



# Different type of copper and nitrogen priming application alters morphology and physiology in *Miscanthus x giganteus*

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## ABSTRACT

This study investigated the effects of copper and nitrogen priming on the morphology, physiology and lignocellulose components of the industrial crop *Miscanthus x giganteus* aiming to improve plant resilience and productivity under stress conditions. Nitrogen and copper priming solutions were applied to rhizomes or leaves of plants at three concentration levels. Nitrogen priming in some variants enhanced biomass production, particularly through foliar application. On the other hand, higher dose of copper applied to rhizomes exhibited concentration- and application-dependent effects on lignin and cellulose content, influencing the structural integrity of cells. Nitrogen priming significantly increased leaf size, mainly in foliar-applied variants, whereas copper treatment had variable morphological effects. Stomatal density increased with nitrogen treatment and decreased with copper. These findings offer insights into optimizing physiological performance under elevated temperatures in *Miscanthus x giganteus* for sustainable biomass production based on intended utilization. We hypothesize that nitrogen priming, particularly via foliar application, enhances growth and physiological performance of *Miscanthus x giganteus*, while copper priming induces stress-related changes in morphology and secondary metabolism depending on the dose and application method.

## Abbreviations

CRII	Carotenoid Reflectance Index 1
Fo	Minimal fluorescence, fluorescence level when electron acceptors are fully oxidized
Fm	Maximal fluorescence, fluorescence level when electron acceptors are fully reduced
Fv/Fm	The total performance index for the photochemical activity (photosystem II), the maximum quantum yield of photosystem II
G	Greenness index
MCARI	Modified Chlorophyll Absorption in Reflectance Index
NDVI	Normalized Difference Vegetation Index
OJIP	Analysis of fast fluorescence induction kinetics of chlorophyll and other key phases of photosynthesis
Φ <sub>Do</sub>	Quantum yield of energy dissipation
Φ <sub>EO</sub>	Quantum yield of electron transport
Ψ <sub>o</sub>	Probability that the electron will move further to transport chain
SR	Simple ratio index

(continued on next column)

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Vi	Relative variable fluorescence at I-Step
Vj	Relative variable fluorescence at J-Step

## 1. Introduction

*Miscanthus x giganteus* (MxG) is a high-yield perennial grass that is widely recognized as a promising crop for the production of sustainable lignocellulosic biomass with many applications. It can provide multiple benefits, such as high dry matter yield, persistent growth, low nutrient requirements, efficient conversion of radiation to biomass, efficient use of nitrogen and water, and good resistance to pests, diseases, and various contaminants [1]. Relatively low requirements for soil quality pre-determine it for cultivation on marginal sites. Also, its cultivation can

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improve soil health via carbon sequestration, rhizosphere microbial stimulation, erosion prevention, or other processes [2]. Furthermore, MxG cultivar is a sterile hybrid not propagated via seeds, which limits invasive spread [3–6].

Climate change, mainly due to increased temperatures and drought, has been imposing harsher conditions even on plants adapted to mild climates. It is thus important to raise our awareness of how abiotic stress affects their physiology. Although miscanthus is generally considered as a stress-tolerant and undemanding crop, suboptimal conditions lower its yields [7,8], as in every crop. Nevertheless, the sensitivity to different abiotic stress factors is highly variable.

Among climatic conditions, especially cold is a problem in miscanthus cultivation. Except for rhizome damage by severe frost in winter, which highly affects plant survival range, miscanthus can also suffer from shoot damage mainly at the beginning and at the end of the growing season [9]. However, frost tolerance increases after the first winter thanks to a rhizome hardening (priming) mechanism [10]. The effect of high temperatures is less explored, but both cold (10 °C) and heat (34 °C) reduced biomass and modified plant physiology in controlled priming experiment [11]. Drought can be another negative factor, despite the fact that MxG is more drought resilient compared to its parental non-hybrid species [12,13]. Yield comparable to non-drought years was recorded in a year with higher temperatures and rainfall shortage in a field study, and even slight increase in biomass was caused by short-term drought under controlled conditions [11]. Nevertheless, the climatic conditions in field affected the duration of vegetation season, represented by leaf area index, and decreased net ecosystem efficiency in the following year, suggesting post-drought effect [14].

Soil conditions can also significantly affect miscanthus growth, which should be especially considered in case of marginal lands. Especially the lack of available nutrients negatively affects miscanthus yields and physiology [15]. The cultivation in poor sandy soil had even higher negative impact than high-level contamination with heavy metals [8]. Nevertheless, exceeding heavy metal content in soil can also result in reduced growth with variable intensity based on element concentration and other soil parameters [1,6,16,17]. MxG is also moderately sensitive to increased salinity [18].

The stressful conditions affecting plant growth are often repetitive, so most plants have developed strategies to cope with them. Plants are able to remember their first encounter with stress (so-called primary stress), so they can then respond more effectively in the future [19,20]. Priming is a process where a regulated dose of primary stress is applied to the plant in the early stages of its development, usually to the seed [21–28], leading to faster activation of defense mechanisms [29]. Since the pathways of plant defense are usually non-specific, the application of one type of stress often results in an overall more resilient individual [29–31].

Many types of priming can be used, i.e. biological (biological agents like bacteria), physical (physical stressors like cold or heat) or chemical, which allows easy application of chemicals in the form of solutions. Various chemicals can be used for priming of seeds to improve growth and stress tolerance [25,28,32–40]. MxG, since it does not produce seeds, can be primed only on the rhizome or leaves. In the present study, we operationally define priming as single, one-time applications of nitrogen or copper solutions to rhizomes or young plants, intended to induce physiological preparedness and stress memory mechanisms rather than to provide continuous nutrient supplementation.

According to our best knowledge, only one rhizome priming experiment has been conducted so far, with the application of biological priming and hydropriming to ginger (*Zingiber officinale*) [30]. In contrast to the use of rhizomes for priming, which is not very common, spraying leaves with micronutrients to increase crop productivity is a well-established method. Compared to applying them to soil, foliar application is often more effective, but excessive application can lead to abnormalities in crop growth and burns [31]. Popular type of chemical priming is called nutripriming, based on improving the availability of

nutrients and water. This method uses pre-treating solutions of nutrients like Mg, Zn, Ca, or others to improve growth, development or flowering. The crucial role of nutripriming is the dose/concentration of the solution and the duration of application [41].

Since different types of chemical priming have already been tested in our previous study with miscanthus with various results [11], we further focused only on the two most promising types, nitrogen and copper. While application of 10mM  $\text{NH}_4\text{NO}_3$  used for watering over a period of 28 days resulted in higher number of tillers, improved biomass yield, and an increase in cellulose content, the similar application of 20  $\mu\text{g/day}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  led to elevated lignin content. The following experiment focused on testing the various concentrations and types of application (rhizomes and foliar) of nitrogen and copper solutions in order to optimize the treatment protocol for practical applications. It was designed as a one-year, small-scale greenhouse experiment to serve as a pilot study investigating the initial physiological and plantation growth responses of MxG to copper and nitrogen priming during the establishment phase. The experiment was conducted under increased temperatures during summer months (up to 45 °C) to simulate rising temperature conditions, which are of increasing relevance in the context of climate change, while maintaining adequate irrigation to isolate the temperature effect from confounding drought stress.

Based on the previous findings of plant responses to nitrogen and heavy metal exposure, we formulated the following hypotheses: (1) nitrogen priming will stimulate biomass production and photosynthetic efficiency, with stronger effects following foliar application; (2) copper priming will induce stress responses manifested in altered lignin biosynthesis and stomatal density, with outcomes depending on both concentration and method of application.

## 2. Materials and methods

### 2.1. Experimental design

A total of 15 experimental variants were prepared (Table 1). One part of the experiment focused on the application of chemicals to plant rhizomes before planting, and the other was devoted to the application of chemicals to the leaves of young plants. The rhizomes part included 6 variants with priming (soaked for 24 h in 1 L of  $\text{NH}_4\text{NO}_3$  or  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  water solutions with various concentrations directly before planting on May 11, 2021) and control "Ctrl H<sub>2</sub>O R" with rhizomes soaked in distilled water according to the similar protocol. Each treatment was prepared in 6 replicates. Analogically, 6 more primed variants along with control "Ctrl H<sub>2</sub>O L" were prepared for foliar application (labelled as "L"). Those variants were treated with 100 mL of solution (or distilled water) by spraying on leaves of young plants (45 days after planting on June 25, 2021). Due to space limitations and practical issues of spraying to ensure consistent spray coverage, each treatment contained only 3 replicates.

**Table 1**  
Experimental setup.

Variant	Treatment	Volume and concentration	Pots
Ctrl	no	no	6
Ctrl H <sub>2</sub> O R	Soaking of rhizome 24 h	1 L of H <sub>2</sub> O	6
N 2.4 R	Soaking of rhizome 24 h	1 L of 2.4 g/L $\text{NH}_4\text{NO}_3$	6
N 12 R	Soaking of rhizome 24 h	1 L of 12 g/L $\text{NH}_4\text{NO}_3$	6
N 24 R	Soaking of rhizome 24 h	1 L of 24 g/L $\text{NH}_4\text{NO}_3$	6
Cu 2.4 R	Soaking of rhizome 24 h	1 L of 2.4 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	6
Cu 12 R	Soaking of rhizome 24 h	1 L of 12 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	6
Cu 24 R	Soaking of rhizome 24 h	1 L of 24 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	6
Ctrl H <sub>2</sub> O L	Spraying of young plant	100 mL of H <sub>2</sub> O	3
N 2.4 L	Spraying of young plant	100 mL of 2.4 g/L $\text{NH}_4\text{NO}_3$	3
N 12 L	Spraying of young plant	100 mL of 12 g/L $\text{NH}_4\text{NO}_3$	3
N 24 L	Spraying of young plant	100 mL of 24 g/L $\text{NH}_4\text{NO}_3$	3
Cu 2.4 L	Spraying of young plant	100 mL of 2.4 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	3
Cu 12 L	Spraying of young plant	100 mL of 12 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	3
Cu 24 L	Spraying of young plant	100 mL of 24 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	3

Additionally, there were 6 more pots with absolutely no treatment (not even water treatment) labelled "Ctrl".

The pot experiment was conducted at the Department of Biology UJEP in Ústí nad Labem (Czech Republic). For all variants, 250 mL of sand drainage and 4 L of AGRO horticultural substrate were used in each pot. The characteristics of the substrate are provided in [Supplemental Table S1](#). No further fertilization was applied during the experiment to isolate the effects of priming treatments. Planting took place on May 12, 2021, and two rhizomes purchased from HVG, a Handels & Vertriebsgenossenschaft cooperative based in Cheb (Czech Republic), were inserted into each flowerpot. All plants were placed in a greenhouse, where they were regularly watered with deionized water, depending on the outside temperature. The plants in the greenhouse were not exposed to such demanding conditions as plants living outdoors (rain, direct sun, herbivores) since the greenhouse is well ventilated and the upper windows are equipped with shading. However, this greenhouse is exposed to the sun and becomes very hot during summer months. In July and August, temperatures in the greenhouse reached up to 45 °C, simulating elevated temperature conditions relevant to climate change scenarios. Unlike field conditions where high temperatures typically coincide with drought, plants were regularly irrigated to isolate the effect of elevated temperature from water deficit stress. During the growing season, the temperature did not fall below 15 °C. In December, when the plants were already browned, the temperature did not fall below 10 °C due to heating. The experiment lasted for one vegetation season and was finished in December by biomass harvest (chapter 2.2).

