Plant, Cell & Environment

WILEY

Plant, Cell & Environment

ORIGINAL ARTICLE OPEN ACCESS

Arbuscular Mycorrhizal Fungi Mitigate Drought-Enhanced Herbivore Performance in Maize

Sheharyar Ahmed Khan[1,2] | Giulia Elena Capella[1,3] | Gaëtan Glauser[4] | Pierre Mateo[1] | Vera Ogi[1] | Marcel van der Heijden[5] | Natacha Bodenhausen[3] | Christelle Robert[1,2]

[1]Chemical Ecology Laboratory, Institute of Plant Sciences, University of Bern, Bern, Switzerland | [2]Oeschger Centre for Climate Change Research, University of Bern, Bern, Switzerland | [3]Department of Soil Sciences, Research Institute of Organic Agriculture FiBL, Frick, Switzerland | [4]Neuchâtel Analytical Chemistry Platform, University of Neuchâtel, Neuchâtel, Switzerland | [5]Department of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland

Correspondence: Natacha Bodenhausen (Natacha.Bodenhausen@fibl.org) | Christelle Robert (Christelle.Robert@unibe.ch)

Received: 24 June 2025 | **Revised:** 11 December 2025 | **Accepted:** 19 December 2025

Funding: Swiss Government Excellence Scholarship from the State Secretariat for Education, Research and Innovation, Grant/Award Number: 2022.0534; ENHANCE project, funded by the Swiss National Science Foundation, Grant/Award Number: 10002724

Keywords: arbuscular mycorrhizal fungi (AMF) | drought | herbivory | maize | sustainable agriculture

ABSTRACT

Drought events are becoming increasingly frequent and intense, posing major challenges to crop productivity. Beyond direct water stress, drought can indirectly affect plants by enhancing herbivore performance. While arbuscular mycorrhizal fungi (AMF) have been proposed to alleviate drought stress and to enhance plant resistance to herbivory, their role in mediating plant responses to the two combined pressures remains poorly understood. Here, we examined the individual and interactive effects of drought, AMF colonisation, and herbivory by *Spodoptera exigua* on maize (*Zea mays*) performance by combining a semi-field experiment with growth chamber assays. Drought reduced maize biomass (by 21.5%) and chlorophyll content (by 8.2%), while AMF improved reproductive traits. In particular, AMF colonisation increased the number of ears (from 1.1 to 1.4) and ear length (from 22.5 to 24.3 cm). Interestingly, drought transiently decreased DIMBOA-Glc levels in maize leaves, an effect that was exacerbated under AMF colonisation. Consistently, drought increased leaf herbivore performance by 32%. However, AMF colonisation mitigated the drought-induced increase in herbivore performance, even though leaf damage levels remained similar, indicating a post-ingestive resistance effect. This study highlights the need to consider multi-stressor interactions to harness AMF benefits in agriculture under increasing drought pressure.

1 | Introduction

Drought is becoming increasingly frequent and intense across many regions due to shifting climate patterns, posing a serious threat to global food security (Farooq et al. 2023; Intergovernmental Panel on Climate Change IPCC 2023; Rezaei et al. 2023). While drought directly impairs plant growth and yield by limiting water and nutrient uptake, it can also increase herbivore pressure, either by weakening plant defences or by improving plant tissue nutritional value. Arbuscular mycorrhizal fungi (AMF) can improve plant drought resilience by enhancing water and nutrient acquisition and by modulating drought-responsive metabolic pathways (Abdalla et al. 2023; Abrar et al. 2024; Chandrasekaran and Paramasivan 2022; Khan et al. 2024; Zaman et al. 2024; Zou et al. 2021). Beyond their effects on drought tolerance, AMF also influence plant resistance or tolerance to herbivory by altering nutrient allocation and secondary metabolite production (Dowarah et al. 2022; H. Yang et al. 2014). However, the role of AMF in mediating plant responses under combined drought and herbivory

Sheharyar Ahmed Khan, Giulia Elena Capella, Natacha Bodenhausen and Christelle Robert are equally contributed.

remains poorly understood. Addressing this knowledge gap is essential for developing sustainable strategies to improve plant resilience in increasingly variable environments.

The increasing frequency and severity of drought events threaten agricultural productivity worldwide, especially in regions already vulnerable to water scarcity (Intergovernmental Panel on Climate Change IPCC 2023; Rosenzweig et al. 2014; Yuan et al. 2024). Among climate-related stressors, drought is one of the most damaging, with the potential to reduce crop yields by over 50% on arable land by 2050 (Vinocur and Altman 2005). The three major cereal crops, maize, wheat, and rice, which together provide over half of the global caloric intake, are particularly sensitive to water stress (Deribe 2025; Farooq et al. 2023; Kheyruri et al. 2024; Mohammadi 2024; Sheoran et al. 2022). Drought leads to impaired photosynthesis, stunted growth, disrupted nutrient uptake, early senescence, and reduced yield (Gupta et al. 2020; Qiao et al. 2024). A meta-analysis showed that a 40% water reduction caused yield declines of up to 21% in wheat and 40% in maize in the field (Daryanto et al. 2017). As drought episodes intensify, safeguarding these staple crops is essential to ensure food security for a growing population.

Beyond its direct effects on plant growth and yield, drought can also indirectly exacerbate plant stress by increasing herbivore pressure (Chávez-Arias et al. 2021). Water limitation can trigger increased tissue concentrations of sugars and amino acids due to osmotic adjustment and weaken or delay activation of defence pathways (Ruan 2014). In crops, drought has been shown to impair the jasmonic acid (JA)- and salicylic acid (SA)-mediated defence responses that normally deter herbivory (Shafi et al. 2024). For instance, the drought-mediated downregulation of JA marker gene expression led to reduced activity of serine protease inhibitors and peroxidase (POD) in tomato plants, increasing growth and damage rate of tomato rust mite (Ximénez-Embún et al. 2017). However, abscisic acid (ABA) the canonical drought hormone, can also synergise with JA-dependent defences and potentiate JA-mediated herbivore defences such as proteinase-inhibitor defences, leading to reduced performance of some chewing herbivores (Nguyen et al. 2016). These results highlight that drought-induced JA-related defences and resistance to herbivory remain unpredictable and likely depend on the species, severity and/or timing of drought, herbivore type, and possibly the plant's genetic background (Liao et al. 2025), and underscore the importance of considering biotic and abiotic stress interactions.

AMF form symbiotic associations with the roots of most terrestrial plants and play a key role in improving plant resilience to drought (Bhupenchandra et al. 2025; Martin and van der Heijden 2024; Wang et al. 2024). AMF penetrates cortical cells of the roots and produce arbuscles where an exchange of nutrients between the two partners takes place. This symbiotic association helps the plant to acquire nutrients, resistance against pathogens, enhanced growth under abiotic stresses (Bhupenchandra et al. 2025; Kumar et al. 2024). The fungi, in return receive carbohydrates and lipids from the plant (Balestrini et al. 2020; Salmeron-Santiago et al. 2022). By extending their hyphal networks into the soil, AMF enhance water uptake beyond the root depletion zone, thereby improving plant hydration under limited water availability (Abrar et al. 2024). Furthermore, AMF increase the acquisition of essential nutrients such as phosphorus, potassium, and micronutrients, which are often less mobile in dry soils (Balestrini et al. 2020; Bhupenchandra et al. 2025). In addition to improving resource uptake, AMF modulate plant physiological responses to drought by promoting osmotic adjustment through the accumulation of solutes like proline and soluble sugars, enhancing antioxidant enzyme activity, and stabilising photosynthetic processes (Begum et al. 2019). These effects help maintain cell turgor, delay senescence, and support root hydraulic conductivity under water-limiting conditions (Abdalla et al. 2023). Studies across various species have demonstrated that AMF-inoculated plants maintain higher biomass, chlorophyll content, and stomatal conductance under drought stress compared to non-mycorrhizal plants (Tang et al. 2022). For instance, mycorrhizal symbiosis can increase the uptake nutrients such as nitrogen, phosphorus and iron as demonstrated in a study in *Pelargonium graveolens* under drought stress (Amiri et al. 2017). Similarly. AMF-inoculated pistachio plants revealed high levels of phosphorus, potassium, zinc and manganese (Bagheri et al. 2012). Several studies have indicated that the association of AMF with plants led to an increase in biomass, rise in net CO_2 assimilation and stomatal conductance (Kakabouki et al. 2023; Ran et al. 2024). The photosynthetic activity indicated by higher levels of photosynthetic pigments and chlorophyll fluorescence parameters were also observed (Bagheri et al. 2019). Under drought stress, AMF can stabilise water relations, improving plant resilience through mechanisms such as increased root hydraulic conductivity when plants are subjected to drought stress (Erice et al. 2024). These improvements in hydraulic conductivity are often linked to AMF-mediated modulation of plant aquaporins, particularly plasma membrane intrinsic proteins (PIPs), whose enhanced expression or activity facilitates water transport across root cells under drought conditions (Quiroga et al. 2017). Through these multifaceted mechanisms, AMF contribute significantly to plant drought tolerance and represent a promising tool for improving crop resilience in water-scarce environments.

AMF can further enhance plant defences against herbivory under ambient conditions (Meier and Hunter 2018). AMF can increase plant vigour and support the synthesis of defensive secondary metabolites by improving nutrient acquisition, particularly of phosphorus and nitrogen (Amani Machiani et al. 2022; Orine et al. 2025). Additionally, AMF can enhance plant resistance to herbivory through both signalling and metabolic pathways. AMF colonisation has been shown to prime or amplify defence signalling pathways, notably those mediated by JA (Rivero et al. 2021). For example, AMF inoculation increased gibberellic acid and JA content in peanut and tomato plants under feeding by *Spodoptera exigua* (L. He et al. 2017). Such AMF-mediated activation of defence pathways can translate into both direct effects on herbivores, through increased toxicity or reduced digestibility of plant tissues, and indirect effects, by enhancing the production of volatiles that attract natural enemies. For example, AMF reconfigure secondary metabolite profiles, increasing phenolic compounds such as quercetin, vanillic acid, rutin, coumaric acid, and kaempferol in quinoa (Benaffari et al. 2024), tannins in *Perilla frutescens* (Y. Liu et al. 2025), as well as benzene- and sulphur-containing defensive compounds in *Solanum nigrum* (Rashidi et al. 2024). In tomato plants, AMF enhanced tolerance to *S. littoralis* even in JA-deficient genotypes, suggesting that mycorrhizae can even compensate for impaired defence signalling (Formenti and Rasmann 2019). In *Asclepias* species, AMF inoculation increased foliar phosphorus levels and conferred greater resistance to monarch butterfly larvae

(Tao et al. 2016). Similarly, AMF associations reduced aphid performance on *Ageratina adenophora* by lowering nymph survival and supporting stronger plant growth (E. Du et al. 2022). These examples demonstrate that AMF can bolster plant defences and mitigate herbivore damage under normal environmental conditions, highlighting their potential as a natural strategy for pest management in agriculture.

While AMF have been shown to improve plant tolerance to both drought and herbivory when studied separately, their role under simultaneous exposure to these stressors remains poorly understood. In real-world agricultural settings, plants often face multiple, interacting stresses rather than isolated ones. It is therefore critical to understand whether AMF can continue to support plant performance and defence when both stressors co-occur. Simultaneous drought and herbivory may profoundly alter AMF-mediated effects because plants must reallocate limited resources between osmotic adjustment, growth, and defence, and because drought and herbivory activate partially overlapping signalling pathways that can interact antagonistically or synergistically. Some studies suggest that AMF can prime plant defence pathways even under abiotic stress, potentially maintaining resistance to herbivores during drought. For instance, *Medicago truncatula* inoculated with *Rhizophagus irregularis* showed increased expression of JA-responsive genes and elevated flavonoid levels under combined drought and insect stress, suggesting that mycorrhizal colonisation can help sustain chemical defences even when plants face water limitation (Adolfsson et al. 2017). However, the benefits of AMF may be highly context-dependent, varying with the timing, severity, and combination of stresses, as well as the plant and AMF genotypes involved. Investigating AMF-plant-herbivore interactions under realistic, multi-stress conditions will enable us to better predict and harness their potential for sustainable crop protection and climate-resilient agriculture.

