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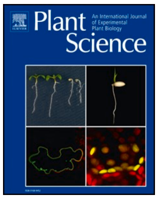

Do plants respond and recover from a combination of drought and heatwave in the same manner under adequate and deprived soil nutrient conditions?

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ABSTRACT

Extreme climatic conditions with extended drought periods and heatwaves are predicted to increase in frequency and severity in many regions of the world. Aside from this, other abiotic stress factors such as nutrient deficiency could pose a serious problem to plants when combined with other stressors resulting in more complex underpinning mechanisms. In the present study, we evaluated the response of *Brassica napus* to single and combined impacts of drought and heatwave (HW) under adequate or deprived (N-A and N-D) soil nutrient conditions. In addition, to get better insights in the plant response to combined stress, a post-stress period, pointing out a degree of the recovery after the cessation of stress, was also included. The results showed a different manner of single drought and heatwave action. The adverse effect of drought on leaf gas exchange was lagged on the growth and became more apparent only after recovery period with no obvious difference between different nutrient levels. Contrary, the growth response of nutrient-deprived plants to single HW was weak and in most cases, insignificant. Heatwave applied simultaneously with drought highly exacerbated the adverse effect of drought both under N-A and N-D conditions. Combined drought and heatwave stress resulted in the sharper decline of A_{sat} and it was attributed to both stomatal and non-stomatal limitations. Interestingly, plants underwent combined drought and HW treatment under N-D conditions showed better aboveground growth recovery, compared to those grown under N-A conditions, while displayed far more diminished photochemistry of photosystem II and badly disturbed the C/N balance. This discrepancy came from the fact that soil nutrient deficiency, by itself, evoked strong stress under control climate conditions resulting in a dramatically slower aboveground growth of nutrient-deprived plant. In turn, although combined drought and HW stress had similar effect on the aboveground growth either under N-A or N-D conditions, the recovery of later one was better. These results highlight the necessity to look at plants' performance under unfavorable environmental conditions beyond the actual event, since it can be depended not only on the duration of exposure but also on the legacy effect after treatment.

1. Introduction

Extreme heat and drought are among the most severe abiotic stressors affecting crop productivity worldwide that are predicted to increase in frequency and severity in many regions of the world due to global climate change [1]. According to IPCC AR5, since 1950, high-temperature extremes (hot days, tropical nights and heatwaves) have become more frequent in a large part of Europe [2]. In the absence of a universal definition, the heatwaves that are one of the most direct consequences of climate change are broadly defined as an extended period of excessively hot weather relative to the local climate [3]. Using statistical–meteorological criteria, Frich et al. [4] and Tebaldi et al. [5] defined a heatwave as at least five consecutive days with maximum temperatures at least 5 °C higher than the climatological norm of the same calendar days. This definition was adopted by the IPCC AR4 [6] and is used by the World Meteorological Organisation (WMO). In the natural environment, heatwaves are often accompanied by the low soil and relative air humidity drawing a drought stress that is a result of an imbalance of the water flux rate between the evapotranspiration demand and water transport into the soil-root system [7,8].

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It has been shown that the plants' growth and performance decline rapidly when water deficit and high temperature occur simultaneously [9–12]. Moreover, occurring simultaneously these adverse conditions alter plant metabolism in a novel manner that cannot be predicted from each of these stressors applied individually [13–15]. An abundance of intricacy of plant responses to combination of two or more abiotic stress conditions results from the fact that co-occurrence of different stressors impose a specific set of requirements on the plant that are mainly controlled by different signaling pathways which may interact or inhibit each other [14,15]. A single abiotic stress induces a plant response tailored to that specific environmental condition while plants encountering combined treatment of different abiotic stressors might require clashing readjustments [16]. The combined drought and heat stress could also evoke the divergent responses of plants. Under heat conditions, for example, plants usually increase their stomatal conductance to cool its leaves by transpiration while, under drought stress, plants tend to close their stomata to avoid unnecessary water loss. Thus, simultaneously applied heat and drought must influence signals controlling gas exchange in the way that, in order to ensure a proper defense, plants have to act and balance stomatal responses between preventing water loss and protecting from over-heating [15,16].

Most of the recent studies have showed that simultaneously applied heat and drought resulted in a more severe stress than when taken individually [17–20]. However, the impact of different abiotic stressors on plant functioning traits do not necessarily lead to an additive response but as a consequence of a synergistic or antagonistic effect of both stress factors drive the novel unique responses [21,22]. For example, a study with experimental plant communities, containing three common herbaceous species (*Plantago lanceolata* L., *Rumex acetosella* L. and *Trifolium repens* L.), showed that, under the combined conditions of heat and drought, the induced adverse effects were mostly more extensive than the sum of each of the stressors applied individually [23]. Meanwhile, in the study with *Eucalyptus globulus*, Correira et al. [13] found that rather than presenting an additive outcome, the heat stress ameliorated part of the negative effect of drought when applied simultaneously. These findings suggest that the interaction between these stressors and the plant responses are species/experiment dependent. It has been shown that for the same European oak species (*Quercus robur*, *Q. petraea*, *Q. pubescens*) the elevated daytime temperature was beneficial when imposed alone but was detrimental when combined with drought [24]. At the same time, heatwaves either as single stressor or combined with drought have a significant detriment effects for other tree and crop species [17–19]. Besides, plants display different responses to different stressors depending on their developmental stage [25].

In general, abiotic stressors, including heat and drought, are controlled by multiple genes, resulting in more complex underpinning mechanisms [7]. Therefore, it is important that both drought and heat would be considered together. In addition, other abiotic stress factors such as nutrient deficiency, soil salinity could also have an influence on heat and drought or may alter plant metabolism, making the study of plant responses to the impact of multiple abiotic stressors even more challenging. It has been shown that nutrient deficiency could pose a serious problem to plants when combined with other stressors [16]. And, conversely, a proper implementation of crop management practices, including a sufficient nutrient supply, can potentially alleviate the harmful effects of drought and heat stress [7,8]. Partly it could be explained by the fact that all the macronutrients, except K and Ca, are integrated into important organic compounds, such as amino acids and proteins (N and S), nucleic acids (N and P), phospholipids (P) and chlorophyll (Mg) [26]. While the micronutrients (such as Cu, Zn, Si and others) are essential for the activation of many enzymes scavenging reactive oxygen species (ROS) and thus alleviating oxidative stress [27]. The application of macronutrients, such as K and micronutrients like Ca, B, Se, and Mn, for example, which are known to modify stomatal function, can help to activate the physiological and metabolic processes contributing to preserving high water potential in tissues thereby increasing tolerance to heat stress [28]. Also, it has been shown that the application of potassium allows faster reopening of leaf stomata following drought-induced closure [29]. On the other hand, several studies have shown that the application of fertilizer has no significant effect on drought stress since water is critical for the mobility and metabolism of most of the nutrient [8,30].

Yet, with the changing climate, the trajectory of terrestrial nitrogen (N) availability is uncertain. Previously it was anticipated that the projected warmer and dryer climates would most likely stimulate the process of soil organic matter decomposition and nitrogen mineralization, thereby enhancing the N availability to plants [31]. However, a very recent review study, dealing with the more certain knowledge of terrestrial N availability, revealed a multiple line of evidence of declining N availability in many unfertilized terrestrial ecosystems [32]. Moreover, the certain environmental factors such as drought may cause nutrient deficiencies, even in fertilized fields, as the physiochemical properties of the soil can lead to a reduced mobility and bioavailability of individual nutrients [26], suggesting necessity to investigate the impact of increasing frequency of heatwaves and extended episodes of drought in conjunction with decreasing nutrient availability.

Rapeseed (*Brassica napus* L.) is the third most important oil crop in the world, following oil palm (*Elaeis guineensis*) and soybean (*Glycine max*) [33]. Because of its richness in polyunsaturated fatty acids, rapeseed oil is considered as a healthy ingredient in people diet. At the same time, oilseed rape meal, a by-product of oil extraction, containing comparable amino acid profile to soybean meal and rich in essential minerals and vitamins is used for animal feed as an alternative to soybean meal [34]. The present study, is, therefore, aimed at the investigation of how the adverse environmental conditions by imposed heatwave and drought will affect this agronomically important crop and to what degree their recovery will be managed after the cessation of these stressors' combination under adequate or deprived soil nutrient conditions.

2. Materials and methods

2.1. Plant material and experimental design

Spring oilseed rape (*Brassica napus* L., var. 'Fenja') were sown in plastic pots (64 cm diameter, 50 cm height) filled with a mixture of field top-soil (taken from Aleksandras Stulginskis University Experimental Research Station, Kaunas district), perlite and fine sand (5:3:2, by volume) with an available nitrogen of 46.7 ± 0.9 kg ha^{-1}. The planted pots were then divided and placed in two climate chambers (2.0 × 2.0 × 2.5 m width, length, height, LESLAT, Lithuania). Until the treatments, plants were grown under the ambient air temperature (AT) conditions of vegetation period that averaged 21.1 ± 0.02 °C/ 14.1 ± 0.02 °C (day/night) (Figs.1A and 2), corresponding to the long-term average for Lithuania, with 14 h/10 h day/night photoperiod (Fig. 1A). A day/night relative air humidity (RH) averaged 56 ± 0.1%/73 ± 0.3% (Fig. 1A), and a daytime CO_2 concentration averaged 406 ± 1.2 μmol mol^{-1}. A light level of about 270 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) was provided by a combination of natural daylight luminescent lamps (Philips, Waterproof OPK Natural Daylight LF80 Wattage 2 × 58 W/TL-D 58 W) and high-pressure sodium lamps (Philips MASTER GreenPower CG T 600 W). Volumetric soil water content (SWC), measured with a Theta Probe ML2x sensor combined with a handset HH_2 moisture meter with a depth of 6 cm (Delta-T Devices Ltd., Cambridge, UK), for the well-watered plants was kept at 30% on average.