## 2.2. Biometric parameters and biomass composition

The basic biometric parameters, shoot height and the number of stems, were determined three times during the vegetation season on July 15, August 17 and September 15, 2021. Height was measured as the length of the longest shoot from the soil surface to the top of the last leaf.

At the end of the vegetation season (December 7, 2021) when the plants were completely browned and withered, they were harvested and weighed for raw biomass. Consequently, four (rhizomes treatment) or three (foliar treatment) samples of whole plants from each variant were dried at 55 °C for 48 h and ground to pass through 1 mm sieve of a knife mill (Brabender Technologie GmbH & Co. KG, Duisburg, Germany). In ground samples, dry matter content was estimated according to European Union standards for sampling and analysis [32]. Neutral detergent fibre with  $\alpha$ -amylase and sodium sulfite corrected for ash (aNDF) was estimated according to standard ISO 16472 [33], ash-free acid detergent fiber (ADF) and acid detergent lignin (ADL) according to standard ISO 13906 [34]. The content of cellulose was then calculated as ADF-ADL and hemicellulose content as NDF-ADF.

## 2.3. Analysis of leaf morphology

From each plant, the third fully developed leaf from the top of the plant was cut off with scissors as close as possible to the stem. The length of the leaf blade was then measured using a meter, and the width was measured in the widest part of the blade using a ruler. The same leaves were further used in the counting of stomata. To count the number of stomata, the microrelief method was used. Stomata reliefs were counted using the BEL capture program (BEL Engineering, Italy). The number of stomata on the upper and lower leaves was recalculated to 1 mm<sup>2</sup>, three measurements per plant.

## 2.4. Fluorescence and reflectance analysis

The physiological state of plants was monitored using analyses of chlorophyll fluorescence and leaf reflectance three times during the vegetation season (at the same time as plant height and the number of stems in chapter 2.2). A total of 186 measurements were made in thriving individuals. Seven plants did not survive the application or died

soon after application (will be assessed later in chapter 3.1). Consequently, one sample of "N 24 L" variant was eliminated from August fluorescence measurement due to invalid results caused probably by improper handling with fluorometer. Analogically, one sample was eliminated from reflectance results variants "N 24 R" and "Cu 12 R" measured in July.

Leaf fluorescence was measured using a FluorPen FP 110 fluorometer (PSI, Drásov, Czech Republic), which is equipped with a blue LED emitter (455 nm), emitting a maximum light intensity of 3000  $\mu\text{mol}/\text{m}^2/\text{s}$ . Detector is PIN photodiode with 667–750 nm bandpass filters. Fluorescence was measured after 15 min of dark adaptation of the samples. The fluorescence transients (OJIP curves) were plotted as the mean values for individual treatments. Various fluorescence indices were calculated according to the manufacturer's instructions ( $F_o$ ,  $F_m$ ,  $V_j$ ,  $V_i$ ,  $F_v/F_m$ ,  $\Psi_o$ ,  $\Phi_{Eo}$ ,  $\Phi_{Do}$ ).

The content of leaf pigments was measured using PolyPen PR410 UVIS (PSI, Drásov, Czech Republic) using a non-invasive method of pigment reflectance (wavelengths between 340 and 780 nm). In addition, various vegetation indices were calculated to assess plant health and relative pigment content (NDVI, SR, G, MCARI, CRI1) according to the instructions of manufacturer.

## 2.5. Statistical analysis

All statistical analyses were performed using R software (ver. 4.3.1; [42]). And data visualization in boxplots (supplementary material) was carried out using the ggplot2 package (ver. 3.4.4; [43]). Data normality was evaluated using the Shapiro–Wilk test. As some groups did not meet the assumption of normality, non-parametric tests were applied. The analysis focused on comparing each priming treatment directly to the related water-treated control group using the Wilcoxon rank-sum test. Besides that, water-treated controls ("Ctrl H<sub>2</sub>O R" and "Ctrl H<sub>2</sub>O L") were compared to non-treated control ("Ctrl") to distinguish the effect of water and chemicals application. Consequently, the graphs used in text were plotted using Microsoft Excel 2019 (OJIP curves) and OriginPro 2024 (line and spider plots).

Additionally, Pearson correlation analyses were performed to examine relationships among measured parameters within each measurement period and between physiological measurements and final harvest parameters.

## 3. Results and discussion

### 3.1. Biomass yield and growth dynamics

The biomass yield is usually the most relevant parameter for potential practical application of the new technology in terms of energy and industrial crops.

The experimental evaluation of the effects of priming treatments administered by soaking and spraying techniques unveiled variable effects on plant development ([Table 2](#), [Supplemental Fig. S1](#)). Surprisingly, the variations were found also in the control groups treated only by distilled water. While rhizomes soaking ("Ctrl H<sub>2</sub>O R") did not result in significant change of biomass yield compared to non-treated control ("Ctrl") and the median value was lower, leaf spraying ("Ctrl H<sub>2</sub>O L") led to the median biomass higher by 38 %, although insignificantly due to the low number of replicates in L variants. This unexpected trend brings up interesting questions about the role of water in leaf physiological processes, possibly related to stomatal opening and the rise in carbon dioxide uptake that comes with it. Given that control plants remained generally healthy with adequate irrigation, the substantial biomass increase following water spraying likely reflects optimization of plant performance under elevated temperatures rather than rescue from severe stress. Alternatively, this effect could be explained by several complementary mechanisms. Leaf spraying might have transiently reduced leaf surface temperature, which in the greenhouse conditions

**Table 2**

Biomass weight and composition and plants survival rate in different treatments; median (IQR, Q3-Q1); an asterisk indicates value significantly different from related water-treated control "Ctrl H<sub>2</sub>O R" or "Ctrl H<sub>2</sub>O L" and plus sign indicates significant difference of water-treated controls from non-treated control "Ctrl" (Wilcoxon rank-sum test,  $\alpha = 0.05$ ); ND = not determined.

Treatment	Biomass [g]	Lignin [% dw]	Cellulose [% dw]	Hemicelluloses [% dw]	Survival rate [%]
Ctrl	87 (28)	8.8 (0.3)	45.2 (1.5)	27.2 (1.0)	100
Ctrl H <sub>2</sub> O R	59 (25)	9.2 (0.5) <sup>+</sup>	45.6 (1.8)	26.3 (0.9)	100
N 2.4 R	78 (36)	10.1 (0.6)	46.6 (2.9)	26.3 (1.8)	100
N 12 R	66 (13)	9.8 (0.8)	45.2 (2.0)	26.8 (2.5)	100
N 24 R	78 (33)	8.9 (0.5)	44.7 (2.1)	25.9 (1.3)	100
Cu 2.4 R	61 (22)	9.7 (1.8)	44.3 (0.6)	25.6 (0.9)	100
Cu 12 R	15 (11)*	7.6 (0.3)*	41.1 (0.8)*	24.0 (4.7)	67
Cu 24 R	ND	ND	ND	ND	17
Ctrl H <sub>2</sub> O L	119 (26)	9.1 (1.3)	46.9 (1.7)	26.2 (0.8)	100
N 2.4 L	135 (31)	9.0 (0.8)	47.8 (1.1)	25.9 (1.0)	100
N 12 L	116 (29)	8.9 (1.1)	47.9 (1.8)	26.1 (1.6)	100
N 24 L	135 (48)	9.0 (0.6)	48.3 (2.9)	26.9 (0.4)	100
Cu 2.4 L	113 (34)	11.3 (1.4)	49.0 (2.4)	25.8 (3.2)	100
Cu 12 L	110 (32)	10.0 (1.7)	46.6 (1.7)	25.5 (1.0)	100
Cu 24 L	108 (16)	9.3 (0.3)	45.8 (0.6)	26.1 (1.7)	100

frequently reached up to 45 °C. It has been shown that decrease of Fv/Fm during midday depression of citrus trees can be mitigated via water spraying [35]. Also, field observations of *Prosopis velutina* revealed that evaporative cooling could reduce canopy leaf temperature by up to 5 °C, depending on water availability. This drop of the temperature has significant physiological consequence beyond simple cooling, as it was shown to increase the carbon uptake by reducing leaf respiration by approximately 15 % in the middle of the growing season [36]. Furthermore, the removal of any accumulated dust or pathogens from leaf surfaces could have improved light interception for photosynthesis. Even though the greenhouse has a specific environment with limited access of external particles, dust and various spores could still accumulate on leaves, decreasing photosynthetic rate [37]. It is also plausible that direct foliar water absorption contributed to maintaining leaf turgor and hydration, optimizing physiological processes during periods of high temperatures.

The precise mechanisms underlying this noticeable growth in miscanthus would dedicate further investigation, as it could offer a simple strategy for enhancing productivity in controlled environments or during specific periods. Given the high temperatures (up to 45 °C) experienced in the greenhouse, factors such as transient leaf cooling and maintenance of turgor through foliar water application might have played more significant role, than what might be expected under milder conditions. These temperature conditions, while elevated, did not induce severe stress in adequately irrigated control plants (chapter 3.4), suggesting that the observed treatment effects reflect optimization of performance rather than stress mitigation. Therefore, further research with increased replication would be beneficial to fully substantiate these trends and explore the underlying mechanisms with greater statistical power.

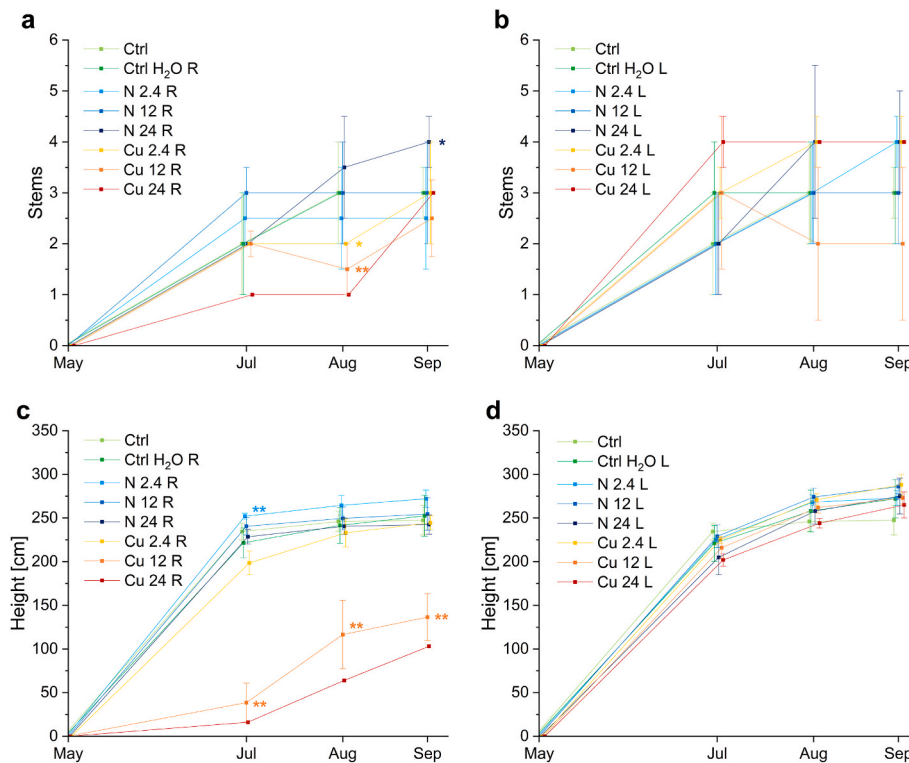
The increased biomass yield after foliar treatment compared to non-treated plants was noted also with nitrogen solutions. However, it did not result in additional improvement compared to ("Ctrl H<sub>2</sub>O L"), so the effect could not be attributed to the role of chemical. In the case of application on rhizomes, nitrogen solutions did not have any significant effect and the biomass yields were comparable to appropriate controls and lower than the corresponding concentration variants applied by leaf spraying.

