha...
(10–22%), these grass species were formed by biological nitrogen. For comparison, *Medicago varia* plants formed during the growing season up to 58% of the total yield (table 3).

5. Conclusion

Biological nitrogen captured from the atmosphere by perennial legumes is an important source of nitrogen for plants. The uptake of nitrogen from the atmosphere depends on the biological characteristics of the species. In the southern forest-steppe zone of Western Siberia, on the meadow chernozem soil, biological nitrogen is used most effectively by *Medicago varia, Galega orientalis, Onobrychis arenaria* and *Astragalus galegiformis*. In the years of maximum productivity, *Medicago varia* and *Onobrychis arenaria* fix up to 379 kg/ha, *Galega orientalis* and *Lotus corniculatus* – to 233, *Astragalus galegiformis* and *Astragalus cicer* – to 193, *Melilotus album*, *Lupinus perennis*, *Trifolium pratense* and *Trifolium hybridium* – to 162 kg/ha of air nitrogen during the growing season. The amount of biological nitrogen fixed by perennial leguminous herbs depends on the ecological conditions of their cultivation. In the northern forest-steppe zone in the solonetz fine accumulation of symbiotic nitrogen is 1.1–4.6 times less than in the meadow-chernozem soil of the southern forest-steppe.

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