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# INFLUENCE OF SOIL CHEMICAL REACTION AND CROP ROTATION ON THE CAPACITY OF REDDISH PRELUVOSOIL FOR FREE (ASYMBIOTICAL) - FIXING OF THE ATMOSPHERIC DINITROGEN

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***Abstract***

*This paper continues other scientific research (Ştefanic and Oprea G., 2010, 2011) aimed to rectify the Waksman and Karunacker method for determining the soil capacity to fix (asymbiotically) atmospheric dinitrogen. Their method ignored that Nt, determined by the Kjeldahl digestion, did not include nitrates, because these volatilized during boiling with concentrated $H_2SO_4$. That error, repeated by all specialists until the present, made Feher (1954), and Rippel-Baldes (1955) and others conclude that atmospheric dinitrogen fixation is insignificant in comparison with the symbiotical one. In this paper we present some results concerning the negative influence of soil acidity, crop-rotation and any monocultures on the microbiological process.*

**Keywords**: *Azotobacter, dinitrogen free-fixation, asymbiotical.*

## INTRODUCTION

Nitrogenous organic matter would have not existed on earth without the biological assimilation of atmospheric dinitrogen because soils arise from rocks containing no nitrogen. The only available source of nitrogen to take into account is derived from the atmosphere due to electric discharges and that is approximated to 3-5 kg/ha/year (Schmalfuss, 1963, cited by Müller, 1965). If the $CO_2$ in the atmosphere is only 0.03% v/v and $N_2$ is 79% v/v, the combination of organic carbon ranges from 5 to 90 times higher. In nature, the assimilation of atmospheric dinitrogen is performed by bacteria containing nitrogenazae (an enzyme capable of reducing molecular dinitrogen to ammonia, with low energy), without human intervention and without harming the environment. When humans decided to supplement plant nutrition with nitrogen, they produced mineral manures of synthesis, with huge consumptions of fossil energy. The study of nitrogen fixation urged scientists to find its source, knowing that it was essential for life on earth. Even Berthelot (1885) published the first results of his research on soil nitrogen relations and acknowledged that fixing it in the soil was the work of microorganisms. In Russia, Winogradsky (1925) found anaerobic sporogenous bacteria in soil, whose role was to fix atmospheric dinitrogen, which he called *Clostridium pasteurianum* and in the Netherlands Beijerinck M., cited by Müller (1965), discovered the bacterium *Azotobacter chroococcum* which was very active in the dinitrogen fixation in soil. Winogradsky (1925) conducted complex microscopy and physiology research on the bacterium. Although the microbiological process of the dinitrogen free (asymbiotical) fixing was elucidated, the laboratory evaluation of the soil samples was uncertain. Bonazzi (1921), cited by Waksman (1932), published a paper which showed the negative effect of soil nitrate in liquid nutritive medium, on the ability to fix atmospheric dinitrogen. In a critical analysis of the soil methods used by his contemporaries, Winogradsky (1925) deemed it unusable for the intended purpose and recommended other methods, i.e. in liquid nutritive medium, that were more suited to highlight the properties of the bacteria to fix atmospheric dinitrogen, cultivating an artificial nutrient environment. In 1924, Waksman and Karunacker, cited by Waksman (1932), experimentally determined the fixation capacity of dinitrogen under laboratory conditions, with 100 g of fresh soil that was sieved, then adjusted to the optimal humidity and $28^0$C, within 30 days. With such methods, in the first half of the twentieth century, various researchers estimated the amounts of $N_2$ fixed in the soil between 5 and 15 kg/ha/year (Feher, 1954; Rippel-Baldes, 1955). This created the general opinion that this soil quality was negligible and, in the second half of the twentieth century until the present, it focused most attention only on dinitrogen fixation by symbiosis with *Rhizobium* and *Bradyrhizobium* bacteria in the nodules of the leguminous plants, estimated to approx. 100 kg N/year, as claimed (Campbell and Less, 1967). ***However, when did the huge amount of combined nitrogen reach the earth and made it possible fore combinations of quaternary substances, and therefore plant and animal protein, to appears? It is known that the rocks that make up the soil did not contain nitrogen. Was it that only the electric discharges from the atmosphere led to the formation of nitrogen oxides, at a rate of 5 kg/year/hectare?*** Literature explains that atmospheric dinitrogen, asymbiotically fixed, occured both in soils and across the globe, where temperature and humidity were achieved, thus explaining the origin of life on earth.

