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# The Influence of Applying Foliar Micronutrients at Nodulation and the Physiological Properties of Common Soybean Plants

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**Abstract:** Legumes, due to their symbiosis with papillary bacteria, can receive nitrogen from the air. The remaining nutrients must be supplied in fertilisers, either soil or foliar. In the pot experiment, we recorded the responses of two soybean cultivars (Annushka, Pompei) to the foliar application of micronutrients (control, Zn, Fe, Cu, Mn, B, or Mo). The physiological properties were expressed as net photosynthetic rate ($P_N$), intercellular $CO_2$ concentration ($C_i$), transpiration rate (E), stomatal conductance ($g_s$), maximum quantum yield of photosystem II ($F_v/F_m$), maximum quantum yield of primary photochemistry ($F_v/F_0$), photosynthetic performance index (PI), and the development of soil plant analyses (SPAD), which were analysed. The effects of individual micronutrients on nodulation, plant growth, and condition were also investigated. Micronutrient fertilisation had a positive effect on plant fresh weight and no negative effect on plant condition. It was shown that elements such as B, Fe, and Mo had the most beneficial effect on nodulation compared to the control, regardless of the cultivar analysed. The application of single-component foliar fertilisers improved the physiological parameters of the plants. The relative chlorophyll content was most favourably affected by the application of Mn, B, and Mo in the Annushka cultivar, and Fe, Mn, and Mo in the Pompei cultivar. Similarly, in the case of chlorophyll fluorescence, the most stimulating effect was found for Mn and B, regardless of the cultivar. In the case of gas exchange, the application of Fe, Mo, and B for the Annushka cultivar and Cu for the Pompei cultivar had the most favourable effect on physiological measurements. The results obtained indicate that the foliar application of the evaluated micronutrients is justified in soybean cultivation and does not disturb the nodulation process.

**Keywords:** *Glycine max* (L.) Merr.; foliar fertilisation; microelements; physiological parameters; nutrients; nodule bacteria



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## 1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most widely cultivated crops. It occupies 50% of the world's legume area and 68% of its world production [1,2]. In Poland, the acreage of soybean cultivation is still small, but interest in this species is growing, which is related to its versatile use as food, animal feed, or in industry.

Productive crops such as soybeans play an important role in crop production due to limited land and water resources, fertiliser costs, and growing environmental concerns [3]. The expansion of agricultural areas at the expense of forests, scrublands, or even lands of destruction requires too much investment or is too risky from environmental and ecosystem perspectives [4–6]. Therefore, the only rational direction for agricultural development is to maximise the yields of the already cultivated areas [7,8]. Technologies related to precision tillage, agrotechnology, and fertilisation are increasingly needed to improve the productivity of agricultural crops without affecting the environment [9]. Furthermore, breeding progress, effective fertilisation, the proper use of crop-protection products, farmers, and their knowledge and practical skills are considered the most important measures to increase yields [10].

Micronutrients are essential for metabolic and cellular processes in plants. Among the most important are zinc (Zn, produce carbohydrates), iron (Fe, chlorophyll synthesis), copper (Cu, seed production), manganese (Mn, photosynthesis process), boron (B, develop proteins and seed setting), molybdenum (Mo, required by nitrogen-fixing bacteria in soybean nodules), and others. Crops have different nutrient requirements and are therefore fertilised according to the needs of the species [11]. Campo et al. [12] and Jarecki [13] have shown that the deficiency of some micronutrients, such as Mo, can reduce the fixation of symbiotic nitrogen. However, some micronutrients (Cu and Mn) applied topically or in a seed dressing can be toxic to *Bradyrhizobium japonicum*. In such a situation, the application of the necessary micronutrients in a foliar spray can be a good solution. Bruns [14] concludes that different fertilisation options, such as single component or micronutrient combinations, can be used in soybean cultivation.

The increase in production per unit of area has resulted in a significant decline in micronutrients in the soil. Micronutrient deficiencies have become a limiting factor in crop productivity in agricultural areas around the world [15]. In situations where plants make it difficult to absorb nutrients from the soil (for example, in cases of extreme drought or when soil nutrient levels are extremely low), foliar fertilisation can be an alternative way to supply plants with the missing macro- and micronutrients. Research has shown that crops in unfavourable habitat conditions take up only 30–40% of nutrients from the soil. Therefore, nutrients are often applied foliarly. Van Roekel et al. [16] point out that achieving maximum soybean yield requires the selection of an appropriate variety and the careful implementation of specific agronomic treatments, including mineral fertilisation with essential nutrients. However, they point out that soybean yield can be limited by various environmental stresses (abiotic and biotic), which cannot always be addressed under field conditions. Although plants need micronutrients in relatively low doses, their deficiencies cause serious disturbances in the physiological and metabolic processes of plants [17]. Fertilisation with single elements should be precisely matched to the needs of the specific plant. This approach allows for the increase in yields in every region of the world. Furthermore, balanced foliar fertilisation positively influences plant physiology without negatively affecting the environment [18–20]. Bacilieri et al. [21] and Di Mauro et al. [22] report that the application of nutrients to the soil, leaves, or to the seed is a well-known treatment in the agro-techniques of many crops. However, depending on the species, fertilisation with specific elements is not always justified. The decision on whether or how foliar fertilisation will be carried out depends on many factors, e.g., habitat, species, irrigation, temperature, soil nutrient availability, etc. Domingos et al. [23] consider that the foliar application of macro- and/or micronutrients is justified during periods of high demand and high nutrient translocation of nutrients to seeds. Nyoki and Ndakidemi [24] confirm that soybeans require both macro- and micronutrient fertilisation. As a result, plants have access to all essential nutrients. Novytska et al. [25] showed that soybean fertilisation is particularly important in low-fertility soils. In case of micronutrient deficiencies, they recommend foliar application during the growing season.

The purpose of this study was to investigate in a potent experiment how the application of individual micronutrients (Zn, Fe, Cu, Mn, B, Mo) in the foliar environment affects the physiological parameters, nodulation, and growth of soybean plants. It was assumed that the foliar application of the evaluated micronutrients would have a more favourable effect on physiological properties and plant growth, while not affecting nodulation.

## 2. Materials and Methods

### *2.1. Plant Material and Pot Experiment Scheme*

Pot experiments were carried out at the University of Rzeszow (Rzeszow, Poland). The soybean seeds were purchased from Agroyoumis Sp. z o.o. (Rudnik nad Sanem, Poland). Two local cultivars that differed in early maturity were selected for the experiment. Annushka, early, and Pompei, medium-late. The composition of the seed coat contained *Bradyrhizobium japinicum* bacteria, so they did not require extra inoculation prior to sowing Pompei.

Soil (Haplic Cambisol—Cmha) [26] was taken from the field belonging to the Experimental Department of the University of Rzeszów in Krasne near Rzeszów (50.065371, 22.072695). Soybeans were not grown at the sampling site. The soil had a slightly acid reaction (pH 1 N KCl 6.15), and the content of phosphorus, potassium and magnesium was high ($P_2O_5$ 180.2 mg·kg$^{-1}$ d.m. of soil, $K_2O$ 231.2 mg·kg$^{-1}$ d.m. of soil, Mg 81.6 mg·kg$^{-1}$ d.m. soil), while those of micronutrients were medium (Zn 11.9 mg·kg$^{-1}$ d.m. soil, Cu 5.37 mg·kg$^{-1}$ d.m. soil, Fe 2736 mg·kg$^{-1}$ d.m. soil, Mn 469 mg·kg$^{-1}$ d.m. soil) or low (B 1.1 mg·kg$^{-1}$ d.m. soil). Soil analysis was performed in the accredited laboratory of the Chemical and Agricultural Station in Rzeszów. Each of the 5 L pots contained 4 kg of dry soil. Soybean seeds were sown on 10 April 2023 and five plants per pot were left in the first stage of the leaf (BBCH 11). The experiment was conducted as a two-factor experiment in four replications ($n$ = 56). The first factor was cultivars (Annushka, Pompei), and the second was a separately foliar application of micronutrients (control, Zn, Fe, Cu, Mn, B, or Mo). Spraying was carried out twice with a hand sprayer at the trifoliate leaf stage (BBCH 19) and at the beginning of budding (BBCH 51) [27].