Plants were fertilized with the complex nutrient (NPK 12-11-18 + microelements) solution. To obtain the final N level of 60 kg ha^{-1} for the nutrient deprived (N-D) treatment, additional fertilizer was added once before drought and heatwave treatments. To obtain the final N level of 160 kg ha^{-1}, for the adequate nutrient (N-A) treatment, half of additional fertilizer was added at the time of sowing and the second was added after 18 days after sowing (DAS) at two-leaf stage. Control plants were kept under the conditions mentioned above throughout the experiment.

The experiment was laid out in a randomized block design under factorial arrangement. Two nutrient levels (N—A, 160 kg N ha^{-1} and N—D, 60 kg N ha^{-1}) were arranged as two independent factors. Then both groups of plants with different nutrient availability (N—A and N—D) were randomly assigned to two water regimes (well-watered and drought-stressed), two temperature treatments (AT, 21/14 °C and HW, 33/26 °C, day/night) and combined drought and heatwave treatment. Three replications (pots) were included in each treatment.

The heatwave (HW, 21/14 *vs.* 33/26 °C, day/night) treatment was imposed after 26 DAS, at three-leaf stage for N-A and two-leaf stage for N-D treatments, BBCH 13 and 12 growth stages, respectively, according to the BBCH (biologische bundesanstalt, bundessortenamt and chemical industry) growth scale [35], for 7 days. At the 1st day of treatment, the temperature in the HW chamber was increased gradually from 21 °C to 33 °C between 8:00 h and 14:00 h in 4 steps: 24 °C at 8:00 h, 27 °C at 10:00 h, 30 °C at 12:00 h, and 33 °C at 14:00 h. Then it was held at 33 °C/26 °C day/night cycle for the next 5 days. At the 7th day of treatment, it was gradually decreased to 21 °C/14 °C day/night cycle in 4 steps between 14:00 h and 20:00 h: 30 °C at 14:00 h, 27 °C at 16:00 h, 24 °C at 18:00 h, and 21 °C at 20:00 h (Fig. 1B–D).

The drought stress to half of the plants in both temperature treatments (AT and HW) was applied by withholding water for the same period of 7 days. After the heatwave was relieved (i.e. the temperature returned to the ambient day/night air temperature cycle of 21/14 °C), the drought-stressed plants were re-watered to the target SWC level of 30% that was maintained further during the 7-day recovery period, according to the indication of the moisture meter (Fig. 3).

The climate program in plant growth chambers was controlled by the IGSS 9–13175 software. The preset values of the air temperature in both growth chambers were controlled manually at each of the chambers operating cupboards (Emerson Network Power S.r.l., Italy, model No. S06UC021V300020FX051260). With the exception of the 7-day heatwave episode in the HW chamber, differences in air temperature and relative humidity between the chambers before the treatments and during the recovery period were negligible ($p >$ 0.05) (Fig. 2A,B). Pots within the same growth chamber were rotated every day to minimize any potential chamber effects on plant performance. Treatment combinations among the chambers were rotated once during the exposure period (on the 4th day) and once during the recovery period (on the 4th day).

2.2. Leaf gas exchange measurements

Light-saturated leaf gas exchange measurements were performed on fully expanded lit leaves (randomly chosen three plants per pot were measured) using a portable photosynthesis system (LI-6400XT, LiCor Biosciences, Lincoln, NE, USA), equipped with a 6 cm^2 leaf cuvette and LED light source (Li-6400-02B LED). At the start of the measurements, the temperature of the leaf cuvette was set to match the temperature in each treatment chamber, either 21 °C for AT or 33 °C for HW treatments. The air flow rate through the assimilation chamber was maintained at 500 μmol s^{-1} and CO$_2$ concentration at 400 μmol CO$_2$ mol^{-1}. During all light-saturated measurements, PAR was set at 1500 μmol m^{-2} s^{-1}. Photosynthesis under saturating light conditions (A_{sat}; μmol CO$_2$ m^{-2} s^{-1}), stomatal conductance (g_s; mol H$_2$O m^{-2} s^{-1}), leaf transpiration (E; mmol H$_2$O m^{-2} s^{-1}), and intercellular CO$_2$ concentration (C_i) were simultaneously measured by the LI-6400XT software. Water use efficiency (WUE; μmol CO$_2$ mmol^{-1} H$_2$O) was calculated as the light-saturated CO$_2$ assimilation rate divided by transpiration rate (A_{sat}/E).

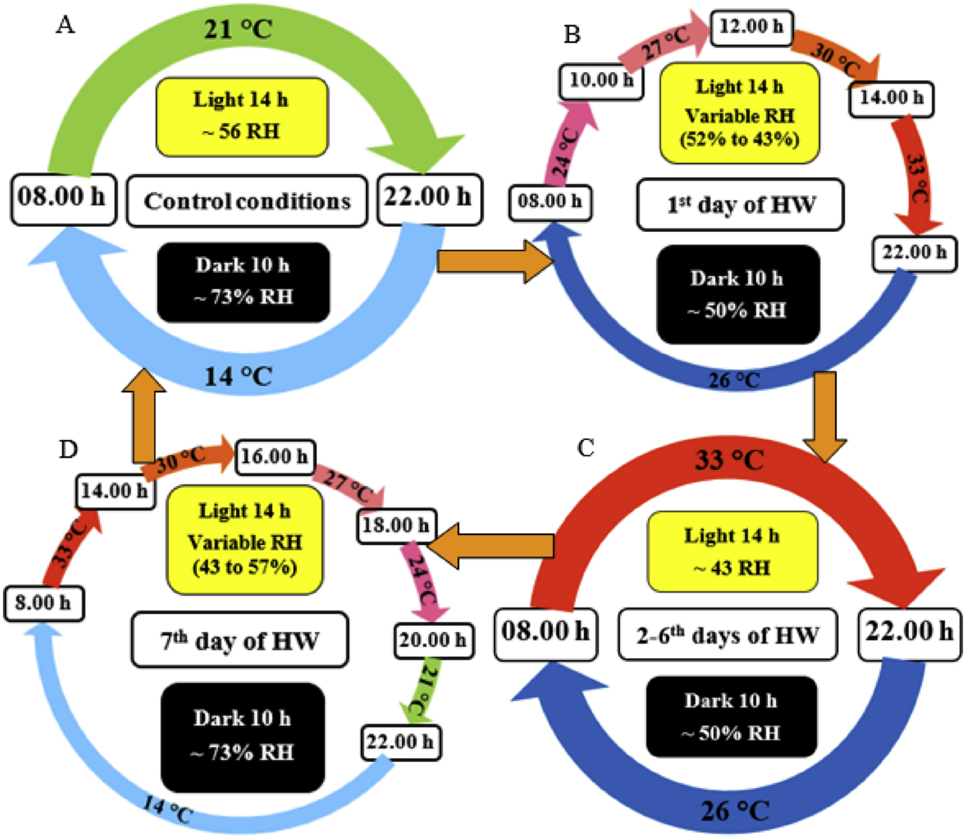

Fig. 1. Experimental design: temperature, relative humidity (RH) and light/dark period before (A), during (B–D) and after (A) heatwave (HW) treatment.

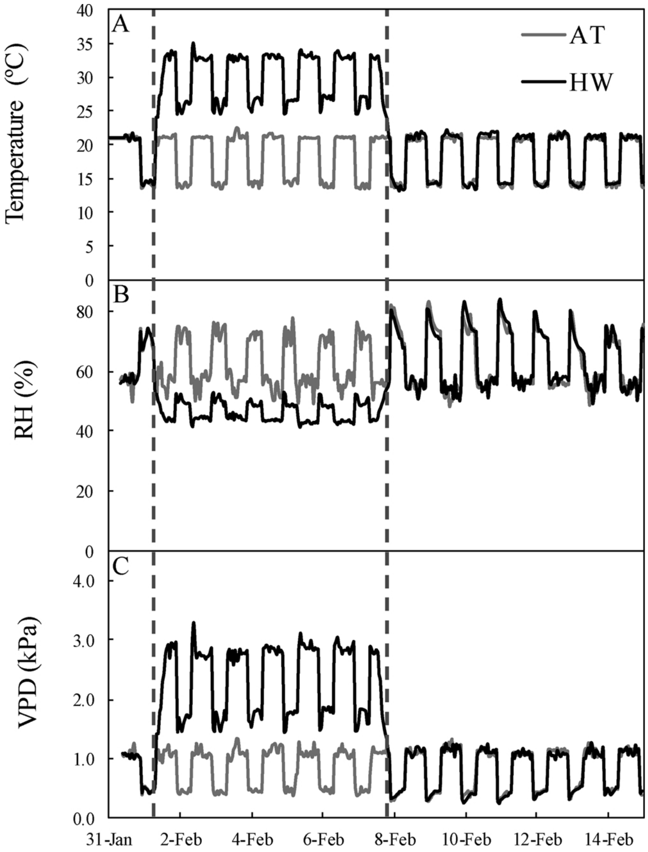

Fig. 2. Environmental conditions: (A) air temperature, (B) relative air humidity (RH) and (C) vapor pressure deficit (VPD) recorded in plant growth chambers throughout the experiment. Treatments: AT – ambient temperature treatment, HW – +12 °C heatwave treatment. The vertical dashed lines indicate a 7-day period during which HW was applied.

