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The effect of climate change on glyphosate control of *Avena fatua*, *Brassica napus*, and *Echinochloa crus-galli*

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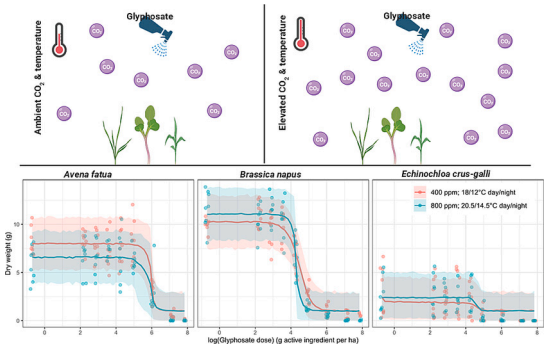
HIGHLIGHTS

- Glyphosate efficacy under elevated CO_2 and temperature was assessed in a dose-response study.
- ED_{50} and ED_{90} values did not change for the grass species barnyard grass and wild oat.
- Oilseed rape, however, required lower glyphosate doses.
- Transcriptome analysis indicated different species' responses to glyphosate and climate change.

GRAPHICAL ABSTRACT



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ABSTRACT

Atmospheric CO_2 concentrations and temperatures have significantly increased, accompanied by substantial changes in precipitation patterns. These changes are anticipated to intensify in the future. In Nordic regions, increasing temperatures can improve growing conditions for some crops by extending the growing season and expanding cultivation northward. Climate changes may also favour some weed species, potentially reducing crop yield and affecting herbicide efficacy. To assess glyphosate efficacy under future climate conditions, we conducted two dose-response experiments on barnyard grass (*Echinochloa crus-galli*- C_4 plant), oilseed rape (*Brassica napus*- C_3 plant) and wild oat (*Avena fatua*- C_3 plant). Plants were grown under ambient conditions (400 ppm CO_2 at 18/12 °C (day/night)) and predicted future conditions (800 ppm CO_2 at 20.5/14.5 °C (day/night)). Glyphosate was applied at 3–4 – leaf–stage in doses of 0, 8.75, 17.5, 35, 70, 140, 420, 1260, and 2520 g active ingredient (a.i.) ha^{-1}, with the highest dose only included in the second experiment. Chlorophyll fluorescence was measured 48 h after spraying. Two days after spraying, oilseed rape exhibited stress symptoms under both growing conditions, while barnyard grass showed symptoms only under future conditions and doses exceeding 6 g a.i. ha^{-1}. Plants were harvested 72 h after spraying for transcriptome analysis and two weeks after spraying to determine dry weight, C%, N% and C/N ratio. The ED_{50} and ED_{90} values did not significantly differ between the two environments for each grass species. However, oilseed rape required significantly higher glyphosate doses to

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reduce dry weight by 50 and 90 % at ambient growing conditions, likely due to the faster translocation of glyphosate. This suggests that glyphosate doses can be reduced in a warmer climate with an elevated CO_2 level. No apparent differences in the C%, N%, or C/N ratio were observed between environments for any species. Transcriptome analysis indicated that all species respond differently to glyphosate and climate change.

1. Introduction

Since the Industrial Revolution, the atmospheric CO_2 concentration has continually increased and is predicted to reach approximately 936 ppm at the end of this century (IPCC, 2007). This elevated CO_2 concentration drives global warming, raising global surface temperatures by an estimated 2.6–4.8 °C by 2100 (IPCC, 2013).

At higher latitudes, the annual precipitation is expected to increase by 8–18 % (Ketzler et al., 2020). In Nordic regions, these climate changes may enhance agricultural production by extending the growing season (e.g., 60–90 days in Norway (Wong et al., 2016)) and allowing cultivation at higher latitudes (ACIA, 2005). Additionally, increased mineralization of organic matter in the soil may elevate soil N content, altering C/N ratio in plants and increasing N-leaching (Ball and Pocock, 1999). Climate changes may also benefit weeds, pests, and diseases, resulting in severe crop yield reductions in northern regions (ACIA, 2005).

Weeds are major contributors to economic loss in crop production worldwide, potentially causing yield reduction of up to 34 % (Oerke, 2006; Pimentel et al., 2000). Crop yield loss is mainly caused by competition with weeds for light, water, and nutrients, greatly influenced by weed density and crop competitive ability. In addition, weeds may reduce yield quality and serve as hosts for crop pests and diseases (Preston, 2014).

Among the key climate variables, elevated CO_2 concentration (e(CO_2)) is a critical component as CO_2 is the carbon source for photosynthesis (Fernando et al., 2016). The magnitude of photosynthesis, growth, and reproductive responses to e(CO_2) varies among plant photosynthetic types (Taub et al., 2008). In C_3 plants (about 94 % of the plant species), the photosynthetic rate approximately doubles under doubling atmospheric CO_2 concentration due to an increase in the RuBisCO (Ribulose-1,5-bisphosphate carboxylase-oxygenase) carboxylation efficiency (Ainsworth and Rogers, 2007; Drake et al., 1997). Elevated CO_2 increases the ratio of CO_2/O_2 in the chloroplast, the site of CO_2 fixation in C_3 plants, favouring the photosynthetic carbon reduction cycle over photorespiration and accelerating growth (Seneweera et al., 2005). In contrast, rising atmospheric CO_2 is not expected to significantly impact the carbon fixation rate, biomass production, and yield in C_4 plants, as they are already photosynthetically saturated at the present CO_2 level (Ainsworth and Rogers, 2007). Competition between weeds and crops can also be affected by increasing CO_2 levels (Patterson and Flint, 1990).

Recent studies have demonstrated that elevated CO_2 and increased temperatures significantly influence plant transcriptomes, leading to alterations in gene expression related to various physiological processes. For instance, in durum wheat (*Triticum durum* Desf.), e(CO_2) induced the upregulation of genes involved in mitochondrial electron transport and secondary metabolism, while genes related to nitrogen assimilation were downregulated (Vincente et al., 2019). It has also been shown that e(CO_2) alters the expression of plant stress-related genes (e.g., abscisic acid-related and salicylic acid-related genes) (Kazan, 2018). Similarly, in maize (*Zea mays* L.), combined e(CO_2) and warming conditions led to differential expression of genes associated with photosynthesis, stress response, and primary metabolism, indicating a complex regulatory network governing adaptation to changing environments (Huang et al., 2019). Understanding gene expression changes triggered by e(CO_2) and its interaction with elevated temperature in crops and weeds is key to evaluating adaptation and fitness in response to climate change.

While e(CO_2) effects have been studied for some crops and weeds (e.g., Varanasi et al., 2016; Toreti et al., 2020; Tørresen et al., 2020), they are under-appreciated from the weed management point of view. Weeds often exhibit greater plasticity and quicker acclimatization to changes in the environment than crop plants. They may also undergo faster adaptive evolution due to intense selective pressures, like herbicide use and characteristics such as short lifecycles, strong dispersal abilities and high genetic variation (Neve et al., 2009). Consequently, climate change may benefit the more adaptable weed species in the crop-weed competition (Pautasso et al., 2010; Ziska et al., 2011). To better anticipate these shifts, there is a need to move beyond a reductionist approach and integrate ecosystem responses to e(CO_2) by utilizing appropriate modelling techniques for better forecasting of e(CO_2) impacts on plant systems (Barnaby and Ziska, 2012). This will enable researchers and enterprises to develop innovative approaches in response to future possible changes in the weed-crop competition patterns and applied weed management methods due to climate change.