The applied dose per hectare was 1 L·ha$^{-1}$ Foliar fertilisers (Intermag sp. z o.o., Olkusz, Poland) contained single micronutrients:

1. MIKROVIT® Zinc—112 g·L$^{-1}$ Zn,
2. MIKROVIT® Iron—75 g·L$^{-1}$ Fe,
3. MIKROVIT® Copper—75 g·L$^{-1}$ Cu,
4. MIKROVIT® Manganese—160 g·L$^{-1}$ Mn,
5. MIKROVIT® Molybdenum—33 g·L$^{-1}$ Mo,
6. BORMAX® Boron150 g·L$^{-1}$ B.

The pot was placed in a growth chamber (model GC-300/1000; JEIO Tech Co., Ltd., Seoul, Republic of Korea) with a humidity of 20 ± 1 °C, a relative humidity of 60 ± 3%, a photoperiod of16/8 h (L/D) and a maximum brightness of about 300 mol m$^{-2}$s$^{-1}$ per day. The humidity of the substrate was maintained at 60% of the water capacity of the field by the daily weight of the bottles. The pot configuration was changed randomly every five days in four rows. The experiment was carried out to the stage of flowering (Annushka: 30 days from the date of sowing, Pompei: 36 days from the date of sowing). In the final phase of the study, the visual condition of the plants (plant health) was assessed. The evaluation was carried out on a 9° scale (where 9° means no leaf damage and 1 ° means total leaf damage). The number of leaves of different morphological appearances (leaf wilting, necrosis, chlorosis, etc.) was evaluated, where 9° = 0–5%, 8° = 6–15%, 7° = 16–25%, 6° = 26–40%, 5° = 41–60%, 4° = 61–65%, 3° = 76–85, 2° = 86–95%, and 1° = 96–100% of the surface of visibly damaged plants. The above-ground parts of the plants were harvested, their fresh weight (FM) was measured on an analytical balance, and the roots were washed with sieves to count papillae. The plant weight of each variation in the experiment is related to the plant weight of the control (100%), according to the equation:

$$FM_{(\%)} = \frac{M_{fp} \cdot 100}{M_c} \quad (1)$$

where:

$FM_{(\%)}$—calculated fresh weights of plants.
$M_{fp}$—weight of the above-ground parts of the plants.
$M_c$—weight of the above-ground parts of the control plants.

### *2.2. Soil Plant Analysis Development*

The relative chlorophyll content was measured using a Minolta SPAD-502 chlorophyll meter and expressed in unmeasured SPAD units (Soil Plant Analysis Development), which are closely related to the chlorophyll content. The instrument measures the difference in leaf light absorption at 650 nm and 940 nm. The proportion of these differences indicates the leaf green index or the chlorophyll content. SPAD was measured twice in each pot, with fully developed leaves 7 days after foliar fertilisation.

### *2.3. Chlorophyll Fluorescence*

The chlorophyll fluorescence in the leaves was measured with a Pocket PEA instrument (Hansatech Instruments, King's Lynn, Norfolk, UK) equipped with black shading clips applied to the leaves away from the tips of the leaves. The following parameters were measured: photosystem II maximum quantum yield (PSII) ($F_v/F_m$), primary quantum yield ($F_v/F_0$), and photosynthesis performance index (PI). Five chlorophyll fluorescence measurements were taken per pot during flowering. The maximum available intensity was 3500 mol (photon), which was applied for a second at a maximum wavelength of 627 nm. The fully developed leaves were adjusted to 30 min of darkness by applying leaf clips to the axial leaves.

### *2.4. The Measurement of Gas Exchange*

Net photosynthetic rate ($P_N$), transpiration rate (E), stomatal conductivity ($g_s$), and $CO_2$ concentration ($C_i$) between cells were measured on two fully expanded leaves (20 measurements per concentration). The LCPro-SD photosynthesis measurement system (ADC Bioscientific Ltd., Herts, UK) was used for the measurement. The flow accuracy of the LCpro-SD plant leaf photosynthesis chamber is about 2% of the range. During the measurement, the light intensity was 300 mol $m^{-2}s^{-1}$ and the temperature in the measuring chamber was 22 °C.

### *2.5. Statistical Analyses*

Statistical analysis was carried out using TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA). A Shapiro–Wilk test was performed to verify the normality of the distribution at $p = 0.05$. In addition. The homogeneity of the variance was also checked. Then, for physiological measurements, an ANOVA test with repeated measurements was performed (with time score as a factor). To determine the significance of the differences for nodulation, an ANOVA test of two factors was performed. A post hoc Tukey test was conducted at an importance level of $p \leq 0.05$ to establish and verify the relationship.

## 3. Results

### *3.1. The Effect of Fertilisation on Fresh Plant Weight and the Plant Health of Soybean Plants*

The application of single-component fertilisers did not adversely affect plant development and visual evaluation before the end of the experiment (Figure 1). Regardless of the element applied, the plants received scores between 8.0 and 9.0. Lower scores (8.0) were recorded for the Annushka control sample and for Pompei, a sample fed with boron. However, these changes were insignificant.

Single-component foliar fertilisers had a positive effect on green plant weight. Each fertilisation variant, regardless of the cultivar, resulted in an increase in plant aboveground weight compared to the control. The highest fresh plant weight was recorded for the Annushka cultivar with fertilisation with boron (155%) and iron (137%). Zn fertilisation resulted in a fresh weight gain of 11% for the Annushka cultivar and 12% for the Pompei cultivar. In the case of Fe, the increase for the Annushka cultivar was 37%, and for the Pompei cultivar it was 10%. During Cu fertilisation, there was an increase in fresh weight compared to the control. This was 11% for the Annushka cultivar and 14% for the Pompei cultivar. The Mn fertilisation resulted in an increase in fresh weight of 15% in the Annushka cultivar and 17% in the Pompei cultivar. In the case of the B-containing fertiliser applied to the Annushka cultivar, the highest increase in green weight was recorded throughout the experiment (55%). In the case of the Pompei cultivar, boron fertilisation did not have the desired result; the fresh weight increase was only 4%. In the case of Mo, the increase in fresh weight in the Annushka cultivar was 21% and in the Pompei cultivar it was 16%.

When comparing the analysed cultivars, it was found that the Annushka cultivar had a better increase in the weight of the plant above ground in response to foliar fertilisation, regardless of the element used.

