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INFLUENCE OF NPK MINERAL FERTILIZATION AND RIES TREATMENT ON RHIZOSPHERIC MICROBIOTA AND STOMATAL TRAITS IN SUGAR BEET (*BETA VULGARIS L.)*

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Abstract. *Sugar beet (*Beta vulgaris *L.) performance is strongly shaped by fertilization-driven interactions between rhizospheric microbiota and leaf anatomical traits. This study investigated the effects of NPK mineral fertilization and RIES treatment on microbial community structure and stomatal characteristics under field conditions. The experiment followed a randomized block design, including an unfertilized control and five NPK variants differing in nutrient ratios, applied as seed treatment, foliar treatment, or their combination. Microbiological analyses quantified total microorganisms, ammonifiers,* Azotobacter*, oligotrophic bacteria, and fungi, while anatomical assessments targeted stomatal density and size on both leaf epidermises. NPK fertilization substantially increased microbial biomass, particularly stimulating ammonifiers and fungi, while sharply reducing oligotrophic bacteria.* Azotobacter *populations were enhanced under moderate fertilization but declined at high nitrogen levels. High P and K supply decreased stomatal density relative to the control, whereas elevated nitrogen exerted a compensatory effect. Stomatal length remained largely unchanged across treatments, while stomatal width increased under balanced nutrient input. Overall, the results indicate that optimizing NPK ratios supports both microbial functionality and leaf-level anatomical traits associated with physiological efficiency. These findings highlight the importance of integrating nutrient management with biological processes to promote sustainable intensification, enhancing both crop productivity and agroecosystem resilience under changing environmental conditions.*

Keywords: *NPK fertilization, RIES treatment, sugar beet, rhizospheric microbiota, stomatal characteristics*

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important industrial crops in Europe, supplying a substantial share of the continent's sugar production and supporting agro-industrial development. Its productivity and physiological performance are strongly shaped by soil fertility, nutrient dynamics, and the activity of rhizospheric microorganisms. Among agronomic practices, fertilization remains one of the most effective measures for enhancing yield and stabilizing production under field conditions (BORCEAN, 1994; BORCEAN et al., 1996; BORCEAN et al., 1997, IMBREA at al., 2011).

Mineral fertilizers containing nitrogen, phosphorus, and potassium (NPK) have a decisive impact not only on nutrient availability but also on the biological activity of the soil. Microbial groups such as ammonifiers, oligotrophic bacteria, *Azotobacter*, and fungi respond differentially to fertilization, triggering functional shifts within the rhizosphere that directly influence plant growth and physiological processes (ALEXANDER and DOIJADE, 1995; AHMAD and SINGH, 1991, IMBREA, 2011).

Leaf anatomical traits — particularly stomatal density and size — are closely related to gas exchange, photosynthetic efficiency, and water-use optimization, and are highly sensitive to both nutrient supply and environmental conditions (AMAYA et al., 1999; CARBONELL et al., 2000; CRNOBARAC et al., 2001). A balanced fertilization regime can enhance both soil biological activity and stomatal functionality, thereby improving overall physiological performance (BORCEAN and PÎRȘAN, 2001, IMBREA, 2014).

The objective of this study was to evaluate the influence of different NPK fertilization levels and RIES treatment on rhizospheric microbiota and stomatal traits in sugar beet. By integrating microbiological and anatomical analyses, this research provides new insights into the interactions between fertilization, soil biological processes, and plant physiology under field conditions.

MATERIAL AND METHODS

Plant material and experimental design

The study was conducted on *Beta vulgaris* L. (sugar beet), using both seeds and plants sampled at different phenological stages. The experiment was arranged in a randomized block design. A non-fertilized treatment served as control, while the fertilized treatments consisted of different NPK combinations, including N50P100K100, N100P100K50, N100P100K100, N50P150K150, and N150P100K100. Fertilizers were applied either as seed treatment, vegetative-stage treatment, or as a combination of both methods.