The vast genetic variation in many weed species enables them to rapidly adapt to environmental changes and overcome weed management strategies. The most well-known example of adaption to weed management is the development of herbicide resistance (Preston, 2014). Although weed management is shifting towards integrated strategies, herbicides remain the mainstay of weed control due to their ease of application and cost-effectiveness (Varanasi et al., 2016). Elevated CO_2 concentrations could influence the efficiency of many herbicides, making weed management a significant challenge for sustainable crop production (Matzrafi et al., 2019). For example, changes in leaf morphology or root/shoot ratio can dilute herbicide uptake and distribution, reducing its efficacy (Ziska et al., 2004). In addition, increased carboxylation of RuBisCo under e(CO_2) lowers its levels in leaves and results in a decline in leaf N content, particularly in C_3 plants (Stitt and Krapp, 1999). Consequently, e(CO_2) reduces the abundance of leaf proteins, the primary targets of herbicide action (Taub et al., 2008). Addressing knowledge gaps about plant performance and herbicide efficiency under e(CO_2) is essential for the development of future weed management strategies (Ziska and Dukes, 2011).

Herbicide efficacy screening can be a lengthy process (Schmidt, 1993). The evaluation of results, either qualitatively (scoring systems) or quantitatively (biomass), usually cannot be performed until two weeks after treatment. However, an early evaluation could be performed using the shape and form of the fluorescence transition curve or Kautsky curve (Christensen et al., 2003). This curve is affected by various stress factors, such as temperature and water stress, pathogens, and herbicides (Daley, 1995; Lazar et al., 1997). A key parameter derived from the Kautsky curve is maximum relative fluorescence (F_v/F_m), which quantifies the relative magnitude of chlorophyll fluorescence (Christensen et al., 2003). This parameter effectively provides a snapshot of a plant's physiological status under stress, offering a potential early indicator of herbicide effects.

Barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv) (C_4 plant) and wild oat (*Avena fatua* L.) (C_3 plant) are well-established weeds in the southern part of Norway (ca 63°N − 57°N) characterized by a temperate climate (Ketzler et al., 2020). Barnyard grass is a particularly troublesome weed in Norwegian cereal (e.g., wheat (*Triticum aestivum* L.) and other arable crops (VKM, 2016). There is a significant risk of the species spreading further north if agricultural production extends north due to climate-related changes. Both species are invasive and regulated in Norway to ensure control and prevent spreading. Expanding their geographical range will reduce crop yields and increase weed control costs (Korres et al., 2016). How and to what extent weeds will evolve in

response to e(CO_2), the type of changes most likely to lead to evolution, and which species are most likely to adapt to changes are essential questions.

Oilseed rape (*Brassica napus* L.) (C_3 plant) is a common crop in Europe. However, its persistent seeds in the soil can give rise to volunteer plants that compete with newly sown crops for resources, thus reducing subsequent crops yields (Gruber et al., 2004). Effective crop management strategies are essential to mitigate the impact of volunteer oilseed rape, particularly in crop rotation systems where its persistence can interfere with optimal agricultural productivity.

We investigate acclimatory changes associated with e(CO_2) and an increased temperature for barnyard grass, wild oat, and oilseed rape, as well as the impact of this climate on glyphosate efficacy. We hypothesized that e(CO_2) and temperature will affect the efficacy of glyphosate and change the C and N content in plants and hence the C/N ratio. We also expected significant transcriptional responses due to climate and the application of glyphosate.

2. Material and methods

2.1. Dose-response experiments

A dose-response study was conducted in climate-controlled greenhouses at the Taastrup campus, University of Copenhagen, Denmark (55°67 N, 12°30 E) from 31 January to 11 March 2024 to assess if a climate with a higher temperature and CO_2 concentration would affect glyphosate efficacy on two noxious grass weeds and a crop. Plants were grown in two greenhouse cells ($Cell_{amb}$ and $Cell_{el}$). $Cell_{amb}$ had a CO_2 concentration of approximately 400 ppm (ambient CO_2, a[CO_2]) and day/night temperatures of 18/12 °C. $Cell_{el}$ had a CO_2 concentration of approximately 800 ppm (elevated CO_2, e[CO_2]) and day/night temperatures of 20.5/14.5 °C. The desired [CO_2] in the cells was sustained by pure CO_2 emission from a bottled tank, released at one point and distributed evenly by internal ventilation. The [CO_2] in the cells was monitored every 6 s by a CO_2 transmitter (Senmatic DGT-volmatic (13 W13), Vaisala Group, Helsinki, Finland). The average daily CO_2 concentration in each cell during the experiments is shown in Fig. 1. In both greenhouse cells, there was 60 % relative humidity, a 16 h photoperiod with >500 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR) supplied by sunlight plus LED lamps (Hortiled® Top Antares, Lux Light International, Germany).

Mature seeds of barnyard grass and wild oat were collected from the Ski region (59.71° N, 10.83° E) in Akershus county and Fredrikstad region (59.22° N, 10.93° E) in Østfold county, Norway, respectively, in September 2023. Seeds of each species were collected from the field by carefully cutting and shaking as many seed-bearing shoots as possible into paper bags. The pooled seeds were then stored in a paper bag under dry conditions at room temperature until they were used in the experiments. Oilseed rape seeds were bought from the Danish market (cv. Silver Shadow; Nordic Seed, Galten, Denmark). In each cell, seeds of each species were germinated in germination boxes with water containing a 0.2 % potassium nitrate (KNO_3) solution to stimulate seed germination. Four seedlings were transplanted into 4 L pots (height: 21 cm; Ø: 18 cm) with sphagnum peat-based growing medium, limed and fertilized with NPK (1 kg m^{-3}) and a pH of 5.5–6 (Pindstrup færdigblanding 2, Pindstrup Mosebrug A/S, Ryomgaard, Danmark). Germination of barnyard grass seeds was started a few days earlier than the two other species to ensure that all species had 3–4 leaves and could be sprayed simultaneously (Table 1). Extra pots with plants were established to safeguard enough uniform plants of each species for the spraying experiment. Pots with plants were moved to a spraying cabinet and sprayed with Roundup Flex-480 (a.i. 480 g L^{-1} glyphosate; Bayer AG, Copenhagen, Denmark) using hydraulic nozzles (Hardi LD-02-100), a spray volume of 150 L ha^{-1}, a pressure of 3 bar, and a speed of 5 km h^{-1}. Upon reaching the 3–4–leaf stage, spraying occurred between 18 and 25 days after germination, depending on the species (Table 1). Four

