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Evaluación de la tolerancia de *Brassica oleracea* L. var. *italica* al estrés por microplásticos y factores abióticos mediante parámetros fisiológicos y perfiles de metabolitos.

Assessment of tolerance in *Brassica oleracea* L. var. *italica* to microplastic and abiotic stresses using physiological parameters and metabolite profiling.

Tesis para optar al grado de Doctor en Ciencias de la Agronomía

MARCELO MARIO ILLANES TAPIA
CHILLAN-CHILE
2025

Profesora Guía: María Dolores López B., PhD.
Dpto. de Producción Vegetal
Facultad de Agronomía
Universidad de Concepción

Profesora Co-Guía: Diego Ángel Moreno, PhD.
Grupo LabFAS.
Dpto. de Ciencia y Tecnología de Alimentos
CEBAS-CSIC
U.A. - Universidad Politécnica de Cartagena

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Profesora Guía

Dra. María Dolores López Belchí
Facultad de Agronomía
Universidad de Concepción

Profesor Guía Externo

Dr. Diego Ángel Moreno
CEBAS-CSIC
U.A. - Universidad Politécnica de Cartagena

Comisión Evaluadora:

Dr. Mauricio Schoebitz Cid
Facultad de Agronomía
Universidad de Concepción

Dr. Nelson Zapata San Martín
Facultad de Agronomía
Universidad de Concepción

Directora de Programa

Dr. Susana Fischer Ganzoni
Facultad de Agronomía
Universidad de Concepción

Marcelo Mario Illanes Tapia, estudiante del Programa Doctorado en Ciencias de la Agronomía, Facultad de Agronomía, Universidad de Concepción, declara ser autor del presente trabajo titulado "**Evaluación de la tolerancia de *Brassica oleracea* L. var. *italica* al estrés por microplásticos y factores abióticos mediante parámetros fisiológicos y perfiles de metabolitos.**"

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RESUMEN

Esta Tesis Doctoral abordó de manera integrada los efectos de los microplásticos (MPs) sobre la fisiología vegetal y la seguridad de los sistemas agroalimentarios, considerando su interacción con estreses abióticos asociados al cambio climático, tales como el aumento de CO_2 y las altas temperaturas. A partir de una revisión crítica y actualizada de la literatura científica y de un ensayo experimental controlado con brotes de *Brassica oleracea* L. var. *italica* (brócoli), se analizaron tanto los riesgos que los MPs representaban para la productividad y calidad de los cultivos, como los mecanismos fisiológicos, enzimáticos y metabolómicos que explicaban sus impactos en las plantas. En el capítulo II se realizó una revisión bibliográfica que evidenció que los microplásticos eran capaces de modificar parámetros esenciales de la fisiología vegetal, afectando la germinación, el desarrollo radicular, la fotosíntesis, la acumulación de metabolitos secundarios y la interacción con microorganismos del suelo. Además, actúaron como vectores de contaminantes inorgánicos y orgánicos persistentes, alterando la expresión génica y generando estrés oxidativo. Los MPs de origen biodegradable (como PLA o PBAT) provocaron respuestas más rápidas e intensas, mientras que los no degradables, como el poliestireno (PS) y el polietileno (PE), tendieron a modificar la estructura del suelo, reducir su porosidad, alterar la retención hídrica y afectar la dinámica microbiana y nutricional a largo plazo. En cultivos del género Brassica, la literatura mostraba una disminución del peso fresco, una reducción de pigmentos fotosintéticos y una acumulación compensatoria

de antocianinas y glucosinolatos, lo que reflejaba una reconfiguración del metabolismo secundario hacia rutas defensivas. A su vez, se observó que la presencia de MPs en el suelo incrementó las emisiones de CO_2 debido a la degradación de plásticos biodegradables y a la estimulación de la actividad microbiana heterótrofa, contribuyendo a la retroalimentación del cambio climático. Por lo tanto, los microplásticos no solo son contaminantes emergentes, sino moduladores ambientales que, dependiendo del tipo de polímero y las condiciones edafoclimáticas, pueden amplificar o mitigar las respuestas de estrés en las plantas. Ante este escenario, se propone avanzar en metodologías estandarizadas para la cuantificación de MPs en matrices agrícolas y en la aplicación de tecnologías ómicas y de aprendizaje automático que permitan identificar biomarcadores de exposición y desarrollar modelos predictivos de impacto fisiológico y ecológico.

El capítulo III de esta tesis se basó en un diseño factorial (2×2×3) que combinó dos niveles de CO_2 (≈500 y 1000 ppm), dos temperaturas (20 y 28 °C) y tres tipos de polímero (sin plástico, poliestireno y polietileno) en brotes de *Brassica oleracea* var. *italica* cultivados bajo condiciones controladas. Se evaluaron parámetros morfológicos, hormonales, enzimáticos y metabolómicos mediante técnicas cromatográficas y análisis multivariados, con el fin de identificar patrones de respuesta y compensaciones entre crecimiento y defensa. Los resultados mostraron que la temperatura fue el factor dominante, regulando las hormonas de crecimiento, la actividad antioxidante y el metabolismo secundario. A 20 °C se observó una mayor acumulación de glucosinolatos, especialmente glucorafanina e indol-3-ilmetilglucosinolato, indicando una activación de rutas defensivas de tipo basal. En contraste, a 28 °C no se registró incremento de estos compuestos, pero sí un aumento significativo de antocianinas y derivados sinápicos, junto con una mayor actividad de catalasa y menor biomasa, lo que refleja una reorientación metabólica hacia mecanismos antioxidantes y un claro trade-off entre crecimiento y defensa. El CO_2 elevado actuó como modulador secundario, favoreciendo la biomasa radicular y la síntesis de auxinas, aunque atenuó la acumulación de glucosinolatos. En tanto, el tipo de polímero mostró efectos específicos. El poliestireno promovió una mayor producción de compuestos fenólicos, mientras que el polietileno redujo la expansión radicular y foliar, con respuestas más marcadas en escenarios cálidos. Los análisis multivariados confirmaron la existencia de dos ejes principales de respuesta fisiológica. El primero, que explicó un 46 % de la varianza total, contrapuso metabolitos defensivos (glucosinolatos, antocianinas y sinapatos) frente a hormonas de crecimiento y biomasa, reforzando la hipótesis del equilibrio crecimiento–defensa. El segundo eje, responsable del 23 % de la varianza, separó los compuestos indólicos de los alifáticos, indicando una reorganización del metabolismo secundario en función de la temperatura y la interacción con los MNPs. Las correlaciones entre parámetros revelaron asociaciones positivas significativas entre la actividad catalasa y el contenido de sinapatos ($r = 0.78$) y antocianinas ($r = 0.74$), así como una correlación negativa con la biomasa aérea ($r = -0.69$), lo que confirma el acoplamiento entre defensa antioxidante y producción de metabolitos fenólicos. Complementariamente, los modelos predictivos desarrollados mediante redes neuronales artificiales (ANN)

permitieron estimar con alta precisión ($R^2 > 0.9$; $RMSE < 0.05$) las concentraciones de glucosinolatos y hormonas de crecimiento a partir de las condiciones ambientales y del tipo de polímero, demostrando la aplicabilidad del aprendizaje automático en la predicción de respuestas bioquímicas bajo escenarios multiestrés. Lo que observamos en este estudio fue que los micro/nanoplásticos actuaron como moduladores, capaces de amplificar o reconfigurar las respuestas fisiológicas inducidas por la temperatura y el CO_2, más que como agentes tóxicos directos.

Estos resultados resaltan la importancia de adoptar enfoques multiestrés y de integrar herramientas de metabolómica y modelamiento predictivo para comprender cómo los contaminantes emergentes afectan la resiliencia y la calidad de los cultivos en un contexto de cambio climático. Desde una perspectiva aplicada, esta tesis aporta un marco experimental y conceptual para el estudio de contaminantes emergentes en agroecosistemas, incorporando herramientas de metabolómica avanzada y modelamiento predictivo como apoyo a la toma de decisiones en agricultura sostenible.

SUMMARY

This doctoral thesis addressed, in an integrated manner, the effects of microplastics (MPs) on plant physiology and the safety of agri-food systems, considering their interaction with abiotic stresses associated with climate change, such as increased CO_2 and high temperatures. Based on a critical and updated review of the scientific literature and a controlled experimental trial using *Brassica oleracea* L. var*. italica* (broccoli) sprouts, both the risks that

MPs pose to crop productivity and quality and the physiological, enzymatic, and metabolomic mechanisms underlying their impacts on plants were analyzed. Chapter II presents a literature review showing that microplastics can alter key parameters of plant physiology, affecting germination, root development, photosynthesis, the accumulation of secondary metabolites, and interactions with soil microorganisms. Furthermore, they act as vectors of persistent inorganic and organic contaminants, modifying gene expression and inducing oxidative stress. Biodegradable MPs (such as PLA or PBAT) triggered faster and more intense responses, whereas non-degradable MPs, such as polystyrene (PS) and polyethylene (PE), tended to modify soil structure, reduce porosity, alter water retention, and affect microbial and nutrient dynamics in the long term. In Brassica crops, the literature reports reductions in fresh weight, decreases in photosynthetic pigments, and compensatory accumulations of anthocyanins and glucosinolates, reflecting a reconfiguration of secondary metabolism toward defensive pathways. Moreover, the presence of MPs in soil was found to increase CO_2 emissions due to the degradation of biodegradable plastics and the stimulation of heterotrophic microbial activity, contributing to climate change feedback. Therefore, microplastics should be viewed not only as emerging pollutants but also as environmental modulators that, depending on polymer type and edaphoclimatic conditions, can amplify or mitigate plant stress responses. In light of this, the thesis emphasizes the need to advance standardized methodologies for MP quantification in agricultural matrices and to apply omics

and machine learning technologies to identify exposure biomarkers and develop predictive models of physiological and ecological impact.

Chapter III was based on a factorial design (2×2×3) that combined two levels of CO_2 (≈500 and 1000 ppm), two temperatures (20 and 28 °C), and three polymer conditions (no plastic, PS, and PE) applied to *Brassica oleracea* var. italica sprouts grown under controlled conditions. Morphological, hormonal, enzymatic, and metabolomic parameters were evaluated through chromatographic techniques and multivariate analyses to identify response patterns and trade-offs between growth and defense. The results showed that temperature was the dominant factor, regulating growth hormones, antioxidant activity, and secondary metabolism. At 20 °C, there was a greater accumulation of glucosinolates, particularly glucoraphanin and indol-3-ylmethyl glucosinolate, indicating activation of basal defensive pathways. In contrast, at 28 °C, glucosinolate levels did not increase; instead, there was a significant rise in anthocyanins and sinapate derivatives, together with higher catalase activity and lower biomass, revealing a metabolic shift toward antioxidant mechanisms and a clear growth–defense trade-off. Elevated CO_2 acted as a secondary modulator, enhancing root biomass and auxin synthesis but attenuating glucosinolate accumulation. The type of polymer produced specific effects: PS stimulated the synthesis of phenolic compounds, while PE reduced root and leaf expansion, with more pronounced effects under heat stress. Multivariate analyses confirmed the existence of two main physiological axes: the first (46 % of total variance) opposed defensive metabolites (glucosinolates, anthocyanins, sinapates) to growth hormones and biomass, reinforcing the growth–defence balance hypothesis; the second (23 %) separated indolic from aliphatic glucosinolates, indicating a reorganization of secondary metabolism as a function of temperature and MP interaction. Correlation analyses revealed strong positive associations between catalase activity and sinapate ($r = 0.78$) and anthocyanin content ($r = 0.74$), and a negative correlation with aerial biomass ($r = -0.69$), confirming the link between antioxidant defense and phenolic metabolism. Additionally, predictive models developed using artificial neural networks (ANNs) accurately estimated glucosinolate and hormone concentrations ($R^2 > 0.9$; $RMSE < 0.05$) based on environmental conditions and polymer type, demonstrating the potential of machine learning to predict biochemical responses under multi-stress scenarios.

In general, the study showed that micro/nanoplastics act as context-dependent modulators, capable of amplifying or reconfiguring physiological responses induced by temperature and CO_2 rather than behaving as direct toxic agents. These findings highlight the importance of adopting multi-stress approaches and integrating metabolomic and predictive modeling tools to better understand how emerging contaminants affect crop resilience and quality in the context of climate change. From an applied perspective, this thesis provides an experimental and conceptual framework for studying emerging pollutants in agroecosystems, integrating advanced metabolomic analyses and predictive modeling as valuable tools for decision-making in sustainable agriculture

CAPÍTULO I

INTRODUCCIÓN Y OBJETIVOS

1. INTRODUCCIÓN

Los sistemas agroalimentarios enfrentan la concurrencia de estreses ambientales, temperatura elevada, salinidad, sequía y variaciones de CO_2, junto con contaminantes emergentes como las partículas plásticas, cuya presencia creciente se ha documentado como moduladora de la fisiología vegetal y potencial riesgo para la calidad e inocuidad de los alimentos. La literatura reciente indica que la interacción entre microplásticos (MPs) y condiciones ambientales, por ejemplo CO_2 elevado y altas temperaturas, puede modificar procesos centrales de la planta (p. ej., conductancia estomática) e inducir estados de estrés oxidativo; además, los MPs facilitan la adsorción de metales traza en tejidos comestibles, con implicancias para la seguridad alimentaria. De forma paralela, enfoques ómicos (genómica, transcriptómica, metabolómica) y herramientas de aprendizaje automático permiten identificar biomarcadores de exposición y analizar bases de datos complejas para anticipar impactos en salud vegetal y rendimiento.

La relevancia de este trabajo radica en que la co-ocurrencia de estreses abióticos y contaminantes emergentes permanece subestudiada en comparación con la respuesta a estreses individuales. Comprender dicha interacción es estratégico por su actualidad, la prevalencia de MPs en matrices agrícolas, su potencial de afectar procesos del suelo y de la planta, y sus posibles aplicaciones en manejo agronómico, trazabilidad e inocuidad, así como en el diseño de políticas públicas. En particular, el aumento de CO_2 atmosférico altera procesos biológicos en plantas, nutrientes del suelo y comunidades microbianas, y su interacción con MPs puede incidir tanto en la fisiología vegetal como en los flujos de carbono del suelo hacia la atmósfera, lo que justifica enfoques integrados para evaluar riesgos y orientar medidas de mitigación.

El problema que aborda esta tesis es la escasez de evidencia integradora sobre cómo los MPs, en interacción con CO_2 elevado y temperatura, reconfiguran rasgos fisiológicos y bioquímicos en cultivos de interés alimentario. El objetivo general fue integrar el conocimiento existente y evaluar de manera experimental las respuestas de brotes de Brassica bajo combinaciones controladas de CO_2, temperatura y material plástico, caracterizando hormonas, metabolitos especializados y marcadores antioxidantes en un marco reproducible y analíticamente integrador. La naturaleza del estudio combina una revisión crítica del estado del arte con un experimento factorial en condiciones controladas; esta aproximación mixta permite, por un lado, sintetizar evidencia sobre vías de ingreso, mecanismos de acción y riesgos de MPs en agroecosistemas y, por otro, contrastar hipótesis sobre efectos combinados clima–contaminantes en un sistema modelo de brásicas.

Para lograr los objetivos, se desarrollaron dos trabajos complementarios. Primero, una revisión que compila y analiza la evidencia sobre estreses concurrentes y MPs, incluyendo avances instrumentales (p. ej., espectroscopía e imágenes hiperespectrales) y analíticos (modelos de aprendizaje automático para identificación y clasificación de partículas). Segundo, un ensayo experimental con brotes de brócoli en un diseño factorial que combinó dos niveles de CO_2 (≈500 y 1000 ppm), dos temperaturas (20 y 28 °C) y tres condiciones de polímero (sin plástico, poliestireno, polietileno). Se cuantificaron rasgos morfológicos radiculares (WinRHIZO), fitohormonas (IAA, IBA, GA_3), actividad catalasa (CAT), capacidad antioxidante total (ORAC) y metabolitos especializados (glucosinolatos, antocianos y derivados sinápicos), con seis repeticiones por tratamiento y análisis estadísticos univariados y multivariantes (PCA/MANOVA) para explorar ejes latentes y trade-offs fisiológicos sin incurrir en sesgos por variables correlacionadas, así también se entrenó una red neuronal artificial.

Esta investigación surge de la necesidad de establecer marcos comparables y trazables para evaluar contaminación emergente en condiciones realistas de co-ocurrencia de estreses, así como de habilitar herramientas predictivas y biomarcadores que sustenten decisiones de manejo y normativas en sistemas agrícolas. La disponibilidad de tecnologías de detección y cuantificación de MPs, junto con herramientas predictivas, ofrece oportunidades para avanzar en vigilancia ambiental y evaluación de riesgos en la interfaz suelo–planta–alimento.

El Capítulo II presenta la revisión de la literatura sobre MPs en agroecosistemas y su interacción con estreses abióticos, incluyendo consideraciones metodológicas y perspectivas futuras. El Capítulo III describe los materiales y métodos del ensayo factorial, seguidos de resultados y discusión integrados.

2. HIPÓTESIS

1. Condiciones ambientales extremas (alta temperatura y alta concentración de CO_2) combinadas con la presencia de partículas contaminantes de microplásticos (MPs) en el suelo, pueden desencadenar respuestas a nivel bioquímico y fisiológico en brotes de crucíferas, en particular, brócoli (*Brassica oleraceae* var. *italica*), que pueden favorecer una mayor síntesis de compuestos del metabolismo secundario (glucosinolatos y compuestos fenólicos).
2. Los modelos predictivos basados en redes neuronales artificiales (ANNs) pueden predecir variables bioquímicas y fisiológicas a partir de factores ambientales y de exosición a partículas plásticas en cultivos de brócoli.