The present study aimed at investigating the individual and combined effects of drought stress, AMF colonisation, and herbivory on maize performance and yield. We first assessed how AMF colonisation by *Rhizophagus irregularis* influenced maize growth, yield, and natural herbivory under ambient and drought conditions in a semi-field experiment. We then conducted a controlled growth chamber assay to evaluate how AMF modulated plant responses to drought and herbivory by the beet armyworm *S. exigua* larvae, a common insect pest worldwide. By integrating physiological, metabolic, and herbivore performance data, our goal was to determine whether AMF can enhance maize resilience under simultaneous abiotic and biotic stress, and to identify potential mechanisms underlying these effects. Specifically, we aimed to test the hypotheses that (i) AMF colonisation mitigates the negative impacts of drought on maize growth and yield, (ii) AMF improves plant defences and decreases herbivore performance, and (iii) these effects interact under combined stress conditions to improve overall plant resilience.

2 | Methods

2.1 | Biological Resources

B73 maize seeds were obtained from Maize GDB germplasm (MGCSC, Urbana, USA) and multiplied by Delley Semences et Plantes (DSP, Delley-Portalban, Switzerland). Inoculum containing sand, soil, roots, and spores of the AMF *Rhizophagus irregularis* (SAF22) as well as a mock inoculum without AMF was produced in the greenhouse, as previously described by Lutz et al. (2023), and were provided by the Swiss Collection of Arbuscular Mycorrhizal Fungi (SAF, Zurich, Switzerland). Briefly, *Plantago lanceolata* was inoculated with SAF22 or no AMF for the mock inoculum, and grown in a mixture of autoclaved soil and sand. Pots were watered regularly and received every second week 20 mL of a modified Hoagland solution (Hoagland and Arnon 1950), containing one quarter of the original P concentration. After 3 months of growth, watering was stopped and the pots dried out. The content of the pots was mixed and used as a source of inoculum. Spore density was approximately 15–30 spores/g. Eggs of *S. exigua* were bought from Frontiers Agricultural Sciences, Newark, NJ, USA and larvae were reared on artificial diet (Lepidoptera diet; Frontier Agricultural Sciences, Newark, NJ, USA). Second-instar larvae were used.

2.2 | Maize Growth and Yield in the Field

The individual and interactive effects of drought and AMF on maize growth and yield were evaluated by conducting a semi-field assay. The experiment was carried out in Ostermundigen (46°57′59.8 ″N 7°29′13.1 ″E), Switzerland between June and October 2024. Weather data was provided by MeteoSwiss (Federal Office of Meteorology and Climatology, Zürich, Switzerland) and are presented in Supporting Information S1: Table S1. Maize seeds (var. B73) were surface sterilised using 15% (v/v) bleach (Potz, Migros, Zurich, Switzerland) in distilled water for 15 min. The seeds were then rinsed with distilled water and pregerminated by placing them on damped filter papers (90 mm; Cytiva, Marlborough, MA, USA) in a plastic box (Semadeni, Bern, Switzerland) in the dark for 3 days. Ten-liter pots (Hortima, Hausen, Switzerland) were covered at the bottom using fabric sheath (Neeser, Reiden, Switzerland) and filled with approximately 11.4 kg of soil (Landerde, Ricoter, Aarberg, Switzerland). The soil chemical profile was analyzed by the laboratory Labour für Boden- und Umweltanalytik (LBU, Steffisburg, Switzerland) (Supporting Information S1: Table 2). For AMF inoculation, we followed the procedure developed by Köhl et al. 2016. Approximately 500 g of the AMF inoculum were added to half of the pots (AMF + , $n = 27$) and mixed with the soil, which corresponds to approximately 4% (w/w). The same amount of mock inoculum was added and mixed with the soil of control pots (AMF-, n = 27). Three pregerminated seedlings were placed 3 cm deep into the soil in individual pots. After 7 days, maize growth was assessed and one seedling (the most central) per pot was kept by manually removing additional seedlings. All plants were watered daily for 2 weeks. After this period, only control plants received water daily (AMF + : $n = 9$, AMF−: $n = 9$), while drought-exposed plants were left unwatered until drought symptoms appeared (leaf wilting score of 4, Sudhakar et al. 2016). Afterwards, all plants were watered once to twice weekly. The watering decision was based on soil dryness (5 cm depth) and leaf wilting symptoms of the RCP8.5 (severe drought) treated plants. Watering of all plants occurred only when RCP8.5 plants showed moderate wilting symptoms (no watering if low to no symptoms), resulting in a watering frequency for all plants of once to twice weekly. The plants

received either 2.3 L (ambient), 1.9 L (RCP2.6) or 1.66 L (RCP8.5) water ($n = 9$ per treatment). The volume of water to add was based on the calculated soil moisture of the current ambient conditions and predicted future climate scenarios RCP2.6 and RCP8.5 with a water content of 23%, 19%, and 16.6% (v/v) respectively (Guyer et al. 2018). All plants received 1% NK fertiliser (NK Flüssigdünger; Biorga, Grossaffoltern, Switzerland) during the eighth and ninth week of the experiment. All pots were covered with 35 L plastic bags (Quick Bag, Galaxus, Zürich, Switzerland) during rain episodes. The 54 pots (2 AMF treatments × 3 drought levels × 9 replicates) were randomly placed in the beds to avoid positional bias.

Plant phenotypic parameters were measured after 60, 85 and 100 days. Relative chlorophyll content of the youngest leaf was measured using Soil and Plant Analysis Development SPAD502 plus (Konica Minolta, München, Germany) around 12 pm for all the plants. The duration of the measurements lasted from 30 min to 1 h. Plant height was measured by using a ruler from the tip of the youngest leaf down to the soil surface. Herbivory damage was measured visually using a score of 1–3, one as the lowest scoring (1. Herbivory of < 5% leaf tissue, 2. Herbivory of 5%–15% leaf tissue, 3. Herbivory of > 15% leaf tissue.). Maize yield was approximated by measuring tassel and cob development after 85 and 100 days of planting the pregerminated seedlings. When two cobs were present, the length of the oldest cob was taken into account for further analyses. The experiment was unexpectedly interrupted when an individual entered the field and collected most maize cobs, resulting in the premature termination of the experiment on Day 118. As a result, and while fresh shoot and root biomass were measured at the termination of the experiment, no final cob parameters are available. Maize youngest leaves were collected on Days 60 and 120 and flash frozen in liquid nitrogen for sugars, hormones and benzoxazinoid analysis. Maize roots were collected on Day 120 for benzoxazinoid analysis and AMF colonisation evaluation.

2.3 | Herbivore Performance Under Growth Chamber Conditions

The impact of AMF and drought on maize resistance to herbivory was investigated by measuring herbivore damage and herbivore performance under laboratory conditions. Maize seeds were sterilised and pregerminated as described above. Germinating seedlings were placed in 3 L pots (Hortima, Hausen, Switzerland) covered at the bottom using fabric sheath (Neeser, Reiden, Switzerland). The pots were filled with either 3.4 kg soil (95% of pot volume; Landerde; Ricoter, Aarberg, Switzerland) mixed with 150 g AMF inoculum (AMF + , $n = 36$) or with 3.4 kg soil (95% of pot volume; Landerde; Ricoter, Aarberg, Switzerland) mixed with 150 g of autoclaved control inoculum (AMF−, $n = 36$). Maize plants were grown in a growth chamber at 23 ± 1°C and 18 ± 1°C with 14/10 h of light and darkness respectively to simulate natural conditions and 60% (v/v) relative humidity. All plants were watered daily for 2 weeks. Because no difference was observed between the two drought levels in the field, only one drought treatment (RCP8.5) was used in this experiment. Half of the AMF+ and AMF− plants were further well-watered on a daily basis. The second half of the plants were watered with 500 mL only upon leaf wilting (score 4) symptoms (RCP8.5). After 60 days, five pre-weighed *S. exigua* larvae were placed in the middle of the shoot tip. Control plants did not receive any insects. All plants were covered with a fleece (cover fleece 1.6 × 20 m; Florada, Hannover, Germany) to prevent larvae from escaping. The pots were randomly placed in the growth chamber. Five days later, *S. exigua* larvae were collected and weighed. Infested plants where no larvae were collected were excluded from the analysis. ImageJ software (version 1.54p, NIH, Bethesda, MD, USA) was used to quantify leaf damage from scanned leaf images. Images were calibrated to scale (1 pixel = 0.055 mm) by setting a known distance in the image with 'Set scale'. Further, the damaged area was selected manually with the 'Polygon selections' tool, and the particles were quantified with the 'Measure' function. The area was then expressed as fed leaf area in cm^2. The youngest leaves and crown roots were collected and flash-frozen in liquid nitrogen to analyze the benzoxazinoid contents. Maize roots were collected and stored at minus 20^{0}C for AMF colonisation assessment by microscopy. The experiment was repeated twice to ensure a sufficient number of replicates per herbivore treatment ($n = 8$). One replicate (ND treatment) was removed as no larvae were recovered at the end of the assay.

2.4 | AMF Colonisation Rates

Root length colonisation was quantified using the magnified intersection method from (McGonigle et al. 1990). Roots were stained following a previously established procedure (Vierheilig et al. 1998). Maize thin roots (diameter 0.5–1 mm) were cut into small segments of approximately 1.5 cm in length and preserved in 50% EtOH (Alcosuisse, Rüti b. Büren, Switzerland). The ethanol was rinsed off using distilled water and the samples were then cleared with 10% w/v KOH (Sigma-Aldrich, Steinheim, Germany) at 80°C in a dry bath (Digital Dry Bath; Labnet, Edison, NJ, USA) for a duration of 30 min. After incubation, the roots were rinsed using distilled water and stained with ink (Pelikan, Hannover, Switzerland) -vinegar solution (5% acetic acid; MBudget, Migros, Zurich, Switzerland) and incubated at 80°C for 30 min. After a final rinse with distilled water, the samples were stored in 50% glycerol (Dr. Bähler Dropa AG, Bern, Switzerland). The root samples were placed on a microscopic slide, mounted with 50% glycerol, and covered with the help of a cover slip. The samples were observed under a Fluorescence epi microscope with camera (Leica DMC6200; Leica Microsystems, Heerbrugg, Switzerland) at the magnification of 200× (magnifying lens * ocular lens). The average number of root segments analyzed for each plant in the field assay was 100, while the average of 60 root segments for each plant was analyzed for the herbivory assay. To exclude contamination in controls, on average 85 root segments were analyzed in the field assay. Each root segment was examined and categorised as either negative, arbuscule, vesicle, or internal hyphae. Colonisation is the percent of non-negative intersections (Bodenhausen et al. 2021).