The variable effect of different application methods was even more pronounced in the case of copper. While the copper foliar treatment did not significantly affect biomass yield, the application of copper solution on rhizomes dramatically reduced it. The effect was highly dose dependent. The lowest dose ("Cu 2.4 R") was comparable to "Ctrl H<sub>2</sub>O R" but the higher copper doses resulted in dramatic decrease of biomass by 76 % ("Cu 12 R") or even mortality ("Cu 24 R"). Since the substrate contained no detectable copper (measured by handheld XRF) prior to

treatment (Supplemental Table S1), the observed phytotoxic effects can be directly attributed to experimental copper applications. These dramatic reductions clearly represent phytotoxic stress responses rather than beneficial priming effects. These results are consistent with published cases of copper-induced phytotoxicity [38–40]. The substantial decrease in plant biomass already seen at the low copper concentrations in miscanthus, indicates that the plants are highly sensitive to copper. This sensitivity may be due to interference with enzyme activity and photosynthesis, as identified in other higher plants [44–47].

The growth dynamics documented by shoot height and the number of stems (Fig. 1 and Supplemental Figs. S3 and S4) provided further context to the biomass results, although the overall treatment trends closely reflected those observed for yield. Plants achieved nearly their final height within the first two months, demonstrating rapid early growth typical for MxG [16]. That effect was even more pronounced in the case of rhizome-treated plants. However, rhizome treatments with higher copper doses markedly suppressed early growth. The surviving plants showed delayed recovery and renewed elongation later in the season, even under high greenhouse temperatures. This rebound suggests partial acclimation following initial copper-induced stress. In contrast, rhizome treatment with the highest nitrogen dose ("N 24 R") promoted tillering by extending the period of new shoot emergence, leading to a higher number of stems in late summer. However, the dose-dependent trend in height of the nitrogen-treated plants was opposite to stems, so the higher number of tillers did not lead to increased biomass yield due to the shorter shoots. Although foliar treatments did not produce statistically significant differences due to the lower number of replicates, interesting temporal patterns were evident: non-treated plants ("Ctrl") exhibited the shortest shoots throughout the experiment. The number of stems in "Cu 12 L" declined between July and August, indicating partial dieback after initial growth, while "N 2.4 L" continued to form new tillers until the end of the season. The prolongation of vegetation season after supplementation with nitrogen was observed already in the previous priming study [11].

To conclude, if seen strictly from the perspective of biomass production, priming rhizomes with nitrogen and copper did not seem to be promising. Nevertheless, the effects on other relevant characteristics, such as biomass composition and plant stress response, were further investigated. Because miscanthus is a perennial crop and the experiment was only short-term, it could not be ruled out that modification of plant physiology and stress resiliency would also lead to changes in plant survival and biomass yield in long-term perspective. However, the long-term evaluation would have to be conducted under field conditions, because miscanthus massive root development limits the durability of pot studies as the limitation of space could be an additional stress factor affecting plants.



**Fig. 1.** Median values and IQR (Q3-Q1) of **a, b**) number of stems and **c, d**) shoot height of **a, c**) rhizome-treated and **b, d**) foliar-treated variants during experiment; asterisks indicate value significantly different from related water-treated control "Ctrl H<sub>2</sub>O R" or "Ctrl H<sub>2</sub>O L" (Wilcoxon rank-sum test, \* $p < 0.05$ , \*\* $p < 0.01$ ), only the data of living plants are included ( $n = 1-6$ ).

### 3.2. Lignin, cellulose and hemicelluloses content

The content of lignocellulose components (lignin, cellulose and hemicelluloses) is one of the main parameters indicating the suitability of biomass for further utilization. Miscanthus is processed in various value chains including heating, construction materials, bioethanol or biogas production, which have different requirements for lignin/holocellulose (cellulose + hemicelluloses) ratio. Thus, both, increase and decrease, of the components content could be beneficial in some cases and selection of the proper treatment protocol can improve the effectiveness of further processing.

As documented in Table 2 and Supplemental Fig. S2, the lignin content was generally very low, as the reported values for miscanthus are usually between 13 and 24 % and hemicelluloses values were in the middle of the reported range 16–34 % [3,11]. On contrary, cellulose content was rather high (reported values 30–49 %).

Water application in control samples had variable effect on lignocellulose components. While rhizomes soaking in distilled water ("Ctrl H<sub>2</sub>O R") caused significant increase in lignin content, leaf spraying ("Ctrl H<sub>2</sub>O L") led to higher value of cellulose, although insignificantly due to the low number of replicates of L samples (Supplemental Fig. S2b). Hemicelluloses contents were unaffected by pure water, no matter the type of application. It is possible, that initial soaking of the rhizome caused short-term hypoxia of this organ, that led to ethylene production, inducing cascade of lignin production [48]. On the other hand, leaf spraying might enhance surface cell hydration, temporarily enhance turgor pressure and activate cellulose synthase [49].

It is known that availability of nutrients, including nitrogen and copper, can substantially influence plant cell wall composition [50–54] which was observed also in our results. The nitrogen treatments, applied in varying concentrations and types of application, demonstrated a range of effects on lignin. The low dose of nitrogen applied on rhizomes ("N 2.4 R") showed a slight increase compared to the water-treated control group ("Ctrl H<sub>2</sub>O R") (Supplemental Fig. S2a), indicating that

nitrogen availability could influence lignin biosynthesis or accumulation in the samples. However, with increasing nitrogen concentration the lignin content continuously decreased to values similar to controls. In our previous study, after repeated soil supplementation with 10mM NH<sub>4</sub>NO<sub>3</sub> lasting for 28 days, the lignin content in miscanthus even decreased by 5 % compared to non-treated control [11] suggesting that excessive nitrogen may downregulate the lignin biosynthetic pathway and result in lower lignin contents as described earlier for poplar and *Eucalyptus* [55,56]. Nevertheless, this pattern was not evident in plants that were sprayed with a nitrogen solution ("N 2.4 L", "N 12 L", "N 24 L"). In these plants, the content of lignin remained rather consistent, similar to plants treated with water ("Ctrl H<sub>2</sub>O L") indicating a possible compartmentalization effect where lignin synthesis is variable in different plant tissues [57,58].

On the other hand, contrarily to our previous results, where NH<sub>4</sub>NO<sub>3</sub> applied in the soil resulted in a dramatic increase in cellulose by 24 % [11], the effect of nitrogen priming applied directly to plant tissues on cellulose and hemicelluloses content was minimal. Nevertheless, analogically to lignin, the decreasing trend in cellulose content with increasing nitrogen dose was observed in rhizome treatment. The response to nitrogen treatments could be indicative of a regulatory mechanism, where nitrogen availability influences carbohydrate allocation and cellulose synthesis, possibly as an adaptive strategy for growth optimization [59,60,61,44].

Conversely, the reaction to copper treatments was significant in both components, lignin and holocellulose, with variable reactions to application method and concentration. The groups treated with copper had a broader spectrum of lignin and cellulose contents than nitrogen-treated. The higher copper concentrations mostly resulted in decreasing lignin and cellulose levels independently of the application. Nevertheless, in comparison with controls ("Ctrl H<sub>2</sub>O R" and "Ctrl H<sub>2</sub>O L") the different treatment methods resulted in different reactions. The rhizome soaking led to a significant decrease of both, lignin and cellulose, in "Cu 12 R". The hemicelluloses content also slightly decreased after rhizomes

treatment, however with much higher data variability (Supplemental Fig. S2c).

On the contrary, following the application of copper to the leaves, slight elevation in lignin content was observed at the lowest concentration ("Cu 2.4 L") (Supplemental Fig. S2a). This shows a defense response, caused by stress due to copper exposure, potentially triggering defense mechanisms including lignification as found in tobacco [45]. This is also consistent with the role of copper as a micronutrient that, when in excess, can lead to toxic effects, thereby inducing the synthesis of lignin as a form of plant defense [46]. Conversely, elevated copper doses, reduced lignin ratio to level similar to control, suggesting that high doses of copper influence more metabolic pathways. The observed variability in lignin content in different treatments underscores the intricate nature of plant metabolic plasticity. It suggests that plants may finely tune their lignin content in response to the availability of certain nutrients or the presence of heavy metals, reflecting an adaptive balance between growth, structural stability and defense [47]. Similarly to lignin, the decreasing trend in cellulose content with increasing copper dose was observed. It suggests reaction scenario potentially involving stress-induced metabolic shifts [62]. Copper, as a heavy metal, might trigger defense mechanisms in plants, leading to altered carbohydrate metabolism and cellulose synthesis [63].

Overall, these findings emphasize the intricate nature of plant responses to environmental factors, specifically nutrient availability, and the importance of considering such variables in studies of plant carbohydrate and lignin metabolism and practical application in crop production. Future research should focus on elucidating the molecular pathways involved in these responses, offering deeper insights into plant adaptation strategies under varying nutrient and stress conditions, and evaluation of the results in field conditions.

### 3.3. Leaf morphology

#### 3.3.1. Leaf width and length

Leaves are the fundamental plant structure, where the most important plant process, photosynthesis, takes place. Therefore, analysis of its properties is essential.

The statistical test results did not indicate significant differences in leaf length and width between the control group and plants of most variants with nitrogen treatment (Table 3). The only exception was the middle concentration applied on rhizomes ("N 12 R"), which resulted in significantly narrower leaves than control. The nitrogen rhizomes treatment had a dose-dependent trend with the narrowest leaves after the lowest dose application ("N 2.4 R"), however the difference from control in this variant was insignificant due to the higher data variability.