3. OBJETIVOS GENERALES

1. Realizar una revisión sistemática e integradora permitiendo caracterizar las tendencias, vacíos y convergencias de la literatura sobre microplásticos (MPs) en agroecosistemas y su co-ocurrencia con estreses abióticos, generando un marco conceptual que oriente líneas de investigación y gestión.
2. Evaluar experimentalmente cómo la interacción entre microplásticos, CO_2 y temperatura reconfigura rasgos fisiológicos y bioquímicos en brotes de brócoli, identificando ejes latentes de respuesta y posibles compensaciones crecimiento–defensa para orientar manejo y evaluación de riesgos en agroecosistemas.

4. OBJETIVOS ESPECÍFICOS

1. Revisar críticamente la literatura sobre la co-ocurrencia de estreses ambientales y microplásticos en cultivos y suelos agrícolas, destacando mecanismos, vacíos y prioridades de investigación.
2. Evaluar la respuesta fisiológica de los brotes y raíces de brócoli con la aplicación de MPs y en combinación con condiciones ambientales extremas (alta temperatura y concentración CO_2).
3. Analizar los efectos sobre la composición de compuestos bioactivos (glucosinolatos, antocianos y derivados sinápicos) en brotes de brócoli, como resultado del desarrollo en condiciones de estrés abiótico individual y combinado (MPs, altas temperaturas y concentración de CO_2 alta).
4. Aplicar análisis multivariante (PCA/MANOVA) y modelos predictivos para identificar ejes latentes y agrupamientos de respuestas en brotes de brócoli bajo distintas condiciones medioambientales.

CAPITULO II

Integrating microplastic research in sustainable agriculture: Challenges and future directions for food production.

Marcelo Illanes[a] , María-Trinidad Toro[b] , Mauricio Schoebitz[c] , Nelson Zapata[a] , Diego A. Moreno[d] , María Dolores Lopez-Belchí[a*]

a Department of Plant Production, Faculty of Agronomy, Universidad de Concepción, Avenida Vicente Méndez, 595, Chillán, Chile.
b School of Nutrition and Dietetics, Faculty of Medicine and Health Science, Universidad Mayor, Temuco, Chile.
c Department of Soils and Natural Resources, Faculty of Agronomy, Universidad de Concepción, Víctor Lamas 1290, Concepción, Chile.
d Laboratorio de Fitoquímica y Alimentos Saludables (LabFAS), CEBAS, CSIC, Campus Universitario de Espinardo-25, Murcia 30100, Spain.

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Abstract

In agroecosystems, plants are frequently subjected to a wide range of environmental stressors that have a substantial influence on plant physiology, crop performance, and food security. Abiotic stress responses to plant crop physiology and performance have been widely studied, but the co-occurrence of stressors, such as emerging contaminants (e.g., pharmaceuticals, plastic particles, or pesticides), combined with environmental conditions, remains understudied. Microplastics (MPs) have been identified as modifiers of plant physiology; therefore, these particles present a risk to the quality and safety of plant food production systems. One relevant question is how these emerging pollutants interact with the increasingly extreme environmental conditions of today. For example, evidence indicates that the interaction of MPs particles with elevated levels of ambient CO2 can modify stomatal conductance. In addition, their interaction with high temperatures may induce increased oxidative stress, whereas drought conditions can adversely affect vegetative growth. Salinity has been shown to alter root development, and MP particles can enhance the adsorption of trace metals onto plant tissues, thereby compromising food safety and increasing health risks. Currently, the application of omics technologies, including genomics, transcriptomics, and metabolomics, offers novel insights into molecular mechanisms that enable the identification of specific biomarkers associated with MP exposure. Furthermore, machine learning algorithms can be employed to analyze complex datasets, enhancing our ability to predict the impacts of MPs on plant health and crop performance under different environmental conditions. These results are significant for agricultural practices and policy formulation. As the prevalence of MPs in the environment continues to escalate, policymakers should address the potential risks these contaminants constitute to food safety and agricultural sustainability. This review compiles and synthesizes the most recent evidence regarding the impact of various stressors on crop quality and performance, with a particular emphasis on the interactions involving different plastic particles present in the environment and evaluates their potential risks to food safety and environmental resilience.

Keywords: Abiotic stress; Combined toxicity; Environmental stress; Exposome; Food security; Plastics

1. Introduction

Environmental stressors and emerging contaminants present significant challenges to plant growth, development, and productivity, eventually affecting food systems and agricultural sustainability. These stressors include drought, salinity, extreme temperatures, nutrient deficiency, heavy metals, pathogens, herbivores, global warming, climate emergency, air, water, and soil pollution, and emerging contaminants, including microplastics (MPs) [1,2]. The effects of these stressors on food systems result in reduced crop yields, compromised food quality, and adverse impacts on animal health. For instance, climate-induced temperature increases alter the distribution of plant and animal species and diminish agricultural productivity. However, uncertainty in plant responses to these stressors requires a comprehensive understanding of their effects on agriculturally important families with high nutritional value. Particularly, growing concern over the impact of environmental stressors on food production in the context of climate change emphasizes the urgency of understanding the interactions between these stressors and MPs that affect plant health and productivity. Furthermore, emerging contaminants (ECs) such as MPs, industrial chemicals and household waste can contaminate food, water, and soil, causing health risks to humans and animals [3–6]. ECs (Fig. 1) are generally used to refer to compounds of different origins and chemical nature, whose presence in the environment is not considered significant in terms of distribution or concentration; consequently, they often go undetected. However, they are now widely identified and have the potential to have an ecological impact as well as adverse health effects [7,8]. A characteristic of these groups of contaminants is that they do not need to be constantly present in the environment to cause negative effects because their high transformation/removal rates can be compensated by their continuous introduction into the environment [9]. It has been established that these compounds enter the environment through different sources, such as domestic and industrial wastewater, waste from treatment plants, hospital effluents, agricultural and livestock activities and septic tanks, which contain many specific organic compounds and ECs that occur at different concentrations in surface waters, and whose environmental quality criteria have not yet been specified [10,11]. In addition, conventional wastewater treatment plants are not designed to remove these pollutants, which is a concern to scientists and environmental regulators [12]. Introduction of novel contaminants, such as pharmaceuticals (Pharms), personal care products (PCPs), per- and polyfluorinated alkyl substances (PFAS), and organophosphate flame retardants (OPFRs), into the environment exhibits an emerging threat to plant health and its physiology. These contaminants can reach plants through various pathways, including wastewater irrigation, atmospheric deposition, and bioaccumulation in the soil and water. Their presence can disrupt various plant processes and lead to detrimental effects on plant growth, development, and health. Scientific research has elucidated the physiological responses of numerous species with agricultural significance to environmental stressors. Nevertheless, the interaction between these novel environmental conditions and emerging contaminants is a comparatively recent area of study. It is hypothesized that the cumulative impact resulting from the interaction of these factors will exceed their individual effects, thus presenting substantial implications for global food production contingent upon the degree of their interaction. The increasing prevalence of these novel contaminants in the environment necessitates further research to understand their potential effects on plant physiology and ecosystem health. This manuscript provides an extensive review of the effects of environmental stressors and emerging contaminants, particularly MPs, on plant health and food production. Additionally, we outline the research directions that are essential for developing sustainable and healthy food systems.

2. Literature review

This research focused on answering and collecting current information regarding the effects of the interaction of micro(nano)plastics and other current environmental stressors, as there is a constant relationship between the soil, air, and water levels between the fragments from the degradation of daily use plastics and the increase in environmental temperature, CO2 concentration, drought, salinity, and heavy metals.

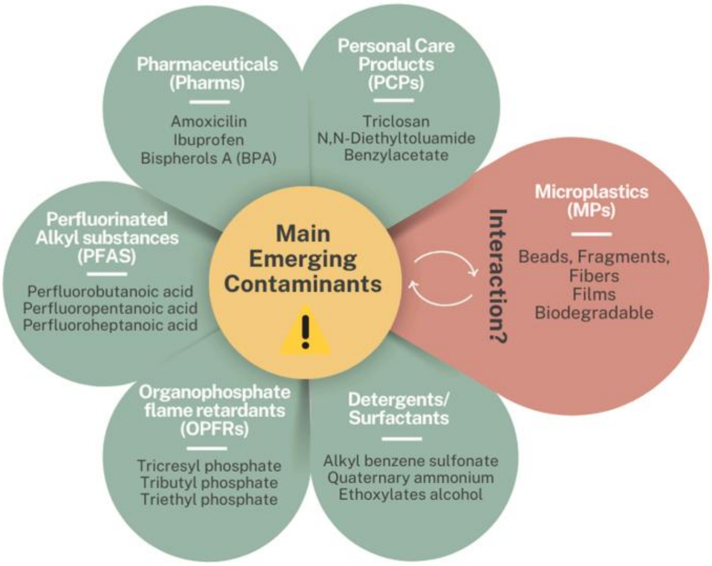


Figure 1. The main emerging contaminants include pharmaceuticals, personal hygiene products, perfluorinated alkyl substances, organophosphate flame retardants, MPs, and detergents/surfactants. Biorender.com.

This interaction, which occurs simultaneously throughout the world, can affect the physiological and biochemical responses of numerous plant species. This literature review aims to identify the largest number of studies on MPs. Some environmental factors were named above, but to make them more specific, most of the information had to focus on the response of some plant species of agricultural importance to obtain answers to what would happen at the ecosystem level. Search terms utilized in this study included “microplastic” or “nanoplastic” or “polyethylene” or “polypropylene” or “polystyrene” or “polyethylene terephthalate” or “biodegradable microplastics” and “high temperature” or “high carbon dioxide” or “drought” or “salinity” or “heavy metals” or “trace metals” or “pollutants” or “environment” or “nature” or “plants” or “physiology” or “biochemistry” or “damage” or “effect” or “consequence.”. All searches were performed in databases, such as ISI Web of Science, PubMed, ScienceDirect, and Scopus. The searches aimed to obtain information no older than 10 years since publication, except in some cases where older publications were added due to their importance in the described topic. Furthermore, only English-language publications were chosen, discarding any research that was neither peer-reviewed nor published. In some cases, results from research conducted in the maritime environment are presented in the tables to support the scarce information that could be obtained from the interaction between these pollutants in the terrestrial environment, always providing the most pertinent information from each publication, such as type of MPs, dose, type of environmental stress, concentration-dose-level, its effect on the plant studied, and the researchers who carried out that research. The complex relationships between crops and environmental stressors are shown in the conceptual structure map (Fig. 2).

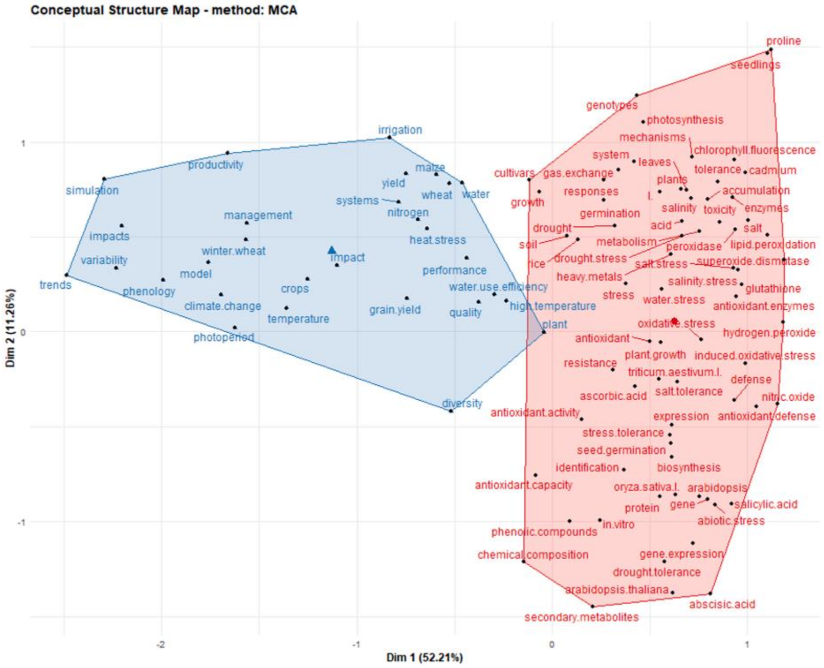


Figure 2. Conceptual structure plot using Multiple Correspondence Analysis (MCA). The database used was Web of Science© (WoS), and the diagram

was created using the RStudio software v.4.0.2, with a biblioshiny interface. A two-dimensional graphic map was prepared with the 100 most relevant keywords, considering their similarities. The results were interpreted on the basis of the relative positions of the points and their distributions along the dimensions. The more similar these words are, and the closer they are to the map, the better they will be represented.

3. Microplastics

Global production of plastics has increased in recent years reaching 360 million tons in 2018 [13], 368 million tons in 2019 [14] and 367 million tons in 2020 [15]. According to commercial statistics, China is the main global producer, accounting for 32 % of the total, in 2020, followed by North America at 19 %, the rest of Asia at 17 %, and Europe at 15 % [15], of which only 9 % is recycled [16], with the consequent environmental and global impact generated by these pollutants. The residues of these plastics, under the influence of mechanical abrasion and environmental oxidation (UV radiation, heat, chemical, and biological processes), break down into smaller particles, resulting in MPs [17]. MPs are particles between 100 nm and 5 mm in size, whereas nanoplastics are smaller (< 100 nm). The other large particles were classified as mesoplastics (5–25 mm) and macroplastics (> 25 mm) (Fig. 3) [18]. The most common plastic particles in the environment are polyethylene (PE), polypropylene (PP), and polystyrene (PS) in the form of fibers, fragments, foam, and flakes [19]. These components are recognized as a new major global change factor, which influences terrestrial ecosystems [20], because they are found in all environments, and the amounts detected in soil have reached 690000 particles kg^{-1} and in freshwater 155000 particles L^{-1} [21]. Plastics, specifically MPs, have gained notoriety in the marine environment, encompassing a large amount of current research and publications, although it is estimated that the terrestrial environment annually receives

4–23 times more plastic debris than the marine environment [22]. MPs originate from the degradation of larger plastic items or from the direct release of synthetic fibers, beads, and pellets. MPs have been detected in various environments, such as oceans, the atmosphere, and soils, and can enter the soil environment by different pathways, such as the application of sewage sludge, compost, mulching, irrigation with wastewater or runoff, and atmospheric deposition [23]. MPs in the soil environment are persistent contaminants for long periods because of their resistance to biodegradation and low mobility [24]. On the other hand, MPs originated from biodegradable plastics tend to affect plants more quickly, as these materials degrade faster in soil. However, there is currently insufficient evidence regarding the long-term detrimental effects of these nondegradable plastics. Accumulation of MPs in plants can have direct ecological effects and implications for agricultural sustainability and food security. These particles can be adsorbed on the root surface or in the case of nanoplastics (NPs), they can be absorbed by roots, plant tissues, fruits or edible parts and, consequently, accumulate and transfer in the food chain [20]. They can indirectly affect plants by altering physicochemical parameters and the soil microenvironment [25], as well as directly affecting plant growth and biomass [26], germination capacity [27], root lignification development and root cell death, accelerated chlorophyll breakdown, and hindered normal electron transfer within the PSII photosystem [28]. Moreover, they can influence nutrient uptake, seedling development, gene expression, and oxidative stress [29]. Similar to plants, MPs have also been found in fish and shellfish, chicken, land snails, some fruits and vegetables, salt, honey, sugar, water, and a restricted number of beverages, including beer and wine [30], as well as in marine animals and humans [31]. The presence of MPs in soil can have a significant impact on plant growth and development. Studies have shown that MPs can alter soil properties, affect plant performance, and influence soil microbial activity [32]. High concentrations of MPs have been found to inhibit root surface area and growth characteristics in plants [33]. MPs PP, reduced the fresh weight, leaf number, and photosynthetic pigment contents of pak choi (*Brassica rapa* L. ssp. chinensis) [34]. In Chinese cabbage (*Brassica chinensis* L.), the application of PS at 10–20 g kg− 1 or at sizes of < 25 and 48–150 μm were observed to significantly reduced fresh weight and growth, but the addition of high-density polyethylene had no effect on fresh weight [35]. MPs can interfere with plant growth through their morphology. For example, exposure to MPs in the soil can disrupt plant growth and function and alter *Zea mays* root morphology, resulting in cell wall damage [36], reducing weight, height, and leaf area in *Lactuca sativa* by applying foliar PS [37], blocking nutrient transport in *Vicia faba* roots (probably blocking cell connections or cell wall pores) [38], and triggering biochemical imbalances affecting photosynthetic, antioxidant, and sugar metabolism in *Cucumis sativus* [39]. In addition, MPs would affect the concentration of bioactive compounds. In fact, secondary metabolism and phytochemical content of crops can also be affected by the presence of MPs. For example, in a study developed by Lopez and collaborators it was demonstrated that the content of glucosinolates (GSL) was negatively affected by the presence of medium doses of MPs in the soil, decreasing from 182 to 124 mg 100 g− 1 in broccoli, while these compounds decreased drastically from 253 to 151 mg 100 g− 1 at the same doses in radish, in contrast, the anthocyanin content increased significantly up to medium doses of MP ranging from 6.28 to 11.44 mg 100 g− 1 in broccoli, while in radish it was from 2.44 to approximately 4 mg 100 g− 1 [40]. MPs can also alter the plant-microbe interactions by changing microbial community composition and function, influencing symbiotic associations with beneficial microbes, or facilitating the transmission of pathogens or toxins [41,42]. Moreover, MPs can act as sources and delivery systems for other contaminants, such as heavy metals, organic pollutants, and additives, further increasing their toxicity to plants. The presence of MPs intensifies the negative effects of environmental stressors on plants in several ways, including physical damage, because MPs can block plant pores and stomata, thereby reducing gas exchange and transpiration. MPs also adhere to plant roots, restricting the uptake of water and nutrients [43]. In addition, they induce oxidative stress because MPs can generate reactive oxygen species (ROS) [44], which can damage plant cells and tissues or cause hormonal disruption [45]. MPs can interfere with the production and function of plant hormones, which can disrupt plant growth and development. In addition, nutrient uptake (MPs can compete with plants for nutrients in the soil) and vectors for pathogens, such as bacteria and fungi [46,47], can increase plant susceptibility to disease. In addition, MPs allow the entry of trace metals into oilseed rape (*Brassica napus* L.), so they can bioaccumulate these compounds inside plants. The results showed that 0.1 % PE MPs in the soil increased the trace metal concentration of oilseed rape plants. For instance, the Cu2+ and Pb2+ of oilseed rape in the combined treatments with contaminants were 1.1 and 1.08 times higher than that in the single treatment with trace metals (Cu100 or Pb50), respectively. Therefore, contaminants have negative effects on autoregulatory processes such as increased malondialdehyde content and deterioration of oilseed rape quality [48].