2.5 | Soluble Sugar Quantification

The quantification of soluble sugars was performed using Ultra High Performance Liquid Chromatography (UHPLC) coupled with Mass Spectrometry (MS) following a protocol adapted from (Barzen-Hanson et al. 2018; J. Yang and Rainville 2019; Zhu et al. 2015). Maize roots and leaves samples were ground to a

fine powder in liquid nitrogen using a mortar and a pestle. Aliquots of 100 ± 1 mg were extracted by adding 500 µL of 50% (v/v) aq. EtOH in 2 mL tubes microtubes (Sarstedt AG and Co. KG, Nümbrecht, Germany). The samples were incubated for 15 min at 78°C in a dry bath, vortexed, and centrifuged at 14,000 rpm at 4°C for 10 min, and the supernatant was transferred to a new tube. This extraction was repeated twice, adding the supernatants of the same sample to the same tube. The samples were diluted 100 times and stored at −20°C until analysis. Fructose, glucose, and sucrose profiling were performed with an Acquity UPLC I-Class system coupled to a single quadrupole mass spectrometer (QDa) equipped with an electrospray source (Waters, Milford, MA, USA). Gradient elution was performed on an Acquity BEH Amide (1.7 µM, 2.1 × 150 mm i.d.; Waters, Milford, MA, USA) column maintained at 85°C, using normal phase chromatography in negative ion mode. The elution conditions were as follows: solvent A consisted of isopropanol (IPA) and aq. 10 mM ammonium formate (50:50 v/v), while solvent B consisted of acetonitrile (ACN), IPA, and aq. 10 mM ammonium formate (90:5:5 v/v). The flow rate was set to 0.7 mL/min. The gradient programme was: 100% solvent B from 0.00 to 2.00 min; a linear gradient from 100% to 60% solvent B from 2.00 to 6.00 min; 60% solvent B from 6.00 to 8.00 min; a rapid linear gradient from 60% to 100% solvent B from 8.00 to 8.10 min; and finally, 100% solvent B from 8.10 to 10.00 min. MassLynx v4.1 SCN923 (Waters, Milford, MA, USA) was used to control the instrument and for data processing. Absolute quantities were determined using standard curves of the corresponding pure compounds. Glucose, fructose, and sucrose standards were bought from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

2.6 | Phytohormone Analyses

Salicylic acid (SA), oxophytodienoic acid (OPDA), jasmonic acid (JA), jasmonic acid-isoleucine (JA-Ile), and abscisic acid (ABA) concentrations were quantified by UHPLC-MS/MS as described by Glauser et al. (2014) with minor adjustments (Gfeller et al. 2023). Aliquots of 85 ± 5 mg ground plant material were extracted by adding 990 µL of extraction solvent, consisting of ethyl acetate (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and formic acid (FA; Thermo Fisher Scientific, Waltham, MA, USA; 99.5:0.5 v/v), and 10 µL internal standard solution (isotopically labelled hormones at 100 ng/mL in water; d_6-SA from Sigma-Aldrich Chemie GmBH, Buchs, Switzerland; d_6-ABA and d_5-OPDA from OlChemIm, Olomouc, Czech Republic; d_5-JA from CDN Isotopes, Quebec, Canada; $^{13}C_6$-Ja-Ile produced in the laboratory of the Neuchatel Platform of Analytical Chemistry). The solution was vortexed (Vortex-Genie 2; Genie, Bohemia, NY, USA) for 10 s before adding 5–10 glass beads for mixing in a mixer mill (MM300; Retsch, Haan, Germany) at 30 Hz for 3 min and subsequently centrifuged at 14,000 rpm for 3 min (Centrifuge 5427 R; Eppendorf, Hamburg, Germany). The supernatants were transferred to 2 mL tubes microtubes. The pellet was re-extracted in 500 µL of extraction solvent and centrifuged as described above. The two supernatants were combined. The solvent was evaporated using a centrifugal evaporator (CentriVap; Labconco, Kansas City, MO, USA) and resuspended in 200 µL of aq. MeOH (50:50 v/v; Thermo Fisher Scientific, Waltham, MA, USA) using vortex and ultrasounds (Ultrasonic bath XUBA1; Grant Instruments Ltd, Royston, UK). The supernatant was filtered through a polytetrafluoroethylene hydrophilic syringe filter (0.22 µm × 13 mm i.d.; BGB, Boeckten, Switzerland) and collected in a clean Eppendorf tube (Microtube CapLock; Nolato, Torekov, Sweden). Hormone profiling was conducted using an Acquity UPLC I-Class (Waters AG, Baden-Dättwil, Switzerland) coupled to a QTRAP 6500+ mass spectrometer (Sciex, Framingham, MA, USA) operated in multiple reaction monitoring (MRM) mode with negative ionisation. Chromatographic separation was performed on an Acquity BEH C18 column (1.7 µM, 2.1 × 50 mm i.d.; Waters, Milford, MA, USA) coupled to a guard column of identical phase chemistry. UHPLC gradient conditions were as follows: solvent A consisted of H_2O and FA (99.95:0.05 v/v), and solvent B consisted of ACN and FA (99.95:0.05 v/v). The flow rate was set to 0.4 mL/min. The injection volume was 1 µL and the column temperature was maintained at 35°C. The gradient programme was: a linear gradient from 5% to 50% solvent B from 0.00 to 5.00 min; a linear gradient from 60% to 100% solvent B from 5.00 to 8.00 min, 100% solvent B from 8.00 to 12.00 min; and re-equilibration at 5% solvent B from 12.00 to 16.00 min. Analyst v.1.7.1 (Sciex, Framingham, MA, USA) was used to control the instrument and for data processing.

2.7 | Benzoxazinoid Profiling

Benzoxazinoid contents were characterised using an Acquity UPLC I-Class system coupled to a single quadrupole mass spectrometer (QDa) equipped with an electrospray source (Waters, Milford, MA, USA) as previously described (Hu et al. 2018). The plant metabolites were extracted from 100 ± 1 mg by adding 1 mL MeOH:H_2O:FA (70:30 v/v, 0.1% FA) and thoroughly vortexed for 10 s. The samples were then centrifuged for 20 min at 1300 rpm at 10°C and the supernatant was collected for analysis. Compounds were separated on an Acquity BEH C18 column (1.7 µM, 2.1 × 100 mm i.d.; Waters, Milford, MA, USA). The flow rate of the mobile phase was maintained at 0.4 mL/min. The injection volume was 1 µL and the temperature of the column was maintained at 40°C. The MS was operated in negative mode, and data were acquired in the scan range (m/z 150–650) using a cone voltage of 10 V. All other MS parameters were left at their default values. The elution conditions were as follows: solvent A consisted of H_2O and FA (99.9:0.1 v/v), while solvent B consisted of ACN and FA (99.9:0.1 v/v). The gradient programme was: 2% solvent B from 0.00 to 1.00 min; a linear gradient from 2% to 40% solvent B from 1.00 to 4.00 min; a linear gradient to 100% solvent B from 4.00 to 6.00 min; 100% solvent B from 6.00 to 8.50 min; a gradient from 100% to 2% solvent B from 8.50 to 8.51 min; and 2% solvent B from 8.51 to 10 min. MassLynx v4.1 SCN923 was used to control the instrument and for data processing. The absolute quantities of HMBOA, DIMBOA, DIMBOA-Glc, DIMBOA-2Glc, HDMBOA-Glc, and MBOA were determined using standard curves of the corresponding pure compounds. MBOA was purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). DIMBOA-Glc DIMBOA-2Glc, and HDMBOA-Glc were isolated from maize plants in our laboratory as previously described (Sutour et al. 2024; Thoenen et al. 2023). DIMBOA and HMBOA were synthesised in our laboratory following

published protocols (Macías et al. 2006). HMBOA-Glc, HMBOA-2Glc, HM_2BOA-Glc, DIMBOA-3Glc, DIM_2BOA-Glc, and HDM_2BOA-Glc for which no analytical standards were available, were quantified by comparison with the standard curve of their closest parent compounds, HMBOA, DIMBOA-Glc, and HDMBOA-Glc. Full names and chemical formulas of measured benzoxazinoids can be found in Supporting Information S1: Supporting Table S3.

2.8 | Statistical Analyses

Statistical analyses and data visualisation were done with R (version 4.4.2; R core team, 2018) using R studio (version 2024.12.0.467; Posit team, 2024). The data was read in with the package readxl (version 1.4.3). For organising and structuring the data the package dplyr (version 1.1.4) was used. The semi-field assay and the herbivory assay followed a fully multifactorial design. Two- and three-Way ANOVAs were used to detect the effects of response variables, depending on the number of variables in the experiment. Explanatory variables were AMF presence or absence, water regimes, and for the herbivore assay presence or absence of herbivores. When significant effects were detected, Tukey's HSD post-hoc tests were performed to identify differences between treatment level, and effect sizes were calculated as Cohen's d using the residual standard deviation from the ANOVA model. Homoscedasticity and normality of distribution of residuals were confirmed visually with the diagnostic plots of base R. If the model fit was not satisfactory, the tested variables were rank transformed prior to analysis. For the insect performance data, no effect of the experimental repetition could be observed, and thus the data of both experiments were combined for analysis. Plots were made using the package ggplot2 (version 3.5.1) and ggpattern (version 1.1.1).

3 | Results

3.1 | Drought Decreased Maize Growth, but AMF Improved Plant Growth and Reproductive Success Independently of Soil Moisture Levels

A semi-field experiment was carried out to assess the effects of drought, AMF, and naturally occurring herbivores in conditions relatable to agriculture (Supporting Information S1: Figure S1A). The addition of AMF increased colonisation from 2.34% to 76.1% in ambient conditions and from 0% to 46.3% and 0.25% to 51.4% under RCP2.6 and RCP8.5 conditions respectively (Figure 1A). Drought further decreased shoot height already at day 60 and the effect intensified after 85 days but not further after 110 days (Supporting Information S1: Figure S1C). Shoot biomass was also decreased under RCP2.6 and RCP8.5 drought conditions (Figure 1B). Drought further decreased leaf chlorophyll contents (Supporting Information S1: Figure S1D). Conversely, AMF presence increased shoot biomass, cob length, and cob number (Figure 1B–D). No interactions between drought and AMF were observed on any of the measured growth and reproductive parameters under semi-field settings (Figure 1). Root biomass was not affected by treatments (Supporting Information S1: Figure S2). Field damage by herbivores was low and did not show a treatment effect (Supporting Information S1: Figure S3).

3.2 | AMF and Drought Modulated Maize Metabolism in Semi-Field Conditions

In leaves, drought triggered transient changes in benzoxazinoids at day 60, reflected by a decrease in DIMBOA-Glc levels and an increase in DIM_2BOA-Glc leaf concentrations (Figure 2A; Supporting Information S1: Figure S4). The AMF-induced decrease in DIMBOA-Glc was stronger under ambient than drought conditions (Figure 2A; Supporting Information S1: Figure S4). AMF colonisation was positively correlated with leaf sucrose and ABA concentrations (Supporting Information S1: Figure S5). At Day 60, AMF colonisation was negatively correlated with DIM_2BOA-Glc (Supporting Information S1: Figure S6). After 120-day, drought stress decreased sucrose and, albeit not significantly, glucose concentrations, but did not affect fructose levels in leaves (Figure 2B; Supporting Information S1: Figure S7). Drought did not affect leaf hormonal levels (Figure 2B; Supporting Information S1: Figure S7).

In roots, the prolonged drought increased fructose, JA, OPDA, SA, HMBOA-2Glc, and HM_2BOA-Glc levels (Supporting Information S1: Figure S8). AMF presence increased fructose, glucose, and sucrose concentrations, and decreased OPDA and total benzoxazinoid levels, particularly through lowered concentrations of HMBOA-Glc and DIMBOA-Glc (Supporting Figure 8). Drought and AMF presence showed an interactive effect on fructose, as AMF-induced increase in fructose levels was stronger in the RCP2.6 drought scenario (Supporting Information S1: Figure S8). A negative correlation between AMF colonisation and HM_2BOA-Glc and DIMBOA-2Glc was observed (Supporting Information S1: Figure S9).

3.3 | AMF Colonisation Limited Drought-Induced Increase in Insect Performance

Drought and AMF showed individual and interactive effects on maize leaf benzoxazinoids in the field (Figure 2). Because the natural herbivore pressure in the field was low (Supporting Information S1: Figure S3), the potential effects of drought and AMF-mediated changes in benzoxazinoids on herbivore performance were assessed under controlled conditions. As in the semi-field assay, drought reduced AMF colonisation, plant height, and shoot biomass (Supporting Information S1: Figure S11). Drought and AMF showed interactive effects on chlorophyll contents, as AMF-induced decrease in chlorophyll content was pronounced only under ambient conditions Supporting Information S1: Figure S11).

After 2 months, plants were subjected to feeding of 5 *S. exigua* larvae for 5 days. The relative growth of the leaf herbivore *S. exigua* was not affected by AMF presence in soil but was slightly increased on plants that were subjected to drought than on plants that grew in ambient conditions (Figure 3A). While the herbivore performed better under drought conditions in the absence of AMF, the effect disappeared in the presence of AMF (Figure 3A). The leaf damage area was not affected by drought nor AMF (Figure 3B), but a significant correlation between the absolute mass gain of larvae and the leaf damage area was observed (Supporting Information S1: Figure S11).