Unlike nitrogen, substantial differences in leaf length and width were found in variants with copper, both rhizome- and foliar-treated. Variant "Cu 12 R" (rhizome soaked) displayed a statistically significant impact in the form of shortening and narrowing of the leaves. On the other hand, the same concentration of copper applied to the leaf ("Cu 12 L") had a completely opposite effect. All leaves after the application of this concentration of copper were longer and wider, although this trend was not statistically significant due to the low number of replicates ( $n=3$ ) and should be interpreted cautiously. Additionally, while rhizomes copper treatment had a clear dose-dependent trend, the foliar treatment was effective only in the middle concentration. The response to different copper applications can provide insights into how copper influences leaf morphology. The reduction in stomatal density observed with copper treatments reflects a stress-induced morphological adjustment rather than a beneficial adaptive priming effect.

These findings indicate that both, the method of application and solution concentration, might play a role in how these treatments affect leaf morphology. The effect of application method could be due to differences in how the plant absorbs and utilizes these nutrients or elements. Especially for nitrogen, it seems that the nutrients are more

**Table 3**

Leaf characteristics of experimental plants; median (IQR, Q3-Q1), asterisks indicate value significantly different from related water-treated control "Ctrl H<sub>2</sub>O R" or "Ctrl H<sub>2</sub>O L" (Wilcoxon rank-sum test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

Treatment	Plants count	Adaxial stomata	Abaxial stomata	Leaf length	Leaf width
Ctrl H <sub>2</sub> O R	6	14.4 (3.1)	43.0 (3.7)	80.5 (13.5)	2.6 (0.2)
N 2.4 R	6	17.7 (4.5)**	45.4 (5.1)	77.5 (9.0)	2.1 (0.5)
N 12 R	6	16.2 (4.0)	39.3 (7.5)	77.6 (12.0)	2.4 (0.5)*
N 24 R	6	16.5 (3.6)	44.5 (6.9)	80.8 (7.5)	2.7 (0.3)
Cu 2.4 R	6	15.4 (4.0)	39.4 (11.1)*	80.0 (14.5)	2.5 (0.6)
Cu 12 R	6	11.6 (4.0)***	30.3 (7.8)***	64.8 (14.0)*	1.7 (0.2)**
Cu 24 R	1	7.7 (2.6)**	27.3 (2.5)**	60.0	1.4
Ctrl H <sub>2</sub> O L	3	16.1 (4.2)	43.6 (5.2)	72.0 (16.0)	2.1 (0.2)
N 2.4 L	3	16.7 (2.9)	45.3 (6.2)	83.9 (13.2)	2.3 (0.5)
N 12 L	3	17.1 (4.0)	44.6 (7.9)	73.2 (14.0)	2.3 (0.5)
N 24 L	3	16.3 (3.9)	42.3 (10.1)	83.2 (11.0)	2.3 (0.7)
Cu 2.4 L	3	15.8 (7.4)	37.8 (5.0)*	80.0 (9.5)	2.3 (0.5)
Cu 12 L	3	17.5 (2.6)	44.3 (4.2)	95.7 (1.1)	2.5 (0.1)
Cu 24 L	3	17.7 (2.2)	45.1 (7.6)	74.0 (5.0)	2.1 (0.3)

effectively utilized in the leaves when absorbed from the soil (via the rhizome) compared to direct foliar application. This phenomenon has been observed in other crops where soil-based nutrient delivery resulted in better nutrient uptake and plant growth compared to direct foliar application. The insignificance of the leaf spraying treatments could also be due to the concentrations used or specific characteristics of miscanthus plants affecting how they absorb and respond to foliar-applied nutrients. In some studies, foliar applications have shown limited effectiveness due to inadequate penetration and absorption of nutrients through the leaf surface [64,65].

#### 3.3.2. Number of stomata

Measuring the number of stomata on the adaxial (top) and abaxial (bottom) leaf surfaces is crucial because they influence vital physiological activities like photosynthesis and leaf gas exchange [66]. While the abaxial surface is typically more involved in gas exchange, the adaxial surface is usually more involved in light-harvesting reactions.

The analysis of stomatal density on both the adaxial and abaxial leaf surfaces under various treatments revealed notable disparities with differential effects of nitrogen and copper (Table 3). This difference and variability, along with presence of outliers suggests a heterogeneous response to stress, which could be potentially a strategy to survive.

The abaxial surface appears to respond better to copper than nitrogen treatment. However, analogically to leaf width and length, only the rhizomes treatment resulted in dose-dependent trend, where the number of bottom stomata continuously decreased with increasing copper concentration. In the case of foliar application, only the lowest dose caused significant decrease compared to control.

The adaxial surface shows high sensitivity to copper only when applied on rhizomes. The number of stomata continuously decreased with increasing copper concentration. The rhizome copper effect is consistent with research suggesting that changes in stomatal density and distribution on the adaxial surface may be a strategy to regulate transpiration and minimize water loss under high temperatures [50,51,66]. On the other hand, stimulatory effect had the application of low nitrogen

dose "N 2.4 R", which resulted in slight but significant increase of stomata. It was reported that plants with increased stomatal density have a greater capacity for nitrogen uptake [52].

These differences between the leaf surfaces show the complex and surface-specific responses of plants to environmental factors. Understanding these dynamics is crucial for comprehending the nuanced physiological adaptations that plants employ in response to different elemental exposures [53]. The distinct reactions to nitrogen and copper treatments emphasize the importance of considering leaf surface specificity in studies of plant environmental responses and stress tolerance [54] and could have potential implications in plant water management under drought stress conditions.

### 3.4. Fluorescence analysis

Analysis of chlorophyll fluorescence is one of the common approaches to investigate plant physiology, focused on photosynthesis and stress reactions. Thus, alterations in the fluorescence levels and derived parameters provide information on the effectiveness of tested treatments in improving plant resiliency.

Fv/Fm (Fig. 3 and Supplemental Fig. S5e) is a widely used index to assess plant stress levels. Its universality has been used as the first step of analysis in this study. The values ranged between 0.75 and 0.81, which

corresponded to the values generally indicated in healthy plants [67, 68]. Additionally, they did not display dramatic changes during the vegetation season. Nevertheless, the significant effect of copper application, especially in the form of rhizome treatment, was documented with this index. Although the significant increase compared to water-treated control was determined only in "Cu 12 R" in July, the dose-dependent increasing trend was documented during the whole vegetation season in rhizomes-treated and in July also in foliar-treated plants. That indicated that primary photochemistry might not be affected despite retarded plant growth of R plants and the copper had a stimulating effect with the highest relevance at the earlier stage of plant development. Oppositely, the nitrogen treatment effect was observed mostly at the end, in September, when the Fv/Fm of rhizome-treated plants continuously increased with nitrogen concentration. Nevertheless, as it was previously noted that this index is not highly sensitive [69], the fluorescence transients and several other derived indices were further determined to investigate the effects on photosynthetic apparatus in more detail.

The mean fluorescence transients of experimental plants (Fig. 2) developed variably during the vegetation season. In July, 9 weeks after experiment establishment, the OJIP curves began to diverge markedly between control and treated plants. Interestingly, also water-treated samples and especially those sprayed on leaves ("Ctrl H<sub>2</sub>O L")

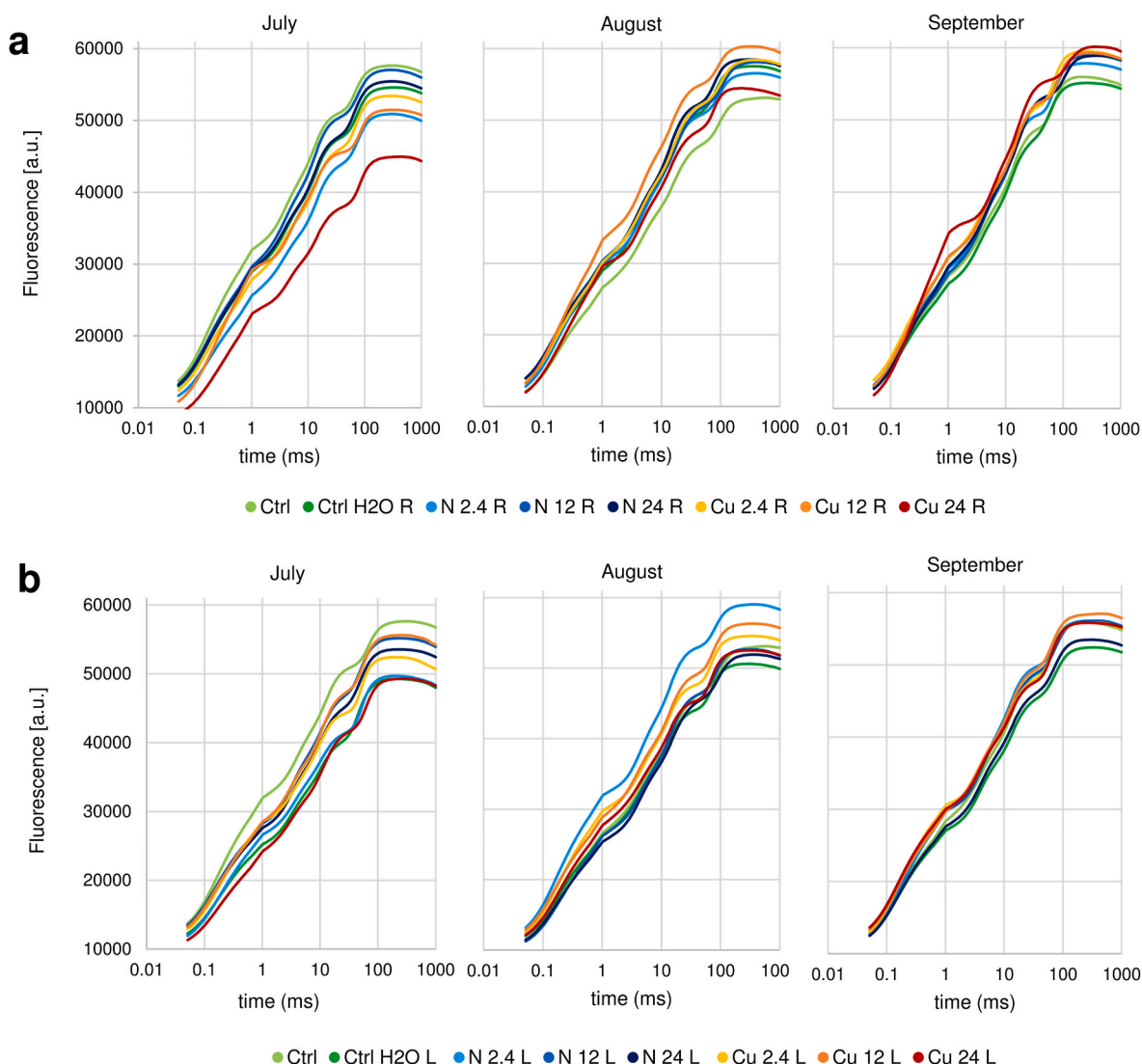
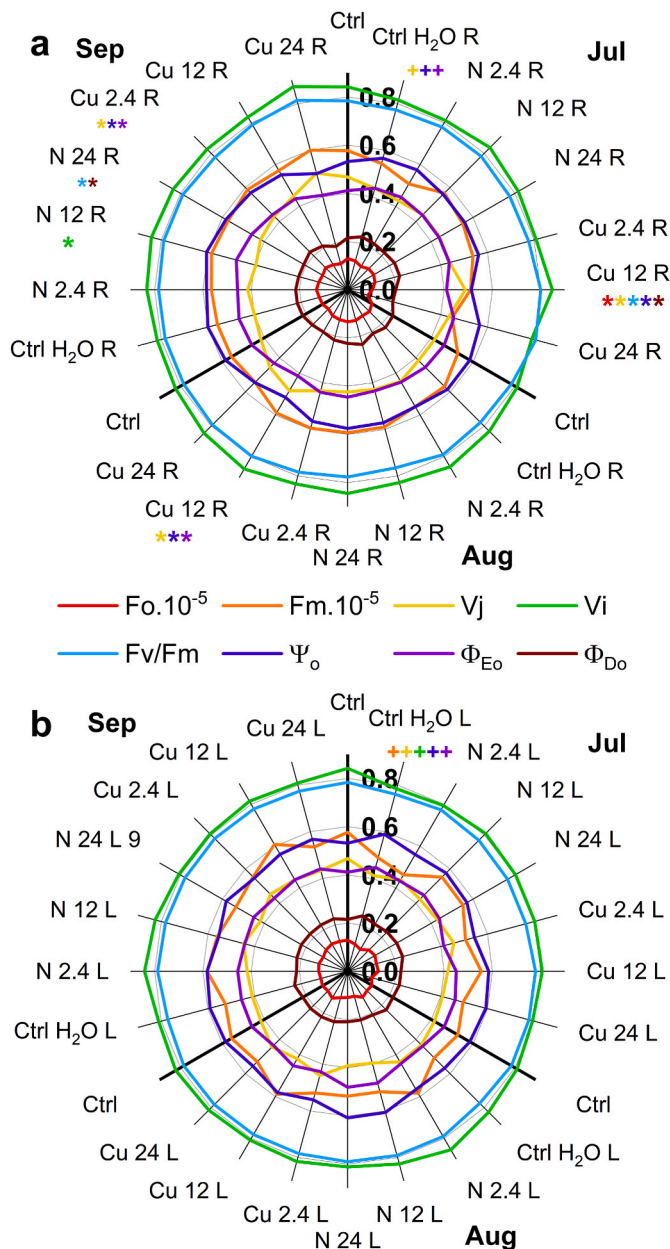