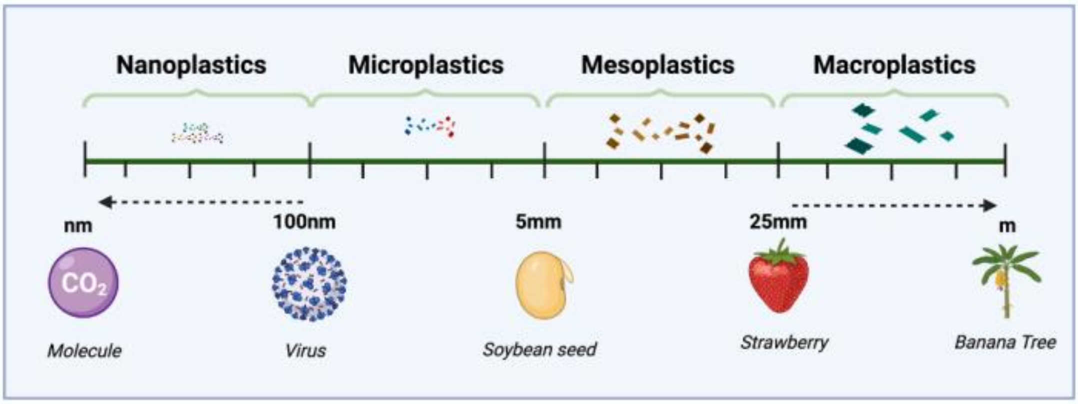


Figure 3. Classification of plastics according to size measured in any/longest dimension. The figure allows sizes to be distinguished based on perspective objects of known sizes to facilitate understanding and comparison. Biorender.com.

Thus, MPs can negatively affect plant growth and development in several ways. They physically damage the roots, hinder water and nutrient uptake, and block stomata, thereby affecting gas exchange. Studies have shown reduced weight, leaf number, and photosynthetic pigments in Pak choi and Chinese cabbage following exposure to MP. MPs also induce oxidative stress and alter gene expression, thereby affecting photosynthesis, antioxidant activity, and sugar metabolism. They disrupt beneficial plant-microbe interactions and act as carriers for other harmful contaminants. Furthermore, MPs amplify the negative effects of environmental stressors such as drought and nutrient deficiency. Therefore, MPs are a significant threat to plant health and ecosystem stability. Hence, conducting studies in areas contaminated with MPs rather than solely in controlled settings and standardizing sampling methods for measuring plastic concentrations in soils are essential for enabling comparative analyses in scientific investigations on this subject.

3.1. Advanced techniques to assess MPs in the environment

Measurements of MPs in the environment requires stable protocols and a defined methodology. The design of the collection process, extraction area, number of samples, and frequency at which these samples are collected from the environment are an essential step, with careful attention given to differentiating the source of each sample [49]. Recent studies have highlighted the need to integrate multiple analytical techniques to accurately identify and quantify MPs in complex agricultural matrices. For instance, a combination of Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) has been effective in characterizing the morphology and chemical composition of MPs extracted from soil [50]. Additionally, thermogravimetry-mass spectrometry (TG-MS) has been used for quantitative analysis of specific MPs, such as polyethylene terephthalate (PET), in soil samples, demonstrating its efficacy in complex environmental matrices [51]. On the other hand, machine learning (ML) algorithms are being applied to enhance the identification and classification of MPs. For example, a study developed a machine learning model for high throughput identification of FTIR spectra, showing its potential for the rapid and accurate assessment of MPs pollution [52]. This approach not only streamlines the identification process but also improves the reliability of the data collected from the environment. Moreover, innovative methodologies, such as impedance spectroscopy, have been introduced for high-throughput quantification of MPs in water and soil samples. This technique allows for rapid measurement of MPs concentration and size without the need for extensive preprocessing, making it particularly suitable for monitoring [53]. Additionally, the use of microscopic image datasets combined with segmentation and detection algorithms has further enhanced the capability to analyze MPs in environmental samples, facilitating more detailed assessments of their prevalence and characteristics [54]. The need for these advanced techniques extend beyond detection; they can be key in policy and management strategies aimed at mitigating MPs pollution in agriculture because obtaining robust data on the sources and concentrations of MPs will guide regulatory frameworks and practices for waste management in agricultural settings. Furthermore, assessing the interactions between MPs and agricultural practices can lead to the development of more sustainable farming techniques that minimize plastic use and enhance soil health.

3.1.1 Fourier transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a prominent non-destructive analytical method employed for the identification and characterization of various materials, including MPs, through the examination of infrared absorption spectra. Nevertheless, FTIR remains less suitable for particles smaller than 10 µm, which restricts its efficacy in analyzing these minute entities [58]. Thakur et al. (2023) utilized FTIR and X-ray diffraction to characterize MPs in soils near industrial areas, successfully identifying distinct polymer types based on their spectral fingerprints. The study highlighted specific peaks in the FTIR spectra corresponding to known MPs polymers, facilitating accurate identification. This approach is particularly valuable in the agricultural context, where understanding the types of MPs present can inform risk assessments related to soil contamination and crop safety. The application of FTIR in agricultural soil studies has also been supported by other authors, who investigated MPs pollution in rice and vegetable soils in Xiangtan, China. The study employed FTIR alongside stereomicroscopy and scanning electron microscopy (SEM) to analyze MPs characteristics, confirming the presence of various polymer types in the soil [59]. In addition, Piehl et al. quantified macro- and MPs contamination in agricultural farmland using FTIR, revealing significant concentrations of polyethylene, polystyrene, and polypropylene [60]. Furthermore, the integration of machine learning with FTIR data has shown promise for enhancing the identification and classification of MPs. For example, Yan et al. developed an ensemble machine learning method to improve the accuracy of MPs identification using FTIR spectra, demonstrating the potential for automated analysis in environmental monitoring [61]. This innovative approach could streamline the assessment of MPs in soils, allowing more efficient data processing and interpretation.

3.1.2 Raman spectroscopy

Raman spectroscopy is a powerful non-destructive technique used to analyze the chemical and physical properties of materials, particularly MPs. Based on the Raman effect, it detects shifts in light scattered by a sample, revealing molecular vibrations and allowing the identification of chemical species and their structures [56]. This method is particularly useful for distinguishing between different types of MPs and studying their size, structure, and additives or impurities [49]. Micro-Raman, an advancement of this technique, allows for the identification of smaller MPs, down to 1 μm, providing higher resolution compared to other techniques like FTIR [57]. However, Raman spectroscopy faces challenges such as interference from fluorescence in environmental samples, which can complicate the interpretation of results. Solutions such as adjusting instrument parameters, pre-light exposure, or chemical digestion to remove surface contaminants can help mitigate these issues [62]. Despite these limitations, Raman spectroscopy is widely used for MPs characterization because of its high specificity for identifying molecular bonds, such as aromatic and C-H bonds [63]. However, it is difficult to identify very small MPs due to diffraction limits and overlapping spectra from various polymers and organic impurities in complex mixtures [64]. Compared with FTIR, Raman spectroscopy has the advantage of being less sensitive to water and can detect chemical groups that FTIR fails to identify, making it useful for distinguishing between different polymers and additives [58,65].

3.1.3 Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS)

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) is an advanced technique used to analyze MPs by thermally degrading polymers into their constituent parts, which are then separated and identified through gas chromatography and mass spectrometry [64]. This method provides detailed chemical information about the polymers, making it effective for identifying and quantifying MPs, even at trace levels [66]. Py-GC/MS is highly sensitive and capable of characterizing complex mixtures of MPs in environmental samples, but it is a destructive technique, meaning it cannot be used for repeated analyses of the same sample [55]. The technique operates at temperatures between 500 and 800◦C to break down plastic polymers, producing thermal degradation products that are analyzed to determine the polymer composition [56]. Py-GC/MS is particularly useful for identifying a wide range of polymers, and it can complement other methods such as FTIR and Raman spectroscopy for more comprehensive analysis [58]. However, the technique requires strict experimental conditions and is influenced by sample impurities, such as organic matter, which may interfere with results [67].

3.1.4 Hyperspectral imaging (HSI)

Hyperspectral Imaging (HSI) is an advanced, non-destructive technique that allows for the detection and quantification of MPs, including those within the nano-plastics range. One of its major advantages over techniques such as FTIR and Raman spectroscopy is that it requires minimal sample pretreatment [55]. Although, increased moisture in the samples can complicate MPs detection by enhancing near-infrared (NIR) reflectance, leading to potential uncertainties [55]. HSI works by capturing spectral information from a sample based on the unique interactions between chemical species and incident light at different wavelengths. This results in distinct spectral signatures that depend on the material's chemical composition, shape, size, and color [57]. This technology enables the simultaneous identification of multiple particles, and when combined with machine learning algorithms, can produce faster results than traditional methods [68]. Despite its advantages, the primary challenge with HSI depends on its operational complexity and the need for extensive data processing [57].

4. Combination of MPs and Environmental Stressors

4.1. Carbon dioxide and MPs

The biological processes of plants, soil nutrients, and microorganisms are affected directly or indirectly by elevated CO_2 levels. Therefore, growing concerns regarding the possible consequences of increased CO_2 on soil processes, especially those related to changes in soil carbon, enzymes, and microbial activities, have stimulated experimental research [84]. Unfortunately, plants require optimal atmospheric temperature and CO_2 concentration for their development. Therefore, when these variables are altered, an internal imbalance is generated that can affect both physiological and biochemical responses. Studies have shown that elevated CO_2 increases plant growth and crop yield by stimulating carboxylation and suppressing the oxygenation activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), particularly in C3 plants, whereas the effects are marginal in C4 plants because Rubisco is already saturated in this CO_2 environment [85]. Thus, the primary effects of elevated CO_2 concentrations on plants include higher rates of photosynthesis, increased water use efficiency, higher productivity, and altered secondary metabolites [86]; however, depending on the doses and the species, there will be different responses to CO_2 concentrations (Table S1). The atmospheric CO_2 concentration also affects leaf transpiration, stomatal conductance, and root exudation. Studies conducting such stimulation in plants have indicated that elevated CO_2 can additionally induce enhanced fluxes of organic compounds into the soil, owing to an increased rate of leaf litter and rhizodeposition [84]. There are two relationships between CO_2 and MPs. First, the effect of the increase in environmental CO_2 and its interaction with the increase in MPs in the soil can affect the physiology and biochemistry of plant species, as well as the effect of MPs on the increase in CO_2 emissions from the soil to the environment by modifying bacterial populations and introducing carbon through the composition of these plastics (Fig. 4). The relationship between CO_2 and MPs has been the focus of recent research as a correlation has been observed between the presence of MPs and increased CO_2 emissions from the soil. These emissions increased when the MPs originated from biodegradable plastics. These films degrade easily in soil, generating MPs and releasing chemical compounds. Table 1 presents recent work that demonstrates how plastic biofilms drastically increase emissions compared to soils without the presence of these components and the effect of the interaction between MPs in the soil and high environmental CO_2 on plants. This is mainly due to the fact that the composition of these biofilms is around 60–80 % carbon. These studies suggest that a combination of CO_2 and MPs can have a negative impact on plant physiology. Additionally, plastic handling in agriculture may increase CO_2 emissions and worsen the climate crisis. This is a concern for global food security because climate change is expected to lead to elevated CO_2 levels and increased MPs pollution.

4.2. High temperatures and MPs

An increase in the global temperature causes a negative response in plants. Previous studies have shown that increased leaf temperatures reduce plant growth and limit crop yields by an estimated 17 % for each degree of increase in average temperature during the growing season, moderately high temperatures (35–40 °C) reduce the rate of photosynthesis, but at temperatures > 45°C, damage to photosystem II (PSII) is generated, which decreases energy production in the plant and affects the functioning of Rubisco [98]. Exposure of plants to extreme temperatures (> 50°C) results in severe tissue damage within minutes and a rapid collapse of cellular organization [99]. High temperatures generate anatomical, morphological, and functional changes in plants (Table S2), such as reduction in cell size, reduced stomatal conductance and stomatal closure, changes in membrane permeability, increased density of stomata and trichomes, and increased xylem vessel size [100,101] and when interacting with different types of MPs in the soil, it can affect different parameters in agricultural crops and soil (Table 2.)

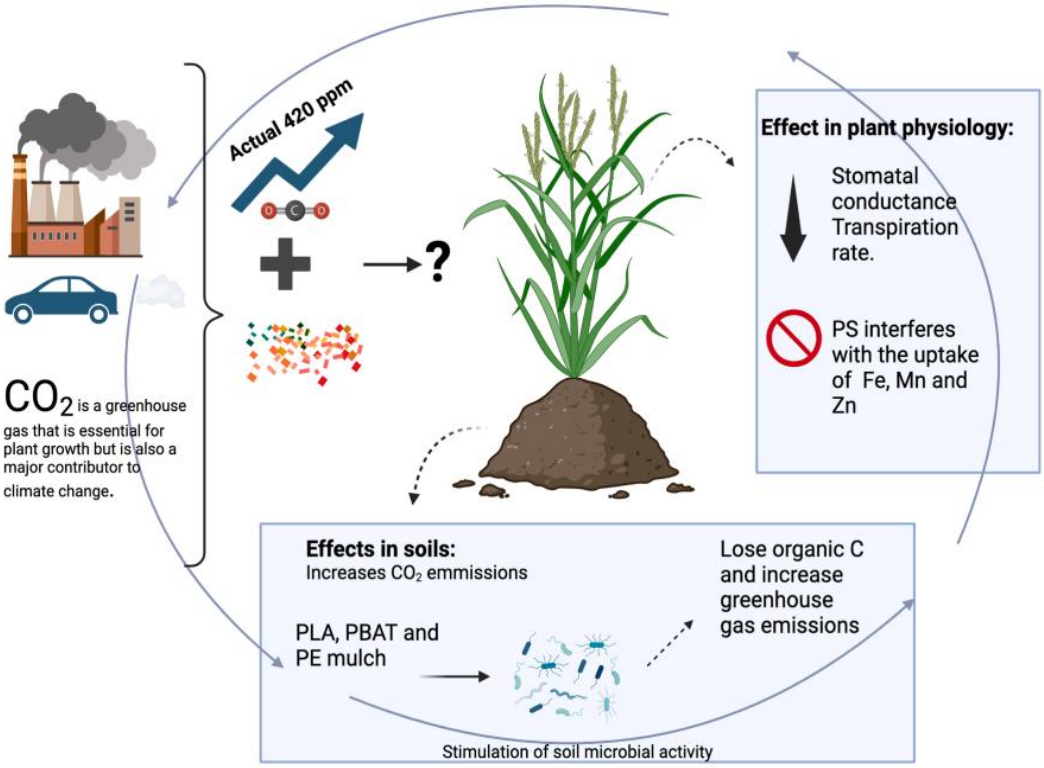
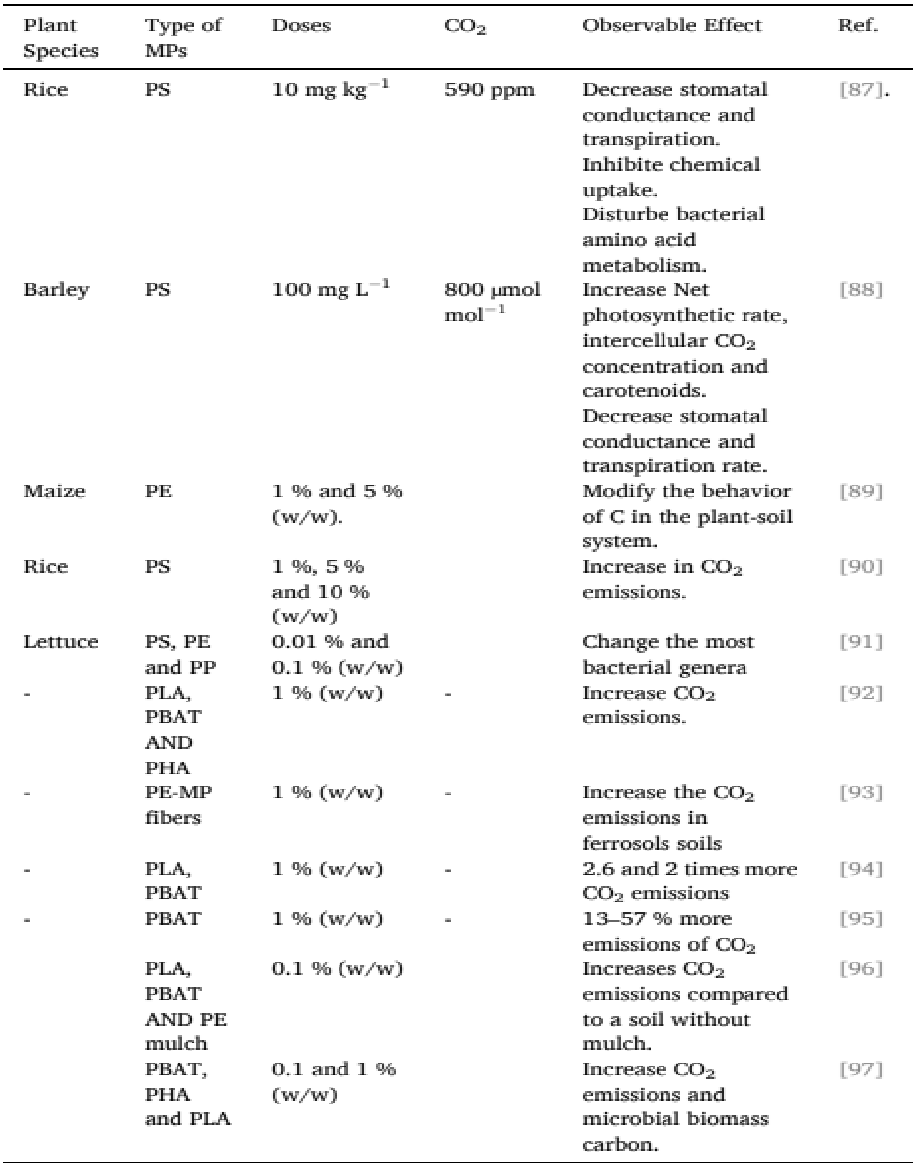


Figure 4. Effect of the interaction between CO_2 and MPs.