2.3. Chlorophyll *a* fluorescence measurements

The measurements of chlorophyll *a* fluorescence (ChlF) were taken with a Handy PEA portable fluorometer (Hansatech, UK). Data were recorded after 30 min of leaf adaptation to darkness in leaf clips. Light intensity reaching the leaf was 1800 μmol (quantum) m^{-2} s^{-1}. The following parameters were monitored: T_{Fm} (time (in ms) to reach maximal fluorescence, F_m), Area (total complementary area between fluorescence induction curve and F_m), PI_{abs} (overall performance index of PSII photochemistry), Ψ_o (probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-), and RC/ABS (density of reaction centers based of absorbed energy). PSII and Q_A^- are photosystem II and the primary quinone acceptor of PSII, respectively. F_v/F_m (maximum quantum yield of PSII) was calculated according to van Kooten and Snell [36]. The detailed formulae and terms used by the JIP-test for the analysis of the fluorescence transient O–J–I–P can be found in Rasineni et al. [37] and Meng et al. [38].

2.4. Determination of photosynthetic pigments

Leaves of plants were harvested and immediately frozen with liquid nitrogen. Frozen and grounded biomass was stored at −80 °C until the analyses. 50–100 mg of leaf tissue was weighted into the 2 ml Eppendorf test-tubes and homogenized (Retsch HM400, Germany) in 1 ml 80% (v/v) ethanol. The supernatant was used for the analysis. The content of chlorophylls a, b, a + b and carotenoids in tissue extract (in ethanol) were determined spectrophotometrically and expressed as mg g^{-1} fresh weight (FW).

2.5. Growth measurements

Half of the pots (24) were harvested after 7-day treatments, and another half of the pots were harvested after the 7-day recovery period. Random five plants per pot from each treatment were cut at the base, divided into leaves, stems and roots and used for the growth measurements. Leaves and stems were weighed immediately after harvest to determine mean fresh weight (FW) of leaves and stems in each treatment. Prior the measurements, roots were hand washed to remove all potting material and drained with a paper towel. For evaluation of leaf area, all leaves per plant were scanned using CanoScan 4400 F (Canon, USA) and then the leaf area was evaluated using GIMP 2.8 software. For the determination of dry weight (DW), foliage, stem and root biomass were dried at 60 °C until a constant DW was obtained and weighed. The shoot and root DW of each sample were calculated into DW per plant and root/shoot ratio and averaged as the values of each replicate.

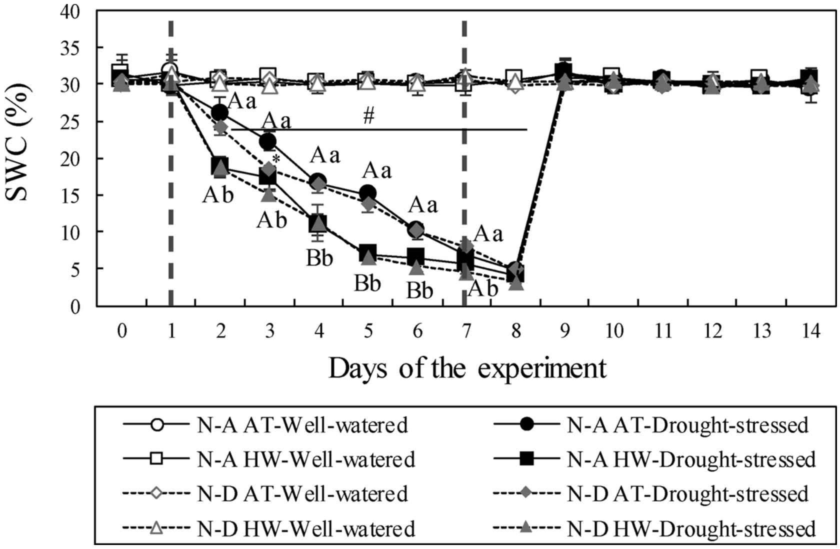

Fig. 3. Measured volumetric soil water content (SWC) at 12 cm depth throughout the experiment. Values are means ± SE ($n = 3$). Well-watered and drought-stressed plants grown under an adequate soil nutrient conditions (N-A) in ambient temperature (AT) are indicated by open and filled black circles, respectively. Well-watered and drought-stressed plants grown under N-A conditions in heatwave (HW) treatment are indicated by open and filled black squares, respectively. Well-watered and drought-stressed plants grown under deprived soil nutrient conditions (N-D) in ambient temperature (AT) are indicated by open and filled grey diamonds, respectively. Well-watered and drought-stressed plants grown under N-D conditions in HW treatment are indicated by open and filled grey triangles, respectively. The vertical dashed lines indicate a 7-day period during which HW treatment was applied. Values are means ± SE ($n = 3$). Different letters above the columns indicate significant difference ($p < 0.05$) among the AT and HW treatments within each day (Fisher's LSD). The capital letters are for the N-A conditions, and the lowercase letters are for the N-D conditions. # above the continuous horizontal line means that, from day 2 of exposure, all drought-stressed plants were significantly different ($p < 0.05$) from control plants, except for N-A plants grown under AT conditions (Fisher's LSD). * indicates a significant difference ($p < 0.05$) between N-A and N-D plants grown at the same temperature (Fisher's LSD).

2.6. Determination of leaf moisture content

The moisture content in leaves of each samples calculated as percentage of fresh weight was determined using the following formula:

$$(L_{FW} - L_{DW})/L_{FW}) \times 100\% \quad (1)$$

where L_{FW} is the fresh weight of leaves; L_{DW} is the dry weight of leaves.

2.7. Determination of total carbon and macro and micronutrients

After being oven-dried at 60 °C to a constant weight, shoot and root samples were pulverized, ground to a fine powder with a mill (Retsch HM400, Germany), and stored in hermetic pokes at room temperature until analysis. For measurement, 1.5–2.0 mg of tissue samples were warped up with tin capsules (9 × 5 mm), and the concentrations of total carbon (C) and nitrogen (N) were determined by combusting in an elemental analyzer Flash 2000 (Thermo Fisher Scientific, Great Britain).

To determine the total contents of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and cuprum (Cu), a milled samples of shoots and roots (~ 0.2 g) were first digested in a mixture of 65% HNO_3 and 30% H_2O_2 solutions (v/v = 8/2) using a high-pressure microwave digestion system (Milestone ETHOS One, Italy). An Optima 8000 ICP-OES (PerkinElmer, USA) was applied for the determination of the contents of the above six nutrients in mineralized samples by inductively coupled plasma–optical emission spectroscopy (ICP-OES).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) followed by the Fisher's LSD *post hoc* all pairwise multiple comparison tests for all measured variables were conducted for each sampling point (i.e., after exposure and recovery periods). Student's *t*-tests were used to compare the differences in air temperature and RH between the AT and HW chambers before the treatments and during the recovery period. Correlation analysis (Pearson) was used to test the relationship between different plant response parameters. The results were considered significant if $p \leq 0.05$. The data normality was checked prior to all the statistical analyses. All statistical analyses were performed by STATISTICA 8 software.

3. Results

3.1. Growth

Nutrient deprivation hampered the growth-related attributes of oilseed rape, significantly decreasing leaf area (LA), shoot, root, and total dry weight (DW) by 76, 51, 49, and 51% ($p < 0.05$), respectively, compared with control (Fig. 4A,C − E). Under both, nutrient adequate (N-A) and nutrient deprived (N-D) conditions, the response of growth-related attributes to the single drought and heatwave (HW), and in particular to the combined effects of these factors, was of the same pattern (Fig. 4A − F). Drought stress alone significantly reduced LA under N-A conditions and significantly increased root/shoot (R/S) ratio under N-D conditions, while other growth parameters did not significantly differ from their respective controls (well-watered plants grown under N-A or N-D conditions in AT treatment). A single HW had a positive effect on growth-related attributes in both N-A and N-D conditions, but the effect was less pronounced in N-D conditions. In contrast, the combined effects of HW and drought significantly reduced rape growth in both N-A and N-D conditions, to a similar degree in both cases. Under N-A conditions, LA, shoot, root, and total DW decreased by 48, 35, 41, and 36% ($p < 0.05$), respectively, but the R/S ratio remained unaffected ($p > 0.05$). In N-D conditions, LA, shoot, and total DW decreased by 60, 35, and 34% ($p < 0.05$), respectively, but root DW did not change significantly ($p > 0.05$), resulting in a 54% ($p < 0.05$) increase in R/S ratio (Fig. 4A − F). Regarding the leaf moisture content (LMC), it was significantly affected by all stress treatments and changed by the same pattern, as did growth parameters, under both N-A and N-D conditions (Fig. 4B).