In the leaves, levels of HMBOA-Glc and DIM_2BOA-Glc increased and HDM_2BOA-Glc decreased under drought

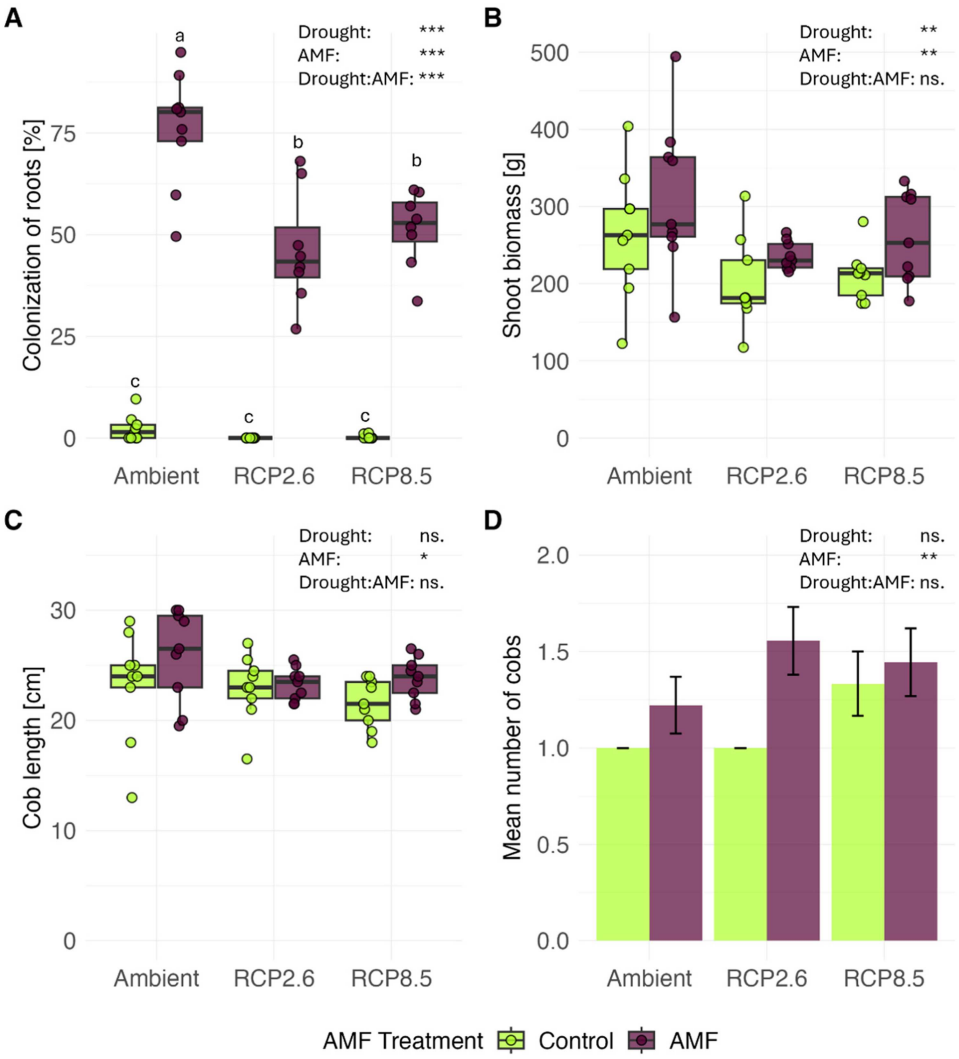

FIGURE 1 | AMF colonisation promotes shoot biomass and cob length independently of moisture conditions. (A) AMF colonisation after 120 days (Cohen's d > 0.8 for all significant differences between treatments), (B) fresh shoot biomass after 120 days, (C) mean cob length after 100 days, (D) number of cobs after 100 days. Boxplots with individual data points or mean ± standard errors are shown ($n = 9$ per treatment). Ambient soil moisture: 23% (v/v); Drought soil moisture: RCP2.6 and RCP8.5: 19% and 16.6% (v/v) respectively. AMF = Arbuscular mycorrhizal fungi, *Rhizophagus irregularis* (SAF22). ANOVA tests were run to analyze differences among treatments, ns, not significant; $= 0.05 < p < 0.10$, $* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.0001$. Tukey's HSD post-hoc test was performed when significant interactions were found. Different letters indicate significant differences between treatments when interactions between AMF and drought were observed. Effect size was calculated using Cohen's d. Data on drought and AMF colonisation effects on leaf chlorophyll contents, shoot height, root biomass, and field damage are provided in Supporting Information S1: Figures S1–S3.

conditions (Supporting Information S1: Figure S12). AMF alone showed no effect, but AMF presence induced an increase in DIMBOA-2Glc under drought, but not ambiant, conditions (Supporting Information S1: Figure S13). Herbivory did not affect benzoxazinoid levels in leaves (Supporting Information S1: Figure S13).

In roots, drought increased the concentration of HMBOA-Glc, HMBOA-2Glc, HM2BOA-Glc, DIMBOA-2Glc, DIM_2-BOA-Glc, and MBOA, while only HMBOA showed a decrease. AMF treatment affected DIMBOA-3Glc through elevated concentrations in AMF+ plants. Interactive effects between drought and AMF were observed for DIMBOA and DIMBOA-2Glc, yet following opposite trends. While DIMBOA levels were lower in AMF+ plants under drought treatment, DIMBOA-2Glc levels were increased in the same conditions. HMBOA-Glc and DIMBOA-Glc were increased under drought conditions when subjected to herbivory (Supporting Information S1: Figure S14).

4 | Discussion

Our study revealed that drought significantly reduced maize vegetative growth, while AMF colonisation improved plant growth and reproductive success independently of soil moisture levels. Under controlled conditions, drought increased herbivore performance, yet this effect was neutralised in AMF-colonised plants, suggesting that AMF may reduce drought-enhanced susceptibility to herbivory. Together, these findings highlight the potential of AMF to support maize reproductive performance and buffer biotic stress under drought.

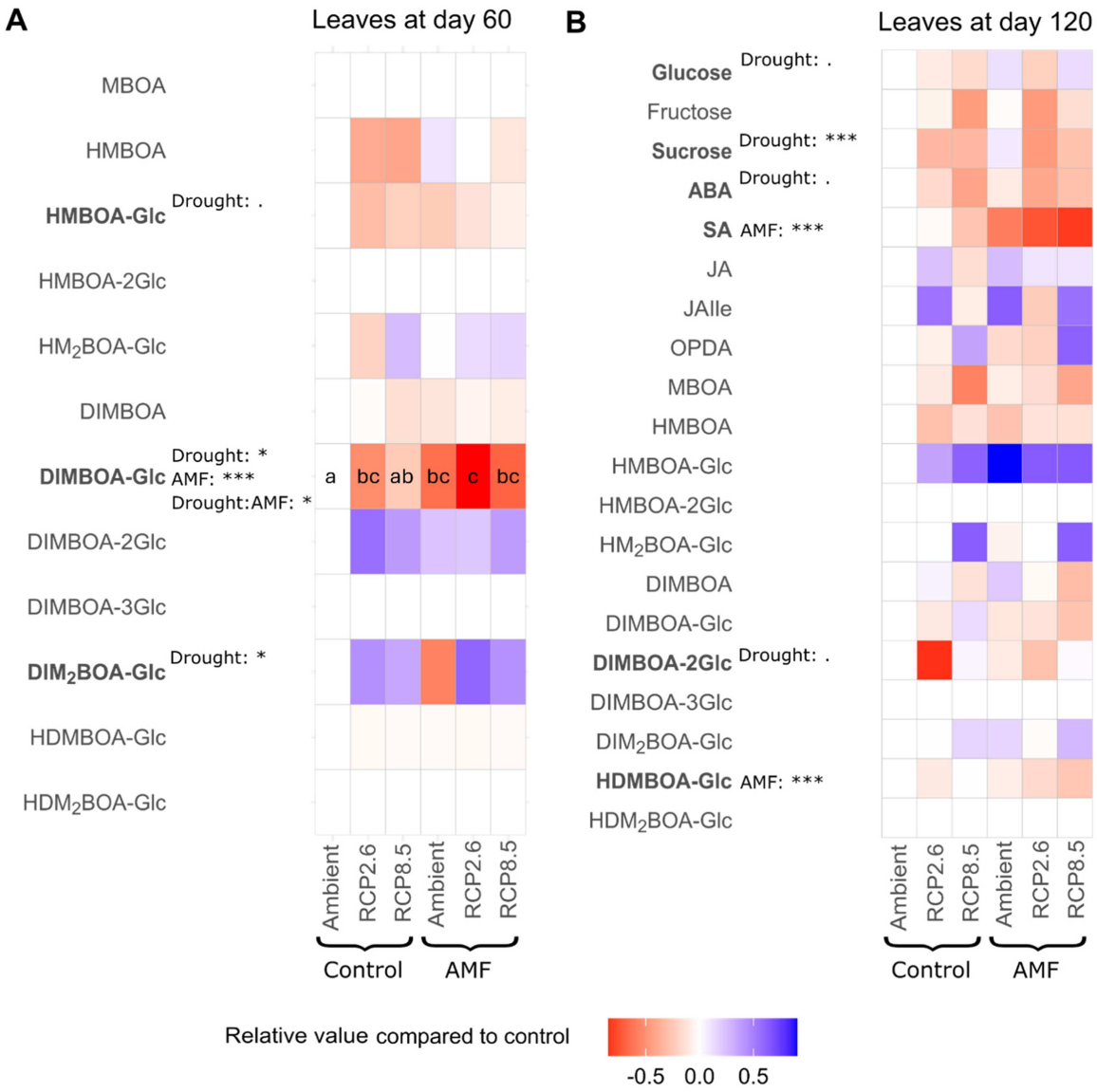

FIGURE 2 | Drought and AMF modulate the maize metabolism. (A) Heatmap of leaf metabolite concentrations relative to concentrations in control plants under ambient conditions after 60 days, (B) Heatmap of leaf metabolite concentrations relative to concentrations in control plants under ambient conditions after 120 days. Ambient soil moisture: 23% (v/v); Drought soil moisture: RCP2.6 and RCP8.5: 19% and 16.6% (v/v) respectively. AMF = Arbuscular mycorrhizal fungi, *Rhizophagus irregularis* (SAF22). Data were log-transformed ($n = 9$ per treatment). Compounds highlighted in bold showed significant differences. Stars indicate significant differences (linear model for each compound): $= 0.05 < p < 0.1$, $* = p \leq 0.05$, $** = p \leq 0.01$, $*** = p \leq 0.001$. Tukey's HSD post-hoc test was performed when significant interactions were found and different letters indicate significant differences between treatments. Histograms for individual compound graphs and roots are shown in Supporting Information S1: Figures S4, S5, and S8. Correlations between AMF colonisation and ABA and between AMF colonisation benzoxazinoids are shown in Supporting Information S1: Figures S6, S7, and S9. Structural equation modelling (SEM) is shown in Supporting Information S1: Figure S10.

Drought stress alone had clear effects on maize growth and metabolism, as well as on herbivore performance. Drought led to significant reductions in maize shoot height, biomass, and chlorophyll content, reflecting impaired photosynthetic capacity. Reductions in chlorophyll under drought are commonly associated with limited CO_2 assimilation caused by stomatal closure. In this study, drought reduced chlorophyll levels without markedly altering ABA concentrations in leaves, suggesting that stomatal or hydraulic limitations, rather than shifts in ABA accumulation, were the primary drivers of the reduced photosynthetic capacity and overall plant vigour. These observations are consistent with previous studies showing that drought reduces maize performance (Deribe 2025), although the extent of these effects can vary depending on genotype, developmental stage, and nutrient availability (Blein-Nicolas et al. 2020; L. Liu et al. 2021). In roots, prolonged drought increased fructose and glucose concentrations, consistent with the known role of soluble sugars in osmotic adjustment and stress tolerance (Anjum et al. 2017; Sepulveda et al. 2022). However, in leaves, drought reduced sucrose and tended to decrease fructose concentrations, while glucose levels remained unchanged. This partially contrasts with studies reporting