Table 1. Effect of interaction of CO2 and microplastics on crop plants, and on environmental CO2 emissions.



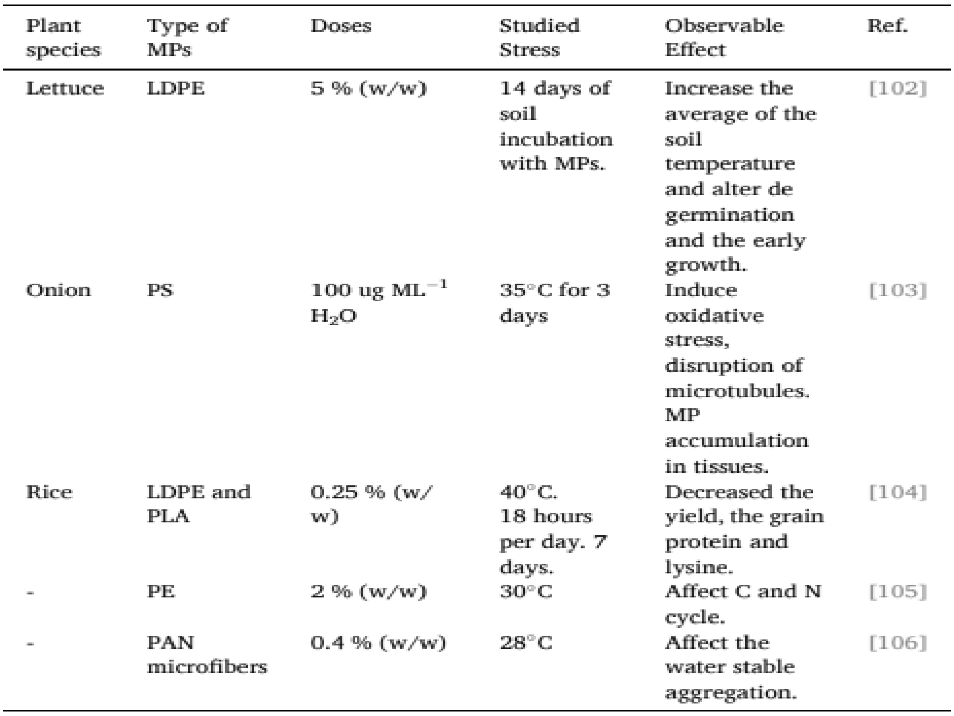
Plant Species	Type of MPs	Doses	CO_2	Observable Effect	Ref.
Rice	PS	10 mg kg^{-1}	590 ppm	Decrease stomatal conductance and transpiration. Inhibite chemical uptake. Disturbe bacterial amino acid metabolism.	[87].
Barley	PS	100 mg L^{-1}	800 μmol mol^{-1}	Increase Net photosynthetic rate, intercellular CO_2 concentration and carotenoids. Decrease stomatal conductance and transpiration rate.	[88]
Maize	PE	1 % and 5 % (w/w).		Modify the behavior of C in the plant-soil system.	[89]
Rice	PS	1 %, 5 % and 10 % (w/w)		Increase in CO_2 emissions.	[90]
Lettuce	PS, PE and PP	0.01 % and 0.1 % (w/w)		Change the most bacterial genera	[91]
-	PLA, PBAT AND PHA	1 % (w/w)	-	Increase CO_2 emissions.	[92]
-	PE-MP fibers	1 % (w/w)	-	Increase the CO_2 emissions in ferrosols soils	[93]
-	PLA, PBAT	1 % (w/w)	-	2.6 and 2 times more CO_2 emissions	[94]
-	PBAT	1 % (w/w)	-	13–57 % more emissions of CO_2	[95]
	PLA, PBAT AND PE mulch	0.1 % (w/w)		Increases CO_2 emissions compared to a soil without mulch.	[96]
	PBAT, PHA and PLA	0.1 and 1 % (w/w)		Increase CO_2 emissions and microbial biomass carbon.	[97]

Some studies indicate that the interaction between MPs in the soil and heatwaves could result in a yield reduction of up to 30% in globally significant crops, such as rice, thereby posing a potential threat to global food supply. Conversely, other studies have demonstrated that even in the absence of crops, when there is an increase in ambient temperature, soil interacts with MPs, adversely affecting the carbon and nitrogen cycles, leading to a decrease in microbial biomass, ultimately resulting in biodiversity loss. More research is needed to better understand the effects of high temperatures and MPs on plants and develop strategies to mitigate the negative impacts of these stressors.

4.3. High temperature, CO_2 and MPs

The combination of high temperatures, CO_2, and MPs in plants is a complex and relatively understudied area of research. However, available evidence suggests that these three stressors can interact in ways that negatively affect plant growth and physiology. There is still no significant amount of evidence of the interaction of these stresses and their effects on plants, and there is only one study published by Obrien et al., which demonstrated the negative effects of tire leachate, high temperatures, and carbon dioxide on duckweed (a plant that grows in aquatic environments), affecting the relationship between the plant and the associated microbiota, especially when tire leachate is included in the equation. However, by increasing the CO_2 levels, the negative effects of tire leachate were reduced. Multiple interacting stressors can affect multiple interacting species, and leachate from tire wear particles can potentially disrupt plant-microbe mutualisms [107]. There is no further evidence of the effect of these combined factors; however, we were able to point out how these stresses affect the behavior and performance of plant species, so it could be deduced that a combined effect would have even greater consequences at the physiological level. However, it is necessary to study the differences in plant species and interactions between these stresses to determine the magnitude of the effect.

Table 2. Effect of interaction of temperature and microplastics on crop plants and soil.

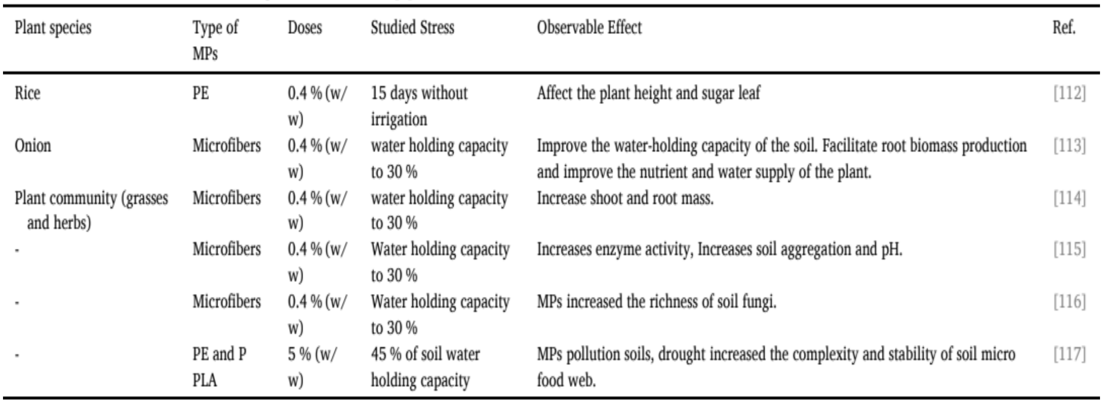


Plant species	Type of MPs	Doses	Studied Stress	Observable Effect	Ref.
Lettuce	LDPE	5 % (w/w)	14 days of soil incubation with MPs.	Increase the average of the soil temperature and alter de germination and the early growth.	[102]
Onion	PS	100 ug ML^{-1} H_2O	35°C for 3 days	Induce oxidative stress, disruption of microtubules. MP accumulation in tissues.	[103]
Rice	LDPE and PLA	0.25 % (w/w)	40°C. 18 hours per day. 7 days.	Decreased the yield, the grain protein and lysine.	[104]
-	PE	2 % (w/w)	30°C	Affect C and N cycle.	[105]
-	PAN microfibers	0.4 % (w/w)	28°C	Affect the water stable aggregation.	[106]

4.4. Drought and MPs

Drought is defined as insufficient rainfall over a prolonged period, causing adverse impacts on living organisms such as vegetation, animals, and people. Precipitation deficits, combined with high vapor pressure deficit (VPD) or increased atmospheric evaporative demand (AED), induce soil moisture deficits that affect plant growth and crop yield [108]. Irrigated crops contribute to approximately 40% of the global food supply for the entire world, making them indispensable for global food security. In addition, climate change has put great pressure on the hydrological cycle. In recent years, warm weather has increased the likelihood and severity of drought, possibly due to decreased precipitation and increased evapotranspiration in some areas of the world. Groundwater levels rapidly decline in these areas [109]. Drought stress has a major impact on the yield of major crops, such as cereals and legumes, which are exposed to progressive water deficits during the flowering and grain filling stages, reducing leaf photosynthesis and the production of photosynthetic assimilates that are directly transferred to the grain. Consequently, the number of grains per ear/pod is considerably reduced and the grain weight is reduced, but to a lesser extent, leading to lower yields [110]. In addition, drought stress caused significant changes in the morphological, physiological, and biochemical properties of the plants (Table S3). For example, it causes stomata closure, decreased leaf water potential and turgor pressure, reduced cell division and flexibility, altered plant coloration and decreased plant biomass, ionic hemostasis in plant cells is affected by drought, while the rate of photosynthesis decreases [111]. The combinations of the reduced water and MPs are listed in Table 3. It is important to understand the mechanisms by which plants respond to drought stress, as well as the effects of MPs on enzymatic activities of plants, which could lead to genotoxicity and oxidative damage [118,119]. MPs can affect the growth of individual plants directly by altering physiological processes and indirectly by influencing soil biota, which in turn affects plant growth [120]. Research on the combined effects of drought and MPs on plants is still in its early stages. However, available evidence suggests that these two stressors can interact and have a significant negative impact on plant growth and physiology. This is a concern for global food security because climate change is expected to lead to both more frequent and severe droughts and increased MP pollution. More research is needed to better understand the effects of these stressors on plants and to develop strategies to mitigate these negative impacts.

Table 3. Effect of interaction of drought and microplastics on crop plants and soil.

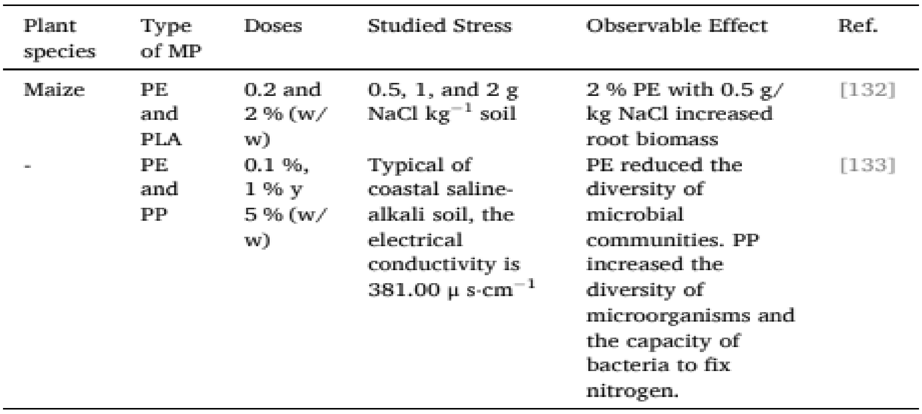


Plant species	Type of MPs	Doses	Studied Stress	Observable Effect	Ref.
Rice	PE	0.4 % (w/w)	15 days without irrigation	Affect the plant height and sugar leaf	[112]
Onion	Microfibers	0.4 % (w/w)	water holding capacity to 30 %	Improve the water-holding capacity of the soil. Facilitate root biomass production and improve the nutrient and water supply of the plant.	[113]
Plant community (grasses and herbs)	Microfibers	0.4 % (w/w)	water holding capacity to 30 %	Increase shoot and root mass.	[114]
-	Microfibers	0.4 % (w/w)	Water holding capacity to 30 %	Increases enzyme activity, Increases soil aggregation and pH.	[115]
-	Microfibers	0.4 % (w/w)	Water holding capacity to 30 %	MPs increased the richness of soil fungi.	[116]
-	PE and P PLA	5 % (w/w)	45 % of soil water holding capacity	MPs pollution soils, drought increased the complexity and stability of soil micro food web.	[117]

4.5. Salinity and MPs

Salinity is defined as the presence of high levels of dissolved salts in the soil or water. Soil salinization has become a major environmental stressor in areas with low rainfall and intensive agricultural production [121]. Climate change-induced drought and increased evaporation deteriorate soil salinity [122]. In irrigated agricultural fields, high soil surface temperatures, poor irrigation, poor drainage [123], and altered rainfall patterns accumulate soluble salts in the active root zones of plants [124]. Other authors have suggested that 20 % of the irrigated area and 6 % of the world's total land are compromised owing to saline conditions, adversely reducing crop yields by up to 70 % [125]. These numbers are increasing owing to unsustainable irrigation management and climate change [126]. Therefore, increased salt in the soil or irrigation water generates negative effects as it decreases the water uptake capacity of the plant while increasing the uptake of Na+ and Cl- . Moreover, excessive salt accumulation in the root zone causes osmotic stress and nutrient imbalance in plant cells. Salt stress negatively affects cell elongation, metabolic processes, and photosynthetic efficiency [127]. Furthermore, it induces ROS accumulation, which severely affects the structure of enzymes, nucleic acids, and lipids [128]. Additionally, it is related to the depreciation of photosynthesis via the inhibition of normal processes within the electron transport chain and oxidative damage, including cell integrity and chlorophyll structure [129]. Examples of the effects of salinity on crops of agricultural importance are presented in Table S4. Moreover, salinity negatively affects the soil by interfering with the normal behaviour of soil microbiota, reducing organic carbon content, reducing crop yields, and leading to desertification [130]. Increased salinization poses special concerns for the global food supply systems. Salinization is likely to have a negative impact on the economy, living conditions, and ecosystem functions of the affected regions [131]. Therefore, there is an urgent need to develop climate adaptation strategies to combat the effects of climate change and salinity on crop yields [124]. Salinity and MPs are two stressors that negatively affect plant growth and physiology (Table 4). Therefore, it can be deduced that if there is an effect on the soil microbiota, it would indirectly affect the plants that grow in those soils; however, there is still a lack of information to confirm these effects at a physiological level.

Table 4. Effect of interaction of salinity and microplastics on crop plants and soil.