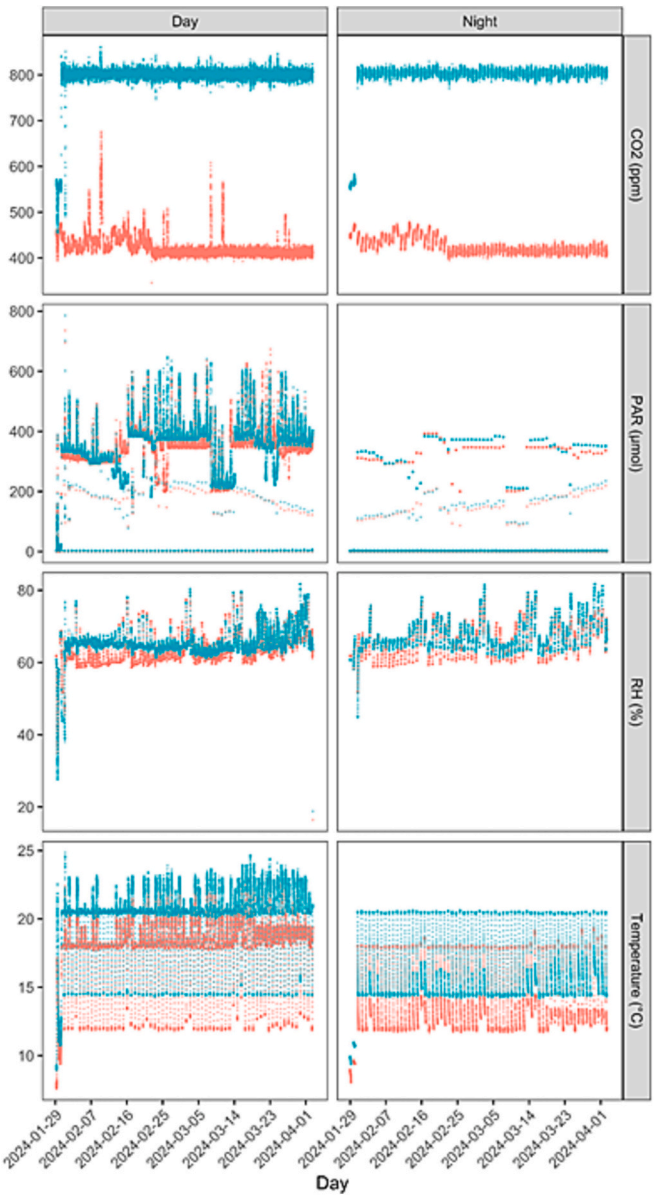

Fig. 1. The actual [CO_2] concentration, light intensity (PAR (µmol)), relative humidity (RH (%)), and temperature (°C) measured in the two environments (red = $Cell_{amb}$ = 400 ppm CO_2, 18/12 °C day/night; blue = $Cell_{el}$ = 800 ppm CO_2, 20.5/14.5 °C day/night) during the experimental period (February–April 2024).

pots with four plants of each species were sprayed with 0, 8.75, 17.5, 35, 70, 140, 420, and 1260 g a.i. ha^{-1}, respectively. After spraying, the pots were placed on tables next to the spraying cabinet for an hour before they were moved back to the greenhouse cells and placed in a randomized design.

Plants were manually watered as needed. The pots were watered with the insecticide GnatrolSC® (Nordisk Alkali, Hvalsø, Denmark) containing the biocontrol agent *Bacillus thuringiensis* subsp. *israelensis* AM65–52 (1.8 × 1011 CFU/L (11,6 % (*w*/w)) corresponding to 123 g L^{-1} to prevent fungus gnats (*Bradysia coprophila* and *Bradysia impatiens*) after transplanting.

Plants were harvested two weeks after spraying at the soil surface and dried in a drying cabinet for 72 h at 75 °C. Afterwards, dry weights were measured. The first experiment included 192 pots (2 greenhouse cells × 3 species × 8 doses × 4 replicates).

The experiment was repeated from 29 February to 9 April 2024,

Table 1
Timetable of the experimental work in 2024. $Cell_{amb}$: CO_2 concentration of 400 ppm (ambient CO_2, a[CO_2]) with respective temperature of 18/12 °C day/night; $Cell_{el}$: CO_2 concentration of 800 ppm (elevated CO_2, e[CO_2]) with respective temperature of 20.5/14.5 °C day/night.

	Experiment 1		Experiment 2	
	$Cell_{amb}$	$Cell_{el}$	$Cell_{amb}$	$Cell_{el}$
Start of germination				
Avena fatua (wild oat), *Brassica napus* (oilseed rape) *Echinochloa crus-galli* (barnyard grass)	02 February 31 January	02 February 31 January	04 March 29 February	04 March 29 February
Transplanting	09 February	09 February	11 March	11 March
Spraying	23 February	20 February	25 March	22 March
Chlorophyll fluorescence measurement	25 February	22 February	27 March	24 March
Sampling for molecular analysis	26 February	23 February	28 March	25 March
Harvest	08 March	05 March	08 April	05 April
Dry weight measurement	11 March	08 March	11 April	08 April

adding one more dose (2520 g a.i. ha^{-1}), resulting in 216 pots (2 greenhouse cells × 3 species × 9 doses × 4 replicates). Table 1 shows the exact timing of the activities.

2.2. Measurements

2.2.1. Chlorophyll fluorescence

Chlorophyll fluorescence was used to measure plant stress. The photosynthetic efficiency of photosystem II in a dark-adapted state (F_v/F_m) was recorded 48 h after spraying using a portable chlorophyll fluorimeter (Handy PEA+, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The measurements were done on one plant's last fully developed leaf per pot. The leaf was dark-adapted for at least half an hour before the measurements. Data were processed with PEA Plus software version 1.30, provided with the Plant Efficiency Analyzer.

2.2.2. Sampling for molecular analysis

One plant per pot was cut above the soil surface 72 h after spraying and quickly transferred to a falcon tube in a Styrofoam box containing liquid nitrogen. Thereafter, the samples were sent to the Norwegian Institute of Bioeconomy Research, Ås, Norway, on dry ice for RNA extraction.

2.2.3. Dry biomass weight

Fourteen days after spraying, the three remaining plants in each pot were cut just above the soil surface, placed in mesh bags, and dried in a drying cabinet for 72 h at 75 °C. Thereafter, dry weights were recorded. Dry biomass was used to calculate the ED_{50} and ED_{90} values, indicating the doses needed to reduce the response (biomass weight) by 50 % and 90 %, respectively.

2.2.4. C and N content

The dry biomass of the plants was used for C and N measurements. The plants were first ground with a mill (Cyclone mill TWISTER, Retsch GmbH, Hann, Germany). C and N were analysed with a FLASH 2000 CHNS-O analyzer (Thermo Fisher Scientific Inc.).

2.2.5. Molecular analysis

The plant samples were ground to a powder in liquid nitrogen. Only samples from doses 0, 70, and 420 g a.i. ha^{-1} were extracted for RNA analyses. RNA extraction was performed using the cetyltrimethylammonium bromide (CTAB)/ sodium dodecyl sulphate (SDS) method (Barbier et al., 2019). All chemicals were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) unless otherwise stated. Approximately 20–30 mg of ground material was placed into a clean 2 mL microcentrifuge tube and 652 µL of extraction buffer [650 µL CTAB buffer: 3 % *w/v* CTAB; 100 mM Tris HCl; 50 mM EDTA; 1.4 M NaCl; 13 mg polyvinylpyrrolidone 40 (PVP40); 1.3 µL 2-mercaptoethanol (BME)] was added to each tube. Tubes were incubated at 65 °C for 15 min with shaking. After the incubation, 40 µL of 10 % *W/V* SDS was added to each tube. The tubes were centrifuged in an Eppendorf Centrifuge 5425 R (Eppendorf.com) for 15 min at 4 °C and max speed. The supernatant was transferred into a new 2 mL microcentrifuge tube, after which 300 µL of 7.5 M lithium chloride (LiCl) was added. The tubes were inverted 35 times to mix and incubated for 2 h at −20 °C. After incubation, tubes were centrifuged for one hour at 4 °C and max speed. The supernatant was removed and discarded. The remaining pellet was washed with 900–1000 µL cold 75 % ethanol and centrifuged for 15 min at 4 °C and max speed. This wash was then repeated. All the ethanol was removed from the tube, and the pellet was entirely air-dried before resuspending in 30 µL 65 °C nuclease-free H_2O (VWR Chemicals, catalogue number 436912C). Total nucleotide concentration and purity were measured on a NanoDrop 2000 (Thermo Fisher Scientific Inc.). Extracted RNA was stored at −80 °C until being shipped to BGI Tech Solutions (Tai Po, Hong Kong) on dry ice for sequencing.