Fig. 2. Mean OJIP curves in a) rhizome-treated and b) foliar-treated variants during experiment.



**Fig. 3.** Median values of chlorophyll fluorescence indices in **a)** rhizome-treated and **b)** foliar-treated variants during experiment; colorful asterisks under variant label indicate value significantly different from related water-treated control "Ctrl H<sub>2</sub>O R"(a) or "Ctrl H<sub>2</sub>O L"(b) and colorful plus signs indicate significant difference of water-treated controls from non-treated control "Ctrl" (Wilcoxon rank-sum test,  $\alpha = 0.05$ ); abbreviations Jul, Aug and Sep refer to the month of measurement.

displayed lower fluorescence values, which was documented by significantly lower Fm (P phase) (Fig. 3b and Supplemental Fig. S5b). In comparison with water-treated controls, especially the negative effect of the elevated levels of copper was significant. This divergence is particularly evident in the J-I-P phases of the curves, which are sensitive indicators of the photosystem I (PS I) electron transport chain efficiency. A very similar effect was observed after exposure of *Spirodela* to copper. The pronounced separation suggests that copper, in higher concentrations, exerts a suppressive effect on PS I, likely through the disruption of electron transport and the alteration of thylakoid membrane integrity [70]. Notably, the highest rhizome copper treatment group ("Cu 24 R") was anomalous, however the data were collected only from one surviving plant, thus the result was insignificant. Similar effect was

observed by Kulikova on soybean seedlings [38]. It is noteworthy that in this variant, the most dramatic changes appeared throughout the season. While in June, plants were very small, during the summer they started to grow very vigorously (chapter 3.1). This rebound could be reflective of a hormetic response, wherein exposure to sub-lethal stressors engenders a compensatory increase in physiological performance [71,72]. Nevertheless, already the middle dose "Cu 12 R" significantly decreased Fo (O phase) (Fig. 3a and Supplemental Fig. S5a). Nitrogen treatment shows the opposite trend with decreasing mean fluorescence values after low dose application and their continuous increase with increasing concentrations. However, Fo and Fm were statistically comparable with water-treated controls in all cases.

Getting closer to the main part of the vegetation season in the middle of August, the disparity in fluorescence induction profiles between treatments was diminishing. It could be pointed out that, in contrast to July, all variants matched or even exceeded "Ctrl" control. This suggests a possible acclimatization of the plants to the imposed stress conditions. This acclimatization could be attributed to the induction of adaptive mechanisms, such as the upregulation of antioxidative defenses or repair processes within PS I [73]. And especially low dose nitrogen leaf treatment ("N 2.4 L") increased fluorescence, although this trend was not statistically significant due to the low number ( $n = 3$ ) of replicates (Supplemental Fig. S5a and b).

The unification of fluorescence transient profiles was even more pronounced at the end of the main vegetation season, in the middle of September, when all variants reached similar fluorescence levels.

Besides fluorescence values, OJIP curves reconstruction and Fv/Fm, the method allows calculation of a wide range of derived indices providing deeper insight into specific photosynthesis phases and processes. Fig. 3 presents the selected indices which reflected the most significant effects among all of the fluorescence parameters provided by PSI FluorPen software.

Based on these, main attention should be drawn to the variant "Cu 12 R" which was highlighted already with the increased Fv/Fm. In biomass yield and lignin content, this treatment resulted in decrease and substantial induction of stress has been discussed at that point. In biomass yield and lignin content (chapter 3.1 and 3.2), this treatment resulted in decrease, clearly indicating that Cu 12 R induced substantial stress rather than beneficial priming effects. On the other hand, the assumption based on higher Fv/Fm that primary photochemistry might not be affected was supported also with significantly decreased  $\Phi_{D0}$ , documenting that the loss of energy was minimal. Nevertheless, significant decrease of  $\Phi_{E0}$  and  $\Psi_0$  implies errors in electron transport resulting in the suppressed growth. Decrease of electron transport becomes apparent during September also in the lower copper concentration ("Cu 2.4 R"), suggesting a gradual effect of copper toxicity overtime. These effects might reflect a disruption in the balance of essential micronutrients [46, 74]. Copper is required for photosynthetic electron transport as a cofactor in plastocyanin, however, in excess, it can catalyze the formation of reactive oxygen species (ROS), leading to oxidative stress and potentially inhibiting PS I repair mechanisms [75]. This toxic effect can be observed in the diminishing fluorescence, representing a compromised structure and function of photosynthesis, particularly PS I under high copper concentrations [44–47].

The nitrogen treatment of rhizomes had an overall positive effect on photosynthetic efficiency, but, analogically to Fv/Fm, the significant effects were determined only in September. Specifically, "N 12 R" showed higher Vi and "N 24 R" lower  $\Phi_{D0}$ , but, in contrast with copper, without decrease of electron transport, suggesting good physiological state of these individuals. Foliar nitrogen treatment did not cause any significant effects. However, the dose-dependent trends were visible in many indices, such as decreasing Vj and Vi and increasing  $\Psi_0$  and  $\Phi_{E0}$  in particular months. Especially the lowest dose "N 2.4 L" displayed substantial shift compared to control "Ctrl H<sub>2</sub>O L" (Supplemental Fig. S5d) including decreased energy dissipation  $\Phi_{D0}$  (Supplemental Fig. S5h). Overall, these changes observed in some plants across the vegetative

season suggested an enhancement in photosynthetic efficiency, likely due to improved nitrogen assimilation. Nitrogen is a fundamental component of chlorophyll, nucleotides, and amino acids, which are crucial for the photosynthetic apparatus [44,55]. The elevated fluorescence in these plants may be indicative of a higher chlorophyll content per unit area, increased antenna size, or greater electron transport rates, all of which contribute to a more robust photosynthetic machinery.

### 3.5. The reflectance of leaf pigments

The reflectance of leaf pigments is another non-invasive method used repeatedly to assess primary leaf pigments, which are responsive to stress, and plant fitness. Analysis of these parameters provides important information to counterpart fluorescence and other analyses to bring bigger picture to the understanding of plant stress reaction and adaptation.

Generally, the reflectance measurements did not reveal many significant effects (Fig. 4). NDVI, the most common reflectance index used for assessing plant health, had values ranging between 0.33 and 0.66 (Supplemental Fig. S6a), which are standard values for plants that are moderately healthy [76] throughout the vegetation season. The only priming treatment affecting NDVI was "N 12 R" in July, which was significantly higher than "Ctrl H<sub>2</sub>O R" indicating a positive effect of nitrogen application, supported also by the dose-dependent increasing trend (Supplemental Fig. S6a). However, in contrary to fluorescence results, it was indicated just in the earlier phase of the vegetation season. Interestingly, NDVI was also significantly decreased after leaf spraying by water in August and September.

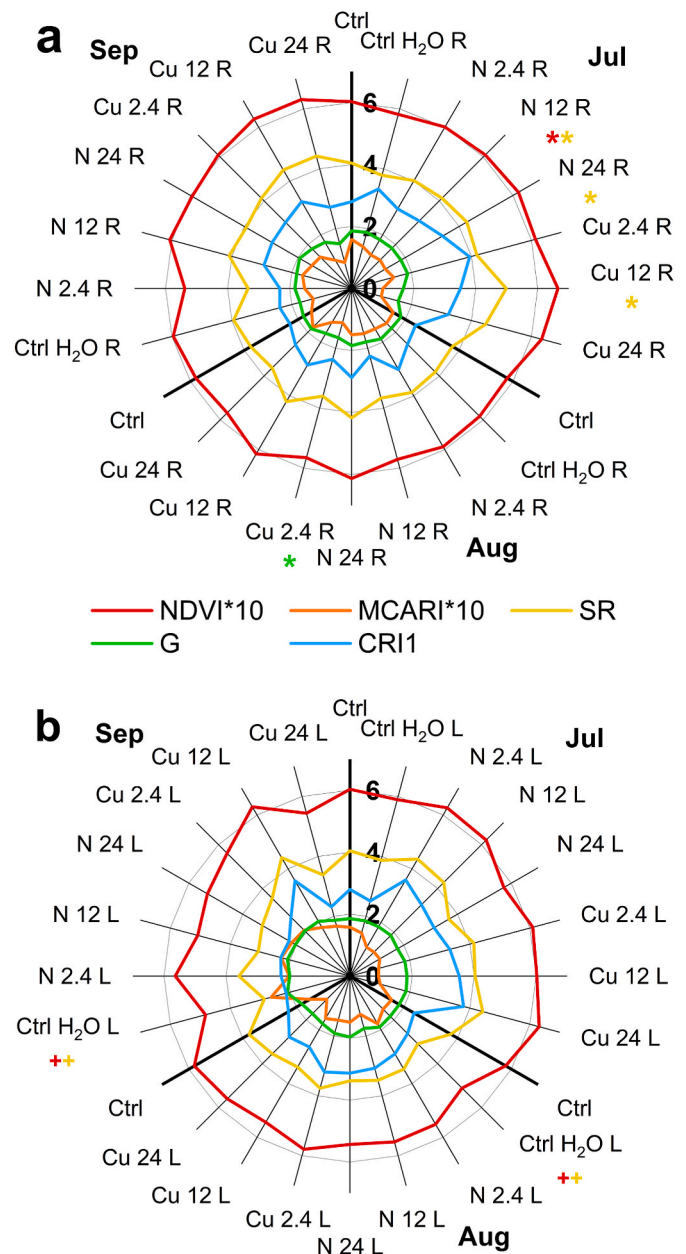
The indices MCARI and G used for assessing the changes in chlorophyll content showed no significant deviations from control with exception of decreased G in "Cu 2.4 R" in August. Similarly, CRI1 reflecting the carotenoids content showed no differences, mostly due to high variability. Nevertheless, interestingly higher CRI1 values compared to control "Ctrl H<sub>2</sub>O L" were detected among all treatments after leaf spraying in July (Supplemental Fig. S6e). Significant changes were spotted in SR parameter in variants "N 12 R", "N 24 R" and "Cu 12 R" during July. Since there were no (except for "N 12 R") significant changes in other reflectance parameters, it seems probable that increased SR was the result of some structural changes of the cells rather than of the leaf pigment content. Absence of direct improvement of chlorophyll or total photosynthetic pigment after nitrogen priming is in contrast to common investigations, where nitrogen added to plants in various forms leads to direct pigment content increase [77]. It has to be noted that priming is not the same as fertilizing; the act of application is a one-time act, taking advantage of initial mild stress that creates the response in subsequent life of the plant.