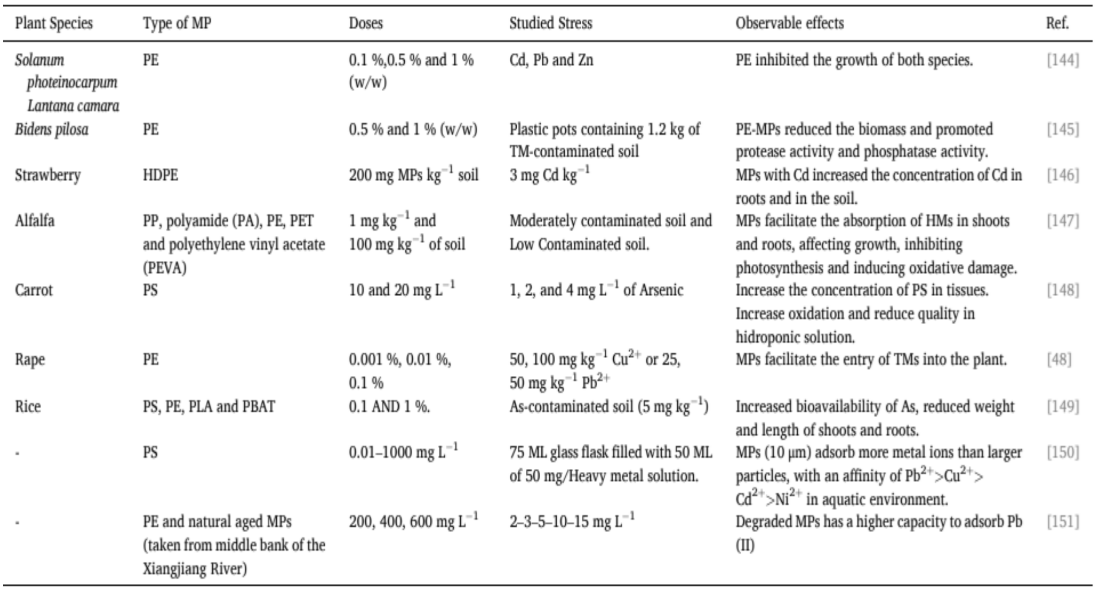


Plant species	Type of MP	Doses	Studied Stress	Observable Effect	Ref.
Maize	PE and PLA	0.2 and 2 % (w/w)	0.5, 1, and 2 g NaCl kg^{-1} soil	2 % PE with 0.5 g/kg NaCl increased root biomass	[132]
-	PE and PP	0.1 %, 1 % y 5 % (w/w)	Typical of coastal saline-alkali soil, the electrical conductivity is 381.00 μ s·cm^{-1}	PE reduced the diversity of microbial communities. PP increased the diversity of microorganisms and the capacity of bacteria to fix nitrogen.	[133]

4.6 Trace metals and MPs

Trace metals (TMs) and metalloids, including arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), cobalt (Co), nickel (Ni), selenium (Se), and zinc (Zn), generally coexist in contaminated soils and can be transferred to plants, animals, and humans through direct absorption or along the food chain. This can seriously threaten ecosystems because of their persistent and non-degradable nature [134]. Their toxic effects have been shown to alter several physiological responses, including reduced seed germination, plant development and growth, leaf area index, photosynthetic rate, membrane stiffness, and integrity, while increasing leaf chlorosis and cell wall damage [135]. TMs contaminate more than 5 million sites covering 20 million hectares of land [136], and interact with other organic pollutants such as hydrocarbons, aromatic compounds, phenols, organochlorine compounds, and pesticides that have been found in high concentrations in soil and sediments. TMs accumulate in the soil and affect the activity of the microbial population, as well as interfere with enzymatic activities, depending on the type of metal present and its chemical affinity. For example, Cd affects ureases, proteases, and basic phosphatases; Pb affects catalases, ureases, invertase, and acid phosphatase; As affects sulfatase and phosphatase; and Cu restricts β-glucosidase, which leads to decreased soil quality and inhibits plant growth [137]. These effects depend on the type of metal and its concentration in the soil solution, as well as the soil properties in which they occur [136]. TMs such as Cd have been observed to alter the function and structure of the plasma membrane and cause cell death [138]. These harmful effects can result in various undesirable alterations, such as the disruption of nutrient and water supplies [135]. The downregulation of thiol transferase processes, epigenetic mediation, and programmed cell death has been demonstrated in *Brassica napus* plants exposed to 200 μmol L^{-1} $NaAsO_2$. Cu and Cr at doses of 200 μmol caused a decrease in fresh weight by 110 and 293%, respectively, in 5-leaf Rap seedlings, decreased root weight, shoot length, and root length, and decreased pigment concentration and photosynthetic activity, which further worsened these values [139]. Cr toxicity in soils reduces fresh and dry root biomass, plant height, root length, stomatal conductance, water use efficiency, and net photosynthesis in *Brassica napus* [140]. Furthermore, the literature indicates that toxic TMs can affect nutrient uptake in *Brassica juncea* (Mustard) and *Brassica rapa* (Turnip), mainly due to competition for common enzyme binding sites, leading to decreased growth, altered morphology, necrosis, chlorosis and decreased photosynthetic activity [141], accumulating in roots, stems, leaves and seeds [142]. Other responses of the species that were subjected to different concentrations of TMs are presented in Table S5. The combination of TMs and MPs can have a synergistic effect on plant growth, meaning that the negative effects of these two stressors are greater when they occur together than when they occur alone. For instance, the combination of MPs and TM can inhibit the growth of shoots and roots in plants, as well as decrease the chlorophyll content (a, b, and total) [143]. The other effects of this interaction are listed in Table 5.

Table 5. Effect of interaction of trace metals and microplastics on crop plants, soil and aquatic environment.



Plant Species	Type of MP	Doses	Studied Stress	Observable effects	Ref.
Solanum photeinocarpum *Lantana camara*	PE	0.1 %,0.5 % and 1 % (w/w)	Cd, Pb and Zn	PE inhibited the growth of both species.	[144]
Bidens pilosa	PE	0.5 % and 1 % (w/w)	Plastic pots containing 1.2 kg of TM-contaminated soil	PE-MPs reduced the biomass and promoted protease activity and phosphatase activity.	[145]
Strawberry	HDPE	200 mg MPs kg^{-1} soil	3 mg Cd kg^{-1}	MPs with Cd increased the concentration of Cd in roots and in the soil.	[146]
Alfalfa	PP, polyamide (PA), PE, PET and polyethylene vinyl acetate (PEVA)	1 mg kg^{-1} and 100 mg kg^{-1} of soil	Moderately contaminated soil and Low Contaminated soil.	MPs facilitate the absorption of HMs in shoots and roots, affecting growth, inhibiting photosynthesis and inducing oxidative damage.	[147]
Carrot	PS	10 and 20 mg L^{-1}	1, 2, and 4 mg L^{-1} of Arsenic	Increase the concentration of PS in tissues. Increase oxidation and reduce quality in hidroponic solution.	[148]
Rape	PE	0.001 %, 0.01 %, 0.1 %	50, 100 mg kg^{-1} Cu^{2+} or 25, 50 mg kg^{-1} Pb^{2+}	MPs facilitate the entry of TMs into the plant.	[48]
Rice	PS, PE, PLA and PBAT	0.1 AND 1 %.	As-contaminated soil (5 mg kg^{-1})	Increased bioavailability of As, reduced weight and length of shoots and roots.	[149]
-	PS	0.01–1000 mg L^{-1}	75 ML glass flask filled with 50 ML of 50 mg/Heavy metal solution.	MPs (10 μm) adsorb more metal ions than larger particles, with an affinity of Pb^{2+}>Cu^{2+}>Cd^{2+}>Ni^{2+} in aquatic environment.	[150]
-	PE and natural aged MPs (taken from middle bank of the Xiangjiang River)	200, 400, 600 mg L^{-1}	2–3–5–10–15 mg L^{-1}	Degraded MPs has a higher capacity to adsorb Pb (II)	[151]

Excessive erosion/aging caused by abrasion with other environmental materials or UV radiation alters the surface of the MPs. The surface of the MPs expands because of these changes, resulting in the formation of active anionic sites. These are the key sites that attract cationic metal pollutants from the environment [152]. MPs can absorb organic pollutants and metals from their surfaces owing to their small size, high hydrophobicity, and high surface-to-volume ratio. MPs and TMs may pose a synergistic pollution risk to the environment, potentially leading to adverse effects on organisms [153]. However, metals also differ in their affinities for the same type of polymer, with Pb having the highest affinity for PS MP adsorption, followed by Cu, Cd, and Ni [152]. The coexistence of metalloids and MPs in soil can alter the toxic effects of TMs and MPs on plant growth [154]. Research has demonstrated that polyethylene terephthalate (PET) particles can act as vectors for the transfer of metals, such as Cd, Pb, and Zn, to the rhizosphere zone of wheat, potentially causing TMs to affect the plant [155]. This can also affect soil fertility, diversity, and microbial functions, thereby posing a potential threat to the multifunctionality of soil ecosystems [156]. On the other hand, in addition to plastics used in agriculture, it has been shown that compost can be a carrier of toxic trace metals bound to MP in agroecosystems. Different toxic metals, such as Cr, Pb, Cu, and Ni, have been associated with MPs in composts [157]. It is noteworthy that interactions between MPs and other contaminants such as TMs in natural environments can be affected by more complex factors such as the presence of humic acid, composition of compounds, and fluctuation of water temperature and pH values [150]. However, research on the combined effects of TMs and MPs on plants is still in the early stages. However, available evidence suggests that these two stressors can interact to have a significant negative impact on plant growth and physiology. This is a concern for global food security, as TMS and MPs pollution are increasing in many parts of the world. More research is needed to better understand the effects of these stressors on plants and develop strategies to mitigate these negative impacts.

5. Future needs for healthy and sustainable agrifood systems

Environmental stress and contaminants in MPs can negatively affect the growth and performance of plants and their food safety, and evidence shows that their interaction can bring negative effects even greater than their effect alone, causing a risk to the sustainable development goals of the 2030 Agenda, such as zero hunger, health and wellbeing, clean water and sanitation, and responsible production and consumption [158]. Thus, there is a need to generate information on the interactions between MPs and environmental stress. Fig. 5 presents the keywords most frequently used by researchers working with MPs between 2022 and 2024. This information allows us to conclude that, in recent years, research on MPs has grown considerably but has focused on its maritime pollutants, pollution in soils and the environment, and its direct and indirect impact on mammals. However, there is very little information on their relationship with high temperatures, increases in CO_2, drought, and salinity; therefore, there is an urgent need to increase knowledge of their possible negative effects on global food security.

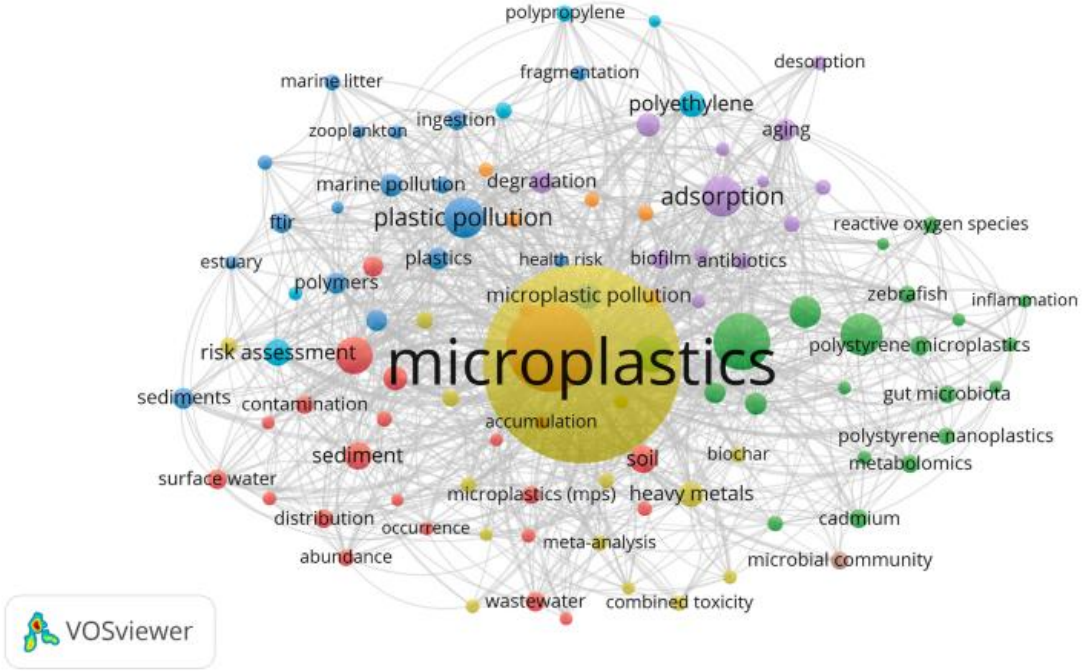


Figure 5. Co-occurrence of the author keywords with a minimum of 40 occurrences (103 met the threshold). Network visualization map of the occurrence of keywords in MPs research between 2022 and 2024. The thickness of the connecting line is directly proportional to the intensity of the coloration between words. The display scale of the circles is based on the number of papers published on the topic. The association percentage is represented by a node and its size. VOSviewer.

5.1. Omics approaches of molecular mechanisms and plant responses to MPs and environmental stressors

Omics technologies (genomics, transcriptomics, proteomics, and metabolomics) are essential for investigating how plants respond to various environmental stressors at the molecular level, including MPs exposure. [69]. The integration of omics technologies facilitates the exploration of plant-microbe interactions in the context of MPs pollution. Studies have demonstrated significant effects of MPs on plant metabolism and gene expression. Exposure to polyurethane membrane shells, a form of MPs, in wheat led to the regulation of genes related to plant growth, such as those in the phenylpropanoid biosynthesis pathway, glycolysis, and oxidative stress responses. These changes in gene expression alter wheat root metabolism and increase oxidative stress, which in turn influences growth. However, biofilm-treated wheat displayed reduced oxidative stress through enhanced jasmonic acid and GABA metabolism [70]. In maize, transcriptomic and metabolomic analyses have revealed that both conventional and biodegradable MPs cause distinct changes in gene expression and metabolic pathways. These MPs regulate secondary metabolite biosynthesis, photosynthesis, carbohydrate metabolism, and plant hormone signaling pathways, including the MAPK signaling pathway, which plays a critical role in stress responses [71]. Lettuce exposed to MPs exhibited changes in the expression of antioxidant enzyme genes. These changes were influenced by the concentration and type of MP, with higher concentrations upregulating genes involved in antioxidant defenses and causing disruptions in various metabolic pathways. Notably, MPs affected glycerophospholipid and sphingolipid metabolism, which are involved in plant stress responses [72]. In rice, exposure to polystyrene MPs disturbed the biosynthesis of amino acids, fatty acids, and nucleic acids, leading to decreased growth and energy production. This disruption was linked to alterations in enzymatic activities and changes in metabolic pathways for plant health [69]. Larger MPs (5 μm) reduced photosynthetic capacity, while smaller MPs (0.1 μm) mitigated some of the negative effects by regulating intercellular CO2 concentration [73].

These studies highlight the broad range of molecular disturbances caused by MPs, including the regulation of antioxidant defenses, stress response pathways, and energy metabolism. Hence, the integration of omics technologies will provide important insights into how MPs exposure affects plant physiology and growth [70,71,73].

5.2. Advancements in machine learning and deep learning for MPs identification and environmental impact assessment

Machine learning (ML) and deep learning (DL) have emerged as powerful tools for analyzing and classifying MPs in environmental samples, offering faster and more accurate methods compared to conventional approaches [74,75]. Traditional ML models, such as random forest (RF), support vector machine (SVM), and k-nearest neighbors (KNN), have been widely used to classify MPs based on spectral data, significantly improving classification accuracy [76]. Furthermore, deep learning, particularly convolutional neural networks (CNNs), have shown remarkable performance in image-based MP identification, although challenges such as overfitting remain [77]. The application of ML in MPs research is driven by its ability to extract meaningful features from complex datasets, such as those obtained from Fourier-transform infrared spectroscopy, Raman spectroscopy, and near-infrared spectroscopy [76]. These techniques, combined with ML algorithms, allow for automated identification and classification of MPs, reducing human error and increasing efficiency [78]. However, ML models rely heavily on the quality and diversity of training datasets, necessitating standardized data-sharing practices to enhance predictive accuracy and generalizability [74] [79] Among ML techniques, RF and SVM have been identified as highly effective for MP classification due to their robust handling of high dimensional data and non-linear relationships [79]. Decision trees (DT) and principal component analysis (PCA) have also been employed to improve feature extraction and classification accuracy, with RF mitigating the risk of overfitting by aggregating multiple DT models [76]. Automated ML platforms, such as H_2O Auto ML, have been introduced to streamline model selection and hyperparameter tuning, thereby minimizing the need for expert intervention [80]. Deep learning, a subset of ML, has further enhanced MP detection by autonomously learning feature representations from data, particularly in image-based analyses [81]. CNN-based models have been applied to classify MPs mixed with organic matter in Raman spectra, achieving high classification accuracy [77]. These advancements demonstrate the potential of ML and DL to revolutionize MP analysis, making it more efficient, scalable, and objective [82]. Despite these advancements, challenges remain, including the need for large, high-quality datasets, improved generalization across diverse environmental conditions, and the development of user-friendly tools for non-experts [83]. Addressing these limitations will be essential to fully leverage ML and DL in assessing MP pollution and its environmental impact.