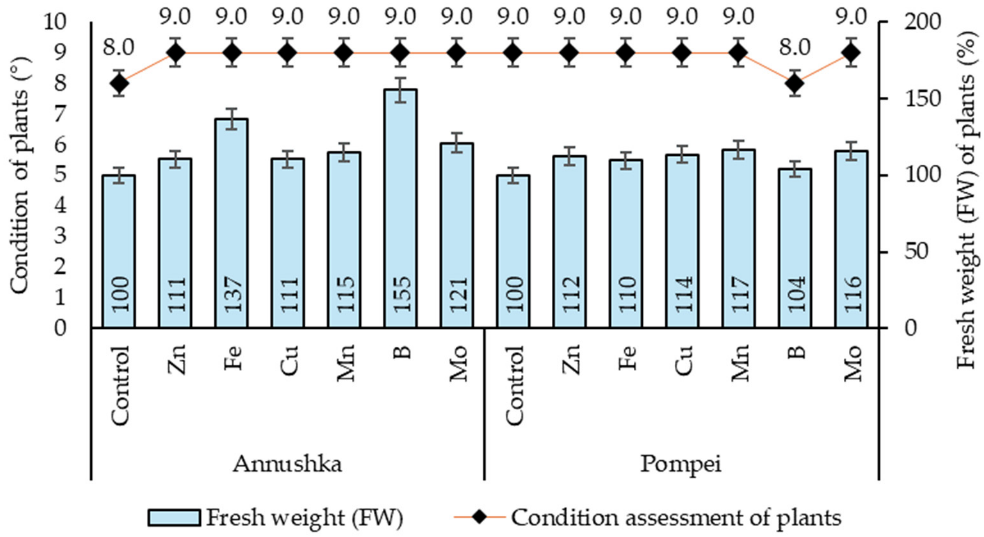



**Figure 1.** Changes in fresh weight (FW) and plant condition (9—most favourable, 1—least favourable) caused by fertilisation with single-component foliar fertilisers, depending on the element applied.

## *3.2. Nodulation*

The number of nodules on the roots varied significantly depending on the foliar fertilisation applied (Figure 2). Compared to the control, each applied foliar fertiliser resulted in an increase in the average number of nodules on the soybean roots. In the case of the Annushka cultivar, the highest number of nodules was recorded for the fertilisation of B (41 nodules), and Fe fertilisation (34 nodules). Slightly lower values were recorded for fertilisation with Mo (28 pcs), Mn (27 pcs.), Cu (26 pcs.), and Zn (23 pcs). Analysing the mean nodulation values obtained for the Pompei cultivar, it was found that fertilisation with Cu (21 units) and Mn (22 units) had no significant effect on soybean nodulation. For the fertilisation with Zn and Fe, the nodulation was 24 units and 26 units, respectively. Only fertilisation with B and Mo had a significant effect on the growth in soybean roots. In both cases, the average number of nodules was 30 pcs.

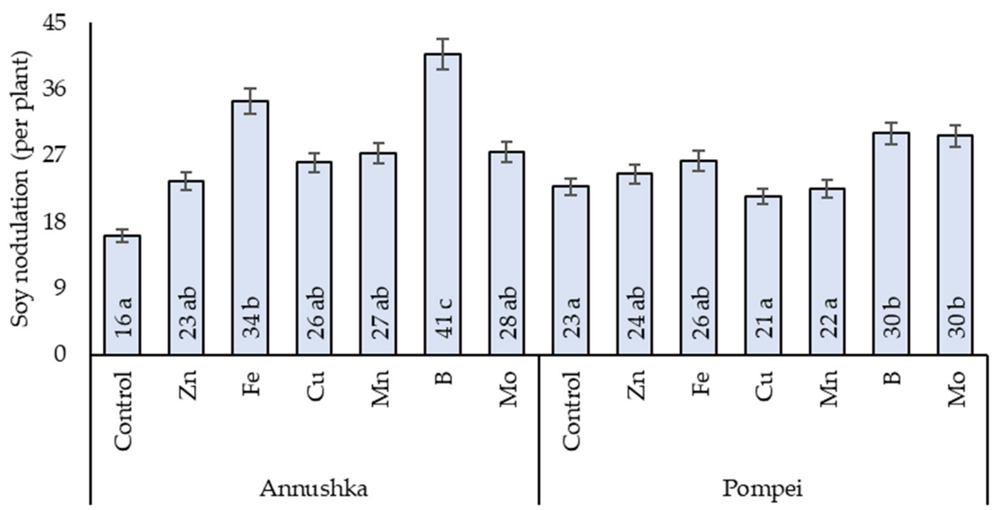



**Figure 2.** Effect of applied foliar fertilisation on the average number of nodules per root per plant. a–c statistically significant differences between values within the cultivar at a confidence level of $p = 0.05$.

*3.3. The Relative Chlorophyll Content of Soybean Leaves*

Figure 3 shows the values of the relative chlorophyll content in soybean leaves depending on the applied foliar fertilisation and the measurement time. In the case of the Annushka cultivar, significant differences were recorded for the fertilisation of Fe, B, and Mo at the first measurement date. On the second measurement date, a significant increase in chlorophyll content expressed in SPAD units was recorded in soybean leaves for all variants of single-component foliar fertilisation. The highest values were obtained with the fertilisation with Mn, B, and Mo.