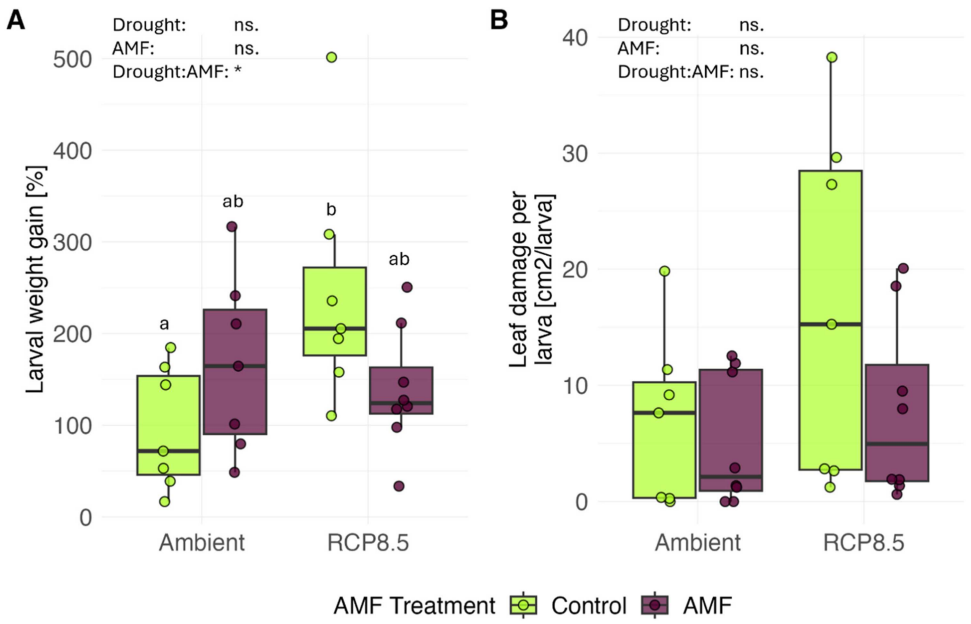

FIGURE 3 | AMF alleviates the drought-mediated increase in insect performance. (A) Relative individual weight gain (Cohen's d between Ambient AMF and RCP8.5 AMF plants is 0.76), (B) Leaf damage per larva. Boxplots with individual data points are shown ($n = 7–8$ per treatment). Ambient soil moisture: 23% (v/v); Drought soil moisture (RCP8.5): 16.6% (v/v). AMF = Arbuscular mycorrhizal fungi, *Rhizophagus irregularis* (SAF22). ANOVA tests were run to analyze differences among treatments: ns: not significant; * = $p < 0.05$. Tukey's HSD post-hoc test were performed when significant interactions were found, and the effect sizes were calculated as Cohen's d. Different letters indicate significant differences between treatments when interactions between AMF and drought were observed. The effect of drought on AMF colonisation under controlled conditions is shown in Supporting Information S1: Figure S11. The correlation between the larval mass gain and leaf damage area is shown in Supporting Information S1: Figure S12. Benzoxazinoid levels in maize leaves and roots are shown in Supporting Information S1: Figures S13 and S14. No fitting SEM model was found. [Color figure can be viewed at wileyonlinelibrary.com]

whole-plant sugar accumulation under drought (Y. Du et al. 2020; Mohammadkhani and Heidari 2008), possibly due to differences in sampling time, tissue type, or drought severity (Gurrieri et al. 2020; Sharma et al. 2019). Despite clear wilting symptoms, drought did not affect ABA levels in roots or leaves, which was surprising given its well-established role in stomatal closure and drought signalling (Kim et al. 2010; Muhammad Aslam et al. 2022). Drought increased levels of JA, its precursor OPDA, and of SA in roots, but not in leaves, reflecting activation of general stress responses. Drought reconfigured the benzoxazinoid leaf profiles. In particular, drought consistently led to decreased levels of DIMBOA-Glc and increased concentrations of DIM_2BOA-Glc in leaves during semi field (day 60) and in both laboratory assays. The observed shift between DIMBOA-Glc and DIM_2BOA-Glc likely mirrors an increased activity of the 2-oxoglutarate-dependent dioxygenase BX13 and/or of *O*-methyltransferase BX7, enzymes responsible for the conversion of DIMBOA-Glc to TRIMBOA-Glc and subsequently to DIM_2BOA-Glc respectively (Handrick et al. 2016). These changes are consistent with the reported reconfiguration of benzoxazinoid profiles under abiotic stress as part of plant defence and stress adaptation (Robert and Mateo 2022; Sutour et al. 2024; Q. Zhang et al. 2020). Similarly, *Zm*BX13 was upregulated under drought conditions, with even stronger induction observed in a maize mutant selected for drought tolerance (C7-2T), suggesting that enhanced BX13 expression may be part of a drought-adaptive metabolic shift (Q. Zhang et al. 2020). In herbivory assays, drought increased the performance of *S. exigua* larvae. The observed increase is unlikely explained by changes in leaf sugar levels, as drought reduced leaf sucrose and fructose, both known to be feeding stimulant and promoting herbivore growth (Ai et al. 2022; S.-S. Zhang et al. 2023). A possible explanation lies in the shift of benzoxazinoid profiles. While DIMBOA-Glc and its aglucone DIMBOA are known to have strong anti-feeding, anti-digestive, or toxic activity on a broad range of generalist herbivores (Wouters et al. 2016), DIM_2BOA-Glc is largely ineffective against caterpillars such as *S. exigua* and *S. littoralis*, though it retains toxicity against aphids (Handrick et al. 2016; Robert and Mateo 2022). The drought-induced decrease in DIMBOA-Glc combined with a relative increase in DIM_2BOA-Glc therefore suggests a reallocation of defences toward phloem feeders, at the expense of resistance to chewing herbivores. In addition, other nutritional or defensive traits not quantified here, such as amino acid composition, nitrogen balance, or inducible defences, may also have contributed to the enhanced growth of *S. exigua* larvae. Overall, our data align with previous studies showing that drought can increase herbivore growth by altering plant nutritional quality (Carvajal Acosta et al. 2023; Duell et al. 2024; Ximénez-Embún et al. 2017).

Under ambient conditions, AMF colonisation alone had significant effects on maize growth, yield, and defences. AMF-inoculated plants showed increased shoot biomass, cob length, and cob number, consistent with the well-established role of AMF in promoting plant growth through improved nutrient acquisition and hormonal modulation (Bhupenchandra et al. 2025). Root fructose and glucose concentrations increased under AMF treatment, suggesting enhanced carbon sink strength and possibly greater metabolic activity in roots,

a pattern also reported in peach and tomato plants colonised by AMF (Ge et al. 2008; Mo et al. 2016). Interestingly, AMF colonisation led to a reduction in root sucrose levels, possibly due to increased sucrose cleavage or altered sugar transport dynamics, as seen in other studies where AMF modulated sugar transporter expression (Ge et al. 2008; Tang et al. 2022). In terms of hormonal signalling, AMF colonisation decreased OPDA in roots and SA in leaves, contrasting with several reports that suggest AMF increase phytohormone levels under stress (Tang et al. 2022). Rather than indicating activation of JA- or SA-dependent priming pathways, this pattern likely reflects AMF-mediated buffering of stress signalling, as both OPDA and SA often function as upstream stress cues. AMF are known to stabilize hormonal homoeostasis and reduce perceived stress, and such buffering could contribute to improved tolerance and resource allocation under drought. In our system, the enhanced resistance to *S. exigua* in AMF-inoculated plants is therefore more plausibly explained by AMF-induced metabolic adjustments than by activation of canonical JA-SA defence priming. AMF suppressed benzoxazinoid levels, including HDMBOA-Glc and DIMBOA-Glc, possibly reflecting a trade-off in which improved nutrient status and physiological condition reduce the need for costly chemical defences. The reduction in constitutive defence compounds under ambient conditions could also imply that AMF-colonised plants rely more on induced defences or tolerance strategies. However, AMF colonisation can also lead to enhanced accumulation of defence metabolites such as DIMBOA under pathogen attack, suggesting a complex context-dependent regulation (Song et al. 2011). In the controlled assay, AMF colonisation did not alter benzoxazinoid levels in leaves under ambient conditions. This difference could reflect environmental or developmental factors, as the semi-field experiment involved a longer growth period and greater exposure to fluctuating conditions, possibly inducing stronger AMF-mediated reprogramming of defence metabolism. Consistently, AMF colonisation alone did not impact *S. exigua* growth in the herbivore assays. This aligns with earlier findings indicating that AMF-mediated resistance is often context-dependent and may require either a co-occurring stress or stronger defence priming signals to translate into reduced herbivore performance. For instance, AMF boosted resistance to *S. littoralis* in JA-deficient tomatoes, an effect that was only pronounced when defence pathways were compromised (Formenti and Rasmann 2019). Finally, the observed negative correlations between AMF colonisation and DIMBOA-$_2$Glc and HM$_2$BOA-Glc may reflect AMF-induced shifts in benzoxazinoid turnover or indicate that these compounds exert antifungal activity and reduce AMF colonisation. Overall, these findings highlight the multifaceted role of AMF in modulating maize metabolism, supporting both growth and fine-tuned defence regulation even in the absence of external stressors.

Interactive effects between AMF and drought on maize physiology and metabolism were limited in the semi-field assay but became more apparent under controlled conditions. Consistently with previous studies, drought reduced AMF colonisation (Orine et al. 2022). In the field, AMF and drought affected maize metabolism largely independently. While AMF was found to support maize growth and yield under drought in some studies (Deribe 2025), the absence of interactions was also reported (Tiepo et al. 2024), likely due to development-specific effects (Abrar et al. 2024). Interactive effects were limited to root fructose levels and leaf DIMBOA-Glc contents. AMF increased root fructose and the effect that was more pronounced under drought conditions. Such context-dependent enhancement of sugar accumulation may indicate that AMF contributes to osmotic adjustment under moderate water stress as suggested in previous studies (Bahadur et al. 2019; Chandrasekaran and Paramasivan 2022). At day 60, AMF reduced DIMBOA-Glc concentrations in leaves more strongly under ambient than drought conditions, suggesting drought constrained the AMF effect. Interestingly, under laboratory conditions, AMF similarly reduced DIMBOA-Glc levels in the leaves, but increased them under drought conditions, resulting in comparable levels of the benzoxazinoid in control and AMF colonised plants. Systemic consequences of below-ground shifts may arise through several AMF-mediated signalling pathways. For example, AMF can affect whole-plant carbon partitioning via modulation of SWEET and SUT sugar transporters, and can alter aquaporin activity, thereby adjusting hydraulic conductivity and influencing shoot water status and metabolism (G.-X. He et al. 2025; Romero-Munar et al. 2024; Salmeron-Santiago et al. 2022). In addition, AMF produce Myc-derived signalling molecules that can trigger systemic transcriptional and metabolic responses independently of local colonisation (Salmeron-Santiago et al. 2022). Beyond the targeted metabolites quantified here, nutritional stoichiometry and vascular transport processes may also contribute to the AMF-mediated rescue of resistance under drought. AMF are known to influence nitrogen acquisition and amino acid metabolism, and to modify plant C:N balance through changes in nutrient uptake and carbon partitioning (Stratton et al. 2022; X. Yang et al. 2023). Moreover, AMF and drought both alter xylem transport capacity and phloem loading, potentially modifying the nutritional composition of leaf tissues in ways that are not captured by our measured traits (de Vries et al. 2021). Although our data do not resolve the precise mechanism, these pathways provide a plausible framework for understanding how AMF-mediated root metabolic reprogramming could contribute to the observed changes in shoot chemistry and herbivore resistance. While AMF alone had no effect on herbivore performance, their presence cancelled the drought-induced increase in *S. exigua* growth observed in non-mycorrhizal plants. The precise mechanisms by which AMF alleviated the drought-induced increase in caterpillar performance remain unclear as interactive effects were limited in the leaves and would not support the observed performance effect. However, AMF altered root metabolism under drought, including changes in soluble sugars, which may reflect adjustments in osmotic balance or nutrient allocation. Such belowground shifts could directly improve overall plant physiological status or indirectly affect leaf nutritional quality and defence signalling. For instance, AMF was found to alter the expression of marker genes involved in Ca^{2+} pathways, sugar transport, malondialdehyde accumulation, antioxidant enzyme activity, and water use under drought (Chen et al. 2025; Deribe 2025; Eftekhari et al. 2025). Although we explored structural equation modelling to test whether drought and AMF effects on larval performance could be statistically explained by the measured leaf traits, none of the candidate models achieved acceptable fit. This indicated that the quantified traits did not fully capture the systemic processes through which AMF modulate plant physiology under drought.

Consistently, the restoration of herbivore resistance by AMF was not fully explained by leaf-level metabolic responses alone, suggesting that the interactive effect on *S. exigua* performance likely arose from whole-plant adjustments rather than from any single foliar trait. Future work integrating metabolomics with physiological measurements such as leaf water potential, phloem loading, or induced defence kinetics will be required to resolve the causal chain linking AMF-mediated root reprogramming to shoot resistance under drought. The AMF-mediated dampening of drought-induced increases in herbivore performance highlights their potential as a valuable biological tool for promoting crop resilience and reducing reliance on chemical pest control in sustainable agricultural systems.