5.3. Other futures considerations

Current strategies to overcome this challenge include different alternatives for mitigating the stress caused by these contaminants and stress factors in both cultivated and wild plants. Biostimulants (BSts) are natural or synthetic substances that help plants to resist stress. BSts have displayed tremendous potential for mitigating the abiotic stressors that climate change has exacerbated without compromising crop production, productivity, and quality, which will fulfil the food and nutritional security requirements [159]. For example, AMF have been reported to be functional microbial-based biostimulants because of their beneficial effects on crops, including modulation of root architecture, improved nutrient uptake, translocation and assimilation of macro- and micronutrients, enhancement of photosynthesis, regulation of plant hormones, and modulation of the microbial community in the rhizosphere [160]. Furthermore, BSt can promote phytoremediation by promoting the absorption of contaminants and increasing the elimination process, as well as helping plants overcome the stress resulting from the presence of these xenobiotics. In other cases, PBs can limit the uptake of contaminant, thus allowing plants to survive the resulting damage [161]. In addition to benefiting plants in physiological processes, some microorganisms such as algae, fungi, and bacteria have been genetically improved (recombinant DNA technology, gene cloning, and genetic modification) to improve the bioremediation ability of microorganisms in the presence of different hydrocarbons and heavy metals, thereby providing promising tools for the recovery of contaminated soils [162]. Another alternative to phytoremediation is the use of plants to clean the environment, because there is evidence that plants can absorb MPs, which can help reduce their concentrations in soil and water [36,163]. Another option is the development of biotechnological approaches such as genetic engineering, genome editing, and RNA-mediated gene silencing using next-generation sequencing. Genome mapping has paved the way for precise and faster genetic modification of plants, which is used to improve plants adapted to an increasing number of challenges brought about by the changing climate [164]. Modification of agricultural practices, such as rational use and reduction, collection, reuse, and innovative recycling are key measures for curbing plastic pollution from agriculture. On the other hand, in addition to using the three Rs principle (Reduce, Reuse, Recycle), it is necessary to collect plastics after use and improve their durability. If we cannot collect some of the plastics we use, we must use less toxic and biodegradable polyethylene to replace conventional polyethylene with high persistence in the environment [165]. Another example is the use of sustainable practices such as organic agriculture, agroforestry, and permaculture. These practices can help to reduce pesticide and fertilizer use, improve soil health, and reduce greenhouse gas emissions. New technologies for sustainable agricultural production, such as vertical farming and hydroponics, can be used to grow food in urban areas and in areas with limited land or water resources. Utilization of insects is another innovative solution; for example, the use of *Tenebrio molitor*, *Zophobas morio*, and wax worms (*Galleria mellonella* L.), which use PS and PE for mineralization as carbon sources, without observable toxic effects. After complete digestion, their droppings can be used as a natural fertilizer because as larvae, they can be reared at high density to excrete nitrogen and chitin-rich droppings that have proven capable of replacing traditional NPK (nitrogen, phosphorus and potassium) fertilizers in a circular economy [166,167]. Artificial intelligence (AI) is another alternative for reducing the environmental impacts of climate change and improving the sustainability of food production. AI involves the development of algorithms and computational models that allow machines to process and analyze large amounts of data, identify patterns and relationships, and make predictions or decisions based on that analysis to generate critical data to refine strategies for the optimal exploitation of the data. resources. Its future aims to optimize food production and consumption and value chains, as well as minimize negative environmental impacts [168,169]. Despite these promising trends, several challenges still need to be addressed to ensure a sustainable future for food. One challenge is the need to increase food production to meet the needs of a growing population. Another challenge is the need to reduce the environmental impact of agriculture. By investing in research and development, we can understand the effects of new contaminants and implement measures to mitigate their effects. Studies in this area are promising and could lead to the development of novel strategies for protecting plants and ensuring food safety. These interactions can lead to a significant decline in biodiversity and ecosystem resilience [170]. Moreover, the sorption of harmful organic pollutants to MPs can enhance their bioavailability and toxicity, further complicating the ecological risks posed by these contaminants [171]. In addition, as MPs are ingested by organisms, they may facilitate the transfer of pollutants through the food chain, raising concerns regarding food safety and human health [172]. The potential of MPs to act as vectors for pathogens and antimicrobial resistance genes adds another layer of complexity to their ecological impact [173]. Future research should prioritize a multi-stressor approach to fully elucidate the interactions between MPs and environmental stressors. This includes examining the effects of MPs across various biological levels from individual organisms to the entire ecosystem. Understanding these interactions is essential for developing effective management strategies and policies aimed at mitigating the impacts of both MPs and environmental stressors on ecosystem health and sustainability The future of healthy and sustainable food production in the face of climate change and anthropogenic pollutants is both complex and challenging. However, the development and research of new trends and emerging technologies offers hope to reverse this uncertain global outlook.

6. Conclusions

Plants in agroecosystems are significantly affected by a variety of environmental stressors that impact on their physiology, crop performance, and food security. Recent studies have shown that the presence of MPs introduces new components into the plants which must be assessed. Different techniques such as FTIR, Raman spectroscopy or PyGC/MS are being applied to identification and quantification of plastic into the agroecosystem. Stress factors, such as high temperatures, salinity, drought, and specifically traces metals associated with MPs in the soil can shift and magnify the physiological response of plants. Although the effect depends on the duration of stress, species, concentration of MPs in the soil, and type of MPs present, this review also highlights that MPs derived from biodegradable plastics tend to affect plants more rapidly because of the rapid degradation of these compounds in the soil. In contrast, non-degradable MPs, such as polystyrene or polyethylene, initially improve soil conditions (enhancing structure and moisture retention), which may initially benefit plant performance. However, there is still insufficient evidence regarding the long-term damage caused by these non-degradable plastics, as well as the need to study various species of agricultural importance and their interactions with current environmental stressors.

It has been demonstrated that the interaction of MPs with elevated CO_2 levels can alter stomatal conductance, whereas high temperatures and drought conditions can increase oxidative stress and negatively affect vegetative growth. In addition, MPs can enhance the adsorption of trace metals onto plant tissues, compromising food safety and elevating health risks, indicating the need for further research on their interactions with various environmental stressors. Finally, the integration of omics technologies will provide valuable comprehension into the molecular mechanisms related to microplastic (MP) exposure, allowing an identification of specific biomarkers. Additionally, machine learning algorithms will analyze complex datasets to predict the effects of MPs on plant health and crop performance across various environmental conditions. The integration of these technologies is essential to consider novel agricultural practices and policy decisions. Hence, further studies on agroecosystems contaminated with MPs and their impact on the food chain are necessary, as well as standardize sampling methods for quantifying plastic concentrations in soils.

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CAPITULO III

Physiological and phytochemical responses of broccoli sprouts to micro-/nanoplastics, elevated CO_2, and heat stress, with predictive insights

Marcelo Illanes[1,2], Felipe Noriega[1,2], María-Trinidad Toro[3], Mauricio Schoebitz[2], Roberto Fustos-Toribio[5] Nelson Zapata[1], Diego A. Moreno[4]*, María Dolores López-Belchí[1]*

[1]Department of Plant Production, Faculty of Agronomy, University of Concepcion, Avenida Vicente Méndez, 595, Chillán, Chile; marceloillanes@udec.cl, fnoriega@udec.cl, nzapata@udec.cl, mlopezb@udec.cl

[2]Department of Soils and Natural Resources, Faculty of Agronomy, University of Concepcion, Victor Lamas 1290, Concepción, Chile; mschoebitz@udec.cl

[3]School of Nutrition and Dietetics, Faculty of Medicine and Health Science, Universidad Mayor, Temuco, Chile; maria.toror@umayor.cl

[4]Laboratorio de Fitoquímica y Alimentos Saludables (LabFAS), CEBAS, CSIC, Campus Universitario de Espinardo-25, 30100 Murcia, Spain. dmoreno@cebas.csic.es

[5]Department of Mining Engineering, Universidad de Concepción, 4030000 Concepción, Chile; robertofustos@udec.cl

* Correspondence: mlopezb@udec.cl; dmoreno@cebas.csic.es

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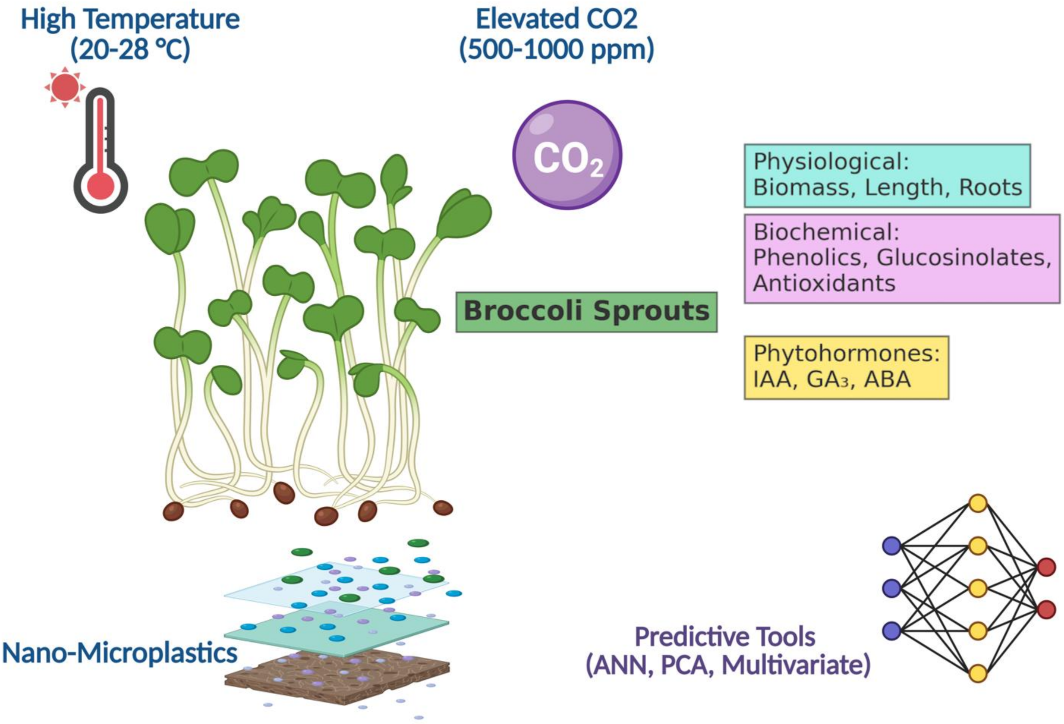
Highlights

- *Broccoli sprouts exposed to micro/nanoplastics, elevated* CO_2*, and high temperature showed marked physiological and biochemical changes.*
- *Temperature was the main stressor enhancing the content of glucosinolates but also affecting the growth (growth–defense trade-off).*
- CO_2 *enrichment had moderate and context-dependent effects, while plastics acted as secondary modulators.*
- *Multi-Stress conditions altered phytohormones, biochemistry, and the phenolic composition of broccoli sprouts.*
- *Neural-network modeling accurately predicted physiological responses, highlighting the value of predictive tools in plant-stress research.*

Graphical Abstract



Abstract

Broccoli sprouts (Brassica oleracea L. var. Italica) are rich in bioactive compounds with recognized health-promoting properties. However, emerging pollutants such as microplastics and nanoplastics (MNPs), together with climate-related stressors like elevated CO_2 and temperature, may alter their metabolic balance. This study evaluated the combined effects of polyethylene-derived MPs and polystyrene-derived NPs under simulated global warming conditions (1000 ppm CO_2, 28 °C) compared with current ambient levels (500 ppm, 20 °C). Temperature emerged as the dominant driver of plant responses. Elevated temperature increased total glucosinolates by 10–71% across treatments, while dry biomass decreased by 1–13%, revealing a clear growth–defense trade-off. CO_2 enrichment exerted moderate, context-dependent effects, stimulating glucosinolate and anthocyanin accumulation at 20 °C but suppressing them under heat. MNPs acted as secondary modulators, inducing indirect yet measurable changes in biomass and phenolic metabolism through interactive effects with CO_2 and temperature.

At moderate temperature, elevated CO_2 enhanced gibberellin content (+25–40%), whereas heat inhibited auxin signaling (−30%), indicating hormonal reconfiguration linked to defense activation. Multivariate and neural-network analyses ($R^2 = 0.86$–0.94) revealed temperature-driven shifts from growth-related phytohormones toward sulfur-rich and phenylpropanoid defenses. Hence, the phytochemical composition of broccoli sprouts proved highly sensitive to combined environmental stressors, with temperature as the primary regulator, CO_2 as a conditional modulator, and plastic particles exerting context-dependent effects on growth and defense metabolism.

Keywords: emergent contaminants, environmental stress, glucosinolates, plant secondary metabolites, predictive modelling

1. Introduction

Micro- and nanoplastics (MNPs) are emerging pollutants in agroecosystems that threaten soil health, crop productivity, and food security. Microplastics are generally defined as plastic particles smaller than 5 mm, which can further degrade into nanoplastics (<100 nm) through physical, chemical, and biological processes—including UV radiation, moisture, and microbial activity. This progressive fragmentation endows nanoplastics with distinct physicochemical properties and potentially greater bioavailability and toxicity. MNPs originate from a variety of agricultural and industrial sources, including the degradation of mulching films, irrigation systems, compost, and sewage sludge [1,2]. Their persistence in soils is particularly concerning because MNPs are not only resistant to degradation but also capable of interacting with plants, microorganisms, and soil components [3]. This dynamic creates multiple pathways through which MNPs can influence ecosystem functions and enter the food chain, raising both ecological and human health concerns [4,5].

Recent studies indicate that plants are able to absorb microplastics, leading to potentially detrimental effects on growth and development. MNPs in soil have been reported to interfere with key physiological processes such as photosynthesis and nutrient metabolism [6,7]. Furthermore, they can disrupt beneficial interactions between plants and arbuscular mycorrhizal fungi, reducing the fungi’s ability to enhance water and nutrient uptake [8].

In parallel, global agriculture is increasingly challenged by abiotic stressors linked to climate change, notably elevated carbon dioxide (CO_2) concentrations and rising temperatures. Atmospheric CO_2 has risen dramatically since pre-industrial times, enhancing photosynthesis and growth in many C3 plants by increasing carbon availability [9]. Under controlled conditions, elevated CO_2 often improves biomass accumulation, water-use efficiency, and tolerance to certain stresses. However, these benefits are frequently offset by heat stress, which negatively impacts key physiological processes such as flowering, fertility, and grain filling [10–12]. High temperatures promote the excessive formation of reactive oxygen species (ROS), causing oxidative damage, reducing chlorophyll content, and impairing photosynthesis [13–16]. The combined effects of elevated CO_2 and heat stress are complex and context-dependent, but they often lead to shortened growth cycles and significant yield losses in major crops [17–19].

Beyond their direct effects on plants, interactions among CO_2, temperature, and MPs add another layer of complexity. Elevated CO_2 can alter plant nitrogen uptake, while high temperatures exacerbate nutrient imbalances, and MPs further disrupt root architecture and nutrient dynamics [20–22]. These stresses may interact synergistically or antagonistically, making difficult predictions of agricultural outcomes. Thus, the combined presence of pollutants and climate-driven stressors represents a multidimensional challenge for sustainable food production.

The Brassicaceae family, which includes economically important crops such as broccoli (*Brassica oleracea* var. italica), kale, and mustard, is a particularly relevant model for studying plant responses to environmental stressors. Broccoli is valued not only for its nutritional properties but also for its high levels of secondary metabolites such as glucosinolates, flavonoids, and phenolic compounds. These bioactive molecules are associated with antioxidant, anti-inflammatory, and anticancer properties, and their biosynthesis is often stimulated under stress conditions [23–25]. Elevated CO_2 has been shown to promote biomass accumulation and glucosinolate production in broccoli and other Brassica species, but high temperatures frequently counteract these benefits by reducing growth and altering metabolite composition [26–28]. Importantly, environmental pollutants like MPs may further modulate these responses, either directly through physiological stress or indirectly by influencing soil–plant interactions [29]. In previous studies, we demonstrated that the glucosinolate content in broccoli and radish decreased with increasing MP exposure in the substrate, highlighting their role as biomarkers of stress [30].

Combining the convergence of MPs pollution, NPs emergence, elevated CO_2, and heat stress represents an unprecedented challenge for agroecosystems. Broccoli, due to its nutritional importance and sensitivity to environmental changes, serves as a valuable model for understanding plant physiological and biochemical responses under these conditions. Exploring the interactions among these stressors is essential not only to safeguard crop yields and food security but also to identify strategies for enhancing plant resilience through genetic, physiological, and agronomic approaches.

Hence, we hypothesize that exposure to MNPs alters the physiological and biochemical performance of broccoli sprouts under combined elevated CO_2 and high temperature, by disrupting growth, hormonal balance, and the accumulation of secondary metabolites. Furthermore, it is expected that elevated CO_2 partially mitigates MNP-induced stress at moderate temperature but fails to counteract its effects under heat stress, revealing a temperature-dependent trade-off between growth and defense. This study aims to assess the biochemical and physiological responses of broccoli sprouts exposed to MPs and NPs under combined scenarios of elevated CO_2 and high temperature. In addition, predictive modeling approaches will be employed to assess interactive stress effects and to provide a mechanistic understanding of plant adaptation under concurrent pollutant and climate-related stressors.

2. Material and methods

2.1. Experimental design

Broccoli sprouts (*Brassica oleracea* L. var. Italica) were grown in glass trays (3 cm high × 10 cm long × 6 cm wide) filled with coconut fiber substrate. Seeds (cv. Waltham-29, California, USA) were obtained from Semillera San Alfonso (Santiago, Chile). Three substrate treatments were established: (i) control with coconut fiber without plastic (WP), (ii) coconut fiber supplemented with polyethylene (PE) microplastics, and (iii) coconut fiber supplemented with polystyrene (PS) nanoplastics. Six replicate trays were prepared per treatment (n = 6). Each tray contained 1 kg of coconut fiber moistened with 700 mL of

Milli-Q water. For plastic treatments, aqueous suspensions of PE MPs or polystyrene (PS) NPs were applied via irrigation to the substrate establishment to achieve a nominal concentration of 500 mg kg^{-1}, dose previously reported as highly phytotoxic [31]. This application was performed only during the initial wetting of the substrate; all subsequent irrigations consisted solely of distilled water with no additional MNPs.

Broccoli seeds were surface-sterilized in 5 gL^{-1} sodium hypochlorite for 2 h, rinsed, and soaked for 24 h in aerated distilled water. Subsequently, 5 g of seeds were sown per tray (day 0). Trays were covered with aluminum foil and incubated in a growth chamber under complete darkness to promote germination. After three days, the foil was removed to allow shoot elongation.

Trays were then transferred to controlled-environment chambers according to treatment combinations. Twelve environmental treatments were applied, combining two temperature regimes (low: 20 °C/18 °C day/night; high: 28 °C/22 °C) with two CO_2 concentrations (500 ppm or 1000 ppm) across the three substrate conditions (WP, PS, and PE). The photoperiod was set at 16 h light /8 h dark with a photosynthetically active radiation (PAR) of 350 µmol m^{-2} s^{-1}. Relative humidity was maintained at 60% during the day and 80% at night. Trays were irrigated with Milli-Q water every two days.

Sprouts were grown for 12 days, harvested, weighed, and immediately frozen at −80 °C for biochemical analyses. A detailed physicochemical characterization of the coconut fiber substrate is provided in the Supplementary Materials (Table S1).