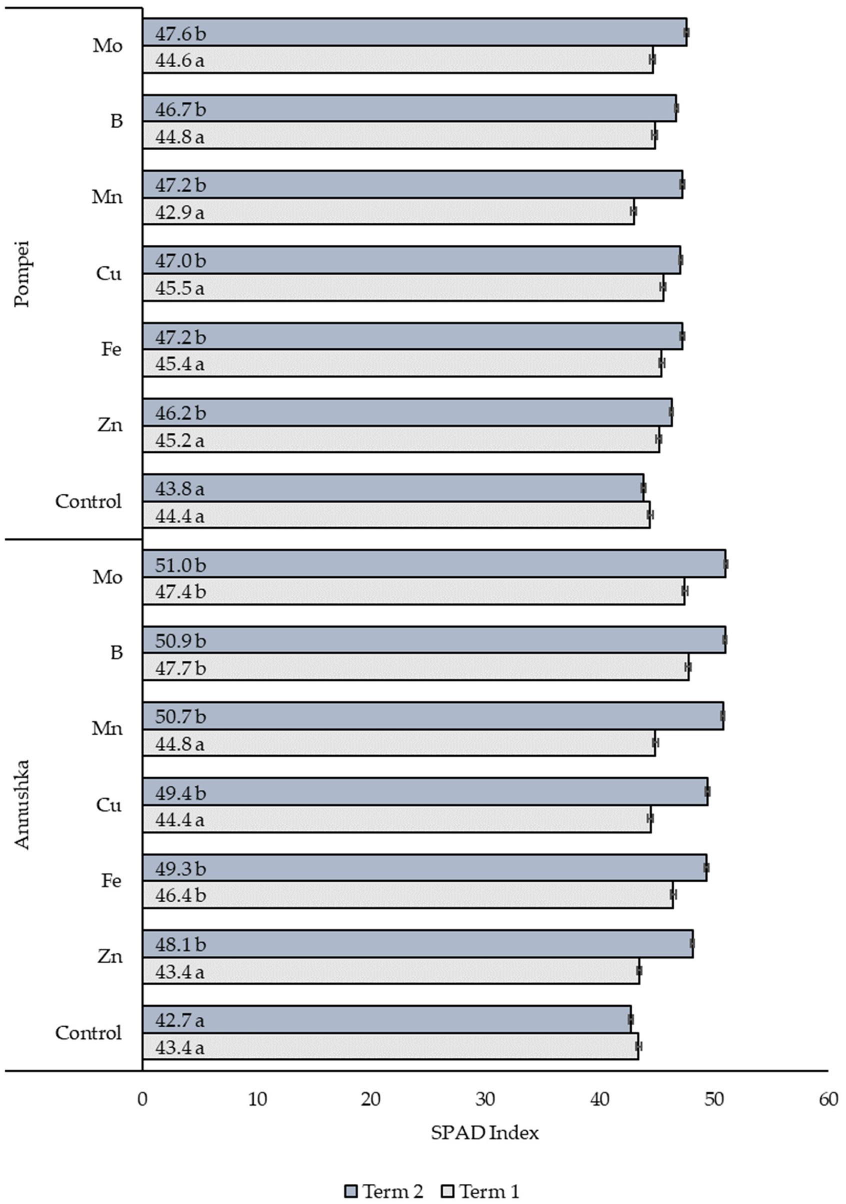
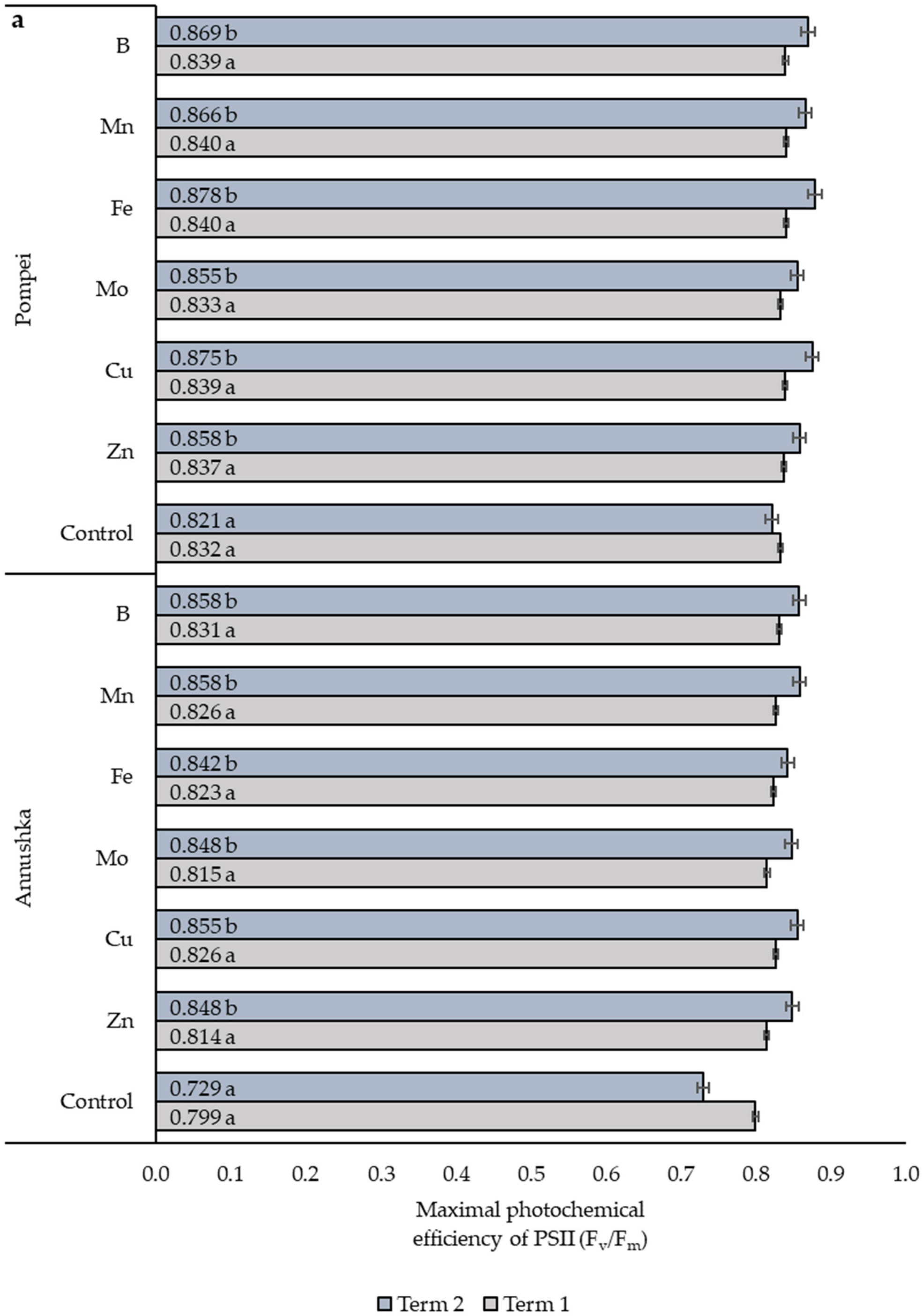



**Figure 3.** Effect of applied foliar fertilisation on mean values of relative chlorophyll content in soybean leaves on successive measurement dates. a–b statistically significant differences between values within the date and cultivar at a confidence level of $p = 0.05$.

When analysing the values obtained for the Pompei cultivar, it was found that there were no statistically significant relationships between the foliar fertilisation at the first measurement date. However, on the second measurement date, statistically significant differences were recorded in all cases analysed. The most stimulating effects were observed for Fe, Mn, and Mo fertilisation.

*3.4. Chlorophyll Fluorescence in Soybean Leaves*

The application of single-component foliar fertilisers had a significant effect on chlorophyll fluorescence expressed as $F_v/F_m$, $F_v/F_0$, and PI (Figure 4). The single-element fertilisation of the foliar at the first measurement date did not significantly affect the value for both Annushka and Pompei cultivars. The values obtained for the individual elements were higher compared to the control, but were not statistically significant. On the other hand, at the second measurement date, the values obtained after the application of the foliar fertiliser were significantly higher compared to the applied control, regardless of the element and the cultivar. Despite significant differences, it is not possible to clearly identify the element that stimulates the $F_v/F_m$ value the most strongly.

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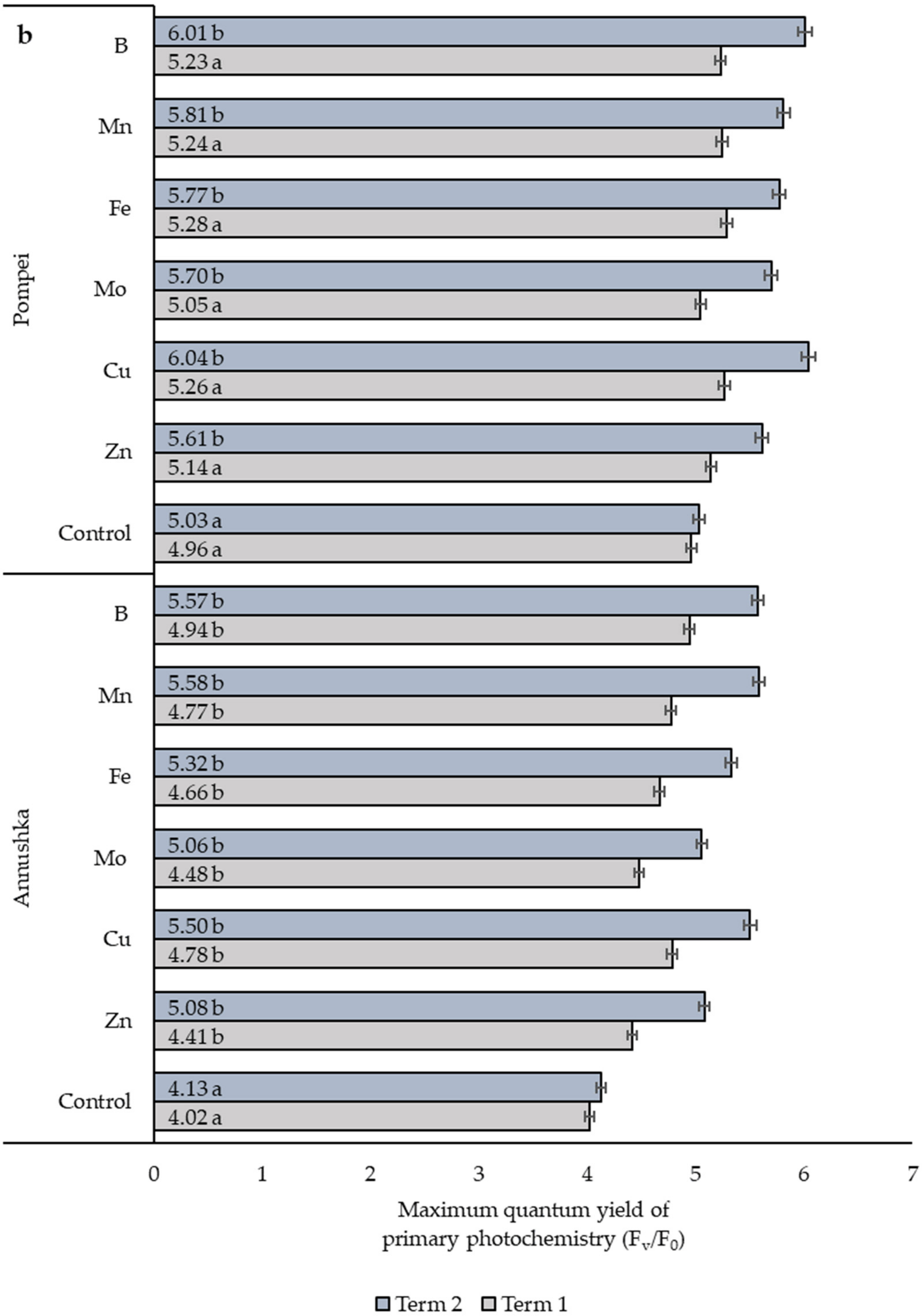