While our study provides novel insights into the role of AMF in maize responses to drought and herbivory, some limitations should be acknowledged. First, the semi-field experiment relied on potted plants, which may have restricted root development and limited the natural spread and functioning of AMF networks compared to open-field conditions. Second, our drought treatment did not include concomitant heat stress, which frequently co-occurs with water limitation in the field and can strongly influence plant-microbe-insect interactions. Finally, we focused on a single maize genotype and one AMF species, whereas responses may vary across genetic backgrounds and fungal partners. These factors should be addressed in future field-based, multi-genotype studies to better capture the complexity of plant responses under agricultural conditions.

5 | Conclusion

This study demonstrates that AMF can enhance maize reproductive success and modulate plant metabolism under both well-watered and drought conditions, with additional benefits under combined abiotic and biotic stress. While drought reduced plant growth and increased herbivore performance, AMF colonisation improved yield-related traits and mitigated drought-induced susceptibility to herbivory. These findings indicate that the AMF-mediated buffering of herbivore performance under drought was not due to any direct suppressive effect on the insect, but instead emerged from AMF-induced adjustments to plant physiology and metabolism that reduced leaf suitability to *S. exigua*. The context-dependency of AMF effects, particularly their modulation of benzoxazinoids and defence signalling under variable environmental conditions, emphasises the need for integrated, multi-factorial studies to understand plant responses in realistic scenarios. From a practical perspective, the ability of AMF to buffer drought-enhanced herbivore pressure offers promising opportunities for sustainable agriculture, reducing the need for external inputs while supporting crop resilience. Future research should aim to elucidate the mechanistic basis of these interactions across diverse plant and AMF genotypes, and under fluctuating field conditions, to better harness the full potential of AMF for climate-smart crop management.

Acknowledgements

We thank Miro Läderach, Sara Hoefer, Samuel Kohler, Ilario Boschetti, and Jonathan Ginzburg for their technical help during sample preparation. We thank Cygni Armbruster for producing the AMF inoculum. We further thank Ian Sanders and anonymous reviewers for their constructive feedback on a previous version of this manuscript. This work was supported by the Swiss Government Excellence Scholarship from the State Secretariat for Education, Research and Innovation (ESKAS-Nr: 2022.0534) and the ENHANCE project, funded by the Swiss National Science Foundation (Nr. 10002724).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are provided as Supporting File (Supporting Information S1: File S1).

References

Abdalla, M., M. Bitterlich, J. Jansa, D. Püschel, and M. A. Ahmed. 2023. "The Role of Arbuscular Mycorrhizal Symbiosis in Improving Plant Water Status Under Drought." *Journal of Experimental Botany* 74, no. 16: 4808–4824. https://doi.org/10.1093/jxb/erad249.

Abrar, M., Y. Zhu, M. Maqsood Ur Rehman, et al. 2024. "Functionality of Arbuscular Mycorrhizal Fungi Varies Across Different Growth Stages of Maize Under Drought Conditions." *Plant Physiology and Biochemistry* 213: 108839. https://doi.org/10.1016/j.plaphy.2024.108839.

Adolfsson, L., H. Nziengui, I. N. Abreu, et al. 2017. "Enhanced Secondary- and Hormone Metabolism in Leaves of Arbuscular Mycorrhizal Medicago Truncatula." *Plant Physiology* 175, no. 1: 392–411. https://doi.org/10.1104/pp.16.01509.

Ai, D., C. Dong, B. Yang, C. Yu, and G. Wang. 2022. "A Fructose Receptor Gene Influences Development and Feed Intake in Helicoverpa Armigera." *Insect Science* 29, no. 4: 993–1005. https://doi.org/10.1111/1744-7917.12984.

Amani Machiani, M., A. Javanmard, R. Habibi Machiani, and A. Sadeghpour. 2022. "Arbuscular Mycorrhizal Fungi and Changes in Primary and Secondary Metabolites." *Plants* 11, no. 17: 2183. https://doi.org/10.3390/plants11172183.

Amiri, R., A. Nikbakht, M. Rahimmalek, and H. Hosseini. 2017. "Variation in the Essential Oil Composition, Antioxidant Capacity, and Physiological Characteristics of Pelargonium Graveolens L. Inoculated With Two Species of Mycorrhizal Fungi Under Water Deficit Conditions." *Journal of Plant Growth Regulation* 36, no. 2: 502–515. https://doi.org/10.1007/s00344-016-9659-1.

Anjum, S. A., U. Ashraf, M. Tanveer, et al. 2017. "Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids." *Frontiers in Plant Science* 8: 69. https://doi.org/10.3389/fpls.2017.00069.

Bagheri, V., M. H. Shamshiri, H. Alaei, and H. Salehi. 2019. "The Role of Inoculum Identity for Growth, Photosynthesis, and Chlorophyll Fluorescence of Zinnia Plants by Arbuscular Mycorrhizal Fungi Under Varying Water Regimes." *Photosynthetica* 57, no. 2: 409–419. https://doi.org/10.32615/ps.2019.048.

Bagheri, V., M. H. Shamshiri, H. Shirani, and H. R. Roosta. 2012. "Nutrient Uptake and Distribution in Mycorrhizal Pistachio Seedlings Under Drought Stress." *Journal of Agricultural Sciences and Technology* 14: 1591–1604.

Bahadur, A., A. Batool, F. Nasir, et al. 2019. "Mechanistic Insights into Arbuscular Mycorrhizal Fungi-Mediated Drought Stress Tolerance in Plants." *International Journal of Molecular Sciences* 20, no. 17: 4199. https://doi.org/10.3390/ijms20174199.

Balestrini, R., C. Brunetti, W. Chitarra, and L. Nerva. 2020. "Photosynthetic Traits and Nitrogen Uptake in Crops: Which Is the Role of Arbuscular Mycorrhizal Fungi?" *Plants* 9, no. 9: 1105. https://doi.org/10.3390/plants9091105.

Barzen-Hanson, K. A., R. A. Wilkes, and L. Aristilde. 2018. "Quantitation of Carbohydrate Monomers and Dimers by Liquid Chromatography Coupled With High-Resolution Mass Spectrometry." *Carbohydrate Research* 468: 30–35. https://doi.org/10.1016/j.carres.2018.08.007.

Begum, N., M. A. Ahanger, Y. Su, et al. 2019. "Improved Drought Tolerance by AMF Inoculation in Maize (Zea Mays) Involves Physiological and Biochemical Implications." *Plants* 8, no. 12: 579. https://doi.org/10.3390/plants8120579.

Benaffari, W., F.-E. Soussani, A. Boutasknit, et al. 2024. "Arbuscular Mycorrhizal Fungi Improve Drought Tolerance of Quinoa Grown in Compost-Amended Soils by Altering Primary and Secondary Metabolite Levels." *Phyton* 93, no. 9: 2285–2302. https://doi.org/10.32604/phyton.2024.055052.

Bhupenchandra, I., S. K. Chongtham, A. G. Devi, et al. 2025. "Unlocking the Potential of Arbuscular Mycorrhizal Fungi: Exploring Role in Plant Growth Promotion, Nutrient Uptake Mechanisms, Biotic Stress Alleviation, and Sustaining Agricultural Production Systems." *Journal of Plant Growth Regulation* 44, no. 12: 6802–6840. https://doi.org/10.1007/s00344-024-11467-9.

Blein-Nicolas, M., S. S. Negro, T. Balliau, et al. 2020. "A Systems Genetics Approach Reveals Environment-Dependent Associations Between Snps, Protein Coexpression, and Drought-Related Traits in Maize." *Genome Research* 30, no. 11: 1593–1604. https://doi.org/10.1101/gr.255224.119.

Bodenhausen, N., G. Deslandes-Hérold, J. Waelchli, A. Held, M. G. A. van der Heijden, and K. Schlaeppi. 2021. "Relative qPCR to Quantify Colonization of Plant Roots by Arbuscular Mycorrhizal Fungi." *Mycorrhiza* 31, no. 2: 137–148. https://doi.org/10.1007/s00572-020-01014-1.

Carvajal Acosta, A. N., A. A. Agrawal, and K. Mooney. 2023. "Plant Water-Use Strategies as Mediators of Herbivore Drought Response: Ecophysiology, Host Plant Quality and Functional Traits." *Journal of Ecology* 111, no. 3: 687–700. https://doi.org/10.1111/1365-2745.14059.

Chandrasekaran, M., and M. Paramasivan. 2022. "Arbuscular Mycorrhizal Fungi and Antioxidant Enzymes in Ameliorating Drought Stress: A Meta-Analysis." *Plant and Soil* 480, no. 1: 295–303. https://doi.org/10.1007/s11104-022-05582-3.

Chávez-Arias, C. C., G. A. Ligarreto-Moreno, A. Ramírez-Godoy, and H. Restrepo-Díaz. 2021. "Maize Responses Challenged by Drought, Elevated Daytime Temperature and Arthropod Herbivory Stresses: A Physiological, Biochemical and Molecular View." *Frontiers in Plant Science* 12: 702841. https://doi.org/10.3389/fpls.2021.702841.

Chen, Y., C. Sun, Y. Yan, D. Jiang, S. Huangfu, and L. Tian. 2025. "Impact of Arbuscular Mycorrhizal Fungi on Maize Rhizosphere Microbiome Stability Under Moderate Drought Conditions." *Microbiological Research* 290: 127957. https://doi.org/10.1016/j.micres.2024.127957.

Daryanto, S., L. Wang, and P.-A. Jacinthe. 2017. "Global Synthesis of Drought Effects on Cereal, Legume, Tuber and Root Crops Production: A Review." *Agricultural Water Management* 179: 18–33. https://doi.org/10.1016/j.agwat.2016.04.022.

Deribe, H. 2025. "Review on Effects of Drought Stress on Maize Growth, Yield and Its Management Strategies." *Communications in Soil Science and Plant Analysis* 56, no. 1: 123–143. https://doi.org/10.1080/00103624.2024.2404663.

Dowarah, B., S. S. Gill, and N. Agarwala. 2022. "Arbuscular Mycorrhizal Fungi in Conferring Tolerance to Biotic Stresses in Plants." *Journal of Plant Growth Regulation* 41, no. 4: 1429–1444. https://doi.org/10.1007/s00344-021-10392-5.

Du, E., Y. Chen, Y. Li, et al. 2022. "Effect of Arbuscular Mycorrhizal Fungi on the Responses of Ageratina Adenophora to Aphis Gossypii Herbivory." *Frontiers in Plant Science* 13: 1015947. https://doi.org/10.3389/fpls.2022.1015947.

Du, Y., Q. Zhao, L. Chen, et al. 2020. "Effect of Drought Stress on Sugar Metabolism in Leaves and Roots of Soybean Seedlings." *Plant Physiology and Biochemistry* 146: 1–12. https://doi.org/10.1016/j.plaphy.2019.11.003.

Duell, E. B., K. A. Baum, and G. W. T. Wilson. 2024. "Drought Reduces Productivity and Anti-Herbivore Defences, but Not Mycorrhizal Associations, of Perennial Prairie Forbs." *Plant Biology* 26, no. 2: 204–213. https://doi.org/10.1111/plb.13604.

Eftekhari, F., M. Sarcheshmehpour, A. Lohrasbi-Nejad, and N. Boroomand. 2025. "Effects of Mycorrhizal and Trichoderma Treatment on Enhancing Maize Tolerance to Salinity and Drought Stress, Through Metabolic and Enzymatic Evaluation." *BMC Plant Biology* 25, no. 1: 687. https://doi.org/10.1186/s12870-025-06729-x.

Erice, G., C. Cano, A. Bago, J. M. Ruíz-Lozano, and R. Aroca. 2024. "Contrasting Regulation of Phaseolus Vulgaris Root Hydraulic Properties Under Drought and Saline Conditions by Three Arbuscular Mycorrhizal Fungal Species From Soils With Divergent Moisture Regime." *Journal of Soil Science and Plant Nutrition* 24, no. 2: 2934–2945. https://doi.org/10.1007/s42729-024-01719-8.