Our findings support the hypothesis that foliar nitrogen application enhances biomass production more effectively than rhizome soaking. Furthermore, the concentration- and method-dependent effects of copper confirm its role as a modulator of stress responses and structural integrity.

### 3.6. Interactions among measured parameters

In addition to individual parameter responses to treatments, interrelationships among various measured traits were performed. To gain insight into the interactions and contributions of various measured parameters to overall plant performance, we analyzed correlations among biomass, morphological traits, lignocellulose composition, and physiological measurements (Supplemental Fig. S7).

This analysis revealed several significant patterns: The height of the plants consistently and positively correlated with the amount of lignin (Supplemental Fig. S7c) in the rhizome-treated plants over all periods. This correlation was especially strong in August and September, at the end of the vegetation season, showing, how higher plants invest in structural reinforcement.



**Fig. 4.** Median values of leaf reflectance indices in a) rhizome-treated and b) foliar-treated variants during experiment; colorful asterisks under variant label indicate value significantly different from related water-treated control "Ctrl H<sub>2</sub>O R"(a) or "Ctrl H<sub>2</sub>O L"(b) and colorful plus signs indicate significant difference of water-treated controls from non-treated control "Ctrl" (Wilcoxon rank-sum test,  $\alpha = 0.05$ ); abbreviations Jul, Aug and Sep refer to the month of measurement.

Final biomass production had a positive relationship with several factors that were measured during entire vegetation season. In rhizome-treated plants, biomass exhibited moderate positive correlations with electron transport efficiency ( $\Phi_{E0}$ ) and the probability of electron transport ( $\Psi_0$ ) in August. This indicates that photosynthetic performance during the peak growing season affected the final yield. Interestingly, biomass also had a positive correlation with different reflectance indices (G, MCARI) in September. This means that the amount of pigment in the leaves at the end of the season showed how healthy the plant was. Parameters like MCARI can therefore be a good predictor for biomass yield.

As expected, leaf length and width, were strongly correlated with each other, but their relationships with other traits changed depending

on the treatment method and time. In foliar-treated plants, leaf length and width exhibited lower correlations compared to rhizome treatments.

The fluorescence parameters  $V_j$  and  $\Psi_o$  exhibited consistently robust negative correlations ( $r = -1.0$ ) in multiple instances. As predicted by the theoretical relationship between these parameters,  $\Phi_{Do}$  (energy dissipation) also had a negative correlation with  $F_v/F_m$ .

### 3.7. Application implication

Obtained results have implications for the practical use of MxG for phytomanagement of marginal lands as well as for the use of biomass. The results show that proper priming (defined here as single, early-stage application) can prepare the young plants to better face sub-optimal conditions. Specifically, foliar application (spraying) with water or low-concentration nitrogen solution (2.4 g/L  $\text{NH}_4\text{NO}_3$ ) on young plants can promote biomass growth under exposure to high-temperature and is a simple, cost-effective technique. Spraying during the morning or evening hours may additionally reduce leaf overheating and transient water stress.

The altered composition of lignocellulose components observed in this study can be utilized to optimize biomass for different applications. An increased lignin content may enhance heating value, which is desirable for direct combustion. It can also improve mechanical strength of the material. A slight lignin increase was observed following low-dose foliar copper application (2.4 g/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), suggesting a potential strategy for producing biomass better suited for energy or construction industry. Conversely, a higher cellulose content is advantageous for applications that rely on cellulose utilization, such as enzymatic hydrolysis and subsequent fermentation. Rhizome soaking in moderate nitrogen concentrations (12 g/L  $\text{NH}_4\text{NO}_3$  for 24 h) can therefore be recommended to increase photosynthetic performance and promote cellulose accumulation.

Priming should be viewed as a single, preparation step rather than a repeated fertilization treatment. Costs of chemicals and labor should be balanced against expected yield benefits, as priming effects are most pronounced under stress conditions such as heat or drought. However, the results of the study should be considered as preliminary. It can be expected that they were significantly affected by the conditions of the greenhouse pot experiment. Despite duration for only one growing season, the pot size could be a limiting factor especially for the best yielding variants. On the other hand, good-quality substrate and regular irrigation preventing additional stress does not correspond to the real conditions in most of the localities, while growing in field. Thus, further field research conducted over multiple years is necessary to validate these priming protocols under variable environmental conditions, to balance realistic effects versus priming costs, and to assess long-term effects on mature stand productivity and stress resilience.

In the Central European context, the expansion of MxG cultivation appears promising, especially on marginal and degraded lands, such as post-mining sites, where conventional agriculture is constrained. As shown by Weger et al., MxG already demonstrates strong adaptation to Central Europe's climate and can yield 15–17 t dw/ha/y under low-input management despite drought episodes [78]. However, based on the example of the Czech Republic, they also highlight that economic barriers remain a key challenge: establishment costs are high, competition from annual crops is strong, and market conditions (subsidies, biomass pricing) significantly affect adoption. In light of this, the priming strategies identified in our study, particularly low-dose foliar nutrient application, may help reduce establishment risk, improve early-stage stress tolerance, and thereby strengthen the economic feasibility of MxG on degraded terrains. Thus, combining agronomic innovations (like priming) with favorable policy incentives and localized biomass value chains could make large-scale MxG cultivation on marginal and degraded lands a realistic prospect.

## 4. Conclusions

This study employed a priming approach (single applications of nitrogen or copper to rhizomes or young plants intended to trigger stress preparedness) to investigate physiological responses in *Miscanthus x giganteus* under elevated temperature conditions (up to 45 °C) relevant to climate change scenarios. Regular irrigation maintained plant health, indicating that results reflect treatment effects on performance under high temperatures rather than classical stress priming responses.

This study highlights the complexity of plant responses to chemical priming in *Miscanthus x giganteus*, particularly under elevated temperature conditions. Notably, a 38 % increase in biomass following foliar water spraying suggests that the physical act of spraying may enhance plant performance independently of nutrient content.

While nitrogen priming did not increase biomass beyond water controls, it did modulate physiological parameters related to photosynthetic efficiency and leaf development, indicating potential for improved stress resilience and metabolic activity with minimal stress indicators. In contrast, copper priming showed strong, dose-dependent phytotoxic stress effects when applied to rhizomes, significantly reducing biomass and stomatal density, while low-dose foliar application moderately increased lignin content, an important trait for tailoring biomass for industrial use.

Correlation analyses revealed that plant performance under elevated temperatures involved coordinated adjustments across physiological, morphological, and compositional traits, with photosynthetic efficiency during mid-season strongly correlating with final biomass production.

These findings underscore the necessity of distinguishing between chemical-specific and application-related effects when designing priming strategies. For practical use, foliar application of water or low-concentration nutrients may provide simple, cost-effective means to enhance biomass production or modulate cell wall properties depending on desired end-use. Future work in field conditions over multiple growing seasons is needed to assess the long-term viability and scalability of priming protocols in bioresource production systems, and to determine whether establishment-phase responses translate into sustained productivity gains in mature *Miscanthus* stands.

### CRedit authorship contribution statement

**Hana Auer Malinská:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Lucie Loukotová:** Visualization, Validation, Formal analysis. **Diana Polanská Nebeská:** Writing – review & editing, Writing – original draft, Validation, Investigation. **Jiří Škvor:** Visualization, Formal analysis. **Josef Trögl:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition.

### Declaration of generative AI and AI-assisted technologies in the writing process

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### Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2025.108538>.

## Data availability

Data will be made available on request.