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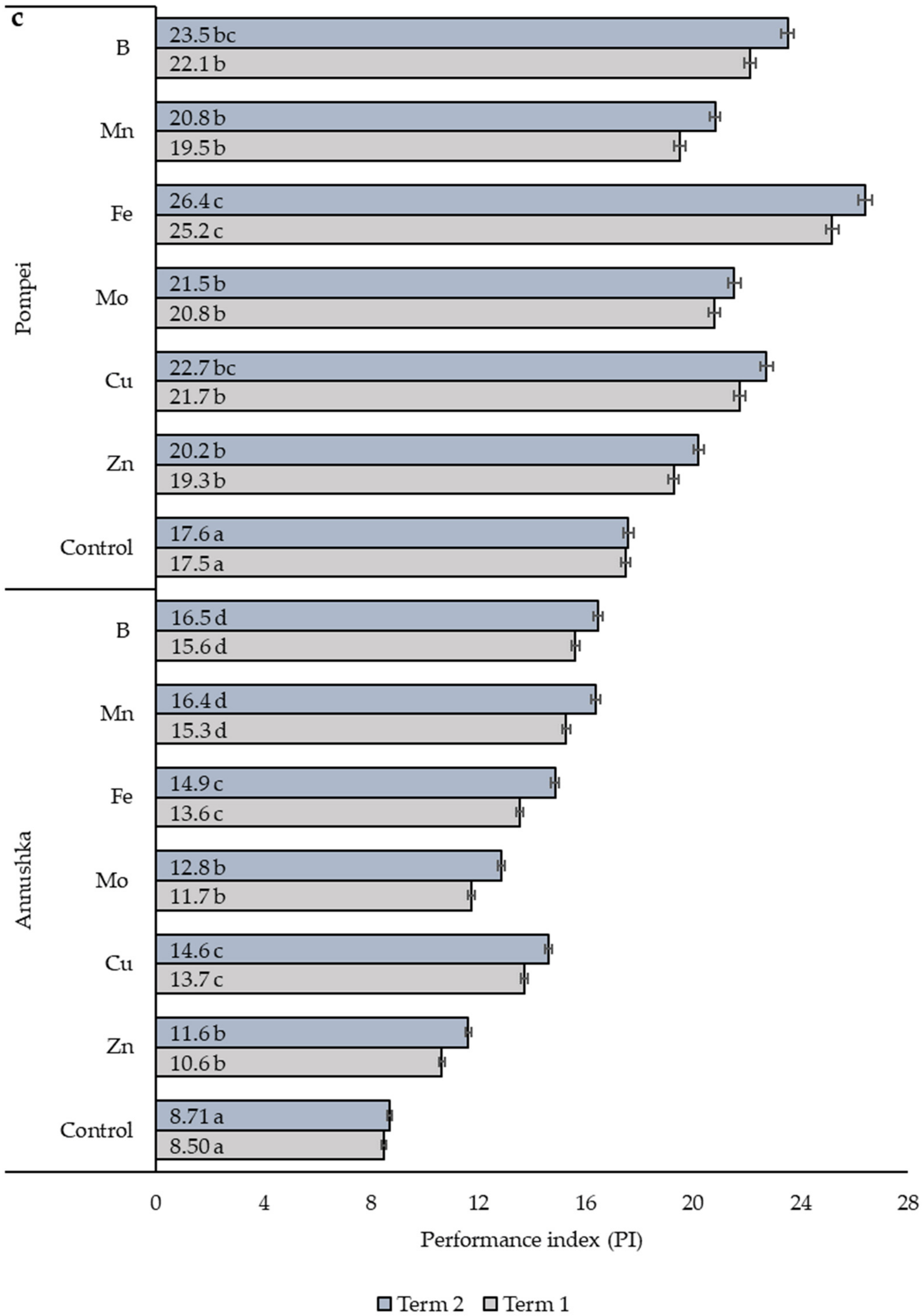


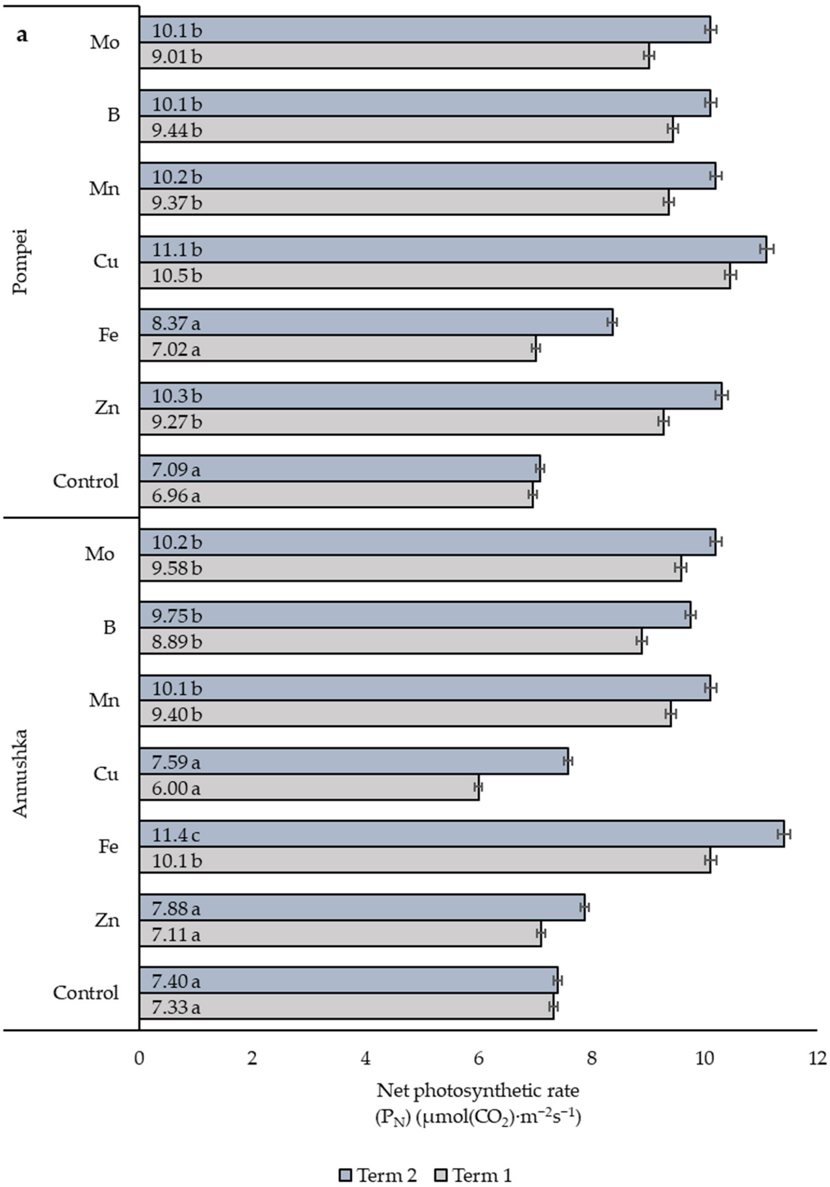

**Figure 4.** Effect of applied foliar fertilisation on chlorophyll fluorescence parameters: (**a**) maximum quantum yield of PSII photochemistry ($F_v/F_m$), (**b**) maximum yield of primary photochemistry ($F_v/F_0$), (**c**) PS II performance index (PI). a–d statistically significant differences between the values within the term and the cultivar at the confidence level $p$ = 0.05.