After the 7-day recovery, the single effect of nutrient deficiency on growth was even more pronounced, in particular by decreasing aboveground growth, which resulted in a significant increased R/S ratio (Fig. 4G − L). In N-A conditions, rape pre-exposed to single drought exhibited retarded growth—although LA, shoot, root, and total DW increased in the post-exposure period, but all of these growth parameters were significantly lower ($p < 0.05$) compared to control plants. At the same time, the values of the growth parameters of N-D plants subjected to single drought remained statistically similar to their control ($p > 0.05$). The effect of single HW on growth was also more pronounced after the recovery period under both N-A and N-D conditions, mainly due to intensified aboveground growth. In contrast, despite the recovery of LMC ($p > 0.05$), the difference between the LA, shoot and total DW values of control and combined heat-drought-exposed plants increased even more, whereas root DW slightly recovered, resulting in a significant increase in R/S ratio (Fig. 4G − L). Meanwhile, in N-D conditions, the aboveground growth of the plants subjected to the combined HW and drought was slightly restored—LA, shoot and total DW no longer differed significantly from their respective controls ($p > 0.05$), while root growth has slowed down ($p < 0.05$).

3.2. Photosynthetic characteristics

At the physiological level, rape grown under conditions of nutrient deficiency alone showed significantly lower values of photosynthetic rate (A_{sat}), stomatal conductance (g_s), water use efficiency (WUE), and Area parameter (total complementary area between fluorescence induction curve and F_m) (Fig. 5A–E and Table 1).

Exposure to single and combined stressors had different influence on the leaf gas exchange and chlorophyll *a* fluorescence (ChlF) parameters though no difference was found between N-A and N-D treatments. Single drought has resulted in significantly ($p < 0.05$) reduced A_{sat}, g_s, transpiration (E), and intracellular CO_2 concentration (C_i) and increased WUE, whereas single heatwave has led to significantly increased A_{sat}, g_s, and E, and decreased WUE ($p < 0.05$). The HW and drought applied simultaneously exacerbated the drought effect and A_{sat}, g_s, E, and C_i were more negatively affected compared to their response to single drought (Fig. 5A − D). Single drought had no significant effect ($p > 0.05$) on ChlF parameters in either N-A or N-D conditions, while single heatwave significantly increased PI_{abs} (PSII performance index), Ψ_o (probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-) and RC/ABS (density of RCs based of absorbed energy) compared to the respective controls ($p < 0.05$). Meanwhile, when plants were exposed to HW and drought simultaneously, Area, PI_{abs} and Ψ_o parameter values decreased significantly ($p < 0.05$) (Table 1). Leaf chlorophyll content remained generally unaffected ($p > 0.05$) in all stress treatments (Fig. 6A−D).

After the recovery period, rape growing under nutrient deprivation continued to show significantly lower levels of A_{sat} and g_s compared to control plants, as well as decreased values of E, Area and RC/ABS parameters ($p < 0.05$) (Fig. 5F−*J* and Table 1). Both leaf gas exchange and ChlF parameter values of plants exposed to single drought and HW after the recovery period were statistically similar to those of the respective controls ($p > 0.05$). With the exception of the significantly lower value of the Area parameter in leaves of plants pre-exposed to drought under N-A conditions ($p < 0.05$) and the higher value of Area in leaves of plants previously subjected to HW under N-D conditions ($p < 0.05$) (Fig. 5F−*J* and Table 1). In contrast, plants previously experienced combined drought and HW stress exhibited significantly lower values for A_{sat}, g_s, E, C_i, and RC/ABS ($p < 0.05$) in N-A conditions and significantly lower values for A_{sat}, g_s, Area, PI_{abs}, Ψ_o

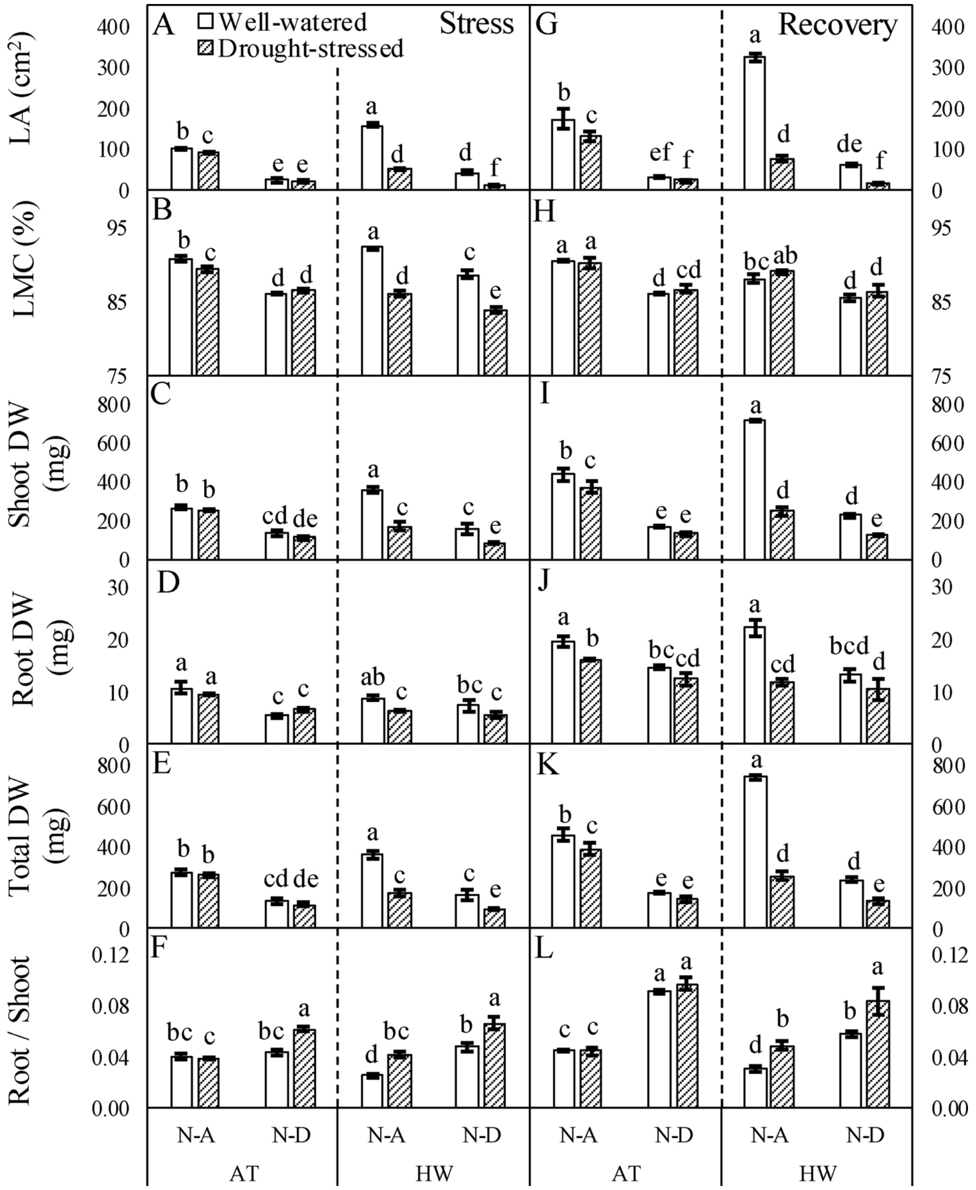

Fig. 4. Leaf area (LA), leaves moisture content (LMC), shoot, root, and total dry weight (DW), and root to shoot ratio of well-watered and drought-stressed plants grown under an adequate (N-A) or deprived (N-D) soil nutrient conditions and under an ambient temperature (AT) or heatwave (HW) treatment at the end of the stress period (A − F) and after recovery (G − L). Values are means ± SE (n = 3). Different lowercase letters above the columns indicate significant difference ($p <$ 0.05) among the treatments (Fisher's LSD).

and RC/ABS ($p <$ 0.05) in N-D conditions with respect to their respective controls (Fig. 5F − *J* and Table 1). In addition, after the recovery period under N-D conditions, plants pre-exposed to the combined drought and HW had significantly higher levels of chlorophylls (Chl a, Chl b, total Chl) in the leaves compared to their control (Fig. 6E − G).

3.3. Carbon (C), nitrogen (N) and C/N ratio

In well-watered rape shoots grown under N-D conditions, the nitrogen concentration was halved compared to that found under N-A conditions ($p <$ 0.05), which resulted in a doubling of the C/N ratio, despite higher C content ($p <$ 0.05). The C/N ratio in N-D rape roots was also significantly higher (about 40%) than in N-A plants, due to significantly lower N concentration in the roots (Fig. 7A − C). Single drought did not significantly affect either N or C content in shoots and roots ($p >$ 0.05), but slightly increased C/N ratio in shoots under N-D conditions ($p <$ 0.05). At the same time, single HW significantly increased N in the shoots under both N-A and N-D conditions but decreased in roots ($p <$ 0.05) and slightly decreased C content in shoots under N-D conditions ($p <$ 0.05). As a result, the C/N ratio in the roots significantly increased in both fertilization cases and decreased in the shoots under N-D conditions ($p <$ 0.05). Plants subjected to combined HW and drought were found to have significantly lower N level in roots under N-A conditions and significantly lower N levels in shoots and roots under N-D conditions. Meanwhile, C content in roots remained unaffected ($p >$ 0.05), slightly increased in shoots under N-A conditions and decreased in N-D conditions ($p <$ 0.05). Consequently, there was a significant increase in C/N ratio in roots under N-A conditions and in shoots and roots in N-D conditions (Fig. 7A − C).