A total of 104 samples were sent to BGI Tech Solutions for DNase treatment, library preparation, sequencing, and bioinformatic analysis. After library preparation, 150 bp paired-end sequencing was performed on BGI's DNBseq platform. After sequencing, BGI performed adaptor trimming and reads were aligned using Bowtie2 (Langmead and Salzberg, 2012) to their respective reference genomes: oilseed rape (*Brassica napus*; GeneBank: GCA_020379485.1; Davis et al., 2023), barnyard grass (*Echinochloa crus-galli*; GeneBank: GCA_900205405.1; Guo et al., 2017). As the *Avena fatua* genome is not available, reads were aligned to the genome of a closely related species (*Avena eriantha*; GenBank: GCA_910589775.1; Tinker et al., 2022). Differential expression analysis was performed with DEseq2 using pairwise comparisons. KEGG annotation of differentially expressed genes was performed using DIAMOND (Buchfink et al., 2021). To determine KEGG pathway enrichment among up- or downregulated transcripts, a hypergeometric test was performed using phyper (observed - 1, total, N - total, n, lower.tail = FALSE)('stats'; R version 4.4). "Total" represents the number of occurrences of a KEGG pathway among all expressed genes within a species, while "observed" refers to the number of occurrences of the same KEGG pathway among up or downregulated genes in specific pairwise comparisons. For more details on BGI's analysis pipeline see Supplemental file 1.

2.3. Statistical analysis

2.3.1. Statistical models

Nonlinear mixed-effects models were fitted to dry weight data using the log-logistic nonlinear function. Let y_{ijklm} be dry weight measured at a glyphosate dose *i*, species *j*, environment *k* (each environment represents a combination of CO_2 and temperature), experiment *l* and replicate *m*. The model formulation is.

$$Y_{ijklm}|a_{jklm} \sim N^{+}\left(\mu_{ijklm}, \sigma^2\right)$$

$$\mu_{ijklm} = a_{jklm} \Big/ \left(1 + \exp.\left\{\gamma_{jk}\left(\log(d_i + 0.5) - \log(\kappa_{jk})\right)\right\}\right)$$

$$a_{jklm} \sim N\left(\alpha_{jk}, {\sigma_a}^2\right)$$

where *N* and N^+ are the normal and the positive truncated normal distributions, respectively; d_i is the i^{th} glyphosate dose; α_{jk}, γ_{jk} and κ_{jk} are the asymptotes, growth rates and inflection points of the nonlinear curves fitted to each species × environment combination, respectively; and a_{jklm} is the experiment × replicate level random effect, included to account for the variability within experiments and replicates.

We used a Bayesian estimation framework to fit the models using the following prior distributions:

$$\alpha_{jk}, \gamma_{jk}, \kappa_{jk} \sim N^{+}(0, 10,000)$$

$$\sigma, \sigma_a \sim U(0, 100)$$

We estimated the models using JAGS (Plummer, 2003) with 10,000 MCMC iterations, with the first 5000 discarded as burn-in with a thinning rate 10. We estimated the 50 % and 90 % effective doses (ED_{50} and ED_{90}, respectively) for each species × environment combinations using the formulas below:

$$ED^{50}{}_{jk} = \kappa_{jk}$$

$$ED^{90}{}_{jk} = \kappa_{jk} \times 9^{\frac{1}{\gamma_{jk.}}}$$

To test for differences, we also computed the 95 % credible intervals for the differences between ED_{50} and ED_{90} within the same species but for different environment levels.

In the Bayesian paradigm, we combine information from the data (the likelihood) and prior information to obtain the posterior distribution for all parameter values. We derived 95 % credible intervals from the posterior distribution, which directly translates to a 95 % probability that the true parameter values are included in that interval. To test whether, for example, the ED_{50} for one species was significantly different from the ED_{50} for another, we computed the 95 % credible interval for the difference between ED_{50} values. If that interval included zero, they were not sufficiently different. If zero was not included, they were significantly different. Therefore, there are no *p*-values to be calculated in this setting.

For the F_v/F_m and C/N, we fitted Gaussian generalized additive models (GAMs) to the F_v/F_m and C/N data, including a random intercept per replicate within the experiment, the effects of species, environment, and the two-way interaction between species and environment, and thin plate regression splines over glyphosate dose for each species × environment combination. The significance of the effects was assessed by using F tests.

For the N and C percentages, we fitted beta generalized additive models (GAMs) to the percentages of N and C data, including a random intercept per replicate within an experiment, the effects of species, environment, and the two-way interaction between species and environment, and thin plate regression splines over glyphosate dose for each species × environment combination. The significance of the effects was assessed by using likelihood-ratio (χ^2) tests.

All analyses were carried out using R (R Core Team, 2024). All Bayesian models were fitted using package R2jags (Su and Yajima, 2024). All GAMs were fitted using the package mgcv (Wood, 2017). All plots were generated using package ggplot2 (Wickham, 2016).

3. Results

In each experiment, the plants were transplanted simultaneously to $Cell_{amb}$ and $Cell_{el}$. Plants in $Cell_{amb}$ reached the 3–4 leaf stage three days later than plants in $Cell_{el}$. Faster growth in an environment with elevated CO_2 and temperatures was expected.

3.1. Photosynthetic efficiency

There was a significant environmental effect on the photosynthetic efficiency of photosystem II for all species ($p = 0.001$). For wild oat, it reflected in higher F_v/F_m values in $Cell_{el}$, but there was no dose effect on F_v/F_m (Table 2, Fig. 3A). The environment in $Cell_{el}$ also improved oilseed rape's photosynthetic efficiency compared to $Cell_{amb}$. Increasing glyphosate doses resulted in significantly reduced F_v/F_m values. A significant interaction effect between dose and environment was recorded for barnyard grass (Table 2). The highest doses reduced F_v/F_m significantly in $Cell_{amb}$, while the F_v/F_m values were almost constant for all glyphosate doses in $Cell_{el}$ (Fig. 3A). There was also a significant interaction between dose and species within environments ($p = 0.001$). Fig. 3A shows how differently the glyphosate doses affected the F_v/F_m of each species for both environments.

3.2. Dry weight

The effect of increasing glyphosate doses was not significantly different in the two environments for the grass species. The ED_{50} and ED_{90} values did not significantly differ between the two environments for each grass species. However, wild oat was less sensitive to glyphosate than barnyard grass (Table 3). In both environments, higher doses were needed to achieve a 50 and 90 % dry weight reduction in wild oat than barnyard grass and oilseed rape (Figs. 2 & 3B). Contrary to the grass species, significantly higher doses were needed to reduce oilseed rape dry weight by 50 and 90 % at ambient growing conditions ($Cell_{amb}$).

3.3. C, N, and C/N ratio

Only the C content (%) was affected by the interaction between species and the environments ($p = 0.042$) but not the N content (%) or C/N ratio ($p = 0.245$ and $p = 0.807$, respectively). However, the interaction effect of environment and dose on C content was only apparent for barnyard grass (Table 2, Fig. 4). Neither N content nor C/N ratio was affected by environment, dose, or the interaction of the two ($p = 0.245$ and $p = 0.807$, respectively). The interaction effect between species and glyphosate dose was only significant for C content (%) within

Table 2
The table shows the p-values associated with the tests for **(A)** the main effects of the environments ($Cell_{amb}$ and $Cell_{el}$), the glyphosate dose and the interaction between the environment and the dose for each species and **(B)** the interaction between the species and glyphosate dose within the environments ($Cell_{amb}$ and $Cell_{el}$), for F_v/F_m and C content (%) where the interaction between the species and environment is significant. The photosynthetic efficiency of photosystem II in a dark-adapted state (F_v/F_m) was measured 48 h after spraying. $Cell_{amb}$: CO_2 concentration of 400 ppm (ambient CO_2, a[CO_2]) with respective temperature of 18/12 °C day/night; $Cell_{el}$: CO_2 concentration of 800 ppm (elevated CO_2, e[CO_2]) with respective temperature of 20.5/14.5 °C day/night.