Analysing the values obtained during the measurement of $F_v/F_0$, it was found that the single-component foliar fertilisation had a significant effect on the value of the analysed parameter at the first and second measurement dates in the case of the Annushka cultivar. In the case of the Pompei cultivar, no statistically significant differences were recorded after applied fertilisation in the first measurement term. On the other hand, on the second measurement date, the values increased and were statistically significant regardless of the element applied. The best effects were recorded for the application of Mo and B in the Annushka cultivar. In the Pompei cultivar, they were Cu, Mn, and B.

The PI value depended on the fertilisation applied and the cultivar. Statistically significant differences were recorded for each analysed element, regardless of the measurement date. The most favourable effects were of B, Mn, Fe, and Cu in the Annushka cultivar and Fe, B, Cu, Mn, and Ma for the cultivar Pompei.

*3.5. Gas Exchange in Soybean Leaves*

In the research carried out, we observed a significant effect of the applied single-component foliar fertilisers on the gas exchange parameters in the soybean leaves (Figure 5). Analysing the data for the $P_N$ parameter, obtained for the Annushka cultivar, it was found that statistically significant differences were recorded for Fe, Mn, B, and Mo fertilisation at the first measurement date. An analogous relationship was observed at the second measurement date. In the case of the Pompei cultivar, statistically significant differences were observed for the fertilisation of Zn, Cu, Mn, B, and Mo at the first measurement date. Similarly, differences were also recorded for the second measurement term.

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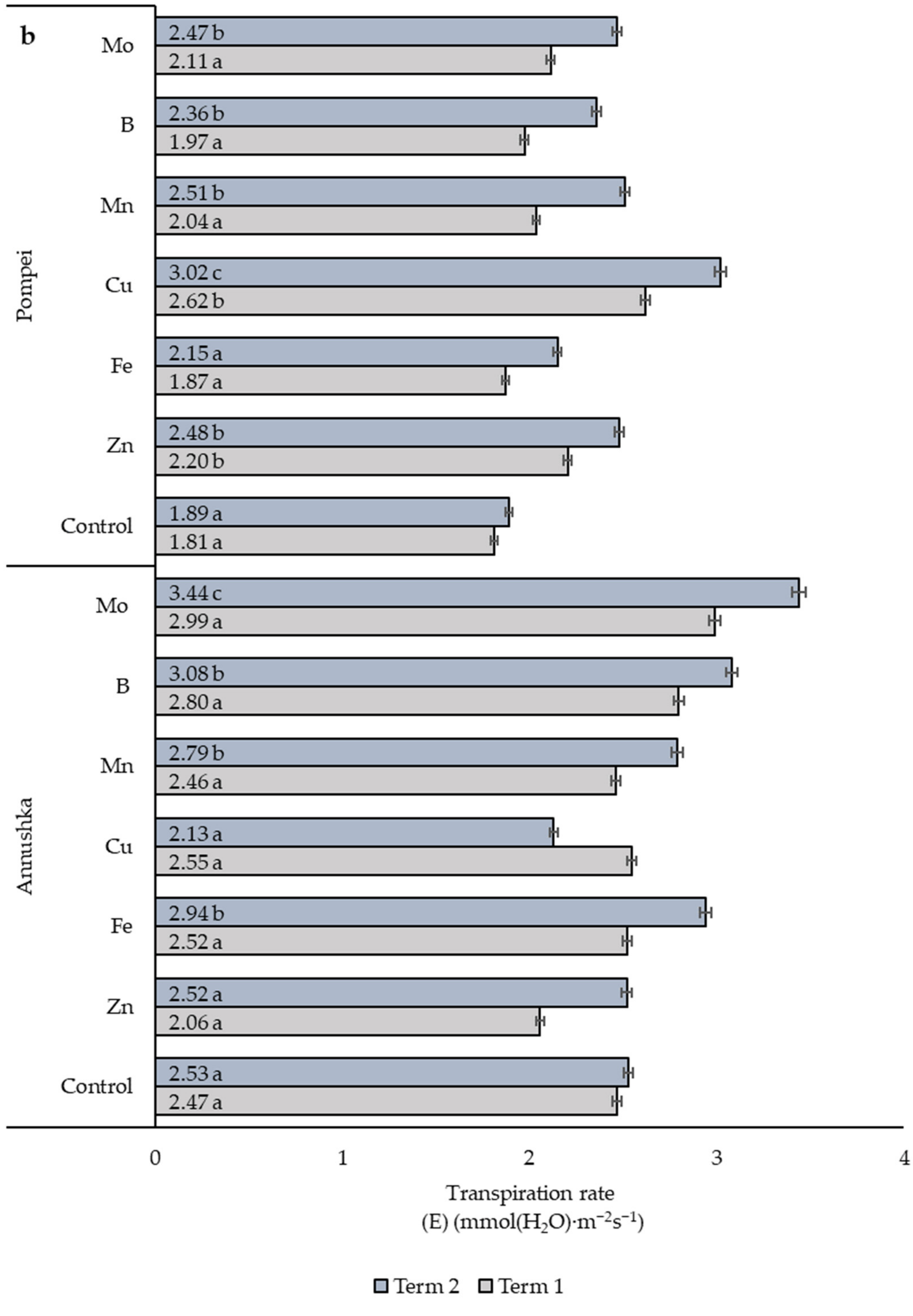