After the recovery period, the N content in the oilseed rape shoots grown solely under nutrient deficiency was further decreased compared to control ($p <$ 0.05), and lower in roots to a similar degree as during exposure (p < 0.05). The C content did not differ significantly from the control plants ($p >$ 0.05), resulting in a further increase in the C/N ratio in the shoots ($p <$ 0.05), while remaining higher in the roots to a

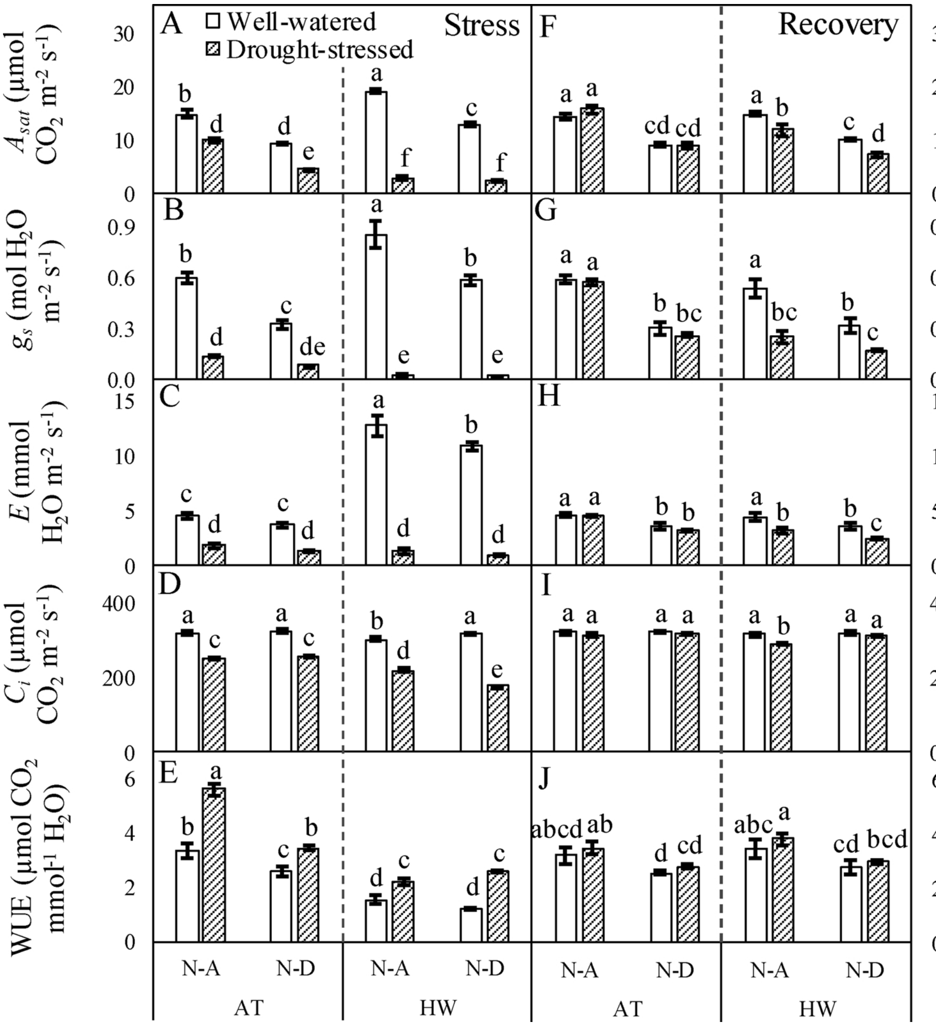

Fig. 5. Light saturated photosynthetic rate (A_{sat}), stomatal conductance (g_s), transpiration (E), intercellular CO$_2$ concentration (C_i), and water use efficiency (WUE) of well-watered and drought-stressed plants grown under an adequate (N-A) or deprived (N-D) soil nutrient conditions and under an ambient temperature (AT) or heatwave (HW) treatment at the end of the stress period (A − E) and after recovery (F − J). Values are means ± SE (n = 9). Different lowercase letters above the columns indicate significant difference ($p < 0.05$) among the treatments (Fisher's LSD).

Table 1

Chlorophyll fluorescence parameters for the well-watered and drought-stressed plants grown under an adequate (N-A) or deprived (N-D) soil nutrient conditions and under an ambient temperature (AT) or heatwave (HW) treatment at the end of the stress period and after recovery. Different lowercase letters for each parameter within the treatments indicate significant difference ($p < 0.05$) among the treatments (Fisher's LSD) (n = 9).

Stress			F_v/F_m	T_{Fm}	Area	PI_{abs}	Ψ_o	RC/ABC
AT	N-A	Well-watered	0.841^{a}	280^{b}	62198^{a}	6.90^{bc}	0.667^{bc}	0.539^{bc}
		Drought-stressed	0.836^{a}	290^{ab}	57373^{a}	5.97^{bcd}	0.652^{c}	0.517^{bcd}
	N-D	Well-watered	0.829^{a}	290^{ab}	47979^{b}	5.39^{cd}	0.642^{c}	0.508^{cd}
		Drought-stressed	0.827^{a}	293^{ab}	43599^{b}	5.10^{cd}	0.636^{cd}	0.492^{cd}
HW	N-A	Well-watered	0.840^{a}	267^{b}	60493^{a}	9.57^{a}	0.730^{a}	0.596^{a}
		Drought-stressed	0.830^{a}	300^{ab}	41996^{b}	4.47^{d}	0.604^{d}	0.494^{cd}
	N-D	Well-watered	0.838^{a}	280^{b}	47755^{b}	7.74^{ab}	0.689^{b}	0.570^{ab}
		Drought-stressed	0.829^{a}	333^{a}	32896^{c}	$4,17^{d}$	0.602^{d}	0.472^{d}

Recovery			F_v/F_m	T_{Fm}	Area	PI_{abs}	Ψ_o	RC/ABC
AT	N-A	Well-watered	0.848^{a}	363^{a}	72093^{ab}	$7,83^{a}$	0.638^{ab}	0.676^{a}
		Drought-stressed	0.833^{a}	367^{a}	64681^{cd}	$6,73^{a}$	0.617^{ab}	0.615^{ab}
	N-D	Well-watered	0.850^{a}	433^{a}	57162^{e}	6.06^{a}	0.621^{ab}	0.591^{b}
		Drought-stressed	0.849^{a}	433^{a}	52806^{e}	$5,63^{a}$	0.590^{b}	0.571^{b}
HW	N-A	Well-watered	0.843^{a}	363^{a}	76772^{a}	$6,42^{a}$	0.615^{ab}	0.597^{ab}
		Drought-stressed	0.842^{a}	433^{a}	70591^{bc}	$6,39^{a}$	0.641^{ab}	0.552^{bc}
	N-D	Well-watered	0.848^{a}	450^{a}	63571^{d}	$5,41^{ab}$	0.602^{ab}	0.546^{bc}
		Drought-stressed	0.830^{a}	500^{a}	43644^{f}	$3,05^{b}$	0.524^{c}	0.472^{c}

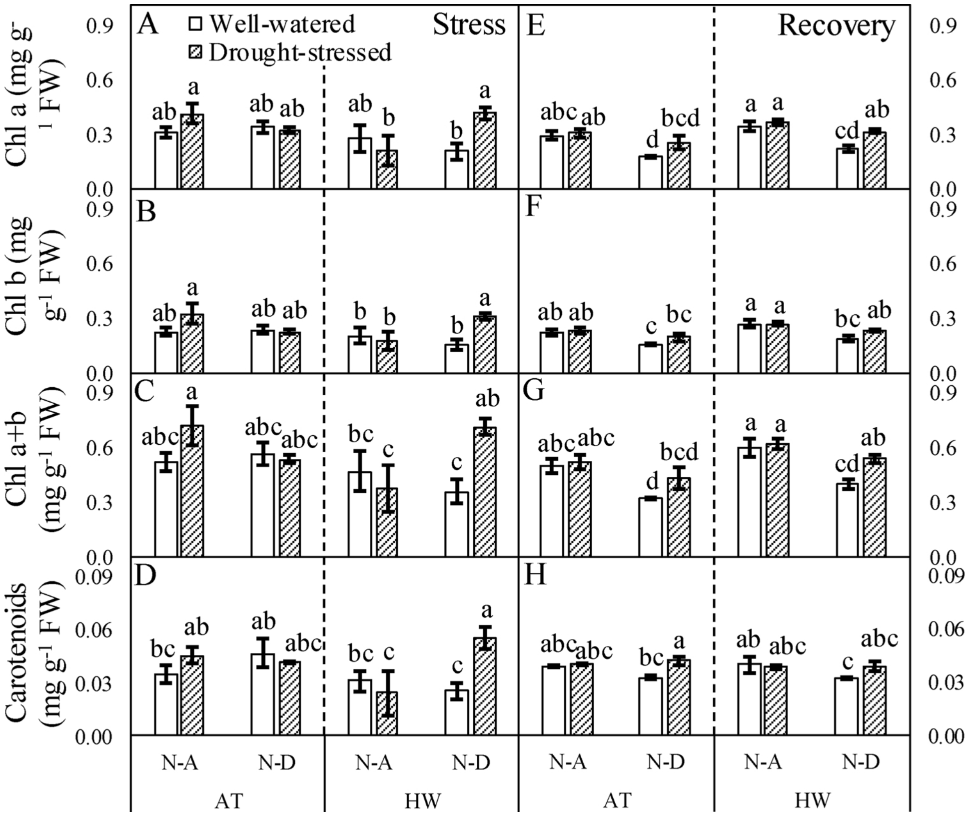

Fig. 6. Chlorophyll (Chl a), chlorophyll *b* (Chl b), total chlorophyll (Chl a + b), and carotenoids contents of well-watered and drought-stressed plants grown under an adequate (N-A) or deprived (N-D) soil nutrient conditions and under an ambient temperature (AT) or heatwave (HW) treatment at the end of the stress period (A – D) and after recovery (E–H). Values are means ± SE ($n = 3$). Different lowercase letters above the columns indicate significant difference ($p < 0.05$) among the treatments (Fisher's LSD).