(A)	Species	Interaction between environment and dose	Main effect of the environment	Main effect of glyphosate dose
F_v/F_m	*Avena fatua* (wild oat)	$p = 0.640$	$p = 0.0013$	$p = 0.983$
	Brassica napus (oilseed rape)	$p = 0.870$	$p = 0.210$	$p < 0.001$
	Echinochloa crus-galli (barnyard grass)	$p < 0.001$	–	–
C content (%)	*Avena fatua* (wild oat)	$p = 0.860$	$p < 0.001$	$p < 0.001$
	Brassica napus (oilseed rape)	$p = 0.101$	$p = 0.588$	$p < 0.001$
	Echinochloa crus-galli (barnyard grass)	$p < 0.001$	–	–

(B)	Environment	Interaction between species and glyphosate dose	Main effect of species	Main effect of glyphosate dose
F_v/F_m	$Cell_{amb}$	$p < 0.001$	–	–
	$Cell_{el}$	$p < 0.001$	–	–
C content (%)	$Cell_{amb}$	$p < 0.001$	–	–
	$Cell_{el}$	$p < 0.001$	–	–

Table 3
Estimated ED_{50} and ED_{90} parameters of the dose (glyphosate) response (plant dry weight) curves (Fig. 3B) based on two experiments and corresponding 95 credible intervals. There was no significant difference between the experiments and therefore data were analysed together. $Cell_{amb}$: CO_2 concentration of 400 ppm (ambient CO_2, a[CO_2]) with respective temperature of 18/12 °C day/night; $Cell_{el}$: CO_2 concentration of 800 ppm (elevated CO_2, e[CO_2]) with respective temperature of 20.5/14.5 °C day/night.

Weed species	Environment	Effective dose (ED-value)	Estimated Dose (a.i. g ha^{-1})	95 % credible intervals	
				2.5 %	97.5 %
Avena fatua (wild oat)	$Cell_{amb}$	50	397	337	421
	$Cell_{amb}$	90	521	426	736
	$Cell_{el}$	50	377	291	423
	$Cell_{el}$	90	582	429	845
Brassica napus (oilseed rape)	$Cell_{amb}$	50	95	85	107
	$Cell_{amb}$	90	220	171	290
	$Cell_{el}$	50	76	71	84
	$Cell_{el}$	90	109	72	160
Echinochloa crus-galli (barnyard grass)	$Cell_{amb}$	50	101	72	138
	$Cell_{amb}$	90	109	74	161
	$Cell_{el}$	50	102	73	136
	$Cell_{el}$	90	107	75	143

environments (Table 2).

3.4. Transcriptome analysis

To better understand the changes occurring in wild oat, oilseed rape, and barnyard grass in response to elevated CO_2 and temperatures and glyphosate, we selected plants exposed to three different glyphosate doses for transcriptional analysis. Sequencing reads were aligned to their respective reference genomes, except for wild oat, which lacks an available reference genome. Instead, the reads for wild oat were aligned to the *A. erinatha* reference genome. Mapping rates to the respective genomes were 65–73 % for wild oat, 88–89 % for oilseed rape, and 87–92 % for barnyard grass. Differentially expressed genes were identified by performing pairwise comparison between samples treated with different glyphosate doses under different climate conditions but within the same species.

Principal component analysis (PCA) was conducted to identify global patterns and assess consistency in differentially expressed genes across the experiments (Supplemental files 2; Fig. 5). In both experiments, climate conditions appeared to have the greatest influence on the wild oat transcriptome, whereas in oilseed rape, the samples were more grouped based on glyphosate dose. For barnyard grass, samples from plants in $Cell_{amb}$ and treated with 420 g a.i. ha^{-1} were most

Fig. 2. Plants' response to increasing doses of glyphosate applied under two different environmental conditions. Doses 1 to 9 correspond to glyphosate doses of 0, 8.75, 17.5, 35, 70, 140, 420, 1260, and 2520 g a.i. ha^{-1}, respectively. Images were taken at harvest time of the second experiment, 14 days after spraying. $Cell_{amb}$: CO_2 concentration of 400 ppm (ambient CO_2, a[CO_2]) with respective temperature of 18/12 °C day/night; $Cell_{el}$: CO_2 concentration of 800 ppm (elevated CO_2, e[CO_2]) with respective temperature of 20.5/14.5 °C day/night.

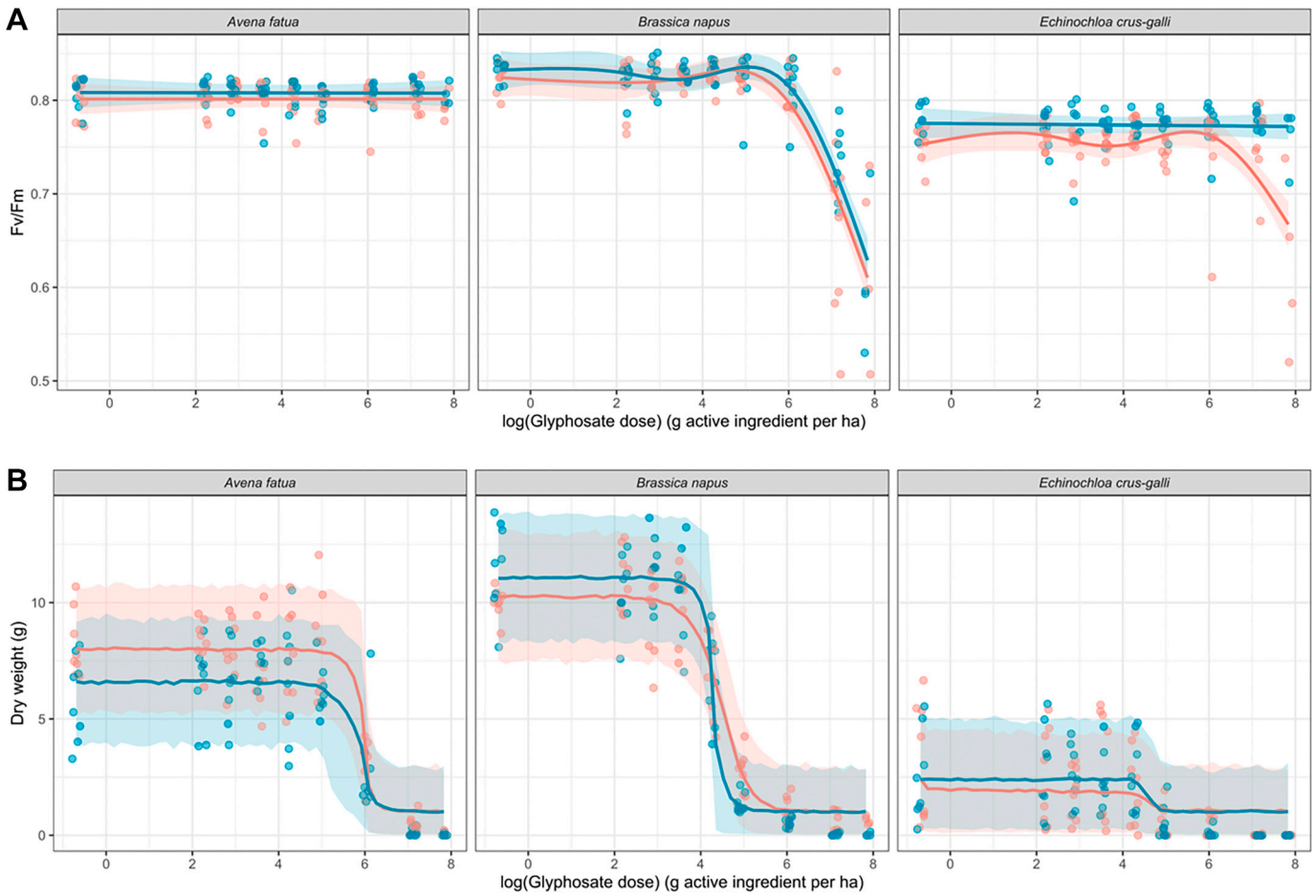

Fig. 3. (A) Photosynthetic efficiency of photosystem II reflected in F_v/F_m values in response to different glyphosate doses and **(B)** Estimated dose-response relationship between glyphosate dose and plant dry weight 14 days after spraying, for *Avena fatua* (wild oat), *Brassica napus* (oilseed rape), and *Echinochloa crus-galli* (barnyard grass) in the two different environments based on joint data from two similar experiments (red = 400 ppm CO_2, 18 °C /12 °C, day/night ($Cell_{amb}$); blue = 800 ppm CO_2, 20.5 °C/14.5 °C, day/night ($Cell_{el}$)). 95 % confidence (A)/credible (B) intervals are shown in coloured shades.

distinguishable from the other samples. However, the strength of clustering and these observed patterns varied between experiments.