Farooq, A., N. Farooq, H. Akbar, Z. U. Hassan, and S. H. Gheewala. 2023. "A Critical Review of Climate Change Impact at a Global Scale on Cereal Crop Production." *Agronomy* 13, no. 1: 162. https://doi.org/10.3390/agronomy13010162.

Formenti, L., and S. Rasmann. 2019. "Mycorrhizal Fungi Enhance Resistance to Herbivores in Tomato Plants With Reduced Jasmonic Acid Production." *Agronomy* 9, no. 3: 131. https://doi.org/10.3390/agronomy9030131.

Ge, L., S. Sun, A. Chen, Y. Kapulnik, and G. Xu. 2008. "Tomato Sugar Transporter Genes Associated With Mycorrhiza and Phosphate." *Plant Growth Regulation* 55, no. 2: 115–123. https://doi.org/10.1007/s10725-008-9266-7.

Gfeller, V., J. Waelchli, S. Pfister, et al. 2023. "Plant Secondary Metabolite-Dependent Plant-Soil Feedbacks Can Improve Crop Yield in the Field." *eLife* 12: e84988. https://doi.org/10.7554/eLife.84988.

Glauser, G., A. Vallat, and D. Balmer. 2014. "Hormone Profiling." In *Arabidopsis Protocols*, edited by J. J. Sanchez-Serrano and J. Salinas, 597–608. Humana Press. https://doi.org/10.1007/978-1-62703-580-4_31.

Gupta, A., A. Rico-Medina, and A. I. Caño-Delgado. 2020. "The Physiology of Plant Responses to Drought." *Science* 368, no. 6488: 266–269. https://doi.org/10.1126/science.aaz7614.

Gurrieri, L., M. Merico, P. Trost, G. Forlani, and F. Sparla. 2020. "Impact of Drought on Soluble Sugars and Free Proline Content in Selected Arabidopsis Mutants." *Biology* 9, no. 11: 367. https://doi.org/10.3390/biology9110367.

Guyer, A., B. E. Hibbard, A. Holzkämper, M. Erb, and C. A. M. Robert. 2018. "Influence of Drought on Plant Performance Through Changes in Belowground Tritrophic Interactions." *Ecology and Evolution* 8, no. 13: 6756–6765. https://doi.org/10.1002/ece3.4183.

Handrick, V., C. A. M. Robert, K. R. Ahern, et al. 2016. "Biosynthesis of 8-O-Methylated Benzoxazinoid Defense Compounds in Maize." *The Plant Cell* 28, no. 7: tpc.00065.2016. https://doi.org/10.1105/tpc.16.00065.

He, G.-X., F.-L. Zheng, Y.-N. Zou, X.-B. Gao, Q.-S. Wu, and C. Guo. 2025. "Mycorrhizal Regulation of Core ZmSWEET Genes Governs Sugar Accumulation in Maize." *Agriculture (London)* 15, no. 16: 1790. https://doi.org/10.3390/agriculture15161790.

He, L., C. Li, and R. Liu. 2017. "Indirect Interactions Between Arbuscular Mycorrhizal Fungi and Spodoptera Exigua Alter Photosynthesis and Plant Endogenous Hormones." *Mycorrhiza* 27, no. 6: 525–535. https://doi.org/10.1007/s00572-017-0771-2.

Hoagland, D. R., and D. I. Arnon. 1950. "The Water-Culture Method for Growing Plants Without Soil." *California, Agricultural Experiment Station, Circular* 347: 32.

Hu, L., C. A. M. Robert, S. Cadot, et al. 2018. "Root Exudate Metabolites Drive Plant-Soil Feedbacks on Growth and Defense by Shaping the Rhizosphere Microbiota." *Nature Communications* 9, no. 1: 2738. https://doi.org/10.1038/s41467-018-05122-7.

Intergovernmental Panel on Climate Change (IPCC). 2023. *Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. https://doi.org/10.1017/9781009325844.

Kakabouki, I., P. Stavropoulos, I. Roussis, A. Mavroeidis, and D. Bilalis. 2023. "Contribution of Arbuscular Mycorrhizal Fungi (AMF) in Improving the Growth and Yield Performances of Flax (Linum Usitatissimum L.) to Salinity Stress." *Agronomy* 13, no. 9: 2416. https://doi.org/10.3390/agronomy13092416.

Khan, W., Y. Zhu, A. Khan, et al. 2024. "Above-And Below-Ground Feedback Loop of Maize Is Jointly Enhanced by Plant Growth-Promoting Rhizobacteria and Arbuscular Mycorrhizal Fungi in Drier Soil." *Science of the Total Environment* 917: 170417. https://doi.org/10.1016/j.scitotenv.2024.170417.

Kheyruri, Y., S. B. H. S. Asadollah, A. Sharafati, A. Rahman, and S. H. Mohajeri. 2024. "Examining the Effects of Meteorological Drought Variability on Rainfed Cultivation Yields in Iran." *Theoretical and Applied Climatology* 155, no. 7: 6427–6444. https://doi.org/10.1007/s00704-024-05013-6.

Kim, T.-H., M. Böhmer, H. Hu, N. Nishimura, and J. I. Schroeder. 2010. "Guard Cell Signal Transduction Network: Advances in Understanding Abscisic Acid, CO2, and Ca2+ Signaling." *Annual review of plant biology* 61(2010: 561–591. https://doi.org/10.1146/annurev-arplant-042809-112226.

Köhl, L., C. E. Lukasiewicz, and M. G. A. van der Heijden. 2016. "Establishment and Effectiveness of Inoculated Arbuscular Mycorrhizal Fungi in Agricultural Soils." *Plant, Cell & Environment* 39, no. 1: 136–146. https://doi.org/10.1111/pce.12600.

Kumar, R., R. K. Meena, Y. Tak, and P. K. Chachaiya. 2024. "Fungi Boost Up Plant Growth by Improving Nutrient Uptake: A Review." *Environment and Ecology* 42, no. 3B: 1364–1368. https://doi.org/10.60151/envec/OVGG9937.

Liao, Z., B. Chen, H. Boubakri, et al. 2025. "The Regulatory Role of Phytohormones in Plant Drought Tolerance." *Planta* 261, no. 5: 98. https://doi.org/10.1007/s00425-025-04671-8.

Liu, L., Y. Xiang, J. Yan, et al. 2021. "Brassinosteroid-Signaling Kinase 1 Phosphorylating Calcium/Calmodulin-Dependent Protein Kinase Functions in Drought Tolerance in Maize." *New Phytologist* 231, no. 2: 695–712. https://doi.org/10.1111/nph.17403.

Liu, Y., J. Jian, L. Xu, et al. 2025. "Arbuscular Mycorrhizal Fungi Change the Growth and Metabolites of Perilla Frutescens, With Subsequent Effects on the Development and Behavior of Spodoptera Exigua." *Journal of Agricultural and Food Chemistry* 73, no. 29: 18186–18197. https://doi.org/10.1021/acs.jafc.5c03530.

Lutz, S., N. Bodenhausen, J. Hess, et al. 2023. "Soil Microbiome Indicators Can Predict Crop Growth Response to Large-Scale Inoculation With Arbuscular Mycorrhizal Fungi." *Nature Microbiology* 8, no. 12: 2277–2289. https://doi.org/10.1038/s41564-023-01520-w.

Macías, F. A., D. Marín, A. Oliveros-Bastidas, D. Chinchilla, A. M. Simonet, and J. M. G. Molinillo. 2006. "Isolation and Synthesis of Allelochemicals From Gramineae: Benzoxazinones and Related Compounds." *Journal of Agricultural and Food Chemistry* 54, no. 4: 991–1000. https://doi.org/10.1021/jf050896x.

Martin, F. M., and M. G. A. van der Heijden. 2024. "The Mycorrhizal Symbiosis: Research Frontiers in Genomics, Ecology, and Agricultural Application." *New Phytologist* 242, no. 4: 1486–1506. https://doi.org/10.1111/nph.19541.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. "A New Method Which Gives an Objective Measure of Colonization of Roots by Vesicular—Arbuscular Mycorrhizal Fungi." *New Phytologist* 115, no. 3: 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x.

Meier, A. R., and M. D. Hunter. 2018. "Mycorrhizae Alter Toxin Sequestration and Performance of Two Specialist Herbivores." *Frontiers in Ecology and Evolution* 6: fevo.2018.00033. https://doi.org/10.3389/fevo.2018.00033.

Mo, Y., Y. Wang, R. Yang, et al. 2016. "Regulation of Plant Growth, Photosynthesis, Antioxidation and Osmosis by an Arbuscular Mycorrhizal Fungus in Watermelon Seedlings Under Well-Watered and Drought Conditions." *Frontiers in Plant Science* 7: fpls.2016.00644. https://doi.org/10.3389/fpls.2016.00644.

Mohammadi, R. 2024. "Effects of Post-Flowering Drought and Supplemental Irrigation on Grain Yield and Agro-Phenological Traits in Durum Wheat." *European Journal of Agronomy* 156: 127180. https://doi.org/10.1016/j.eja.2024.127180.

Mohammadkhani, N., and R. Heidari. 2008. "Drought-Induced Accumulation of Soluble Sugars and Proline in Two Maize Varieties." *World Applied Sciences Journal* 3, no. 3: 448–453.

Muhammad Aslam, M., M. Waseem, B. H. Jakada, et al. 2022. "Mechanisms of Abscisic Acid-Mediated Drought Stress Responses in Plants." *International Journal of Molecular Sciences* 23, no. 3: 1084. https://doi.org/10.3390/ijms23031084.

Nguyen, D., I. Rieu, C. Mariani, and N. M. van Dam. 2016. "How Plants Handle Multiple Stresses: Hormonal Interactions Underlying Responses to Abiotic Stress and Insect Herbivory." *Plant Molecular Biology* 91: 727–740. https://doi.org/10.1007/s11103-016-0481-8.

Orine, D., E. Defossez, F. Vergara, H. Uthe, N. M. van Dam, and S. Rasmann. 2022. "Arbuscular Mycorrhizal Fungi Prevent the Negative Effect of Drought and Modulate the Growth-Defence Trade-Off in Tomato Plants." *Journal of Sustainable Agriculture and Environment* 1, no. 3: 177–190. https://doi.org/10.1002/sae2.12018.

Orine, D., H. Saha, G. Glauser, A. Biere, and S. Rasmann. 2025. "Microbial Interactions Influence the Chemical Defense of Wild and Cultivated Tomato Species." *Journal of Chemical Ecology* 51, no. 2: 47. https://doi.org/10.1007/s10886-025-01598-y.

Qiao, M., C. Hong, Yw Jiao, S. Hou, and H. Gao. 2024. "Impacts of Drought on Photosynthesis in Major Food Crops and the Related Mechanisms of Plant Responses to Drought." *Plants* 13, no. 13: 1808. https://doi.org/10.3390/plants13131808.

Quiroga, G., G. Erice, R. Aroca, F. Chaumont, and J. M. Ruiz-Lozano. 2017. "Enhanced Drought Stress Tolerance by the Arbuscular Mycorrhizal Symbiosis in a Drought-Sensitive Maize Cultivar Is Related to a Broader and Differential Regulation of Host Plant Aquaporins Than in a Drought-Tolerant Cultivar." *Frontiers in Plant Science* 8: 1056. https://doi.org/10.3389/fpls.2017.01056.

Ran, Q., S. Zhang, M. Arif, X. Yin, S. Chen, and G. Ren. 2024. "Effects of Arbuscular Mycorrhizal Fungi on Carbon Assimilation and Ecological Stoichiometry of Maize Under Combined Abiotic Stresses." *Journal of Plant Ecology* 17, no. 2: rtae010. https://doi.org/10.1093/jpe/rtae010.

Rashidi, S., A. R. Yousefi, and A. Mastinu. 2024. "Mycorrhizal Symbiosis Can Change the Composition of Secondary Metabolites in Fruits of Solanum Nigrum L." *Chemistry & Biodiversity* 21, no. 7: e202400208. https://doi.org/10.1002/cbdv.202400208.

Rezaei, E. E., H. Webber, S. Asseng, et al. 2023. "Climate Change Impacts on Crop Yields." *Nature Reviews Earth & Environment* 4, no. 12: 831–846. https://doi.org/10.1038/s43017-023-00491-0.