similar degree as during the exposure ($p < 0.05$) (Fig. 7D – F). Under both N-A and N-D conditions, the N and C content of single drought-affected rape shoots and roots, as well as the C/N ratio, did not differ significantly from the respective controls after the recovery period ($p > 0.05$), except for a slight decrease in N content of shoots in N-A conditions ($p < 0.05$). Meanwhile, in rape shoots pre-exposed to HW alone, the N content increased even more under N-A conditions and remained elevated to a similar degree under N-D conditions compared to the respective controls ($p < 0.05$). C content was not different in shoots or roots ($p > 0.05$). In turn, the C/N ratio in the shoots decreased significantly in N-A conditions and slightly recovered in the N-D condition, although it remained significantly lower compared to control plants ($p < 0.05$). In both cases of fertilization, the root C/N ratio between HW-exposed and control plants did not differ after recovery ($p > 0.05$) (Fig. 7D – F).

Under N-A conditions, the N content of the combined HW and drought-affected rape shoots was slightly elevated compared to the control ($p < 0.05$) and decreased even more in the roots after recovery

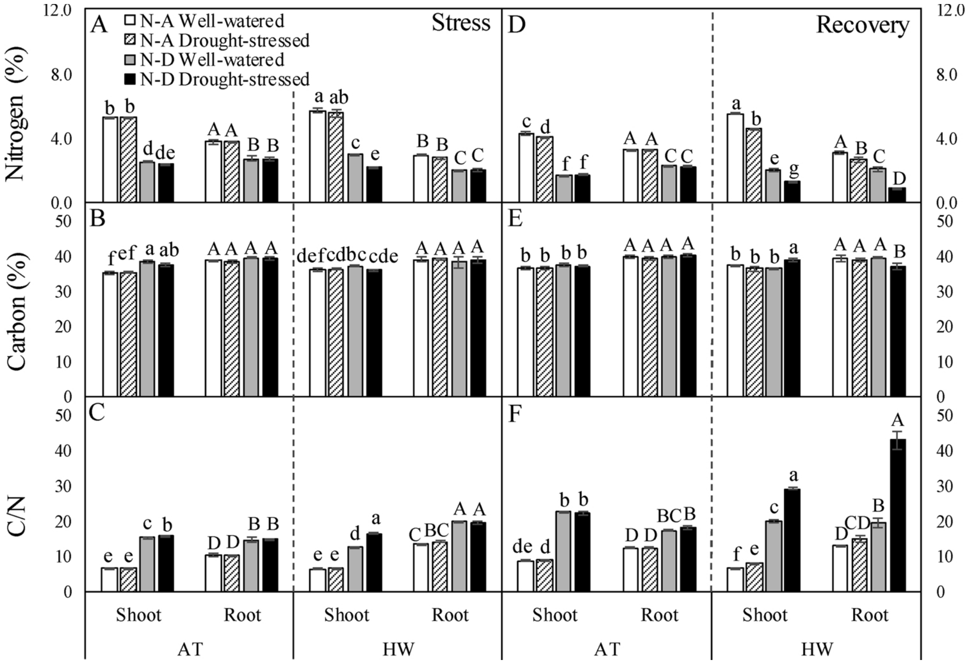

Fig. 7. Variation of carbon and nitrogen concentrations and carbon-to-nitrogen ratio (C/N) in shoots and roots of well-watered and drought-stressed plants grown under an adequate (N-A) or deprived (N-D) soil nutrient conditions and under an ambient temperature (AT) or heatwave (HW) treatment at the end of the stress period (A – C) and after recovery (D – F). Values are means ± SE ($n = 3$). Different lowercase letters above the columns indicate significant difference ($p < 0.05$) among the treatments (Fisher's LSD).

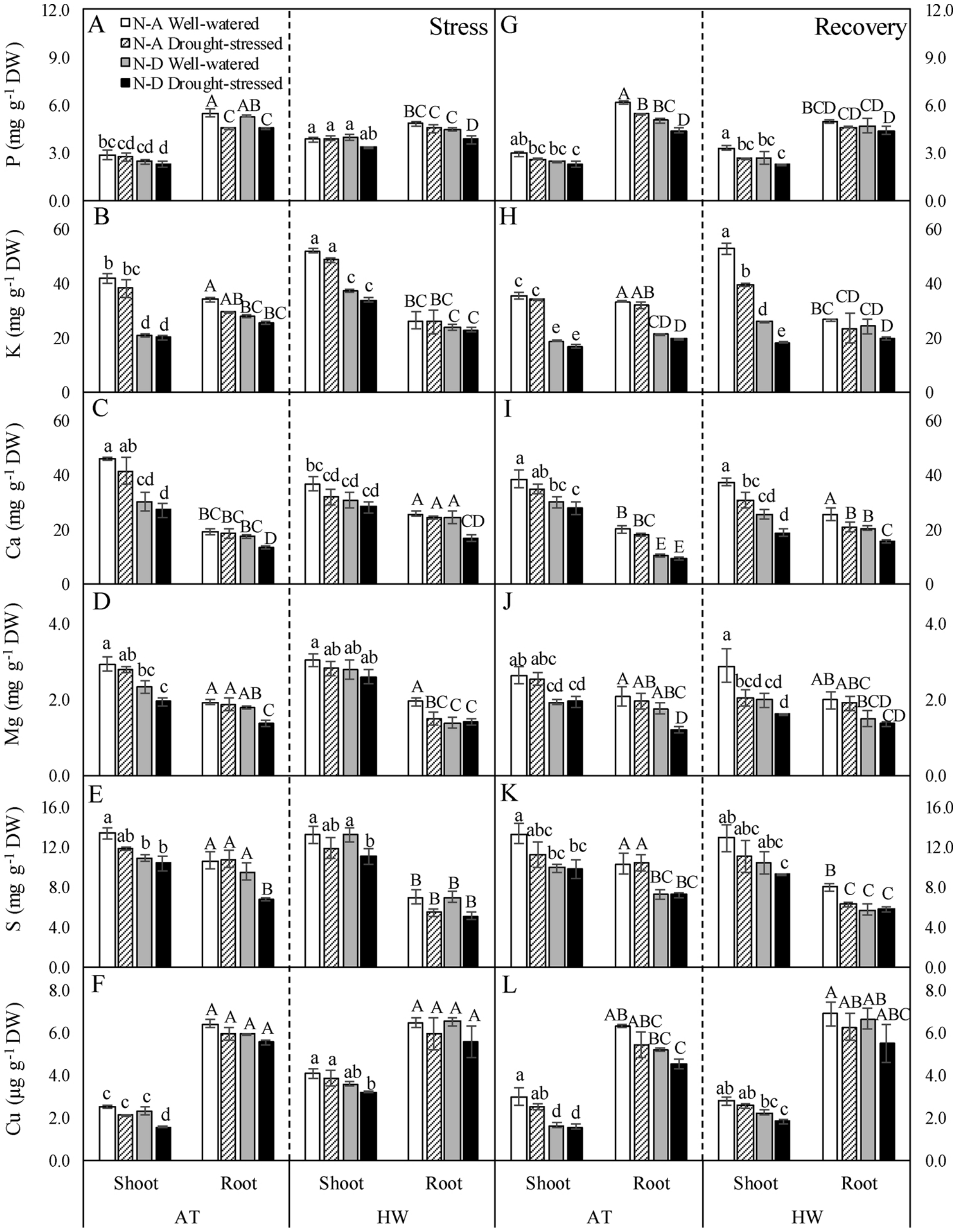

Fig. 8. Variation of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), and copper (Cu) concentrations in shoots and roots of well-watered and drought-stressed plants grown under an adequate (N-A) or deprived (N-D) soil nutrient conditions and under an ambient temperature (AT) or heatwave (HW) treatment at the end of the stress period (A − F) and after recovery (G − L). Values are means ± SE ($n = 3$). Different lowercase letters above the columns indicate significant difference ($p < 0.05$) among the treatments (Fisher's LSD).

($p < 0.05$). As well as further decreased in both shoots and especially in roots under N-D conditions ($p < 0.05$). The C content in shoots and roots did not differ from control plants under N-A conditions ($p > 0.05$), whereas in shoots increased slightly and in roots decreased under N-D conditions ($p < 0.05$). Consequently, after recovery, the C/N ratio in N-A conditions for previously combined HW and drought-exposed rape shoots and roots did not differ from their control ($p > 0.05$), whereas in N-D conditions significantly increased in both shoots (up to 30%) and especially roots (almost one and a half times) compared to their controls (Fig. 7D−F).