Microbiological analysis

Soil samples were collected from the rhizosphere of sugar beet plants at characteristic growth stages. The density of the main microbial groups (total microorganisms, ammonifiers, *Azotobacter*, oligotrophic bacteria, and fungi) was determined by cultivation on selective media. Results were expressed as colony-forming units (CFU) per gram of dry soil.

Anatomical measurements

Fully developed young leaves were manually collected for anatomical determinations. Epidermal fragments were prepared through bleaching and mounting for microscopy. Stomatal density (no. mm^{-2}), length (µm), and width (µm) were measured using a light microscope equipped with an eyepiece micrometer. Measurements were taken separately on the adaxial (upper) and abaxial (lower) epidermis.

Statistical analysis

The data were subjected to statistical processing, including the calculation of mean values and standard errors. Significant differences were evaluated using the least significant difference (LSD) test at the 5% and 1% significance levels. Results were interpreted relative to the control and among treatment variants.

RESULTS AND DISCUSSIONS

The results obtained in the microbiological research (Table 1) highlight the impact of mineral fertilization on the structure and dynamics of microbial communities in the sugar beet rhizosphere. There is a significant increase in the total number of microorganisms with the application of NPK fertilizers, the highest values being recorded in the high-dose variants, which demonstrates the general stimulation of microbial activity by the exogenous nutrient intake.

The populations of ammonifiers show a positive response directly proportional to the level of fertilization, which reflects the intensification of the mineralization processes of the organic nitrogenous compounds. On the other hand, nitrobacteria are favored under moderate fertilization conditions, but register a reduction in density at high doses of mineral nitrogen, which suggests an inhibitory effect determined by the increased availability of easily assimilated nitrogen and the reduction of the need for biological fixation. Oligotrophic bacteria, characterized by adapting to environments with limited resources, significantly diminish their presence with the enrichment of the soil in nutrients, confirming their tendency to be replaced by copiotrophic microorganisms in ecosystems subjected to intensive fertilization. As far as fungal communities are concerned, the data show a constant and pronounced stimulation of them by the application of NPK, with maximum values at high doses, which can be correlated both with the proliferation of saprophytic fungi and with the possible intensification of mycorrhizal interactions beneficial for the plant. Overall, mineral fertilization determines a substantial increase in microbial biomass and an accentuation of nitrogen transformation processes, at the same time as structural changes in the composition of microbial communities, which tend to favor groups consuming available resources to the detriment of those adapted to oligotrophic conditions.

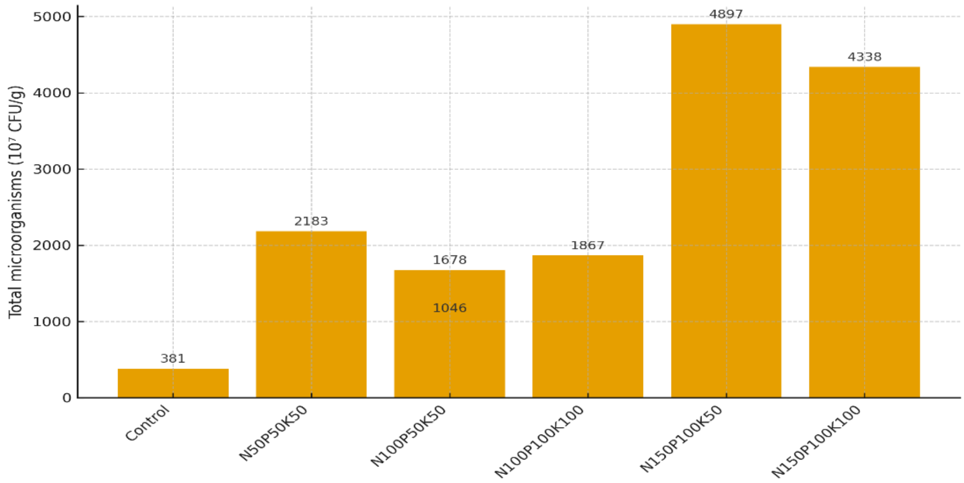

Figure 1. Influence of fertilization on the total number of microorganisms in sugar beet rhizosphere