## References

- [1] Z. Zgorelec, N. Bilandzija, K. Knez, M. Galic, S. Zuzul, Cadmium and Mercury phytostabilization from soil using *Miscanthus × giganteus*, *Sci. Rep.* 101 10 (2020) 1–10, <https://doi.org/10.1038/s41598-020-63488-5>, 2020.
- [2] L.E. Erickson, V. Pidlisnyuk, Phytotechnology with biomass production, phytotechnology with biomass prod. <https://doi.org/10.1201/9781003082613>, 2021.
- [3] J. Clifton-Brown, K.-U. Schwarz, D. Awty-Carroll, A. Iurato, H. Meyer, J. Greef, J. Gwyn, M. Mos, C. Ashman, C. Hayes, L. Huang, J. Norris, C. Rodgers, D. Scordia, R. Shafiei, M. Squance, T. Swaller, S. Youell, S. Cosentino, R. Flavell, I. Donnison, P. Robson, Breeding strategies to improve miscanthus as a sustainable source of biomass for bioenergy and biorenewable products, *Agronomy* 9 (2019) 673, <https://doi.org/10.3390/agronomy9110673>.
- [4] T.R. Hodgkinson, M. Klaas, M.B. Jones, R. Prickett, S. Barth, *Miscanthus*: a case study for the utilization of natural genetic variation, *Plant Genet. Resour.* 13 (2015) 219–237, <https://doi.org/10.1017/S147926211400094X>.
- [5] S. Rusinowski, J. Krzyżak, K. Sitko, H.M. Kalaji, E. Jensen, M. Pogrzeba, Cultivation of C4 perennial energy grasses on heavy metal contaminated arable land: impact on soil, biomass, and photosynthetic traits, *Environ. Pollut.* 250 (2019) 300–311, <https://doi.org/10.1016/j.envpol.2019.04.048>.
- [6] N. Wanat, A. Austruy, E. Joussein, M. Soubrand, A. Hitmi, C. Gauthier-Moussard, J.-F. Lenain, P. Vernay, J.C. Munch, M. Pichon, Potentials of *Miscanthus × giganteus* grown on highly contaminated Technosols, *J. Geochem. Explor.* 126–127 (2013) 78–84, <https://doi.org/10.1016/j.jgexplo.2013.01.001>.
- [7] H. Burdová, D. Nebeská, K.S. Al Souki, D. Pilnaj, Z. Kwoczynski, S. Kríženecá, H. Auer Malinská, M. Vaněk, P. Kurán, V. Pidlisnyuk, J. Trögl, *Miscanthus × giganteus* stress tolerance and phytoremediation capacities in highly diesel contaminated soils, *J. Environ. Manag.* 344 (2023) 118475, <https://doi.org/10.1016/J.JENVMAN.2023.118475>.
- [8] D. Nebeská, H.A. Malinská, M. Vaněk, J. Popelka, S. Adamec, S. Ušfak, R. Honzík, J. Trögl, Nutrients deficiency affects *Miscanthus × giganteus* physiology and essential metals uptake more intensively than soil contamination, *Ind. Crops Prod.* 189 (2022) 115845, <https://doi.org/10.1016/J.INDCROP.2022.115845>.
- [9] S. Fonteyne, I. Roldán-Ruiz, H. Muylle, T. De Swaef, D. Reheul, P. Lootens, A review of frost and chilling stress in miscanthus and its importance to biomass yield, *Perenn. Biomass Crop. a Resour. World* (2016) 127–144, [https://doi.org/10.1007/978-3-319-44530-4\\_12](https://doi.org/10.1007/978-3-319-44530-4_12).
- [10] A. Plazek, F. Dubert, F. Janowiak, T. Krepski, M. Tatrzńska, Plant age and in vitro or in vivo propagation considerably affect cold tolerance of *Miscanthus × giganteus*, *Eur. J. Agron.* 34 (2011) 163–171, <https://doi.org/10.1016/J.EJA.2011.01.002>.
- [11] H.A. Malinská, M. Vaněk, D. Nebeská, D. Šubrt, M. Brestič, J. Trögl, Plant priming changes physiological properties and lignin content in *Miscanthus × giganteus*, *Ind. Crops Prod.* 174 (2021) 114185, <https://doi.org/10.1016/J.INDCROP.2021.114185>.
- [12] J.C. Clifton-Brown, I. Lewandowski, F. Bangerth, M.B. Jones, Comparative responses to water stress in stay-green, rapid- and slow senescing genotypes of the biomass crop, *Miscanthus*, *New Phytol.* 154 (2002) 335–345, <https://doi.org/10.1046/J.1469-8137.2002.00381.X>.
- [13] J.J. De Vega, A. Teshome, M. Klaas, J. Grant, J. Finnan, S. Barth, Physiological and transcriptional response to drought stress among bioenergy grass *Miscanthus* species, *Biotechnol. Biofuels* 14 (2021) 1–13, <https://doi.org/10.1186/S13068-021-01915-2/FIGURES/4>.
- [14] E. Joo, M.Z. Hussain, M. Zeri, M.D. Masters, J.N. Miller, N. Gomez-Casanovas, E. H. DeLucia, C.J. Bernacchi, The influence of drought and heat stress on long-term carbon fluxes of bioenergy crops grown in the Midwestern USA, *Plant Cell Environ.* 39 (2016) 1928–1940, <https://doi.org/10.1111/PCE.12751>.
- [15] H. Malinská, V. Pidlisnyuk, D. Nebeská, A. Erol, A. Medžová, J. Trögl, Physiological response of miscanthus × giganteus to plant growth regulators in nutritionally poor soil, *Plants* 9 (2020) 194, <https://doi.org/10.3390/plants9020194>.
- [16] A. Nurzhanova, V. Pidlisnyuk, S. Kalugin, T. Stefanovska, M. Drimal, *Miscanthus × giganteus* as a new highly efficient phytoremediation agent for improving soils contaminated by pesticides residues and supplemented contaminants, *Commun. Agric. Appl. Biol. Sci.* 80 (2015) 361–366.
- [17] G. Dražić, J. Milovanović, S. Stefanović, I. Petrić, Potential of *Miscanthus × Giganteus* for heavy metals removing from industrial DepoSol, *Acta Reg. Environ. Times* 14 (2017) 56–58, <https://doi.org/10.1515/AREE-2017-0009>.
- [18] E. Stavridou, A. Hastings, R.J. Webster, P.R.H. Robson, The impact of soil salinity on the yield, composition and physiology of the bioenergy grass *Miscanthus × giganteus*, *GCB Bioenergy* 9 (2017) 92–104, <https://doi.org/10.1111/GCBB.12351>.
- [19] N.J. Atkinson, P.E. Urwin, The interaction of plant biotic and abiotic stresses: from genes to the field, *J. Exp. Bot.* 63 (2012) 3523–3543, <https://doi.org/10.1093/JXB/ERS100>.
- [20] H.K. Lichtenthaler, Vegetation stress: an introduction to the stress concept in plants, *J. Plant Physiol.* 148 (1996) 4–14, [https://doi.org/10.1016/S0176-1617\(96\)80287-2](https://doi.org/10.1016/S0176-1617(96)80287-2).
- [21] I. Akram, M. Tahir, M. Abdullah Saleem, T. Ahmad, M. Naz, M. Ahmad, Seed priming effects of copper sulphate on growth characteristics of green gram (*Vigna radiata* L.), *J. Glob. Innov. Agric. Soc. Sci.* 8 (2020) 23–25, <https://doi.org/10.22194/jgiass.8.884>.
- [22] N. Begum, H. Gul, M. Hamayun, I.U. Rahman, F. Ijaz, Z. Iqbal, A. Afzal, M. Afzal, A. Ullah, S. Karim, Seedling Growth of Brassica rapa Under NaCl Stress, *Middle East J. Sci. Res.* 22 (2014) 879–885, <https://doi.org/10.5829/IDOSI.MEJSR.2014.22.06.12470>.
- [23] K. Bera, P. Dutta, S. Sadhukhan, Seed priming with non-ionizing physical agents: plant responses and underlying physiological mechanisms, *Plant Cell Rep.* 41 (2021) 53–73, <https://doi.org/10.1007/S00299-021-02798-Y>, 2021.
- [24] J. Bhardwaj, A. Anand, S. Nagarajan, Biochemical and biophysical changes associated with magneto priming in germinating cucumber seeds, *Plant Physiol. Biochem.* 57 (2012) 67–73, <https://doi.org/10.1016/j.plaphy.2012.05.008>.
- [25] M. Farooq, M. Irfan, T. Aziz, I. Ahmad, S.A. Cheema, Seed priming with ascorbic acid improves drought resistance of wheat, *J. Agron. Crop Sci.* 199 (2013) 12–22, <https://doi.org/10.1111/j.1439-037X.2012.00521.x>.
- [26] R. Johnson, J.T. Puthur, Seed priming as a cost effective technique for developing plants with cross tolerance to salinity stress, *Plant Physiol. Biochem.* 162 (2021) 247–257, <https://doi.org/10.1016/j.plaphy.2021.02.034>.
- [27] A. Shelar, S.H. Nile, A.V. Singh, D. Rothenstein, J. Bill, J. Xiao, M. Chaskar, G. Kai, R. Patil, Recent advances in nano-enabled seed treatment strategies for sustainable agriculture: challenges, risk assessment, and future perspectives, *Nano-Micro Lett.* 151 (15) (2023) 1–37, <https://doi.org/10.1007/S40820-023-01025-5>.
- [28] K. Umnajkitikorn, M. Fukudome, T. Uchiumi, N. Teamroong, Elevated nitrogen priming induced Oxinitro-Responses and water deficit tolerance in rice, *Plants* 10 (2021) 381, <https://doi.org/10.3390/PLANTS10020381>.
- [29] U. Conrath, Chapter 9 priming of induced plant defense responses, *Adv. Bot. Res.* 51 (2009) 361–395, [https://doi.org/10.1016/S0065-2296\(09\)51009-9](https://doi.org/10.1016/S0065-2296(09)51009-9).
- [30] T. Sumitha, K.C. Jisha, Efficiency of priming methods in crop establishment of Ginger (*Zingiber officinale* Rosc.), *J. Stress Physiol. Biochem.* 19 (2023) 96–101.
- [31] A. Khan Amanullah, A. Jan, Z. Shah, B. Ahmad, S. Khalil, A. Ali, Hidayatullah, F. Ahmad, A. Nawaz, Foliar application of nitrogen at different growth stages influences the phenology, growth and yield of maize (*Zea mays* L.), *Soil Environ.* 32 (2013) 135–140.
- [32] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, in: , 2009. <https://eur-lex.europa.eu/eli/reg/2009/152/oj/eng>.
- [33] ISO 16472:2006 - Animal feeding stuffs - Determination of amylase-treated neutral detergent fibre content (aNDF), in: , 2006. <https://www.iso.org/standard/37898.html>.
- [34] ISO 13906:2008 - Animal feeding stuffs - Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents, (2008), <https://www.iso.org/standard/43032.html>.
- [35] M.J. Hu, Y.P. Guo, Y.G. Shen, D.P. Guo, D.Y. Li, Midday depression of photosynthesis and effects of mist spray in citrus, *Ann. Appl. Biol.* 154 (2009) 143–155, <https://doi.org/10.1111/J.1744-7348.2008.00282.X>.
- [36] C.L. Kibler, A.T. Trugman, D.A. Roberts, C.J. Still, R.L. Scott, K.K. Caylor, J. C. Stella, M.B. Singer, Evapotranspiration regulates leaf temperature and respiration in dryland vegetation, *Agric. For. Meteorol.* 339 (2023) 109560, <https://doi.org/10.1016/J.AGRFORMET.2023.109560>.
- [37] L. Li, G. Mu, Similar effects as shade tolerance induced by dust accumulation and size penetration of particulates on cotton leaves, *BMC Plant Biol.* 21 (2021), <https://doi.org/10.1186/S12870-021-02926-6>.
- [38] A.L. Kulikova, N.A. Kuznetsova, V.P. Kholodova, Effect of copper excess in environment on soybean root viability and morphology, *Russ. J. Plant Physiol.* 58 (2011) 836–843, <https://doi.org/10.1134/S102144371105013X>.
- [39] H. Lequeux, C. Hermans, S. Lutts, N. Verbruggen, Response to copper excess in *Arabidopsis thaliana*: impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile, *Plant Physiol. Biochem.* PPB 48 (2010) 673–682, <https://doi.org/10.1016/J.PLAPHY.2010.05.005>.
- [40] L.L. Martins, M.P. Mourato, Effect of excess copper on tomato plants: growth parameters, enzyme activities, chlorophyll, and mineral content, *J. Plant Nutr.* 29 (2006) 2179–2198, <https://doi.org/10.1080/01904160600972845>.
- [41] Á. Iglesias-Ganado, J. Martín-García, J. Poveda, M.F. López-Sainz, T. Sánchez-Gómez, O. Santamaría, Improvement of Wheat and Barley Cultivation Through Seed Priming with UV, Ozone, and Nutripriming (Fe, Zn, and B), *Appl. Sci.* 15 (2025) 9988, <https://doi.org/10.3390/AP15189988>.
- [42] R Core Team. R: A Language and Environment for Statistical Computing, 2023. <https://www.R-project.org/>.
- [43] H. Wickham. ggplot2: Elegant Graphics for Data Analysis, 2009, <https://doi.org/10.1007/978-0-387-98141-3>.