Ștefanic and Oprea (2010) observed the discordance between the evaluation of the importance of atmospheric $CO_2$ assimilation, from the atmosphere, and the evaluation of dinitrogen free-fixed from the atmosphere, which could not explain the formation of terrestrial and aquatic biomass. The study of literature allowed them to conclude that determining the amount of $N_2$ free-fixed (asymbiotical) in experiments on soil, the method for Nt determining (Kjeldahl, 1883) did not contain nitrates from the soil samples nor ammonium nitrified during incubation, because nitrates were lost by boiling the samples in concentrated sulfuric acid. Research for determining the amount of dinitrogen free-fixed by following the classical-Kjeldahl method, was performed in Romania (Laslo et al., 1956; Eliade and Chirita, 1977). In 2010, Ștefanic and Oprea corrected this method (Kjeldahl-classic) by summing Nt (classic Kjeldahl method) with N-nitrate (di-nitrophenol method) after and before the incubation of the soil samples for 30 days, and determed the difference between these amounts, restoring the true measure of the $N_2$ free-fixing process in the biogeochemical circuit.

## MATERIALS AND METHODS

The free-fixation of atmospheric dinitrogen (asymbiotical) by some bacteria and nitrifying bacteria is sensitive in soil acidity in which they develop, as Winogradsky (1925) determined by fundamental research. The soil samples were sampled from the reddish preluvosoil of the experimental field belonging to the research station of Moara Domneasca, Ilfov County.

To assess the bacterial potential of free-fixation of atmospheric dinitrogen and of nitrification bacteria during the incubation of reddish preluvosoil (characterized by pH 5-5.6), an experiment was conducted in vegetation pots for determining the influence of the amendment, with equivalent 5t/ha $CaCO_3$, to create environmental favourable conditions for atmospheric dinitrogen free-fixation (asymbiotical). The experiment was performed in 1-litre plastic bottles with control - the unamended soil (with CaO) with the variants:

$V_1$ - control 1 - soil without CaO equivalent to 5 t/ha $CaCO_3$;

$V_2$ - control 2 - soil amended with CaO equivalent to 5 t/ha $CaCO_3$;

$V_3$ - soil under wheat crop (monoculture) amended with CaO equivalent 5 t/ha $CaCO_3$;

$V_4$ - soil under corn crop (monoculture) amended with CaO equivalent to 5 t/ha $CaCO_3$;

$V_5$ - soil under soybean crop rotation (rotation of 3 years) amended with CaO equivalent 5 t/ha $CaCO_3$;

$V_6$ - soil under sunflower crop (rotation of 4 years with a jumping sole) amended with equivalent CaO 5 t/ha $CaCO_3$.

The method of determining the atmospheric di-nitrogen free-fixed in the soil sample during incubation, as made by Waksman and Karunacker, cited by Waksman (1932) and corrected by Ștefanic and Oprea (2010), had three parts:

**1.** The soil sample with optimum moisture content (40-60% of the maximum capillary water) was incubated at 28 $^{0}C$ for 30 days.
**2.** Before and after incubation, the content of total nitrogen (Nt) was determined in the soil sample by the classical Kjeldahl method (1883) and N-$NO_3$, by means of fenol-disulfonic acid (Chamot et al., 1911, cited by Borlan and Hera, 1973).
**3.** The initial amount of nitrogen in the soil sample not incubated (Nt+$NO_3^-$) was extracted from the final amount of nitrogen in the soil incubated for 30 days (N-Nt+N-$NO_3^+$), resulting in the total real quantity of nitrogen free fixation (asymbiotical) in the soil. The laboratory findings from atmospheric free-fixing dinitrogen were statistically processed by the multiple test method (Tukey, 1953; Snedecor, 1965).