To gain deeper insights into these transcriptional responses, we performed KEGG pathway enrichment analysis on differentially expressed genes identified from pairwise comparisons guided by the PCA (Supplemental files 3 & 4). For wild oat, pairwise comparisons were between samples treated with the same glyphosate dose but grown under different climate conditions (Supplemental Figs. S1 & S2). In the case of oilseed rape, pairwise comparisons examined samples grown under the same climate conditions but treated with different glyphosate doses (Supplemental Figs. S3 & S4). For barnyard grass, samples grown under $Cell_{amb}$ and treated with 420 g a.i. ha^{-1} glyphosate were compared to all other treatments (Supplemental Figs. S5 & S6).

In wild oat, enriched KEGG pathways showed greater similarity among differentially expressed genes in comparisons of the lower glyphosate doses (0 & 70 g a.i. ha^{-1}) than those in the higher dose (420 g a.i. ha^{-1}) (Supplemental Fig. S1 & S2). Many of the KEGG pathways enriched among upregulated genes in samples under $Cell_{el}$ compared $Cell_{amb}$ at the two lowest glyphosate doses were associated with protein biosynthesis and DNA replication (Supplemental Fig. S1 & S2). Conversely, enriched KEGG pathways among downregulated genes included those related to carbon fixation and glycolysis. At the high glyphosate dose, enriched KEGG pathways among upregulated genes in wild oat plants under $Cell_{el}$ included those related to photosynthesis, carbon metabolism, stress responses and protein modification (Supplemental Fig. S1 & S2). Among downregulated genes, enriched KEGG pathways were related to amino acid and nucleotide biosynthesis, as well as carbon metabolism. However, there was limited overlap in enriched KEGG pathways between experiments (Supplemental file S3&4).

For oilseed rape, enriched KEGG pathways in comparisons between different glyphosate doses within the same environment showed more overlap across experiments (Supplemental Figs S3. & S4). KEGG pathways related to protein biosynthesis are enriched among upregulated genes in glyphosate-treated samples compared to untreated samples under both climate conditions (Supplemental Figs. S3 & S4). Additionally, many KEGG pathways enriched among downregulated genes in these comparisons were related to photosynthesis, carbon metabolism, and secondary metabolism (Supplemental Figs. S3 & S4).

Finally, we explored enriched KEGG pathways among differentially expressed genes in barnyard grass by comparing samples treated with 420 g a.i. ha^{-1} glyphosate under $Cell_{amb}$ to those that were treated with lower doses of glyphosate under the same climate conditions, as well as to samples treated with 420 g a.i. ha^{-1} glyphosate under $Cell_{el}$ (supplemental Figs. S5 & S6). Among upregulated genes in these comparisons, enriched KEGG pathways were related to protein and RNA biosynthesis and RNA processing. KEGG pathways enriched among downregulated genes were related to photosynthesis and carbon metabolism. Notably, similar KEGG pathway enrichments were found in comparisons of samples treated with 420 g a.i. ha^{-1} glyphosate under different climate conditions, indicating that they are enriched samples treated with 420 g a.i. ha^{-1} glyphosate at $Cell_{el}$ compared to samples

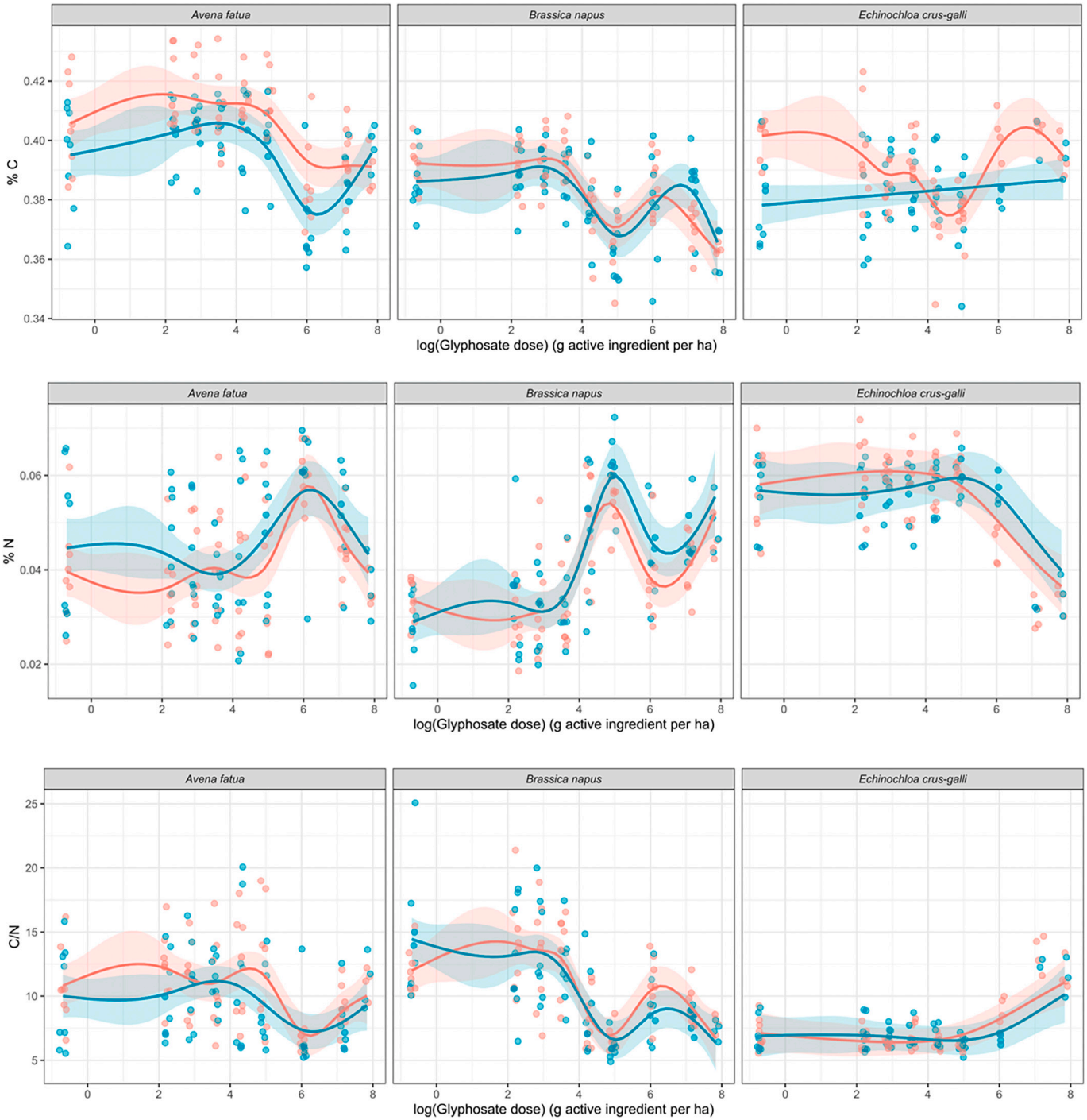

Fig. 4. C and N content percentage, and C/N ratio changes in response to different glyphosate doses in *Avena fatua* (wild oat), *Brassica napus* (oilseed rape), *Echinochloa crus-galli* (barnyard grass) dry plant tissue harvested 14 days after spraying in two different environments based on joint data from two similar experiments (red = 400 ppm CO_2, 18 °C /12 °C, day/night ($Cell_{amb}$); blue = 800 ppm CO_2, 20.5 °C/14.5 °C, day/night ($Cell_{el}$)). 95 % confidence intervals are shown in coloured shades.

treated with the same dose at $Cell_{amb}$.