Rivero, J., J. Lidoy, Á. Llopis-Giménez, S. Herrero, V. Flors, and M. J. Pozo. 2021. "Mycorrhizal Symbiosis Primes the Accumulation of Antiherbivore Compounds and Enhances Herbivore Mortality in Tomato." *Journal of Experimental Botany* 72, no. 13: 5038–5050. https://doi.org/10.1093/jxb/erab171.

Robert, C. A. M., and P. Mateo. 2022. "The Chemical Ecology of Benzoxazinoids." *Chimia* 76, no. 11: 928–938. https://doi.org/10.2533/chimia.2022.928.

Romero-Munar, A., M. Muñoz-Carrasco, R. Balestrini, et al. 2024. "Differential Root and Cell Regulation of Maize Aquaporins by the Arbuscular Mycorrhizal Symbiosis Highlights Its Role in Plant Water Relations." *Plant, Cell & Environment* 47, no. 11: 4337–4353. https://doi.org/10.1111/pce.15029.

Rosenzweig, C., J. Elliott, D. Deryng, et al. 2014. "Assessing Agricultural Risks of Climate Change in the 21st Century in a Global Gridded Crop Model Intercomparison." *Proceedings of the National Academy of Sciences* 111, no. 9: 3268–3273. https://doi.org/10.1073/pnas.1222463110.

Ruan, Y.-L. 2014. "Sucrose Metabolism: Gateway to Diverse Carbon Use and Sugar Signaling." *Annual Review of Plant Biology* 65, no. 2014: 33–67. https://doi.org/10.1146/annurev-arplant-050213-040251.

Salmeron-Santiago, I. A., M. Martínez-Trujillo, J. J. Valdez-Alarcón, et al. 2022. "An Updated Review on the Modulation of Carbon Partitioning and Allocation in Arbuscular Mycorrhizal Plants." *Microorganisms* 10, no. 1: 75. https://doi.org/10.3390/microorganisms10010075.

Sepulveda, L., E. Ponce, R. Pedreschi, and I. F. Cuneo (2022). Osmotic Adjustment and Cell Wall Composition Allow Maize Fine Roots to Maintain Functionality and Growth During Drought. http://ouci.dntb.gov.ua/en/works/4aa2d6g4/.

Shafi, I., M. Gautam, and R. Kariyat. 2024. "Integrating Ecophysiology and Omics to Unlock Crop Response to Drought and Herbivory Stress." *Frontiers in Plant Science* 15: fpls.2024.1500773. https://doi.org/10.3389/fpls.2024.1500773.

Sharma, A., B. Shahzad, V. Kumar, et al. 2019. "Phytohormones Regulate Accumulation of Osmolytes Under Abiotic Stress." *Biomolecules* 9, no. 7: 285. https://doi.org/10.3390/biom9070285.

Sheoran, S., Y. Kaur, S. Kumar, S. Shukla, S. Rakshit, and R. Kumar. 2022. "Recent Advances for Drought Stress Tolerance in Maize (Zea Mays L.): Present Status and Future Prospects." *Frontiers in Plant Science* 13: fpls.2022.872566. https://doi.org/10.3389/fpls.2022.872566.

Song, Y. Y., M. Cao, L. J. Xie, et al. 2011. "Induction of Dimboa Accumulation and Systemic Defense Responses as a Mechanism of Enhanced Resistance of Mycorrhizal Corn (Zea Mays L.) to Sheath Blight." *Mycorrhiza* 21, no. 8: 721–731. https://doi.org/10.1007/s00572-011-0380-4.

Stratton, C. A., S. Ray, B. A. Bradley, J. P. Kaye, J. G. Ali, and E. G. Murrell. 2022. "Nutrition Vs Association: Plant Defenses Are Altered by Arbuscular Mycorrhizal Fungi Association Not by Nutritional Provisioning Alone." *BMC Plant Biology* 22, no. 1: 400. https://doi.org/10.1186/s12870-022-03795-3.

Sudhakar, P., P. Latha, and P. V. Reddy. 2016. *Phenotyping Crop Plants for Physiological and Biochemical Traits*. Academic Press.

Sutour, S., V. C. Doan, P. Mateo, et al. 2024. "Isolation and Structure Determination of Drought-Induced Multihexose Benzoxazinoids From Maize (Zea Mays)." *Journal of Agricultural and Food Chemistry* 72, no. 7: 3427–3435. https://doi.org/10.1021/acs.jafc.3c09141.

Tang, H., M. U. Hassan, L. Feng, et al. 2022. "The Critical Role of Arbuscular Mycorrhizal Fungi to Improve Drought Tolerance and Nitrogen Use Efficiency in Crops." *Frontiers in Plant Science* 13: fpls.2022.919166. https://doi.org/10.3389/fpls.2022.919166.

Tao, L., A. Ahmad, J. C. de Roode, and M. D. Hunter. 2016. "Arbuscular Mycorrhizal Fungi Affect Plant Tolerance and Chemical Defences to Herbivory Through Different Mechanisms." *Journal of Ecology* 104, no. 2: 561–571. https://doi.org/10.1111/1365-2745.12535.

Thoenen, L., C. Giroud, M. Kreuzer, et al. 2023. "Bacterial Tolerance to Host-Exuded Specialized Metabolites Structures the Maize Root Microbiome." *Proceedings of the National Academy of Sciences* 120, no. 44: e2310134120. https://doi.org/10.1073/pnas.2310134120.

Tiepo, A. N., M. H. Fávaro, T. S. Amador, et al. 2024. "Associative Bacteria and Arbuscular Mycorrhizal Fungus Increase Drought Tolerance in Maize (Zea Mays L.) Through Morphoanatomical, Physiological, and Biochemical Changes." *Plants* 13, no. 12: 1667. https://doi.org/10.3390/plants13121667.

Vierheilig, H., A. P. Coughlan, U. Wyss, and Y. Piché. 1998. "Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi." *Applied and Environmental Microbiology* 64, no. 12: 5004–5007. https://doi.org/10.1128/AEM.64.12.5004-5007.1998.

Vinocur, B., and A. Altman. 2005. "Recent Advances in Engineering Plant Tolerance to Abiotic Stress: Achievements and Limitations." *Current Opinion in Biotechnology* 16, no. 2: 123–132. https://doi.org/10.1016/j.copbio.2005.02.001.

de Vries, J., J. B. Evers, T. W. Kuyper, J. van Ruijven, and L. Mommer. 2021. "Mycorrhizal Associations Change Root Functionality: A 3D Modelling Study on Competitive Interactions Between Plants for Light and Nutrients." *New Phytologist* 231, no. 3: 1171–1182. https://doi.org/10.1111/nph.17435.

Wang, Q., M. Liu, Z. Wang, J. Li, K. Liu, and D. Huang. 2024. "The Role of Arbuscular Mycorrhizal Symbiosis in Plant Abiotic Stress." *Frontiers in Microbiology* 14: fmicb.2023.1323881. https://doi.org/10.3389/fmicb.2023.1323881.

Wouters, F. C., B. Blanchette, J. Gershenzon, and D. G. Vassão. 2016. "Plant Defense and Herbivore Counter-Defense: Benzoxazinoids and Insect Herbivores." *Phytochemistry Reviews* 15, no. 6: 1127–1151. https://doi.org/10.1007/s11101-016-9481-1.

Ximénez-Embún, M. G., J. J. Glas, F. Ortego, J. M. Alba, P. Castañera, and M. R. Kant. 2017. "Drought Stress Promotes the Colonization Success of a Herbivorous Mite That Manipulates Plant Defenses." *Experimental and Applied Acarology* 73, no. 3: 297–315. https://doi.org/10.1007/s10493-017-0200-4.

Yang, H., Y. Dai, X. Wang, Q. Zhang, L. Zhu, and X. Bian. 2014. "Meta-Analysis of Interactions Between Arbuscular Mycorrhizal Fungi and Biotic Stressors of Plants." *The Scientific World Journal* 2014, no. 1: 746506. https://doi.org/10.1155/2014/746506.

Yang, J., and P. D. Rainville. 2019. Quantification of Mono and Disaccharides in Foods. Waters Application Note. https://www.waters.com/nextgen/us/en/library/application-notes/2019/quantification-mono-disaccharides-in-foods.html.

Yang, X., Y. Ma, J. Zhang, H. Bai, and Y. Shen. 2023. "How Arbuscular Mycorrhizal Fungi Drives Herbaceous Plants' C: N: P Stoichiometry? A Meta-Analysis." *Science of the Total Environment* 862: 160807. https://doi.org/10.1016/j.scitotenv.2022.160807.

Yuan, X., S. Li, J. Chen, et al. 2024. "Impacts of Global Climate Change on Agricultural Production: A Comprehensive Review." *Agronomy* 14, no. 7: 1360. https://doi.org/10.3390/agronomy14071360.

Zaman, F., M. U. Hassan, W. A. Khattak, A. Ali, M. F. Awad, and F.-S. Chen. 2024. "The Pivotal Role of Arbuscular Mycorrhizal Fungi in Enhancing Plant Biomass and Nutrient Availability Under Drought Stress Conditions: A Global Meta-Analysis." *Science of the Total Environment* 955: 176960. https://doi.org/10.1016/j.scitotenv.2024.176960.

Zhang, Q., H. Liu, X. Wu, and W. Wang. 2020. "Identification of Drought Tolerant Mechanisms in a Drought-Tolerant Maize Mutant Based on Physiological, Biochemical and Transcriptomic Analyses." *BMC Plant Biology* 20: 315. https://doi.org/10.1186/s12870-020-02526-w.

Zhang, S.-S., P.-C. Wang, C. Ning, et al. 2023. "Sucrose Taste Receptors Differ in Larval and Adult Stages of a Moth." *eLife* 12: eLife.91711.1. https://doi.org/10.7554/eLife.91711.1.

Zhu, B., F. Liu, X. Li, et al. 2015. "Fast Quantification of Endogenous Carbohydrates in Plasma Using Hydrophilic Interaction Liquid

Chromatography Coupled With Tandem Mass Spectrometry." *Journal of Separation Science* 38, no. 1: 34–41. https://doi.org/10.1002/jssc.201400899.

Zou, Y.-N., Q.-S. Wu, and K. Kuča. 2021. "Unravelling the Role of Arbuscular Mycorrhizal Fungi in Mitigating the Oxidative Burst of Plants Under Drought Stress." *Plant Biology* 23, no. S1: 50–57. https://doi.org/10.1111/plb.13161.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Figure 1: Maize height and leaf chlorophyll contents in the field. **Supporting Figure 2:** Maize root biomass in the field at day 120. **Supporting Figure 3:** Herbivore damage development over time in the field. **Supporting Figure 4:** Drought effects on sucrose and of AMF on SA and HDMBOA-Glc in maize leaves in the field at day 120. **Supporting Figure 5:** Drought and AMF effects on DIMBOA-Glc in maize leaves in the field at day 60. **Supporting Figure 6:** AMF colonization correlated with sucrose and ABA levels in maize leaves in the field at day 120. **Supporting Figure 7:** AMF colonization correlated with DIM_2BOA-Glc levels in maize leaves in the field at day 60. **Supporting Figure 8:** AMF affected soluble sugar levels in maize roots in the field at day 120. **Supporting Figure 9:** AMF colonization had correlated with HM_2BOA-Glc and DIMBOA-2Glc levels in maize roots in the field. **Supporting Figure 10:** Structural equation modeling (SEM) model of the semi-field assays **Supporting Figure 11:** Drought reduced AMF colonization under controlled conditions in herbivory assay 2. **Supporting Figure 12:** Absolute larvae mass gain and leaf damage area are positively correlated in herbivory assays. **Supporting Figure 13:** Drought increase HMBOA-Glc and DIM_2BOA-Glc and decreased HDM_2BOA-Glc levels in maize leaves in herbivory assay 2. **Supporting Figure 14:** Drought increased HMBOA-Glc, HMBOA-2Glc, HM_2BOA-Glc, DIMBOA-2Glc, DIM_2BOA-Glc and MBOA levels in maize roots in herbivory assay 2. **Supporting Table 1:** Meteorological data during the semi-field assay. **Supporting Table 2:** Soil chemical profile. **Supporting Table 3:** Benzoxazinoid names and chemical formulas. Supporting File 1. Raw data of this study.