## RESULTS AND DISCUSSIONS

In our case, the chemical reaction of soil pH was 5.64. The results in Table 1 show that the experiment in the plots treated with CaO (equivalent to 5 t/ha $CaCO_3$), chemical reaction of 0-10 cm soil depth increased to pH 6.21. The free fixation (asymbiotical) of atmospheric dinitrogen was stimulated from N=14.52 to N=44.58 mg/100 g soil d.m. As shown in Table 1, under acidic conditions, the free-fixation process of dinitrogen from the atmosphere was hindered. By improving the soil reaction, the response was an amount of about 30 mg N/100 g soil d.m., which related to one hectare, in the layer of 0-10 cm, represented 450 kg N/hectare, in a period of 30 days of soil incubation. This amount of 450 kg/ha fixed in the soil for a month was 4.5 times higher than the optimum mineral fertilization administrated per hectare for crop production in a vegetation cycle. Compared with the ammonium nitrate administered per hectare for agricultural production, nitrogen as a biological product was achieved every second, minute, hour, and day, without affecting the chemical reaction of natural soil, and entered the biogeochemical nitrogen circuit, enriching the organic form of nitrogen reserves in the soil. From this point of view, we noted the negative effects of chemical ("optimal") fertilization, that led to soil acidification and enhanced humus mineralization. Also, Table 1 shows that, during the incubation of soil samples, the production of nitrates (by nitrifying bacteria), resulted by ammoniaoxidization, was higher compared to the initial (0.5 to 1 mg of N-$NO_3^-$), in the unamended version.
In the variant with soil reaction adjusted by calcium oxide, there was an increase in the nitrification capacity greater than the unamended variant, from 0.5 to 1.5 mg of N-$NO_3^-$, corresponding to the potential increase of atmospheric dinitrogen fixation.

Table 1. Influence of amendment with CaO in vegetation pots with reddish preluvosoil, on the process of free-fixing (asymbiotically) of atmospheric dinitrogen ($N_2$)

| Soil sample - *experience characteristic average* | Dinitrogen free-fixation of atmospheric $N_2$ (*with and without incubation 30 days)* | pH ($H_2O$) | Nt (Kjeldahl) mg/100 g soil | | Nitrate mg/100 g soil | | Total $N_2$ - free fixed (asymbiotical) | |
|---|---|---|---|---|---|---|---|---|
| | | | Organic nitrogen + $NH_4$ | F-I | N-$NO_3$ | F-I | mg/100 g sol ( col. 5+7) | kg/ha (col. 8 x 15) |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| $V_1$ - Control unamended with CaO | Initial | 5.65 | 188.36 | 14.02 | 0.5 | 0.5 | b 14.52 | 217.90 |
| | Final | | 202.38 | | 1.0 | | | |
| $V_2$ - Control amended with CaO | Initial | 6.21 | 161.30 | 43.08 | 1.5 | 1.5 | a 44.58 | 66.73 |
| | Final | | 204.39 | | 3.0 | | | |

LSD 5%=19.12

The data in Table 2 show that soil samples amended with CaO in plots planted with wheat and maize in monoculture, free-fixing atmospheric dinitrogen, were significantly higher in the soil sample collected under monoculture maize (59.31 mg N/100 g soil d.s.) were placed in the group of significance denoted by **a**, compared with the no-tilled soil sample and unamended (control) denoted by the letter **c** with 14.52 mg N/100 g soil. Regarding wheat monoculture, we found that there were significant differences compared with the control.

Table 2. Determination of atmospheric dinitrogen free-fixation (asymbiotical) potential under the influence of crop monoculture and crop rotations in reddish preluvosoil, amended with CaO, in vegetation pots, in the experimental field of Moara Domneasca, Ilfov County

| Soil sample | Dinitrogen free-fixation of atmospheric $N_2$ (*with and without incubation 30 days)* | pH | Nt (Kjeldahl) mg/100 g soil | | Nitrate mg/100 g soil | | Total $N_2$ - free fixed (asymbiotical) | |
|---|---|---|---|---|---|---|---|---|
| | | | Organic nitrogen + $NH_4$ | F-I | N-$NO_3$ | F-I | mg/100 g sol (col. 4+6) | kg/ha (col. 7 x 15) |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Control without CaO | Initial | 5.65 | 188.36 | 14.02 | 0.5 | 0.5 | **c** 14.52 | 217.80 |
| | Final | | 202.38 | | 1.0 | | | |
| Wheat monoculture | Initial | 6.16 | 129.54 | 12.62 | 1.0 | 1 | **c** 13.62 | 204.30 |
| | Final | | 142.17 | | 2.0 | | | |
| Corn monoculture | Initial | 5.97 | 123.03 | 58.30 | 4.0 | 1 | **a** 59.31 | 889.65 |
| | Final | | 181.34 | | 5.0 | | | |
| Soybean 3 years rotation | Initial | 5.79 | 117.22 | 46.08 | 0.5 | 2.5 | **b** 48.58 | 728.70 |
| | Final | | 163.31 | | 3.0 | | | |
| Sunflower - 4 years rotation | Initial | 6.08 | 131.35 | 73.59 | 0.5 | 0.5 | **a** 74.09 | 1111.35 |
| | Final | | 204.94 | | 1.0 | | | |