3.4. Other macro and micronutrients

Compared to well-watered N—A rape, N—D rape had significantly lower potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) concentrations in the shoots and lower K concentration in the roots at the end of exposure ($p < 0.05$) (Fig. 8A–F). At single drought exposure, nutrient levels in shoots were similar to their respective controls in both N—A and N—D conditions ($p > 0.05$), except for significantly lower copper (Cu) concentration in N—D conditions. However, both groups of rape with different fertilization, affected by single drought, showed significantly lower phosphorus (P) concentrations in the roots, and lower Ca, Mg and S concentrations in the roots ($p < 0.05$) under N—D conditions. Rape exposed to single HW had significantly higher P, K and Cu concentrations in shoots and Ca in roots ($p < 0.05$), but lower P and S concentrations in roots compared to the respective control plants ($p < 0.05$) regardless the nutrient level in the soil. Increases in P and K content in shoots and Ca in roots were more expressed in N—D conditions, whereas in roots, P concentration decreased more in N—D conditions and S decreased more in N—A conditions (Fig. 8A–F). Changes in macro and micronutrient content after exposure to combined drought and HW were similar to that after single HW treatment. However, compared to the individual effects of drought and HW, the combined effects of HW and drought in terms of nutrient depletion were in most cases more damaging (e.g. P in roots) and less pronounced in terms of increases in nutrient contents (e.g. K in shoots) than in HW alone (Fig. 8A − F).

In nutrient-deficient rapes, after the recovery period, shoot concentrations of all nutrients, except P, were significantly lower compared to that grown in sufficient nutrient-rich soil ($p < 0.05$), but they had significantly lower P, K, Ca, and S concentrations in the roots ($p < 0.05$) (Fig. 8G − L). In single-drought-affected rapes, nutrient shoot concentrations did not differ from the corresponding controls, indicating recovery ($p > 0.05$), whereas P levels in roots remained significantly lower ($p < 0.05$). During the recovery period, K shoot concentration in single HW pre-exposed rapes increased ($p < 0.05$) under both nutrient level conditions, though under N−D conditions this increase was less pronounced. Concentrations of other nutrients in the shoots were not significantly different from the control plants under N−A conditions ($p > 0.05$), while Cu concentration remained higher in N−D conditions compared to their control ($p < 0.05$). Ca concentration in rape roots of both groups (N−A and N−D) remained significantly higher and even more under N−D conditions ($p < 0.05$). In N−D conditions, the concentrations of other nutrients in the roots did not differ significantly with respect to their control ($p > 0.05$), whereas in N−A conditions P, K and S concentrations were still significantly lower ($p < 0.05$) and P even stronger (Fig. 8G − L). In N−A conditions, rape previously exposed to combined HW and drought after recovery possessed significantly higher K but lower Cu concentrations in shoots and significantly lower P, K and S concentrations in roots compared to controls ($p < 0.05$). At the same time, N−D oilseed rape possessed higher Cu concentration in shoots and Ca in roots, but lower P concentration in roots with respect to their control ($p < 0.05$). As with exposure, after the recovery period, the increases of nutrient concentrations were less pronounced for the combined effects than for the HW alone, and the reductions were larger than for the individual drought and HW effects (Fig. 8G − L).

4. Discussion

Plants growth and physiological responses to drought, HW and their combined treatment under adequate and deprived soil nutrient conditions

In the present study, the single effect of drought in most cases had no significant effect on morphological parameters of oilseed rape (Fig. 5A − F), but was clearly seen at the physiological level in term of down regulated leaf gas exchange under both N-A and N-D conditions (Fig. 5A − D). A decrease in stomatal conductance (g_s) is well known as the first and foremost responsive event of almost all the plants to water deficiency. This physiological adjustment enables to maintain hydraulic functions avoiding water loss trough transpiration (E) but checks CO_2 intake from the environment into the cellular spaces of the leaf (C_i) leading to the reduced assimilation [7,39,40]. As there was no significant impact on ChlF parameters nor on the content of photosynthetic pigments (Table 1 and Fig. 6A − D), more acute decrease in g_s than in A_{sat} with a parallel decline in C_i indicates that the reduced CO_2 assimilation in single drought-stressed plants was specifically attributed to the direct stomatal rather that the biochemical limitations. These are in accordance with the study of Praxedes et al. [41], where the long-term drought effects on robusta coffee (*Coffea canephora*) were investigated, and consist with the results of our previous study with *Brassica napus*, where single and combined effects of drought and heatwave under ambient and elevated CO_2 levels were investigated, growing plants under N-A conditions [42]. Our results have shown that decrease in g_s had a more inhibitory effect on E than on CO_2 diffusion into the leaf tissues resulting in highly increased water use efficiency (WUE), especially under N-A conditions (Fig. 5B − E). Up-regulation of WUE is also a common plants' response to drought [7,18,19,42,43]. However, significantly decreased leaf moisture content (LMC) under N-A conditions (Fig. 4B), points out that these physiological adjustments in response to drought were insufficient to prevent water loss.

Compared to N-A plants, the growth responses of N-D plants to single HW treatment were weaker and, in most cases, insignificant. Nevertheless, the responses of leaf gas exchange and ChlF parameters were quite similar under N-A and N-D conditions. In both cases, there was significant increase in PI_{abs} (Table 1) that is an indicator of the vitality of photosynthesis [44] and presents the combined measurement of RC/ABS, the maximum quantum yield of primary photochemistry (Φ_{Po}) and Ψ_o. Therefore, the obtained results demonstrate that the imposed heatwave alone stimulated the light-saturated photosynthesis through enhanced photochemistry of photosystem II by increasing the photochemical energy use [45]. In addition, the well-watered plants grown in HW treatment both under N-A and N-D conditions displayed lower value of time taken to reach the maximal fluorescence (T_{Fm}), compared with controls, though not significantly, suggesting the possibly enhanced photosynthetic electron transport and/or optimized the total electron accepting capacity of leaves [46]. Furthermore, despite significant increases in g_s and E and a decrease in WUE (Fig. 5B,C,E), LMC increased under both N-A and N-D conditions relative to their respective controls (Fig. 4B), indicating that a single HW did not influence oilseed rape water relation, allowing them to cool their leaves via transpiration and thus prevent overheating.

Compared to single drought, the significantly lower value of SWC in combined drought and HW treatment (Fig. 3) suggests that intensified effect on gas exchange could be the reason of increased vapor pressure deficit (VPD) that often accelerates evapotranspiration, thereby aggravating soil water depletion and further lowering g_s [47,48]. However, a stronger decrease in A_{sat} under combined stress could be also related to WUE, which is one of the most significant parameters in crop response to loss of osmoregulation, that was regulated in an opposite manner than under single drought treatment or had no significant response (Fig. 5E). A disruption in osmotic potential in the combined effects of drought and HW is also confirmed by the significant decrease in LMC under both N-A and N-D conditions and even a strong negative interaction ($r = -0.90, p < 0.05$) was found between WUE and LMC in the case of combined effects. In addition, plants subjected to combined drought and HW treatment both under N-A and N-D conditions have had down-regulated ChlF parameters, as demonstrated by significantly lower values of Area, PI_{abs} and Ψ_o (Table 1). Area is proportional to the pool size of the electron acceptors Q_A (the primary quinone acceptor of PSII) on the reducing side of PSII. It has been arguable that if electron transport from reaction centers to the quinone pool size is blocked, Area will be dramatically reduced [49,50], suggesting that combined drought and HW treatment decreased the activity of PSII by inhibiting the electron transfer rates at the donor side. Aside from this, the plants' leaf temperature subjected to combined drought and HW both under N-A and N-D conditions was 2–2.5 °C higher, compared with those grown under HW alone (data not shown). Under combined drought and heat conditions, the plants' leaf temperature can be higher up to 2–5 °C [42,51–53], compared with that of plants subjected to heat stress alone, because of reduced g_s and consequent transpirational cooling. It has been shown that, once the thermal threshold is exceeded, the non-stomatal limitations could occur, adding in a decrease of carbon assimilation [15,54,55]. Therefore, our results suggest that, in addition to stomatal limitations, a sharper decline in A_{sat} under combined stress could be also attributed to non-stomatal limitations on photosynthesis that translated into considerably lower aboveground growth (Figs. 5A and 4A,C). Apart from the down-regulated ChlF parameters, all other possible reasons of exacerbated A_{sat} under combined treatment discussed here, i.e. the faster soil drying induced by higher evapotranspiration through higher VPD, the loss of osmoregulation through reduction in WUE, and increased plants' leaf temperature, are discussed in the above-mentioned our recent study [42] in more detail.

Effects of drought, HW and their combined treatment on C, N, C/N and other mineral nutrient status in shoots and roots under adequate and deprived soil nutrient conditions

Besides water deficiency itself, drought has also a great influence on the nutrient relations in the plants as most of the nutrients are provided with water [8,27]. Drought stress not only reduces the availability of nutrients in the soil but also decreases their transport to the upper parts of the plant [56–59] due to decreased stomatal conductance and consequent transpiration [41,60–62]. Drought stress could lead to a sharp decrease in N content in the leaves of plants [27]. A significant positive correlation ($r = 0.85$, $p < 0.05$) between transpiration of drought-stressed plants grown in AT treatment and N content in their shoot was also found in our study, supporting this tendency. About 50% of all N in the leaf is directly involved in photosynthesis as either enzymes or chlorophyll [63]. Thus, insufficient N supply can decrease the plants' photosynthetic performance by reducing the leaf area (as it was detected in our study) and photosynthesis rate as well as accelerating leaf senescence [27,64]. Higher activity of PSII and A_{sat} was also characteristic for rapes grown under N-A conditions (Table 1 and Fig. 5A,F). Further, drought generally results in carbon/nitrogen (C/N) imbalance, associated with drought-induced leaf senescence, which is reflected in the increased C/N ratio [65]. The C/N balance is one of the most important factors in the regulation of plant metabolism, growth and development and rather than C or N alone play an important role in regulating leaf senescence [66–68]. It has been reported that the leaf senescence can be triggered by a higher C/N ratio [67,69]. Thus, a likely reason of slightly increased C/N ratio in shoots of single drought-stressed plants grown under N-D conditions (Fig. 7C) is accelerated leaf senescence, since both the water deficit and N deficiency in soil are the good triggers to promote it.