2.2. Preparation of polyethylene (PE)-MP and and polystyrene (PS)-NP suspensions.

Two types of plastic derivatives were used in this study: NPs derived from PS and MPs derived from PE. PS NPs were obtained from clean polystyrene boxes. The material was degraded using acetone at a ratio of 200 mL of acetone per 1 kg of polystyrene. The resulting polystyrene-acetone solution was transferred to laminar flow chambers to allow partial evaporation of acetone. Subsequently, the mixture was placed in an oven at 40°C for 7 days to ensure complete dehydration. The dry polystyrene material was then pulverized using a ball mill in 2-minute cycles until a nanoscale powder was obtained. PE were produced from high-density polyethylene (HDPE) pellets acquired from Sigma-Aldrich (St. Louis, MO, USA). The pellets were artificially aged for three months under ultraviolet (UV) radiation to simulate the environmental degradation. After aging, the pellets were mechanically ground using a laboratory ball mill to obtain the MPs particles. Particle size was analyzed by Laser analyzer Mastersizer 3000 (Malvern Instruments, Malvern, UK) and a Zetasizer Advanced Ultrared Label (Malvern Panalytical Ltd., UK), with granulometric parameters obtained via Gradistat v8.0. In addition, Fourier-transform infrared spectroscopy (FTIR) images of particles were collected using an FTIR spectrometer and microscope system (Spotlight 400, Perkin Elmer). Finally, particles were analyzed by scanning electron microscopy (SEM, Vega3 Easyprobe SBU, Tescan). Samples (whole and cross-sections) were mounted on stubs with double-sided adhesive tape, gold-coated, and examined at 15 kV and 20 mm working distance. Topographical images were acquired at magnifications from 830× to 7370× (resolving structures in the 50 nm–5 µm range) and from 49× to 1350× (20 µm–1000 µm), enabling characterization at both micro- and nanoscale levels (Figure 1S).

2.3. Assessment of Morphological Parameters in Broccoli Sprouts exposed to MNPs

Sprout growth parameters (fresh weight (g) and total length (cm)) were evaluated for each treatment group exposed to plastic particles. Twelve days after sowing, sprout length was measured from the base of the hypocotyl to the apical tip. Seedlings were subsequently scanned using the WinRHIZO Reg software (Regent Instruments Inc., Québec, Canada) to obtain detailed morphological information. After scanning, seedlings were manually divided into two parts: the radicle and the hypocotyl (aerial portion of the sprout). To remove any remaining substrate or plastic residues, radicles were rinsed three times with distilled water, air-dried for 24 h, and stored in labeled paper envelopes. Root morphological traits—including radicle length, surface area, mean diameter, and volume—were then quantified using WinRHIZO software. Six replicates were prepared per treatment.

2.4. Extraction and quantification of Phytohormones in broccoli sprouts exposed to MNPs

Phytohormone extraction were assessed following Pan *et al*. 2010 [32], with modifications optimized for our laboratory conditions. For extraction, 0.5 g of ground tissue was placed in 15 mL tubes containing 10 mL of extraction solvent (2-propanol/H_2O/concentrated HCl, 2:1:0.002, v/v/v). Samples were shaken at 100 rpm for 30 min at 4 °C. Subsequently, 20 mL of dichloromethane was added, and tubes were shaken again for 30 min under the same conditions. The mixtures were then centrifuged at 13,000 × *g* for 5 min at 4 °C to achieve phase separation. From the lower phase, 9 mL were carefully collected with a micropipette, transferred to a round-bottom flask, and evaporated to dryness by rotary evaporation at 40 °C. The residue was reconstituted in 4 mL of solvent, followed by the addition of 1 mL of methanol to obtain a final volume of 5 mL. The resulting solution was filtered through 0.22 μm PVDF syringe filters and transferred into vials for HPLC analysis. Six replicates were prepared per treatment (n = 6). The results were expressed as mg 100 g^{-1}.

2.5. Antioxidant response of sprouts exposed to microplastics: Catalase (CAT) activity

Catalase (CAT) extraction and activity were assessed following Kalir et al. (1984) [33] and Badiani et al. (1990) [34], with modifications optimized for our laboratory conditions. The assay quantified the amount of H_2O_2 decomposed by CAT over a defined time interval. Freshly germinated tissue (0.5 g) was homogenized in 5 mL of 25 mM HEPES buffer (pH 7.8), filtered through four layers of gauze, and centrifuged at 11,500 rpm for 20 min. A 0.5 mL aliquot of the supernatant (enzyme extract) was then mixed with 0.75 mL of 25 mM sodium phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7), 0.75 mL of 0.8 mM Na-EDTA, and 1 mL of H_2O_2 (nM). The mixture was gently agitated, and CAT activity was determined spectrophotometrically (Orion Aquamate 8000 UV-VIS Spectrophotometer) by recording the decrease in absorbance at 240 nm over 3 min, corresponding to H_2O_2 consumption in the reaction medium. Six replicates were prepared per treatment (n = 6). The results were expressed as $\mu mol \cdot min^{-1} \cdot g^{-1}$ FW.

2.6. Extraction of Glucosinolates from broccoli sprouts exposed to MNPs

For glucosinolate extraction, 100 mg of freeze-dried broccoli sprout powder was mixed with 1 mL of 70% methanol (v/v). The samples were vortexed briefly and immediately placed in a thermostatically controlled water bath at 70°C for 20 min. During incubation, the samples were agitated every 5–10 min using a vortex mixer to enhance compound extraction. After heating, the tubes were transferred to ice for 5 min to stop the extraction process. The samples were centrifuged at 3000 rpm for 30 min, and the supernatant was carefully collected. The extracts were filtered through a 0.22 µm PVDF membrane filter and transferred to amber vials for subsequent analysis by LC-MS.

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2.7. Chromatographic Identification and Quantification of Glucosinolates

Glucosinolates were identified and quantified using HPLC-DAD-ESI-MSn (Agilent Technologies, Waldbronn, Germany), based on their UV–Vis absorption spectra, retention times, and characteristic fragmentation patterns (MS and MS^n), following the protocol described by Baenas et al. (2016) [35]. Chromatograms were acquired at 227 nm, and quantification was achieved using glucoerucin and glucobrassicin as external standards for aliphatic and indolic glucosinolates, respectively (Sigma-Aldrich, St. Louis, MO, USA). All analyses were conducted in sextuplicate, and the results were expressed as mg per 100 g dry weight (DW).

2.8. Extraction and Quantification of Phenolic Compounds from broccoli sprouts exposed to MNPs

For the extraction and quantification of phenolic compounds, 100 mg of freeze-dried plant material was used per sample. Extraction was performed using 1 mL of a solvent mixture (25:1:24, v/v/v) composed of methanol, water, and formic acid. Samples were first vortexed to homogenize the material, then subjected to ultrasonication for 1 h, followed by 24 h of extraction at 4°C. After extraction, the samples were vortexed again to ensure uniformity and centrifuged at 3000 rpm for 30 min. The supernatant was carefully collected and filtered through a 0.22 µm PVDF membrane (Millex V13, Millipore, Bedford, MA, USA), and the final extracts were stored in amber vials for subsequent chromatographic analysis. All solvents used for extraction were of analytical grade and obtained from Merck (Darmstadt, Germany).

Phenolic profiles were determined using an Agilent HPLC 1100 series system equipped with a photodiode array detector and a tandem mass spectrometer (Agilent Technologies, Waldbronn, Germany). The system included a binary pump (model G1312A), autosampler (model G1313A), degasser (model G1322A), and photodiode array detector (model G1315B), all operated with ChemStation software (Agilent, version 08.03). Chromatographic separation was performed on a Luna C18 column (250 × 4.6 mm, 5 µm, 100 Å; Phenomenex, Torrens, CA, USA), protected with Security Guard Cartridges PFD C18 (4 × 3.0 mm). The mobile phases were deionized water/formic acid (99:1, v/v; phase A) and methanol (phase B). The flow rate was set to 0.9 mL/min with an injection volume of 20 µL.

Mass detection was carried out with an ion trap spectrometer (model G2445A) equipped with an electrospray ionization (ESI) interface and controlled by LCMSD software (Agilent, version 4.1). The optimized ionization parameters were capillary temperature 350 °C, voltage 4 kV, nebulizer pressure 65 psi, and nitrogen flow 11 L/min. Full-scan spectra were recorded across an m/z range of 100–1200. Collision-induced fragmentation was performed in the ion trap using helium as the collision gas with voltages ramped from 0.3 to 2 V.

Quantification was performed by external calibration with authentic standards freshly prepared each day*p*-coumaric acid (320 nm), (320 nm), quercetin-3-O-rutinoside (360 nm), cyanidin-3-O-glucoside (520 nm), and sinapic acid (320 nm). Six replicates were prepared per treatment (n = 6), and results were expressed as milligrams per 100 gram of dry weight (mg 100 g^{-1} dw).

2.9. Antioxidant capacity in sprouts exposed to MNPs

The oxygen radical absorbance capacity (ORAC-FL) assay [36] were used to measure free radical scavenging activity, with minor modifications. Analyses were performed in black 96-well microplates (Nunc, Roskilde, Denmark) using a Synergy H1 multimode microplate reader (BioTek, Winooski, VT, USA). Trolox was used as the calibration standard. In each well, 25 µL of sample extract, standard, or blank and 150 µL of fluorescein solution were added, and the plate was incubated at 37 °C for 30 min. The reaction was initiated by adding 25 µL of AAPH solution, and fluorescence was recorded every minute for 60 min at excitation and emission wavelengths of 485 nm and 520 nm, respectively. Antioxidant capacity was calculated from the difference in the area under the fluorescence decay curve between the sample and the blank. Results were expressed as mg Trolox equivalents per 100 g dry weight (mg TE 100 g^{-1} DW), using six replicates.

2.10. Statistical and multivariate analyses of broccoli sprouts exposed to MNPs under varying environmental conditions.

Biochemical and morphophysiological variables (glucosinolate profiles by subtype and totals; sinapic-derivative phenylpropanoids and anthocyanins—including totals; phytohormones IAA/GA_3/IBA; and dry weight) were analyzed with univariate inference such as Analysis of Variance (ANOVA) followed by Tukey's post hoc test was used; results were deemed significant at $\alpha = 0.05$ ($p < 0.05$) and were computed in R (v4.0.5). To evaluate the effects of the experimental factors—microplastic type, CO_2 concentration, and temperature— on plant biochemical and physiological variables, we implemented a multivariate statistical and predictive-modeling framework in R (v4.0.5). Correlation analysis was first conducted by computing a Pearson correlation matrix between bioactive compounds (individual and total glucosinolates, phenolics, sinapate derivatives, and phytohormones) and physiological parameters (dry weight), an exploratory step that characterized linear associations among variables and informed subsequent dimensionality reduction. Principal Component Analysis (PCA) was then performed on mean-centered data to summarize multivariate variability and reduce redundancy; principal components were retained based on explained variance and inspection of the scree plot, biplots were generated to visualize variable contributions, and the first six components were selected for downstream analyses. Assumption checks preceded parametric modeling: multivariate normality was assessed with Mardia's test, where skewness ($p = 0.38$)

indicated no serious departure while kurtosis ($p = 0.047$) suggested mildly heavier tails; accordingly, although strict multivariate normality was not fully met, we proceeded with Multivariate Analysis of Variance (MANOVA) using Pillai’s trace criterion given its robustness under modest assumption violations, treating the first six principal components as dependent variables to test the effects of the experimental factors on the principal dimensions of the system. Artificial Neural Network (ANN) modeling was finally employed via a multilayer perceptron with three input variables (microplastic type, CO_2, and temperature), three fully connected hidden layers, and six output nodes corresponding to the selected principal components; training was carried out using backpropagation, and predictive performance was quantified with root-mean-square error (RMSE) and the coefficient of determination (R^2).

3. Results and discussion

3.1. Morphological Response to Combined Stressors

Assessing CO_2 × Temperature treatments, the impact of MNPs was most pronounced in hypocotyl traits (Fig. S2). The radicle length remained unaffected indicating that the harvested biomass (yield) was not linked to axial root elongation. At 500 ppm CO_2/20 °C, fresh weight increased under PE compared with PS or WP, while sprout length (hypocotyl + cotyledon) and dry weight showed only minor variations. At 1000 ppm CO_2/20 °C, PS reduced dry weight relative to PE, with WP showing intermediate values and no clear effects on fresh weight or sprout length. Under elevated temperature (28 °C), plastics appeared to buffer heat stress. At 28 °C, PS—and to a lesser extent PE—supported shoot biomass relative to WP (fresh weight: +56.6 % at 500 ppm and +30.1 % at 1000 ppm CO_2; dry weight at 1000 ppm: +16.7 %), while PE was generally intermediate. At 20 °C, differences were smaller and not always consistent across CO_2 levels. Fresh weight responded more strongly than dry weight, and root length showed no consistent plastic-specific pattern (Fig. S2), indicating that plastic effects were primarily linked to hypocotyl allocation and/or water relations rather than root foraging.

These results are consistent with reports showing that plant responses to MPs can be neutral or even transiently positive, largely due to changes in substrate physical properties (e.g., porosity, evaporation, water retention) rather than direct particle toxicity [37–39]. Indeed, non-degradable polymers such as PE and PS often elicit heterogeneous outcomes depending on particle type, size, concentration, soil texture, and exposure duration [40,41]. In Brassicaceae sprouts, LDPE has been shown to induce dose-dependent effects on biomass and secondary metabolites such as glucosinolates, with neutral or positive outcomes at low doses but predominantly negative responses at medium to high levels. Symplastic transport of aggregated MPs has been demonstrated in rapeseed [42], while in pak-choi, PE (virgin and aged) exhibited dose- and stage-specific effects, altering growth traits [43]. Previous works support the interpretation that the short-term thermal buffering we observed reflects substrate-mediated physical effects, whereas higher doses, prolonged exposure, or greater system complexity tend to shift the balance toward adverse consequences for plant physiology and soil biota.

3.2. Phytohormonal Signaling (IAA, GA_3, IBA)

Phytohormonal profiles revealed that indole-3-acetic acid (IAA) remained relatively stable across treatments and environments (0.19–0.49 µg g^{-1} DW; Fig. 1), with only minor, non-systematic fluctuations. This stability is in line with previous reports showing context-dependent auxin responses, while warming can enhance auxin levels and signaling in *Arabidopsis* seedlings (e.g., 28 °C vs. 20 °C) [44], in broccoli sprouts, temperature, rather than CO_2, appears to exert a stronger influence on hormonal composition, including IAA [27]. In contrast, gibberellic acid (GA_3) exhibited the clearest environment × polymer interaction, with mean concentrations peaking at 14.6 µg g^{-1} DW under PS at 1000 ppm CO_2/20 °C, compared to 11.7 µg g^{-1} in WP and 7.4 µg g^{-1} in PE. Under heat (28 °C), GA_3 declined to ≈ 7–10 µg g^{-1} DW (−30–40 %), confirming thermal repression of GA biosynthesis. This agrees with evidence that heat stress suppresses GA production and signaling, and that fine-tuning GA pathways is key for thermotolerance [45]. The effects of elevated CO_2 (eCO_2) were moderate and context-dependent—enhancing GA_3 at 20 °C but not at 28 °C—consistent with studies showing that eCO_2 can reconfigure hormonal pathways, including auxins and gibberellins, in a species- and tissue-specific manner [46] and promote GA/auxin-mediated adjustments of root growth [47]. Indole-3-butyric acid (IBA) remained at low concentrations (0.006–0.041 µg g^{-1} DW) without significant variation across conditions (Fig. 1), which is plausible given that under low-to-moderate stress, hormonal signaling is often preserved and microplastic-induced shifts are subtle [48]. Altogether, these quantitative patterns highlight temperature as the dominant regulator of hormonal balance -particularly GA$_3$-, while CO$_2$ and microplastics act as secondary modulators with strongly environment-dependent effects.

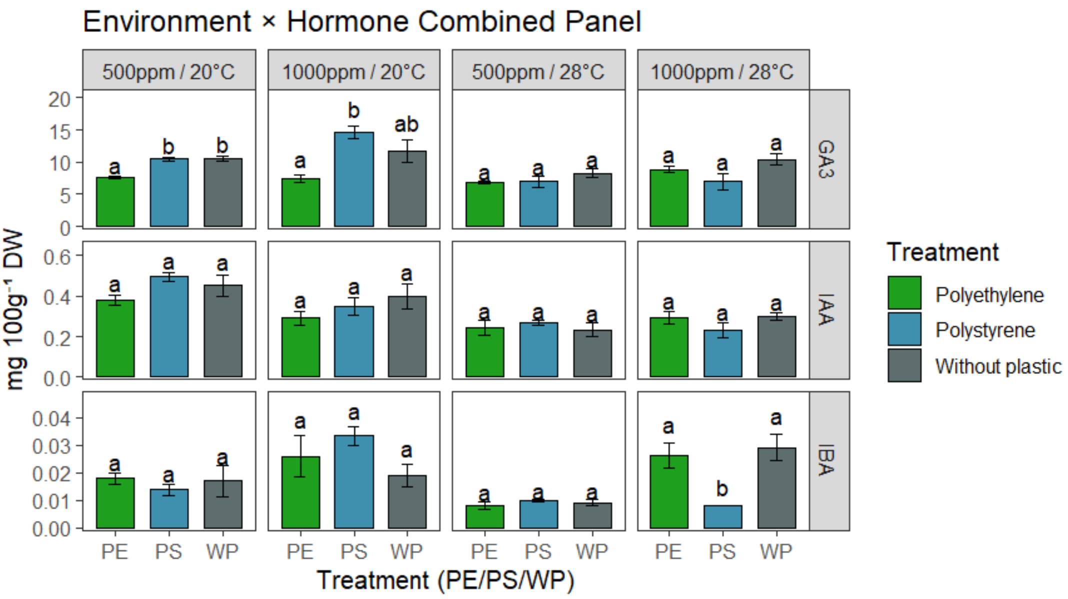


Figure 1 Changes in phytohormone concentrations under varying environmental conditions.