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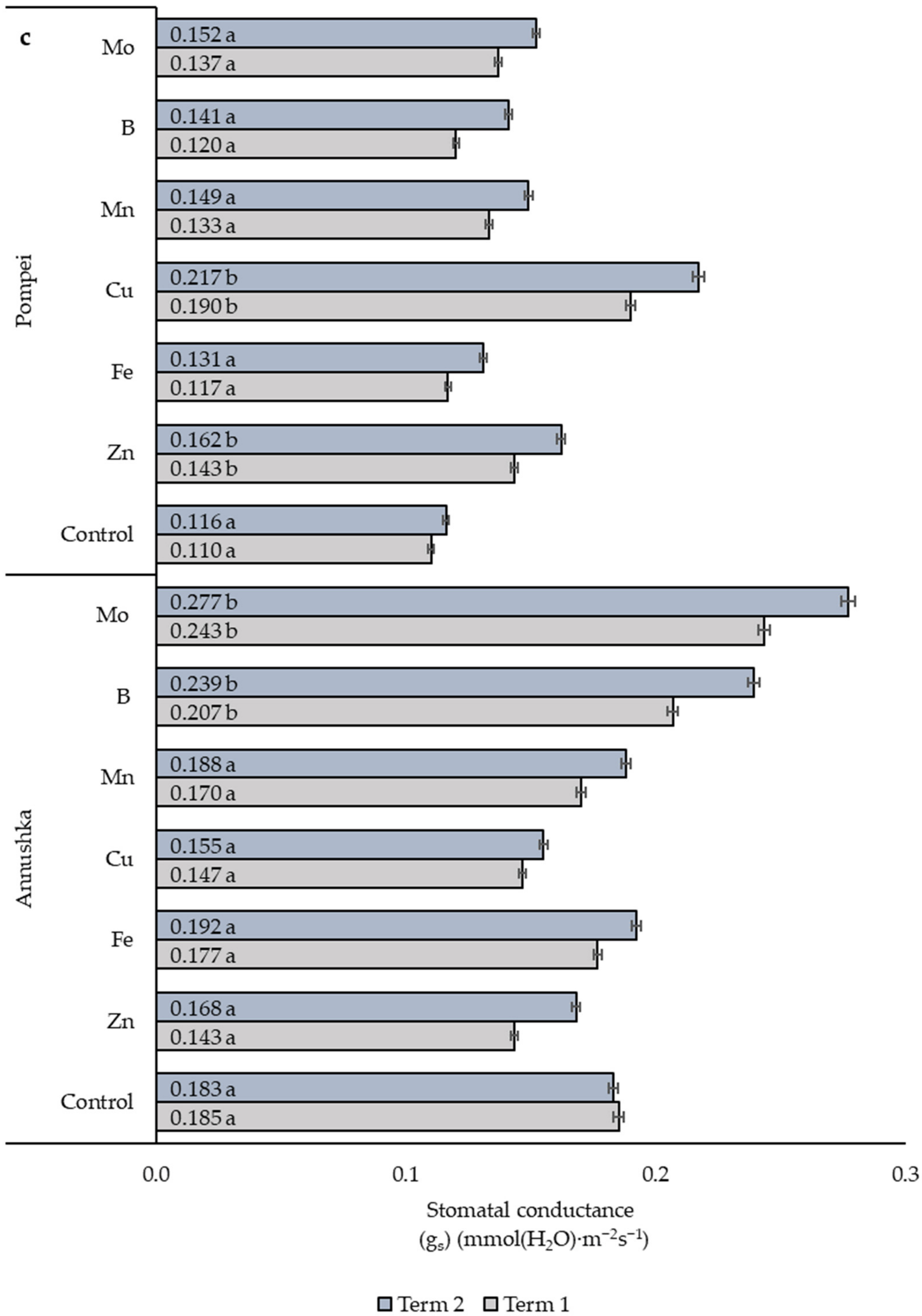


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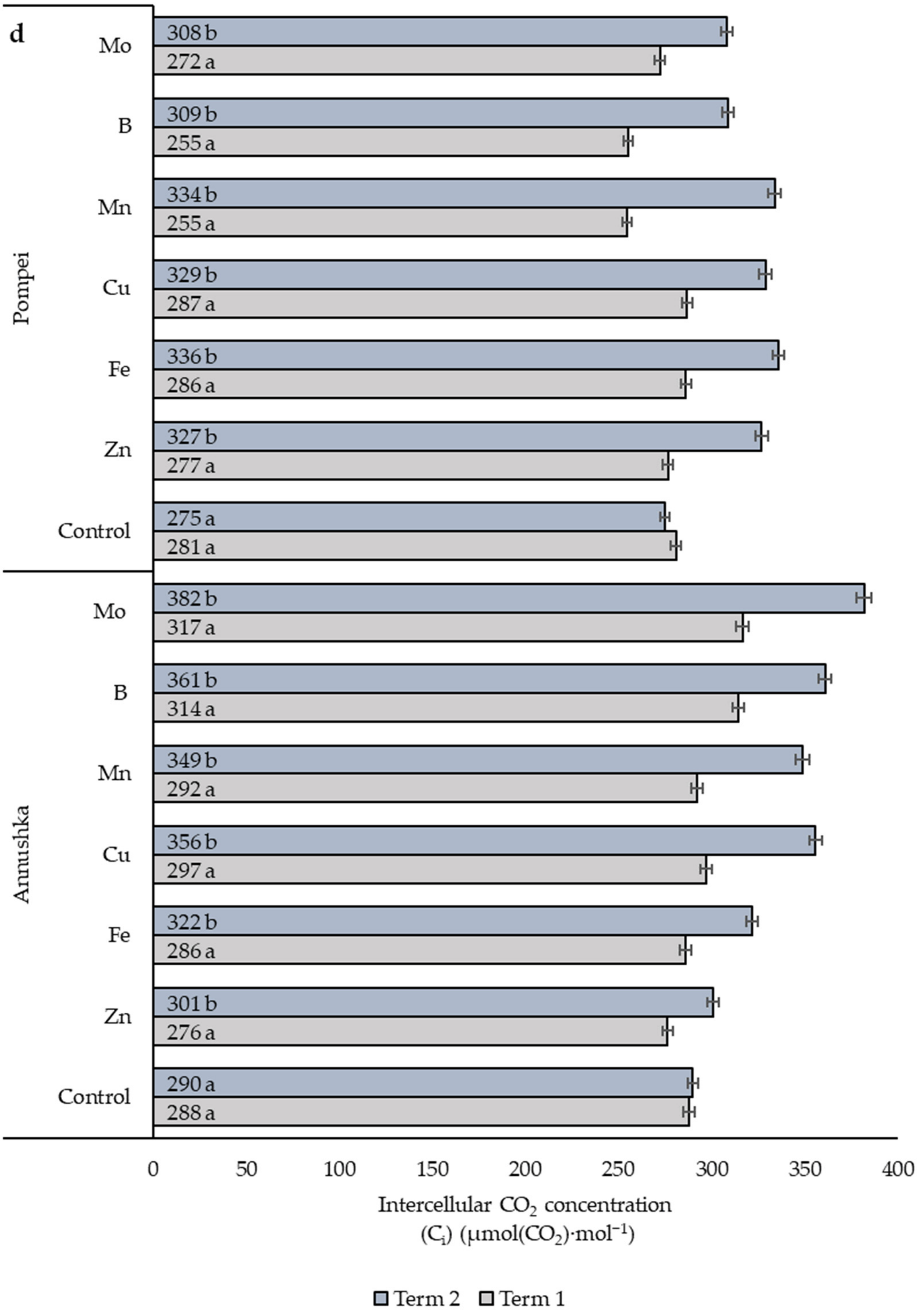


**Figure 5.** Effect of applied foliar fertilisation on soybean gas exchange parameters: (**a**) net photosynthetic rate ($P_N$), (**b**) transpiration rate (E), (**c**) stomatal conductance ($g_s$), (**d**) intercellular $CO_2$ concentration ($C_i$). a–c statistically significant differences between values within a term and at a confidence level of $p = 0.05$.

Analysing the values obtained for the E parameter, in the case of the Annushka cultivar, no significant effect of single-component foliar fertilisation was recorded at the first measurement date. On the second measurement date, the E values increased, but statistically significant differences were recorded only for Fe, Mn, B, and Mo. In the case of

Pompei, a significant effect of foliar fertilisation was recorded for Zn and Cu on the first measurement date. On the second measurement date, statistically significant differences were recorded for the fertilisation of Zn, Cu, Mn, B and Mo fertilisation.

For the $g_s$ parameter, for the Annushka cultivar, the foliar sprays of the single-component fertilisers did not produce a satisfactory result. A positive and significant effect of fertilisation was recorded only for B and Mo at the first and second measurement dates. In the case of the Pompei cultivar, fertilisation with Zn and Cu had the expected effect.

In the case of $C_i$, single-component foliar fertilisation on the first measurement date did not produce significant results in the Annushka and Pompei cultivar. On the second measurement date, a stimulating effect was observed for all the elements analysed.

## 4. Discussion

The increase in crop yields per area has resulted in a significant depletion of micronutrients in the soil. Micronutrient shortages have become a limiting factor in crop productivity on agricultural land around the world [15]. The amount of yield obtained depends, among other things, on efficient photosynthesis and the transport and distribution of assimilates.