4. Discussion

4.1. Photosynthetic efficiency

Wild oat showed significantly higher photosynthetic efficiency of photosystem II at e(CO_2) and elevated temperatures reflected in the higher F_v/F_m values in $Cell_{el}$ than in $Cell_{amb}$. However, increasing glyphosate doses did not affect the photosynthetic efficiency 48 h after spraying (Fig. 3A). Chlorophyll *a* fluorescence is a highly sensitive, non-destructive, and reliable tool to quickly measure photosynthetic efficiency, particularly of the water-plastoquinone oxidoreductase Photosystem II (PSII) (Stirbet and Govindjee, 2011). Changes to the chlorophyll fluorescence Kautsky curve and its derived parameters can be used to determine the effects of photosystem I and II inhibitors and herbicides on plants before they are visible (Christensen et al., 2003).

Glyphosate is not a photosynthetic inhibitor per se. It is an inhibitor

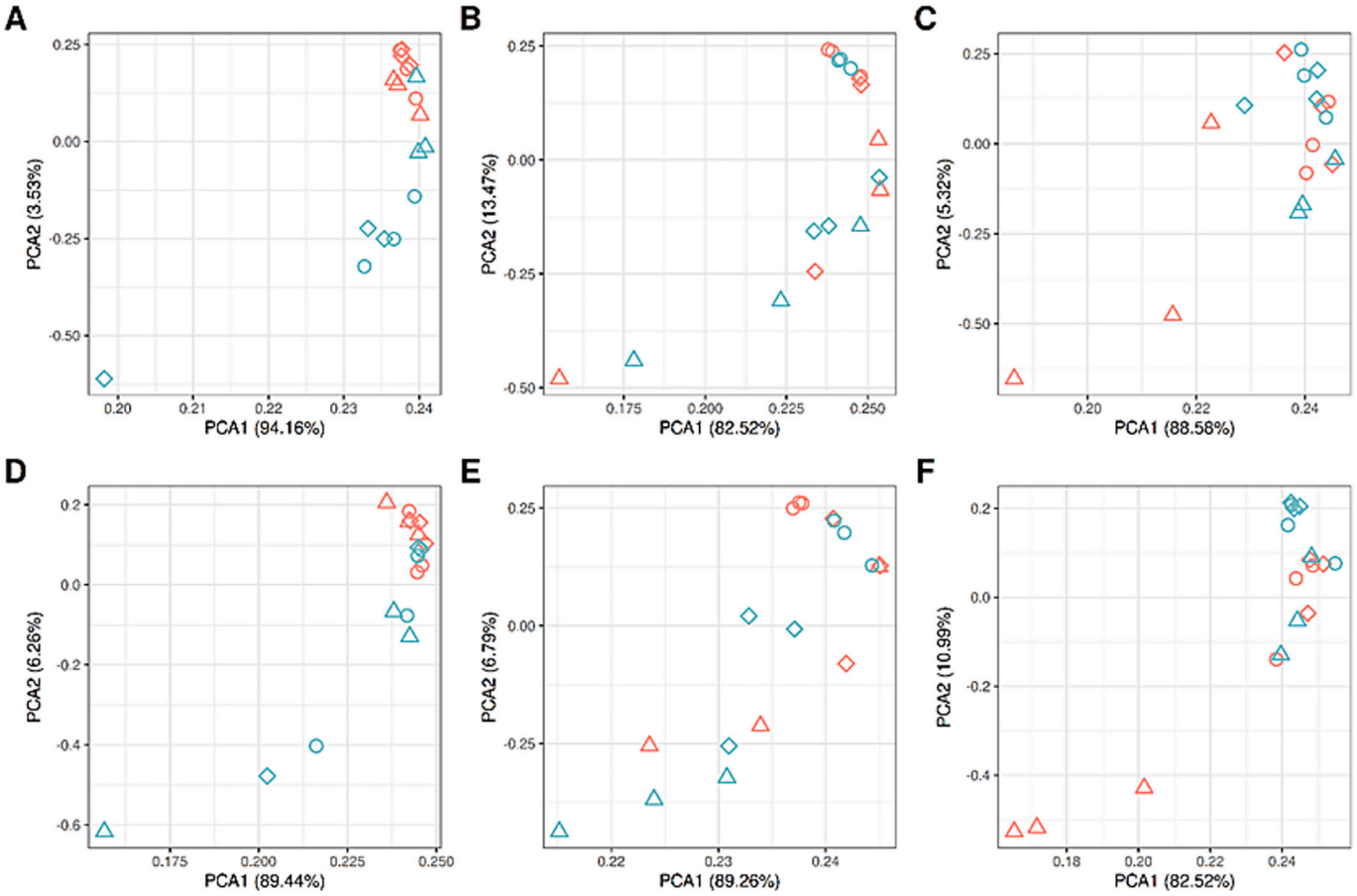

Fig. 5. Principal component analysis (PCA) plot of transcriptional response to elevated CO_2 and different glyphosate doses. The aboveground portion of *Avena fatua* (wild oat) (A & D), *Brassica napus* (oilseed rape) (B & E) and *Echinochloa crus-galli* (barnyard grass) (C & F) was sampled 72 h after glyphosate treatment in two similar experiments (Experiment 1 = A-C; Experiment 2 = D —F) for transcriptomics (n = 3). Colors indicate climate conditions (red = 400 ppm CO_2, 18 °C /12 °C, day/night ($Cell_{amb}$); blue = 800 ppm CO_2, 20.5 °C/14.5 °C, day/night ($Cell_{el}$)). Shapes indicate glyphosate dose (circle = 0 a.i. g ha^{-1}; diamond = 70 a.i. g ha^{-1}; triangle = 420 a.i. g ha^{-1}).

of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase and blocks the synthesis of aromatic amino acids. The binding of glyphosate to EPSP synthase in the cytosol leads to unregulated diversion of erythrose-4-phosphate (E4P) into the shikimate pathway, and thus to an inhibition of the reduction of ribulose bisphosphate (RBP) (Coruzzi and Last, 2000). Therefore, glyphosate can mediate the over-reduction and photo-inhibition of PS II while inhibiting the activation of the vital carotenoids required to protect PSII from photo-inhibition. The observed effect of glyphosate can be explained by the photo-inhibition of PSII, following the over-reduction of the photosynthetic apparatus and a restriction in the activation of protective carotenoids. One reason for the lack of effect of increasing doses of glyphosate on the photosynthetic efficiency of wild oat in our experiment could be that F_v/F_m was measured too early after spraying, and the measurement should have been repeated at later stages because glyphosate only indirectly affects photosynthesis. The indirect ROS effect occurs when sprayed plants stop producing essential amino acids. Silva et al. (2014) evaluated *Raphanus sativus* L. plants for chlorophyll *a* fluorescence at 48, 72, 96, and 120 h after treatment with different glyphosate doses (0–900 g a.i. ha^{-1}), and reported that glyphosate application led to a decrease in the F_v/F_m ratio. This effect was more pronounced at doses above 600 g a.i. ha^{-1} and at 120 h (5 days) after application, where a 72.3 % reduction in the F_v/F_m ratio was observed compared to the control. However, when Kirkwood et al. (2000) used the F_v/F_m parameter to measure the effect of glyphosate on fluorescence, they detected some effects only one day after treatment.