Regarding the phosphorus, this essential mineral nutrient has one unique characteristic in terms of its low availability due to slow diffusion and high fixation in soils [70]. Thus, a decrease of P absorption in roots of drought-stressed plants (Fig. 8A) could be the reason of its low mobility, which is aggravated even more under drought conditions [71]. Generally, P uptake, transport and redistribution has been shown to decrease under drought [27,39,72,73]. The uptake and/or translocation of K, Ca and Mg also decreases under drought conditions [27,59,74,75], leading to a decrease of their content in leaves in the order of K > Mg > Ca [27]. Supplementary added potassium in the growth medium can increase the plants' resistance to drought mainly through its functions in stomatal and osmoregulation, since the stomatal opening mechanism is governed by the K^+ concentration [73,76,77]. Regarding sulphur, it has been shown an increased or not changed its content in plants' leaves, stems and roots [75,78], while no information, up to date, is available regarding the effects of drought on the uptake and distribution of Cu in higher plants.

It has been shown that the effects of single heat on plant nutrient content varies among nutrients and plant species, however for both chronic and acute heat stress (heatwaves) reductions in their content are evident more often than increases or no effect [79,80]. Since the imposed HW alone did not affect significantly the root DW (Fig. 4C) neither under N-A nor under N-D conditions, based on the few recent studies, the likely reason of decreased content of nutrients in roots under single HW is a decrease in nutrient-uptake and -assimilation protein in roots [80,81]. Whereas, the increase of the content of several nutrients in shoots of well-watered plants grown in HW treatment either under N-A or N-D conditions could be related with the markedly increased stomatal conductance and subsequent transpiration rate (Fig. B,C), and thus enhanced nutrient transport from roots to shoot. It was found that, for example, Cu content in shoot of well-watered plants grown under single HW treatment was significantly positively correlated with transpiration ($r = 0.83$, $p < 0.05$).

Very little data are available regarding the C and N relations in plants grown under the heatwave conditions. The recently conducted study of Mainali et al. [82] with the stress-tolerant C_4 grass, *Andropogon gerardii*, have showed that heating increased transfer of recently-fixed C from shoot to roots and soil but decreased efficiency of N uptake by roots. In the present study, the changes in C/N ratio in roots under single HW treatment were solely attributed to the changes in N content, regardless of soil nutrient status (Fig. 7A − C). It was found that plant belowground C/N ratios were negatively correlated with plant belowground N content ($r = -0.98$, $p < 0.01$), while had no significant relationship with plant belowground C content. Similarly, plant aboveground tissues C/N ratios were more strongly associated with aboveground tissues N than C content ($r = -0.99$ for N *vs.* 0.82 for C, $p < 0.001$ and 0.05, respectively).

No information, on our knowledge, up to date, is available regarding the combined effect of drought and heat on the C/N status in plants. A strong negative correlation between C/N and N content in shoot ($r = -0.99$, $p < 0.01$), in parallel with no significant relationship between C/N and C content, showed that slightly increased C/N ratio in combined stress underwent plants' shoots grown under N-D conditions, was more strongly related with the decrease in N content rather than C content. The C/N ratio in plants' roots underwent combined drought and HW stress was significantly increased under both N-A and N-D conditions, because of decreased N content in roots ($r = -0.97$, $p < 0.01$). Recently conducted study of Luo et al. [83] showed that the dynamics of plant nutrient content in roots and shoots under the imposed stress could be attributed with the biomass reallocation and a nutrient dilution effect in the plant-soil system, associated with plant size [84]. It has been shown that the altered climates and soil fertility results in variation in C and other mineral nutrient composition [85,86] because various plant structures and metabolic processes have distinct and divergent requirements for C and other nutrients. Nevertheless, in accordance with the results of our study, Luo et al. [83] showed that, in the responses to the shifted environmental conditions, plant C/N ratios were not or less correlated with corresponding plant C but were negatively correlated with plant N dynamics. The similar results regarding the changes in C/N ratios under the shifted environmental and climatic conditions were also found in two meta-analyses by Yang and Luo [87] and Yang et al. [88].

4.1. Recovery under adequate and deprived soil nutrient conditions

After 7-day recovery, the growth-related attributes of plants pre-exposed to single drought both under N-A and N-D conditions displayed sharper decreases from their respective controls, compared to stress period (Fig. 4G,I − K), meaning that during the post-stress period they possessed the lower growth than during stress period, especially those grown under the N-A conditions. At the same time, under both N-A and N-D conditions, all parameters of the leaf gas exchange showed full recovery, compared with their respective controls (Fig. 5F − *J*). In contrast, the aboveground and total DW of plant previously subjected to single HW both under N-A and N-D conditions were still higher and even to a greater extent, compared to controls (Fig. 4G,I,K), while their leaf gas exchange also returned to the control plants (Fig. 5F–J). Therefore, these results evidence a lagged effect of water shortage and heat on growth, compared to gas exchange, pointing out an interest to look at plants' performance under unfavorable or favorable environmental conditions beyond the actual event. The lagged effects of drought and heatwaves on plants' growth and leaf physiology have also been found in other recent studies [17,89,90], including our previous recent study with oilseed rape [42], showing that both compensation for, or offsetting of, growth suppression following heat stress might be possible [17].

Plants underwent combined drought and HW treatment under N-A conditions exhibited the far worse growth and the leaf gas exchange recovery than those pre-exposed to single drought (Figs. 4G,I–L and 5 F − *J*), which is in line with previous researches by other authors [17,18] and our other recent study with oilseed rape [42]. Interestingly, the recovery of aboveground growth-related attributes of plants undergone combined drought and HW treatment under N-D conditions in most cases did not differ significantly from that previously experienced the single drought stress (Figs. 4G,I–L). However, the formerly, after recovery, had still significantly lower activity of photosynthetic apparatus (Table 1) and, compared to other treatments, clearly diverged regarding the C/N status (Fig. 7D − F). A strong significantly negative relationship between the C/N ratio and N content in these plants ($r = -0.99$, $p < 0.001$) was found. An explanation of the discrepancy between the aboveground growth and physiological responses of N-D plants undergone combined drought and HW treatment is that soil nutrient deficiency, by itself, evoked strong stress under control treatment resulting in a considerably slower aboveground growth of N-D plant, compared to control plants grown under N-A conditions (Fig. 4A,G and C,I). Since the combined drought and HW stress had a very similar effect on the aboveground growth either under N-A or N-D conditions (Fig. 4A − F), in turn, after recovery, the later ones showed less differences from their respective controls. However, compared to control plants grown under N-A conditions, during the recovery period, N-D ones invested more into roots growth (Fig. 4D,*J*). Thus, after recovery, root DW of plants underwent combined drought and HW treatment in N-D conditions was significantly decreased, compared to their respective control, while no significant difference between them was found at the end of the stress period.

5. Conclusions

Overall, the results of this study showed a different manner of single drought and heatwave action on growth and physiology of spring oilseed rapes. Drought as single stressor had a significant negative effect on photosynthetic response of *B. napus*, which was mainly attributed to stomatal limitations, however, the adverse effect of drought on leaf gas exchange was lagged on the growth that became more apparent only after recovery period. No obvious differences were seen between the plants grown under nutrients adequate and nutrients deprived conditions in the growth and physiological response to single drought stress. Contrary, a heatwave alone enhanced a photochemistry of PSII, resulting in higher A_{sat} under both adequate and deprived soil nutrient conditions, though the growth responses of nutrient-deprived plants to single heatwave treatment were weaker and, in most cases, insignificant. Heatwave applied simultaneously with drought highly exacerbated the adverse effect of drought on growth and physiology both under N-A and N-D conditions. A sharper decline in A_{sat} under combined drought and HW treatment was attributed to both stomatal and non-stomatal limitations. Plants underwent combined drought and HW treatment under N-D conditions showed better aboveground growth recovery, compared to those grown under N-A conditions, while displayed far more diminished photochemistry of PSII and badly disturbed the C/N balance. This discrepancy could be explained by the fact that soil nutrient deficiency, by itself, evoked strong stress under control conditions resulting in a dramatically slower aboveground growth of nutrient-deprived plant, compared to control plants grown under adequate soil nutrient conditions. Since combined drought and HW stress had similar effect on the aboveground growth either under N-A or N-D conditions, in turn, after recovery, the later ones showed less difference from their respective control.

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Declaration of Competing Interest

The authors (Austra Dikšaitytė, Akvilė Viršilė, Jūratė Žaltauskaitė, Irena Januškaitienė, Marius Praspaliauskas, Nerijus Pedišius) declare that they have no conflict of interest.

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