Table 1

Influence of RIES treatment on the total number of microorganisms, ammonifiers, Azotobacter, oligotrophic bacteria, and fungi in the rhizospheric soil of sugar beet

Variant	Total microorganisms (10^7)	Ammonifiers (10^7)	Azotobacter (10^1)	Oligotrophic bacteria (10^7)	Fungi (10^4)
Control	381.1	346.0	228.1	749.9	17.2
$N_{50}P_{50}K_{50}$	2183.3	476.3	283.7	1004.7	7.2
$N_{100}P_{50}K_{50}$	1677.6	118.4	369.3	1710.6	10.6
$N_{100}P_{100}K_{100}$	1866.6	416.6	380.2	3094.8	9.0
$N_{100}P_{50}K_{50}$	1046.4	598.6	400.1	1619.8	5.3
$N_{150}P_{100}K_{50}$	4896.9	722.9	368.2	829.0	2.0
$N_{150}P_{100}K_{100}$	4337.9	752.6	422.7	1406.3	6.3

The influence of RIES treatments on the number and size of stomata in sugar beet grown at different fertilisation levels are shown in Tables 2, 3 and 4.

The analysis of the influence of mineral fertilization (NPK) on the density of the stomata on the epidermis of sugar beet leaves shows a significant variation depending on the dose and ratio of elements applied. In the upper epidermis, the mean values show a reduction in the number of stomata compared to the control in most fertilized variants, the lowest densities

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being observed in the $N_{50}P_{150}K_{150}$ treatment, which suggests a restrictive influence of high doses of phosphorus and potassium on the differentiation of the stomata. At the same time, it is noted that the treatment $N_{150}P_{100}K_{100}$ determines a stomatal density close to the control, indicating that higher doses of nitrogen can partially attenuate the inhibitory effect of other combinations of nutrients. In the lower epidermis, where the density of the stomata is generally higher, the trends are similar: the maximum values are recorded in the control and in the high-dose nitrogen ($N_{150}P_{100}K_{100}$) variant, while the combination $N_{50}P_{150}K_{150}$ again produces a pronounced reduction. On average, the data indicate that moderate fertilization tends to reduce the number of stomata compared to the control, and intense nitrogen fertilization has a compensatory effect, maintaining relatively high values. These results suggest that the ratio of nutrients influences the process of leaf organogenesis and stomata formation, with possible implications on gas exchange and photosynthetic efficiency of sugar beet plants.

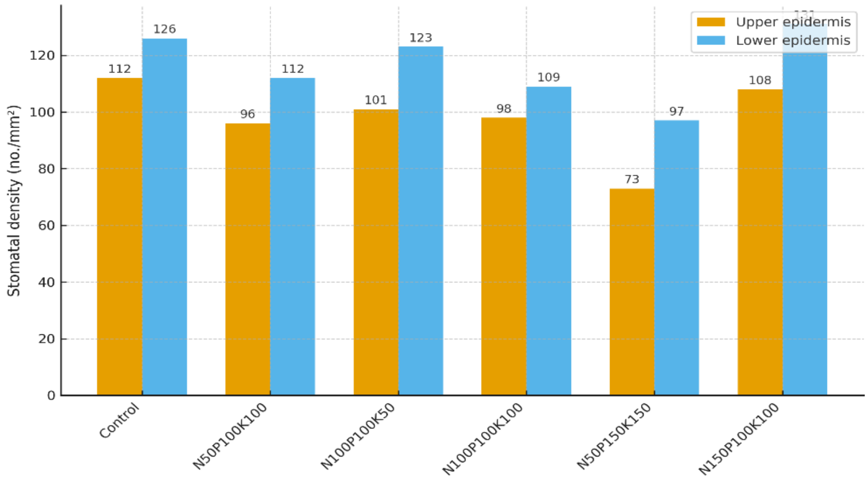

Figure 2. Influence of fertilization on stomatal density on upper and lower epidermis of sugar beet leaves