Additionally, the wild oat seeds were collected on a field where the farmer had severe problems controlling it, indicating that plants may have been less sensitive to glyphosate than expected. However, the plants were not tested for glyphosate resistance. Glyphosate-resistant wild oat plants have been observed, e.g., in Australia since 2017 (Chauhan, 2022). The other C_3 plant (oilseed rape) had significantly higher photosynthetic efficiency at e(CO_2) and elevated temperature ($Cell_{el}$), but the highest glyphosate doses (>6 g a.i. ha^{-1}) resulted in a significant drop after 48 h.

The same occurred for the C_4 plant barnyard grass in $Cell_{amp}$, but in $Cell_{el}$ the parameters of the dose-response models did not change significantly. In general, we would expect that faster growth would result in faster translocation of glyphosate in the plants and, hence, faster symptoms of plant stress. However, the C_4 plants in $Cell_{el}$ did not grow faster because the photosynthesis is already optimized at ambient CO_2 concentration (Fig. 3).

4.2. Dry weight

It was expected that both C_3 plants (wild oat and oilseed rape) would produce more biomass in two weeks under $Cell_{el}$ due to more efficient photosynthesis, but that was not observed for wild oat (Fig. 3B). There was a great variation in the data (Fig. 3B) which may blur the climate effect. Light intensity and duration are also essential factors for plant production, and although both experiments were done in the same cells, the light condition varied between the two experiments as one was started in February and the other in March, where the day length increased (Table 1, Fig. 1).

Matzrafi et al. (2019) studied the glyphosate response of the two C_3

plants *Chenopodium album* L. and *Conyza canadensis* (L.) Cronquist, under ambient and increased temperature and elevated CO_2 conditions. Reduced glyphosate sensitivity was observed in both species in response to increased temperature, elevated CO_2 level, and the combination of both factors. Increased temperature had a greater effect on plant survival than elevated CO_2 levels. In combination, high temperature and elevated CO_2 level resulted in loss of apical dominance and rapid necrosis in glyphosate-treated plants. None of these symptoms were observed for the species in our experiments. Matzrafi et al. (2019) also used ^{14}C-marked glyphosate to study the translocation of the herbicide. In plants subjected to high temperatures and elevated CO_2 levels, glyphosate was more rapidly translocated from treated leaf to shoot meristems and roots than in plants grown subject to lower temperatures and lower CO_2 level. These results suggest that altered glyphosate translocation and tissue-specific sequestration may be the basis of reduced plant sensitivity to glyphosate.

4.3. C, N, and C/N ratio

The effect of elevated CO_2 and temperatures on C and N percentages of dry plant material 14 days after spraying varied significantly without a specific trend in the data due to significant interactions (Table 2; Fig. 4). The same happened for the C/N ratio. Other studies have shown that elevated CO_2 and temperatures can significantly affect the mineralization of organic matter and N compounds in the soil (Ball and Pocock, 1999; Stuart et al., 2011), which may affect the C and N content in plants and the C/N ratio. Leaf nitrogen and carbon composition are affected by the accumulation of carbohydrates resulting from photosynthetic enhancement or the relative tissue source–sink carbon demands (Körner et al., 1995). Many researchers have reported changes in the nutrient contents of plant parts, particularly nitrogen (Cotrufo et al., 1998). Broad literature surveys have consistently found average decreases in nitrogen of approximately 10–15 % in plants grown under elevated CO_2 (Taub and Wang, 2008). Fitter and Hay (2002) reported that elevated CO_2 and temperature lead to a decline in plant tissue nitrogen concentration. In contrast, the carbon content of plant organs increases, and thus, the relative proportion of carbon to nitrogen is considerably higher under elevated CO_2 and temperature conditions. However, in our short-term experiments with plants in small pots, no clear trend was observed across data from all three weed species.

4.4. Transcriptional changes

Transcriptomic and KEGG pathway enrichment analyses revealed distinct responses to glyphosate and environmental conditions across the three species studied. In wild oat treated with the two lower glyphosate doses and grown under $Cell_{el}$, pathways related to protein biosynthesis and DNA replication were upregulated, while pathways related to photorespiration were downregulated. This supports the observation that wild oat had better photosynthetic efficiency when grown under $Cell_{el}$ (Fig. 3A). However, the limited overlap in KEGG pathway enriched between experiments prohibits drawing definitive conclusions about the impacts of climate conditions on wild oat response to high glyphosate doses.

In oilseed rape, the enrichment of protein biosynthesis pathways with increasing glyphosate doses suggests an attempt to counteract glyphosate-induced amino acid synthesis inhibition. Additionally, the downregulation of photosynthesis and secondary metabolism pathways supports the conclusion that the plants are stressed and that glyphosate inhibits the shikimate pathway, which produces amino acids essential for chlorophyll biosynthesis and secondary metabolites.

The transcriptome analysis also indicates that barnyard grass has greater tolerance to glyphosate under elevated CO_2 and temperature. Evidence for this conclusion is the upregulation of stress-related pathways and downregulation of photosynthesis in plants under $Cell_{amb}$ compared to plants under $Cell_{el}$ at the high glyphosate dose. This conclusion is also supported by the lower photosynthetic efficiency in barnyard grass plants treated with high glyphosate doses under $Cell_{amb}$ than under $Cell_{el}$ (Fig. 3A).

5. Conclusion

1. Stress symptoms reflected in the photosynthetic efficiency of photosystem II (F_v/F_m values) two days after spraying were not significant for wild oat in ambient or elevated CO_2 and temperatures, and for barnyard grass at elevated CO_2 and temperatures. At doses >6 g active ingredient ha^{-1}, significant stress could be measured for oilseed rape in both growing conditions and for barnyard grass at ambient conditions. It may be too early for some plant species to measure stress affecting photosynthetic efficiency 2 days after spraying because glyphosate only indirectly affects photosynthesis.
2. At elevated CO_2 and increased temperatures, the dry matter production increased for oilseed rape but not for wild oat and barnyard grass. The ED_{50} and ED_{90} values did not significantly differ between the two environments for each grass species. Wild oat was less sensitive to glyphosate than barnyard grass. Significant higher doses were needed to reduce oilseed rape dry weight by 50 and 90 % at ambient growing conditions indicating that glyphosate doses can be reduced in a warmer climate with an elevated CO_2 level, probably because glyphosate is faster translocated in the oilseed rape plants.
3. There were no apparent trends of changes in the C and N percentages in plant dry matters, and the C/N ratio was caused by an elevated CO_2 concentration and increased temperatures, probably because of the short duration of the experiments under controlled conditions.
4. Transcriptional responses of wild oat, oilseed rape and barnyard grass to glyphosate and elevated CO_2 and temperature differ. In wild oat, climate conditions have the greatest impact on the transcriptome, whereas, in oilseed rape, glyphosate dose exerts the most pronounced effect. For barnyard grass, elevated CO_2 and temperature appear to alleviate the effects of high glyphosate treatment.

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CRediT authorship contribution statement

Zahra Bitarafan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Melissa Hamner Mageroy:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Rafael de Andrade Moral:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Najmeh Salehan:** Investigation. **Kristian Schmidt Nielsen:** Investigation. **Christian Andreasen:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data and R scripts are made available at www.github.com/rafamoral/climate_effects. Transcriptome data can be accessed on NCBI: SAMN46559202 (wild oat), SAMN46559204 (barnyard grass), and SAMN46559206 (oilseed rape). The graphical abstract is created and available in BioRender (Bitarafan, Z. (2025) https://BioRender.com/ahthw1l).

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