In our study, the effect of single-component foliar fertilisation (Zn, Fe, Cu, Mn, B, Mo) on the physiological properties, nodulation, and green weight of soybean plants was analysed in a pot experiment. The fertilisation had a positive effect on the number of nodules on the soybean roots. Nodulation in the Annushka cultivar was most positively affected by foliar fertilisation with B and Fe; in the Pompei cultivar, B and Mo had the best effect compared to the control. Prusinski et al. [28] consider that, of the agrotechnical treatments, properly balanced fertilisation has a great influence on nodulation in addition to seed inoculation. Adeyeye et al. [29] and Adjetey and Mbotho [30] report that nodulation is not always correct in agricultural practice, which is attributed to various factors. They consider environmental stress or agronomic errors to be the most common cause. However, nodulation can be limited by many factors, including deficiencies of certain micronutrients; for example, Mo. Bacilieri et al. [21] showed that soybean biomass was mainly influenced by the cultivar. On the contrary, foliar fertilisation had little effect on plant biomass. Foliar fertilisation with B and Fe increased the weight of the green plant in the Annushka cultivar, which was not confirmed in the Pompei cultivar. Kim et al. [31] showed that micronutrient fertilisation had no effect on the increase in yield. In our study, single-component foliar fertilisers had a positive effect on green plant weight.

Research conducted by other authors on various crop species shows the effectiveness of micronutrient fertilisation in building plant resistance [32–34]. However, Sutradhar et al. [35] showed that in nutrient-rich soils, fertilisation with B, Mn, and Zn is unnecessary. Also, Gonçalves et al. [36] proved that the B fertilisation of soybean had less effect than expected. Therefore, fertilisation with this element was necessary [37]. In contrast, Sharma et al. [38] demonstrated that foliar Fe fertilisation is justified because it increases the bioavailability of this element in soybean seeds. The deficiency of this element also affects chlorophyll, and this can be observed as leaf chlorosis [39]. Almeida et al. [40] demonstrated that high doses of Cu and Zn did not have toxic effects on soybean crops. Yasari and Vahedi [41] showed that foliar Zn fertilisation had good results in soybean crops. In our study, we observed a positive effect of Zn fertilisation on the parameters analysed, but the effects we obtained did not coincide with our assumptions, which was similar in the case of Fe and Cu fertilisation. Fertilisation with Zn, Fe, and Cu positively influenced the values of the SPAD index in the Annushka cultivar, but a similar effect was not observed in the Pompei cultivar. Gheshlaghi et al. [42] showed that Zn fertilisation was justified during the drought period. This element significantly increased the chlorophyll b content and improved the quality of soybean seeds. Oliveira et al. [43] showed that foliar Zn fertilisation was effective in soybeans, but only when soil Zn availability was low. Furthermore, the cultivars showed different responses to Zn fertilisation. Heidarian et al. [44] showed the best results after combined fertilisation with Zn + Fe fertilisation, and Joorabi et al. [45] showed that Zn fertilisation increased antioxidant enzyme activities, changed physiological parameters, and reduced the effects of drought stress on soybean plants. Moreira et al. [46] shows that, in addition to increasing grain yield and dry matter, the application of Cu increases the photosynthetic rate, stoma conductivity, the internal $CO_2$ concentration, transpiration rate, and the chlorophyll content. However, the effectiveness of Cu use varies considerably between different cultivars [47].

Determining cell membrane permeability can be used as a physiological indicator of leaf activity [48–51]. Since SPAD values are fast and accurate methods for measuring chlorophenols [52,53], they are applied to scientific research and agricultural practices. In our own research, it was found that SPAD values depended on foliar fertilisation and increase with the duration of the experiment compared to the control. This proved that all micronutrients tested had a positive effect on the potential chlorophyll content. In the experiment of Bacilieri et al. [21], the measurements of the SPAD index depended on foliar fertilisation, cultivar, and the experimental location. Therefore, they claim that the effectiveness of foliar fertilisation is determined by many factors, including the level of the agro-technique used. In an earlier study, Jarecki et al. [54] confirmed that SPAD index measurements were positively affected by inoculation and foliar fertilisation. Better-nourished plants (higher SPAD) gave a higher yield, which they confirmed with a positive correlation (r = 0.83). Heitholt et al. [55] proved that SPAD values were higher when soybeans were fertilised with Cu, Mn, or Zn. Bacilieri et al. [21] showed that the measurement of the SPAD index varied between the cultivars tested and depended on the foliar fertilisation applied. Therefore, they argue that soybean cultivars should be selected according to local habitat conditions and the level of agrotechnology.

Various abiotic factors such as temperature, light, water, gases, and minerals are essential for the plant's lifecycle. In response to these factors, plants react to various physiological, biochemical, and morphological adaptations that can be observed. Chlorophyll fluorescence is an important tool for studying photosynthesis, and it is worth investigating its effects on the physiological indicators of plant growth. On the other hand, photosynthesis is a fundamental physiological activity necessary to shape crop yield, and photosynthesis strength is closely related to yield level [56,57]. Chlorophyll content and chlorophyll fluorescence are important markers of plant photosynthetic status [58,59]. Geng et al. [60] concluded that the fluorescent parameters of chlorophyll can reflect the absorption and conversion of light energy, energy transfer, distribution, and photosynthesis in plants, which are important indicators for the study of plant physiology. This is an effective method to analyse plant health and photosynthetic capacity under normal or stressful conditions [61,62]. In our study, we found that foliar fertilisation with single-component pre-preparations did not negatively affect the values of the analysed gas exchange and chlorophyll fluorescence parameters. Bărdas et al. [63] showed that foliar fertilisation would increase physiological parameters in soybean plants. Kobraee [64] showed that the application of Ze, Fe, and Mn had a significant effect on the chlorophyll concentration in the leaves of soybeans. They recorded the highest chlorophyll measurement after the combined application of Zn and Fe, Zn and Mn, or Fe and Mn, in appropriate proportions. Liu et al. [65] reported that Mo and B fertilisation affected the growth of the root system, biomass, and photosynthetic rate in soybean plants. In addition to this, they showed that the tested cultivars showed different sensitivity to Mo and B deficiency. However, the authors indicate that the increase in the relative chlorophyll content of the plant leaves is a result of the defence of the plant against stress. Therefore, it seems that these parameters cannot be simply translated into yield, which is the result of the interaction of a wide variety of factors and depends not only on the amount of assimilates produced, but also on their distribution and consumption [66,67].

Considering the benefits of leaf fertilisation, providing nutrients through leaf fertilisations is a good strategy to complement soil fertilisation. In addition, it is environmentally friendly because nutrients can be applied in controlled amounts and at specific times of plant growth. Foliar fertilisation with single-component fertilizers can be successfully applied to soybean crops when taking into account their nutritional needs due to soil richness, crop quality, and other agro-environmental factors.

## 5. Conclusions

The cultivars analysed reacted differently to foliar-applied micronutrients. In general, better single-component foliar fertilisation was observed at the second measurement date. The physiological parameters of the plants were most favourably influenced by Mo fertilisation for the Annushka cultivar and Cu fertilisation for the Pompei cultivar. All micronutrients tested were found to have a positive effect on the potential chlorophyll content. Foliar fertilisation with B and Fe increased the green weight of the plants, and the number of borers was most positively affected by foliar fertilisation with B and Fe (Annushka) and B and Mn (Pompei). Therefore, in this study, it was not possible to unequivocally choose an optimally performing micronutrient. It would be important to repeat the experiment under intensive plant stress conditions, when plants show a significant need for support from foliar fertilisers. For crop growth under good and nutrient-adapted conditions, the foliar feeding of micronutrients may not be economically justified.

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