Table 2

Influence of RIES treatment on stomatal density (number/mm²) on the upper and lower leaf epidermis of sugar beet

Variant	Upper epidermis Ø	Treated plants	Seeds+plants treated	Mean	Lower epidermis Ø	Treated plants	Seeds+plants treated	Mean
Control	127	108	101	112	142	124	113	126
$N_{50}P_{100}K_{100}$	112	90	87	96	118	115	103	112
$N_{100}P_{100}K_{50}$	123	92	88	101	140	123	105	123
$N_{100}P_{100}K_{100}$	118	91	85	98	121	109	96	109
$N_{50}P_{150}K_{150}$	86	63	69	73	103	87	92	97
$N_{150}P_{100}K_{100}$	143	98	82	108	163	134	95	131

The analysis of the influence of mineral fertilization on the length of the stomata on the epidermis of sugar beet leaves (Table 3) shows a moderate variability depending on the dose and NPK ratio. The mean values show that, on both the upper and lower epidermis, the length of the stomata is maintained in a relatively close range (approximately 30–33 µm), without extreme deviations from the control. However, certain treatments determine specific trends: the application of variants with high doses of phosphorus and potassium ($N_{50}P_{150}K_{150}$) is associated with slightly increased values, while fertilization with excess nitrogen ($N_{150}P_{100}K_{100}$) leads to a reduction in the average length on the upper epidermis, indicating a possible inhibitory effect on the process of elongation of the stem cells. Also, the combination $N_{100}P_{100}K_{50}$ generates a more obvious decrease, reflected in the lower average values compared to other variants. Overall, the data suggest that the balanced ratio of nutrients tends to maintain the length of the stomata at a level close to the control, while nutritional imbalances, either due to excess nitrogen or high doses of P and K, can negatively influence dental morphometry. These variations, although statistically moderate, may have physiological implications on the efficiency of gas exchange and sweating regulation in sugar beets.

Table 3

Influence of RIES treatment on stomatal length (µm) on the upper and lower epidermis of sugar beet leaves

Variant	Upper epidermis Ø	Treated plants	Seeds+plants treated	Mean	Lower epidermis Ø	Treated plants	Seeds+plants treated	Mean
Control	30.4	33.7	32.4	32.2	30.7	33.1	32.8	32.2
$N_{50}P_{100}K_{100}$	31.7	32.1	33.0	32.3	31.1	32.4	33.2	32.2
$N_{100}P_{100}K_{50}$	30.4	26.0	33.8	30.1	30.0	26.2	33.5	29.9
$N_{100}P_{100}K_{100}$	30.4	33.5	32.8	32.2	30.5	34.0	32.0	32.2
$N_{50}P_{150}K_{150}$	31.9	29.5	34.0	31.8	32.8	31.3	33.7	32.6
$N_{150}P_{100}K_{100}$	27.5	32.9	34.9	31.7	28.0	33.6	33.8	31.8

The determinations on the width of the stomata in sugar beet (Table 4), depending on the application of the different NPK fertilization variants, show moderate variations between treatments, both at the level of the upper and lower epidermis. The mean values indicate that the control has a stomatal width of 21.5 µm on the upper epidermis and 22.7 µm on the lower epidermis, constituting the benchmark for comparison. In general, fertilization tends to cause a slight increase in the width of the stomata, with maximum values recorded in the $N_{100}P_{100}K_{100}$ and $N_{150}P_{100}K_{100}$ variants, where the average exceeds 23 µm. This effect is more pronounced in the lower epidermis, where the width of the stomata is constantly greater than that of the upper epidermis, confirming the anatomical and functional particularities of sugar beet leaves. On the other hand, the $N_{100}P_{100}K_{50}$ variant led to lower values, close to the control, suggesting that an insufficient intake of potassium can limit the process of cell expansion in the dental system. Overall, the results show that balanced fertilization and especially the increased intake of nitrogen and potassium favors the width of the stomata, which can contribute to optimizing their opening and, implicitly, to the efficiency of gas exchange and photosynthetic processes.