- [44] R.R. Rivai, T. Miyamoto, T. Awano, R. Takada, Y. Tobimatsu, T. Umezawa, M. Kobayashi, Nitrogen deficiency results in changes to cell wall composition of sorghum seedlings, *Sci. Rep.* 11 (2021) 23309, <https://doi.org/10.1038/s41598-021-02570-y>.
- [45] A.S. Tugbaeva, A.A. Ermoshin, I.S. Kiseleva, Biochemical responses to the long-term impact of copper sulfate (CuSO<sub>4</sub>) in tobacco plants, *Int. J. Mol. Sci.* 24 (2023) 15129, <https://doi.org/10.3390/IJMS242015129>.
- [46] I. Yruea, Copper in plants, *Braz. J. Plant Physiol.* 17 (2005) 145–156, <https://doi.org/10.1590/S1677-04202005000100012>.
- [47] J.C.M.S. Moura, C.A.V. Bonine, J. de Oliveira Fernandes Viana, M.C. Dornelas, P. Mazzafera, Abiotic and Biotic Stresses and Changes in the Lignin Content and Composition in Plants, *J. Integr. Plant Biol.* 52 (2010) 360–376, <https://doi.org/10.1111/j.1744-7909.2010.00892.x>.
- [48] X. Han, Y. Zhao, Y. Chen, J. Xu, C. Jiang, X. Wang, R. Zhuo, M.-Z. Lu, J. Zhang, X. Han, Y. Zhao, Y. Chen, J. Xu, C. Jiang, X. Wang, R. Zhuo, M.-Z. Lu, J. Zhang, Lignin biosynthesis and accumulation in response to abiotic stresses in woody plants, *For. Res.* 2 (2022) 9, <https://doi.org/10.48130/FR-2022-0009>.
- [49] E.C. Tredenick, G.D. Farquhar, Dynamics of moisture diffusion and adsorption in plant cuticles including the role of cellulose, *Nat. Commun.* 12 (2021) 5042, <https://doi.org/10.1038/S41467-021-25225-Y>.
- [50] Z. Xu, G. Zhou, Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass, *J. Exp. Bot.* 59 (2008) 3317, <https://doi.org/10.1093/JXB/ERN185>.
- [51] C.D. Muir, M.Á. Conesa, J. Galmés, Independent evolution of ab- and adaxial stomatal density enables drought tolerance without deleterious effect on nutrient uptake, *New Phytol.* 208 (2015) 336, <https://doi.org/10.1111/NPH.13598>.
- [52] C. Hepworth, T. Doheny-Adams, L. Hunt, D.D. Cameron, J.E. Gray, Manipulating stomatal density enhances drought tolerance without deleterious effect on nutrient uptake, *New Phytol.* 208 (2015) 336, <https://doi.org/10.1111/NPH.13598>.
- [53] T. Lawson, M.R. Blatt, Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency, *Plant Physiol.* 164 (2014) 1556–1570, <https://doi.org/10.1104/PP.114.237107>.
- [54] S. Casson, J.E. Gray, Influence of environmental factors on stomatal development, *New Phytol.* 178 (2008) 9–23, <https://doi.org/10.1111/J.1469-8137.2007.02351.X>.
- [55] E.L.O. Camargo, L.C. Nascimento, M. Soler, M.M. Salazar, J. Lepikson-Neto, W. L. Marques, A. Alves, P.J.P.L. Teixeira, P. Mieczkowski, M.F. Carazzolle, Y. Martinez, A.C. Deckmann, J.C. Rodrigues, J. Grima-Pettenati, G.A.G. Pereira, Contrasting nitrogen fertilization treatments impact xylem gene expression and secondary cell wall lignification in Eucalyptus, *BMC Plant Biol.* 14 (2014), <https://doi.org/10.1186/S12870-014-0256-9>.
- [56] C. Hunter, Z. Sun, S.D. Mansfield, M. Shahbaz, M. Pilon, S.M. Gleason, The effects of copper deficiency on lignification, xylem vessel structure, and hydraulic traits in hybrid poplar, *Physiol. Plantarum* 175 (2023) e14006, <https://doi.org/10.1111/PPL.14006>.
- [57] J. Barros, H. Serk, I. Granlund, E. Pesquet, The cell biology of lignification in higher plants, *Ann. Bot.* 115 (7) (2015) 1053–1074, <https://doi.org/10.1093/AOB/MCV046>.
- [58] A.M. Boudet, Lignins and lignification: selected issues, *Plant Physiol. Biochem.* 38 (2000) 81–96, [https://doi.org/10.1016/S0981-9428\(00\)00166-2](https://doi.org/10.1016/S0981-9428(00)00166-2).
- [59] S. Landi, S. Esposito, Nitrate uptake affects cell wall synthesis and modeling, *Front. Plant Sci.* 8 (2017), <https://doi.org/10.3389/fpls.2017.01376>.
- [60] E. Murozuka, K.H. Laursen, J. Lindedam, I.F. Shield, S. Bruun, J. Magid, I.S. Møller, J.K. Schjoerring, Nitrogen fertilization affects silicon concentration, cell wall composition and biofuel potential of wheat straw, *Biomass Bioenergy* 64 (2014) 291–298, <https://doi.org/10.1016/J.BIOMBIOE.2014.03.034>.
- [61] X. Ruan, H. Du, X. Zhan, X. Cong, F. Shi, J. Li, Z. Luo, Z. Dong, Physiological and metabolic responses to nitrogen availability of rice (*Oryza sativa* L.) cultivars with differ in nitrogen efficient, *Plant Biotechnol. Rep.* 17 (2023) 67–78, <https://doi.org/10.1007/S11816-023-00817-W/METRICS>.
- [62] B. Printz, S. Lutts, J.F. Hausman, K. Sergeant, Copper trafficking in plants and its implication on cell wall dynamics, *Front. Plant Sci.* 7 (2016), <https://doi.org/10.3389/FPLS.2016.00601/PDF>.
- [63] T. Liu, C. Shen, Y. Wang, C. Huang, J. Shi, New insights into regulation of proteome and polysaccharide in cell Wall of *Elsholtzia splendens* in response to copper stress, *PLoS One* 9 (2014), <https://doi.org/10.1371/JOURNAL.PONE.0109573>.
- [64] P. Wójcik, Uptake of mineral nutrients from foliar fertilization (review), *J. Fruit Ornament. Plant Res.* 12 (2004) 201–208 (in Polish with English summary).
- [65] D. Oosterhuis, Foliar fertilization: mechanisms and magnitude of nutrient uptake, 2009.
- [66] S. Wall, S. Violet-Chabrand, P. Davey, J. Van Rie, A. Galle, J. Cockram, T. Lawson, Stomata on the abaxial and adaxial leaf surfaces contribute differently to leaf gas exchange and photosynthesis in wheat, *New Phytol.* 235 (2022) 1743–1756, <https://doi.org/10.1111/NPH.18257>.
- [67] H.K. Lichtenthaler, S. Burkart, Photosynthesis and high light stress, *Bulg. J. Plant Physiol.* 25 (1999) 3–16.
- [68] E.H. Murchie, T. Lawson, Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications, *J. Exp. Bot.* 64 (2013) 3983–3998, <https://doi.org/10.1093/JXB/ERT208>.
- [69] M. Živčák, M. Brestič, K. Olšovská, P. Slámka, Performance index as a sensitive indicator of water stress in *Triticum aestivum* L., *Plant, Soil Environ.* 54 (2008) 133–139, <https://doi.org/10.17221/392-pse>.
- [70] K.J. Appenroth, J. Stöckel, A. Srivastava, R.J. Strasser, Multiple effects of chromate on the photosynthetic apparatus of *Spirodela polyrrhiza* as probed by OJIP chlorophyll a fluorescence measurements, *Environ. Pollut.* 115 (2001) 49–64, [https://doi.org/10.1016/S0269-7491\(01\)00091-4](https://doi.org/10.1016/S0269-7491(01)00091-4).
- [71] E. Matkowski, K. Sitko, M. Szopiński, Ż. Gieroń, M. Pogrzeba, H.M. Kalaji, P. Zieleznik-Rusiniowska, Hormesis in plants: the role of oxidative stress, auxins and photosynthesis in corn treated with Cd or Pb, *Int. J. Mol. Sci.* 21 (2020) 2099, <https://doi.org/10.3390/IJMS21062099>.
- [72] C. Poschenrieder, C. Cabot, S. Martos, B. Gallego, J. Barceló, Do toxic ions induce hormesis in plants? *Plant Sci.* 212 (2013) 15–25, <https://doi.org/10.1016/j.plantsci.2013.07.012>.
- [73] S.I. Allakhverdiev, V.D. Kreslavski, V.V. Klimov, D.A. Los, R. Carpentier, P. Mohanty, Heat stress: an overview of molecular responses in photosynthesis, *Photosynth. Res.* 98 (2008) 541–550, <https://doi.org/10.1007/S11120-008-9331-0>.
- [74] B. Alaoui-Sossé, P. Genet, F. Vinit-Dunand, M.L. Toussaint, D. Epron, P.M. Badot, Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion contents, *Plant Sci.* 166 (2004) 1213–1218, <https://doi.org/10.1016/J.PLANTSCL.2003.12.032>.
- [75] L.B. Pena, C.E. Azpilicueta, S.M. Gallego, Sunflower cotyledons cope with copper stress by inducing catalase subunits less sensitive to oxidation, *J. Trace Elem. Med. Biol.* 25 (2011) 125–129, <https://doi.org/10.1016/J.JTEMB.2011.05.001>.
- [76] What is NDVI (normalized Difference Vegetation Index)?, (n.d.). <https://geoparc.tech/blog/what-is-ndvi-normalized-difference-vegetation-index/> (accessed June 14, 2025).
- [77] F. Oner, Effects of nitrogen doses on stomatal characteristics, chlorophyll content, and agronomic traits in wheat (*Triticum aestivum* L.), *PeerJ* 12 (2024) e18792, <https://doi.org/10.7717/PEERJ.18792/SUPP-1>.
- [78] J. Weger, J. Knápek, J. Bubeník, K. Vávrová, Z. Stražil, Can *Miscanthus* fulfill its expectations as an energy biomass source in the Current conditions of the Czech Republic?—Potentials and barriers, *Agriculture* 11 (2021) 40, <https://doi.org/10.3390/AGRICULTURE11010040>.