LSD 0.1%=23.44

This distinction between the influence of plant species could be explained by the mode in which the soil was covered by plant: maize covered less and has deeper roots than wheat and a significant difference in terms of time and duration of soil employment in active vegetation period. Sunflower grown in rotation for four years with skipped sole (sunflower - wheat - corn-corn and alfalfa, skipped sole) significantly stimulated the fixation of atmospheric dinitrogen (**a** 74.09 mg N/100 g soil d.s.) as maize monoculture (**a** 59.31) with a longer vegetation period of nitrogen consumption. In soy-bean, in 3 year rotation (soy-bean - wheat-corn), it was still higher than plot control, although we obtained a lower fixing atmospheric dinitrogen (**b** 48.58). The validity of the atmospheric dinitrogen fixation process was proven every month, from April to Aeptember, as noted in Table 3, published by Ștefanic et al. (2012), noting the potential decrease during the months after soy-bean root nodules began to decompose and *Azotobacter* passed into nitrogen heterotrophic nutrition. The free fixing (asymbiotic) of dinitrogen from the atmosphere was greater than the quantity per hectare in the control (217.80 x 5 months = 1089 kg/ha), compared to about 100 kg per hectare symbiotic nitrogen attached (during the vegetation legumes), considered by the literature. Our results obtained on reddish preluvosoil confirmed the results (Table 3) published by Ștefanic et al. (2010, 2011, 2012).

Table 3. Monthly dynamics of atmospheric dinitrogen free-fixed (asymbiotic) under soybean crop (Significant difference, **D** = 2.141, multiple test, Snedecor, 1968), cited after Ştefanic et al. (2012)

| Months | Amount $Nt+N\text{-}NO_3^-$ | Amount $Nt+N\text{-}NO_3^-$ | Free-fixed $N_2$ | Fixed $N_2$ kg/ha (calculated layer 0-10 cm) |
|---|---|---|---|---|
| | Before incubation | After incubation | | |
| | N mg/100 g sol | | | |
| April | 170.036 | 182.712 | **c** 12.678 | 190.170 |
| May | 171.038 | 185.385 | **b** 14.005 | 210.075 |
| June | 169.700 | 184.043 | **a** 16.343 | 245.145 |
| July | 169.700 | 197.721 | **a** 14.682 | 220.230 |
| August | 183.039 | 192.389 | **d** 9.350 | 233.133 |
| September | 174.702 | 179.722 | **e** 5.020 | 75.300 |

## CONCLUSIONS

Correcting reddish preluvosoil acidity by using CaO in the soil samples collected in plots planted with wheat and maize in monoculture and rotation of 3 and 4 years, favoured the increase in the free fixation of atmospheric dinitrogen. This is explained by the fact that bacteria *Azotobacter* (mainly) and other species of bacteria with a lower potential of atmospheric dinitrogen free-fixation, are adapted to neutral pH of soil.

Free-fixation (asymbiotical) of atmospheric dinitrogen is significantly higher in the soil sample collected from maize monoculture (N = 59.31 mg/100 g soil), compared with the soil sample collected from wheat monoculture (N=13.62 mg/100 g soil).

Sunflower grown in rotation for four years (wheat- sunflower-maize-maize and alfalfa-jumpingsole) significantly stimulates fixation of atmospheric dinitrogen (74.09 mg N/100 g soil d.s.). Soil under soy-bean from crop rotation of 3 years (soy-bean- wheat- maize) fixes more atmospheric dinitrogen (48.58 mg N/100 g soil d.s.) than the control sample - no tillage soil (14.52 mg N/100 g soil d.s.). However, this was lower than in the soil samples collected from the sunflower crop (74.09 mg N/100 g soil d.s.) and in monoculture maize (N=59.31 mg/100 g soil d.s.). Our experiments confirm the different importance given by other researchers who believe that the high number of *Azotobacter chroococcum* is in concordance with good soil fertility.

Free fixation of atmospheric dinitrogen exceeds the quantity reported per hectare (1089 kg/ha in 5 months), compared with about 100 kg N per hectare, symbiotically fixed, wronlyg considered by literature.

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