Table 4

Influence of RIES treatment on stomatal width (µm) on the upper and lower epidermis of sugar beet leaves

Variant	Upper epidermis Ø	Treated plants	Seeds+plants treated	Mean	Lower epidermis Ø	Treated plants	Seeds+plants treated	Mean
Control	20.9	22.1	21.7	21.5	21.8	23.6	22.8	22.7
$N_{50}P_{100}K_{100}$	22.1	22.0	23.4	22.5	22.2	23.8	23.6	23.2
$N_{100}P_{100}K_{50}$	21.6	19.6	24.0	21.7	21.3	20.3	24.5	22.0
$N_{100}P_{100}K_{100}$	22.1	23.7	23.2	23.0	21.8	24.8	23.6	23.4
$N_{50}P_{150}K_{150}$	22.6	21.4	23.3	22.4	23.0	22.4	24.0	23.1
$N_{150}P_{100}K_{100}$	20.7	23.8	24.7	23.1	20.5	24.3	25.0	23.3

CONCLUSIONS

Mineral fertilization significantly stimulates the microbial biomass in the sugar beet rhizosphere.

Ammonifiers and fungi respond most positively to the increase in the NPK dose.

Nitrobacteria are favored by moderate fertilization, but excess mineral nitrogen reduces their efficiency.

Oligotrophic bacteria are inhibited by high nutrient intake, which shows the change in the ecological balance of the soil towards copiotrophic microorganisms (which prefer rich environments).

High doses of NPK lead to a reduced diversity, but an increased abundance of certain groups (ammonifiers, fungi).

The results demonstrate that mineral fertilization significantly influences the stomata density of sugar beets, generally reducing it compared to the control, with more pronounced effects at high doses of phosphorus and potassium. In contrast, the high intake of nitrogen attenuates this reduction, suggesting a compensatory role of nitrogen in maintaining the capacity for gas exchange and photosynthesis.

The results indicate that the length of the stomata in sugar beet is relatively stable, but may be affected by imbalances in the NPK ratio. Excess nitrogen or high doses of phosphorus and potassium can reduce the size of the stomata, which could limit the efficiency of gas exchange and the regulation of sweating. From an applicative perspective, maintaining a balanced nutrient ratio is essential to preserve the optimal morphometric parameters of the stomata, with a direct impact on photosynthesis, water consumption and, implicitly, on crop productivity.

The increase in the width of the stomata under the influence of balanced fertilization, especially in variants with adequate nitrogen and potassium intake, suggests an improved ability of the dental system to regulate gas exchange and sweating. From an agronomic perspective, this highlights the importance of maintaining an optimal NPK ratio in fertilization to support the physiological processes of sugar beet and contribute to higher yields.

The results obtained on the influence of mineral fertilization (RIES) on the stomatal system of sugar beet show that the dose and NPK ratio cause changes both in terms of density and dimensions of the stomata. The number of stomata tends to decrease compared to the control under high doses of phosphorus and potassium, while nitrogen fertilization has a compensatory effect, maintaining relatively high values. The length of the stomata is generally maintained at intervals close to the control, but is reduced under the influence of excess nitrogen or nutritional imbalances, suggesting a sensitivity of the cell elongation process. Instead, the width of the stomata is stimulated in particular by balanced nitrogen and potassium fertilization, which can facilitate the opening of pores and the intensification of gas exchange. Overall, it can be concluded that a balanced NPK ratio is essential for maintaining optimal anatomical parameters of the dental system, with direct implications on photosynthetic efficiency, sweat regulation and, implicitly, on sugar beet productivity.

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