WP: without plastic; PS: with polystyrene; PE: with polyethylene. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

3.3. Antioxidant Enzyme Activity (Catalase) in broccoli sprouts

CAT activity showed no significant differences among PE, PS, and the WP control across environments (Fig. S3). Absolute activities were moderate, with no differences between treatments by environment (~1.48–17.02 $\mu mol \cdot min^{-1} \cdot g^{-1}$ FW). Temperature exerted a stronger influence than polymer type (mean CAT activity at 20 °C, at 500 and 1000 ppm CO$_2$) exceeded the corresponding values at 28 °C, with the most pronounced decline under PE. Shifts associated with elevated CO$_2$ were minor and inconsistent. In general, CAT activity did not differentiate between MPs treatments at the sampling time, and its modulation was primarily driven by temperature rather than CO_2. In this study, we prioritized CAT activity as an enzymatic marker of H_2O_2 detoxification because of its very high catalytic capacity and central peroxisomal role during germination and early growth in Brassica [49,50]. As a high-throughput route for H_2O_2 removal, CAT provides a robust readout of ROS balance in seedlings and is less susceptible than peroxidase-based pathways to transient inhibition or fluctuations in reductant supply [51,52].

3.4. Bioactive compounds in broccoli sprouts exposed to micro/nanoplastics

3.4.1 Glucosinolates

Temperature emerged as the main determinant of glucosinolate accumulation in sprouts (mg 100 g^{-1} DW). Across CO_2 regimes, raising the temperature from 20 to 28 °C nearly doubled glucosinolate levels (≈1000–1200 vs. ≈500–800 mg 100 g^{-1} DW) (Table 1). By contrast, increasing CO_2 from 500 to 1000 ppm produced only modest effects: at 20 °C concentrations were slightly lower at 1000 ppm, whereas at 28 °C CO_2 exerted no significant influence. The role of MPs was minor and environment dependent. A treatment contrast was detected only under 500 ppm CO_2 / 20 °C, where PE plants accumulated more glucosinolates than WP (PS intermediate). In all other conditions, polymer identity did not differentiate responses. In general, warming strongly enhanced glucosinolate content, elevated CO_2 had at most a mild attenuating effect at 20 °C and a neutral effect at 28 °C, and microplastics exerted only secondary, context-specific influences.

Table 1 Glucosinolate content by environment and polymer in broccoli sprouts (mg g^{-1} DW).

GSL compounds	Temperature = 20 °C						Temperature = 28 °C					
	CO_2 = 500 ppm			CO_2 = 1000 ppm			CO_2 = 500 ppm			CO_2 = 1000 ppm		
	WP	**PS**	**PE**	**WP**	**PS**	**PE**	**WP**	**PS**	**PE**	**WP**	**PS**	**PE**
GRA	122.7 ± 14.4a	197.6 ± 44b	208.1 ± 17.9b	176.4 ± 26.9a	134.5 ± 60.0a	330.5 ± 44.1b	303.5 ± 21.7b	184 ± 69.3a	147.4 ± 33.6b	388.6 ± 11.9a	327.1 ± 30a	363.1 ± 10.3a
HGB	59.7 ± 15.9a	73.3 ± 22.3a	71 ± 21.5a	60.2 ± 3.1a	70.9 ± 17.8a	68.7 ± 7a	92.3 ± 7.6a	92.7 ± 24.9a	58.9 ± 7.2a	57.7 ± 7.4a	63.4 ± 5.0a	68.5 ± 11.9a
GER	208 ± 21a	246 ± 15a	252 ± 42a	162 ± 13a	179 ± 20a	202 ± 15a	385 ±.44a	540 ± 108a	423 ± 74a	306 ± 43a	376 ± 94a	292 ± 3a
GBS	92.4 ± 12a	94.5 ± 8.1a	110.9 ± 9.6a	89.5 ± 12.4a	93.9 ± 6.2a	76.4 ± 6.8a	72.8 ± 7a	109.9 ± 24.2a	85.4 ± 9a	62.8 ± 9a	56.4 ± 7.8a	72.1 ± 7.1a
MGB	116.6 ± 7.1ab	108.8 ± 14.1a	146.2 ± 19.4b	78.8 ± 1.7a	92.4 ± 13.9a	95.9 ± 22.6a	122.4 ± 8.9a	164.6 ± 14b	149.7 ± 22.8ab	144.2 ± 8.2a	143.8 ± 22.7a	139.5 ± 1.6a
Total GSL	600 ± 51a	720 ± 56ab	788 ± 89b	567 ± 24a	571 ± 104a	773 ± 71b	977 ± 60a	1091 ± 234a	864 ± 96a	934 ± 96a	982 ± 136a	945 ± 9a

WP: without plastic; PS: with polystyrene; PE: with polyethylene. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

Our results indicate that temperature is the primary determinant of glucosinolate content: at 28 °C, concentrations were consistently higher than at 20 °C, while the effects of CO_2 were weak or absent, and the presence of MPs (PE, PS, WP) acted as a secondary, environment-dependent factor (a single contrast PE > WP observed only under 500 ppm CO_2 / 20 °C). This pattern aligns with the literature reporting marked thermal sensitivity of the GSL pathway and class-/tissue-specific profile shifts, including increases under certain high-temperature conditions in cultivated Brassica [53,54], and cooling-specific modulations in other species [55,56]. Responses to elevated CO_2 are context-dependent: CO_2 enrichment can stimulate the pathway (e.g., in broccoli sprouts; [28] or attenuate it via nitrogen dilution and altered C/N balance [57], consistent with the lack of a robust CO_2 effect in our system. Finally, although reductions in GSL under microplastic exposure have been reported [31], along with metabolic alterations associated with particle uptake [58], the signal under our conditions was minimal, suggesting that, within the tested ranges, MPs did not durably reprogram GSL metabolism. Collectively, these findings indicate that warming dominates GSL homeostasis, whereas CO_2 and microplastics exert smaller and more context-dependent effects.

Beyond total amounts, the class balance shifted in a predictable way: at 28 °C all treatments converged to an aliphatic-dominant profile (~70:30 aliphatic:indolic) (Fig. 2), whereas at 20 °C the composition diverged, with PE showing a higher aliphatic share and PS retaining comparatively more indolics.

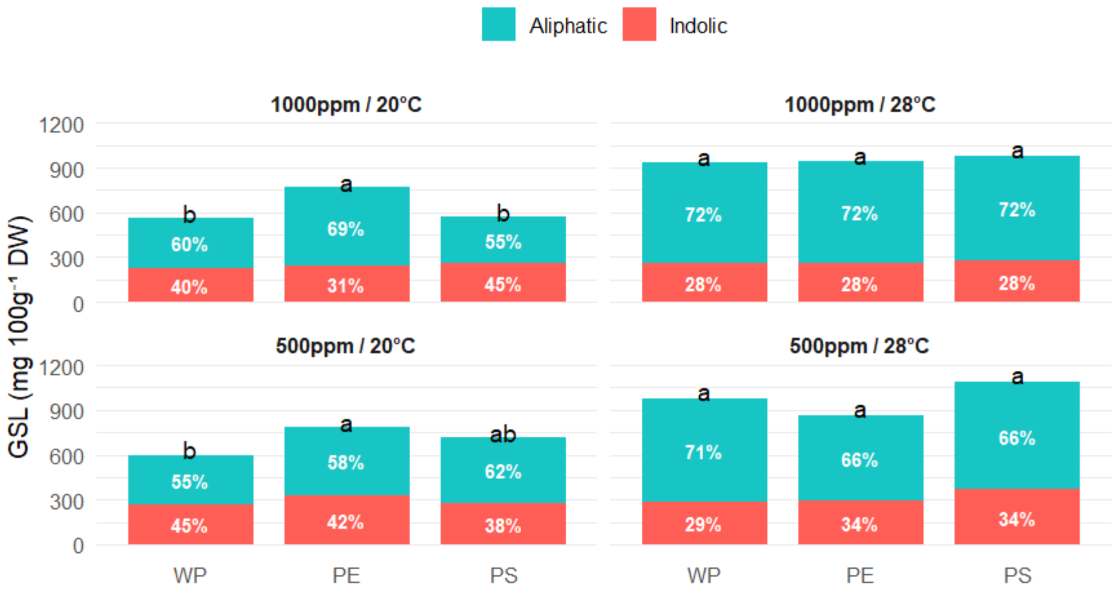


Figure 2. Concentration of aliphatic and indolic glucosinolates under varying environmental conditions.

WP: without plastic; PS: with polystyrene; PE: with polyethylene. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

This pattern is consistent with a temperature, and redox-driven activation of the aliphatic branch (MYB28/MYB29) under warming, whereas cooler conditions maintain indolic signaling through MYB34/MYB51/MYB122 and its crosstalk with auxin. Polymer identity acted only as a weak modifier of this baseline. PE promoted sulfur-rich aliphatic allocation via physical effects on soil structure and water status, while PS aligned with a mild biotic-like/oxidative shift toward indolic metabolism [55,59–61]. In soils, microplastic influences typically arise from changes in porosity, aggregation, and water retention rather than strong chemical cues, which explains the small and environment-dependent contrasts observed here [37,38].

3.4.2 Phenolic compounds: Anthocyanins and sinapic acid derivatives

Across environments, anthocyanin concentrations showed limited sensitivity to polymer identity and CO_2 (Table 2). A single environment-specific effect was detected at 500 ppm CO_2 / 28 °C, where PS > WP, with PE intermediate, indicating a light polymer-dependent stimulation of anthocyanin accumulation under warming at ambient CO_2. At 20 °C (both CO_2 levels) and at 1000 ppm CO_2 / 28 °C, anthocyanin levels did not differ among PE, PS, and WP. Changes attributable to elevating CO_2 from 500 to 1000 ppm were small and unsystematic, whereas temperature effects were context dependent. Similarly, sinapic acid-derived phenylpropanoids remained statistically unchanged across the treatments (Table 3). These results indicate that, under the tested conditions, the phenylpropanoid pathway leading to anthocyanins provides a more sensitive readout to warming in combination with polymer exposure, whereas sinapic derivatives appear comparatively buffered, and CO_2 enrichment exerts only a minor modulatory effect.

The environment-specific panels indicated that anthocyanins varied little with polymer identity or CO_2, with a single environment-specific difference (PS > WP at 500 ppm CO_2 / 28 °C), whereas sinapic acid-derived phenylpropanoids remained statistically unchanged. This pattern is consistent with reports on broccoli, which showed that total anthocyanins are relatively heat-resilient, while higher temperatures increase other phenolics (including sinapic, ferulic, and *p*-coumaric acids), suggesting a redistribution of phenylpropanoid flux rather than a uniform decline in anthocyanins [62]. In kale, temperature has been shown to fine-tune the sinapate branch: disinapoyl-gentiobiose increases at higher temperatures, whereas lower temperatures favor complex sinapoyl-acylated flavonols, supporting the buffering effect observed here for total sinapates [63,64]. The effects of elevated CO_2 are generally context-dependent. Although CO_2 enrichment can stimulate phenolic accumulation or antioxidant capacity in Brassicaceae, responses are not uniform across tissues and conditions, consistent with the absence of a systematic CO_2 effect in our study [28,65]. Finally, while microplastic exposure can alter the phytochemical profile of Brassica sprouts, including shifts in anthocyanins, the direction and magnitude of the response depend on the dose, polymer type, and developmental stage, such that small or null impacts, such as those observed here, are plausible under low-to-moderate stress [31,48]. Our results align with the view that temperature is the dominant modulator of phenylpropanoid metabolism in Brassica species, whereas CO_2 and microplastics act as secondary, strongly environment-dependent modulators.

Table 2. Anthocyanin composition by environment and polymer in broccoli sprouts (mg 100 g^{-1} DW).

Anthocyanin compounds	**Temperature = 20 °C**						**Temperature = 28 °C**					
	CO_2 = 500 ppm			**CO_2 = 1000 ppm**			**CO_2 = 500 ppm**			**CO_2 = 1000 ppm**		
	WP	**PS**	**PE**	**WP**	**PS**	**PE**	**WP**	**PS**	**PE**	**WP**	**PS**	**PE**
Cy 1+2	0.0001 ± 0.0a	0.0004 ± 0.0a	0.0005 ± 0.0a	0.0002 ± 0.0a	0.0001 ± 0.0a	0.0002 ± 0.0a	0.0010 ± 0.0b	0.0031 ± 0.0a	0.0028 ± 0.0a	0.0020 ± 0.0a	0.0012 ± 0.0a	0.0013 ± 0.0a
Cy 3	0.0003 ± 0.0a	0.0002 ± 0.0a	0.0005 ± 0.0a	0.0008 ± 0.0a	0.0006 ± 0.0a	0.0008 ± 0.0a	0.0019 ± 0.0b	0.0036 ± 0.0a	0.0020 ± 0.0b	0.0016 ± 0.0a	0.0012 ± 0.0a	0.0008 ± 0.0a
Cy 4	0.0079 ± 0.0a	0.0087 ± 0.0a	0.0100 ± 0.0a	0.0096 ± 0.0a	0.0128 ± 0.0a	0.0140 ± 0.0a	0.0110 ± 0.0a	0.0117 ± 0.0a	0.0123 ± 0.0a	0.0086 ± 0.0a	0.0103 ± 0.0a	0.0083 ± 0.0a
Cy 5	0.0030 ± 0.0a	0.0036 ± 0.0a	0.0041 ± 0.0a	0.0042 ± 0.0a	0.0044 ± 0.0a	0.0053 ± 0.0a	0.0043 ± 0.0a	0.0059 ± 0.0a	0.0062 ± 0.0a	0.0040 ± 0.0a	0.0048 ± 0.0a	0.0033 ± 0.0a
Total Anthocyanins	0.0112 ± 0.0a	0.0129 ± 0.0a	0.0150 ± 0.0a	0.0148 ± 0.0a	0.0179 ± 0.0a	0.0204 ± 0.0a	0.0182 ± 0.0a	0.0243 ± 0.0a	0.0233 ± 0.0a	0.0162 ± 0.0a	0.0175 ± 0.0a	0.0136 ± 0.0a

WP: without plastic; PS: with polystyrene; PE: with polyethylene. Cy 1 and 2: Cy-3-(sinapoyl)diglc-5-glc + Cy-3-(feruloyl)dglc-5-glc; Cy 3: Cy-3-(p-coumaroyl)(sinapoyl)dglc-5-glc; Cy 4: Cy-3-(sinapoyl)(sinapoyl)dglc-5-glc; Cy 5: Cy-3-(sinapoyl)(sinapoyl)dglc-5(malonyl)glc. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

Table 3. Sinapics composition by environment and polymer in broccoli sprouts (mg 100 g^{-1} DW).

Sinapic compounds	Temperature = 20 °C						Temperature = 28 °C					
	CO_2 = 500 ppm			CO_2 = 1000 ppm			CO_2 = 500 ppm			CO_2 = 1000 ppm		
	WP	**PS**	**PE**	**WP**	**PS**	**PE**	**WP**	**PS**	**PE**	**WP**	**PS**	**PE**
Isorhmn-glc	0.02 ± 0.01a	0.02± 0.00a	0.02 ± 0.004a	0.02 ± 0.00a	0.03 ± 0.00b	0.02 ± 0.004a	0.02 ± 0.006a	0.02± 0.001a	0.02 ± 0.003a	0.01 ± 0.001b	0.02 ± 0.003c	0.000 ± 0a
Kae-pC-SG	0.21 ± 0.08a	0.23± 0.02a	0.25 ± 0.018a	0.14 ± 0.07a	0.18 ± 0.00a	0.18 ± 0.025a	0.21 ± 0.017a	0.23 ± 0.009a	0.21 ± 0.019a	0.20 ± 0.015a	0.19 ± 0.035a	0.19 ± 0.014a
SG	3191± 39b	418 ± 46a	273 ± 26a	2856 ± 458a	2977 ± 39a	2627± 352a	2569 ± 755b	3517 ± 307b	265 ± 97a	172 ± 149a	358 ± 82a	333 ± 137a
DSG	467 ± 44a	528 ± 285a	850 ± 284a	765 ± 213b	658 ± 114b	236 ± 113a	285 ± 92a	1181 ± 336b	192 ± 77a	466 ± 294a	2838 ± 2573a	3801 ± 838a
SFG	733 ± 14b	867 ± 62b	193 ± 19a	142 ± 18b	193 ± 27b	621 ± 212a	287 ± 143a	570 ± 318a	272 ± 41a	271 ± 266a	230 ± 94a	131 ± 125a
UKN-1	1080 ± 38a	249 ± 27b	821 ± 257a	859 ± 54a	1053 ± 289a	813 ± 184a	315 ± 156a	483 ± 160a	280 ± 82a	3013 ± 2829a	359 ± 622a	1075 ± 463a
TSG	314 ± 45a	862 ± 237b	461 ± 103a	253 ± 31a	1118 ± 183a	3672 ± 2505a	321 ± 27b	228 ± 86a	161 ± 32a	104 ± 92a	1675 ± 1455a	3309 ± 3667a
DSFG	5439 ± 79a	5416 ± 313a	5713± 842a	4958 ± 672a	4039 ± 2500a	2077 ± 2686a	5704 ± 1711b	6225± 296b	2251 ± 178a	6480 ± 2537a	1604 ± 1410a	2562 ± 2579a
UNK-2	283 ± 38a	337 ± 31a	4036 ± 3470a	305 ± 78a	258 ± 102a	334 ± 112a	298 ± 48b	241 ± 88b	8005± 182a	209 ± 222a	150 ± 137a	182 ± 98a
Total Sinapics	11508 ± 211a	8678 ± 770a	12347 ± 4172a	10137 ± 883a	10297 ± 2450a	10380 ± 859a	9779 ± 1411b	12446 ± 796a	11426 ± 238a	10713 ± 4645a	7215 ± 21229a	11392 ± 1847a

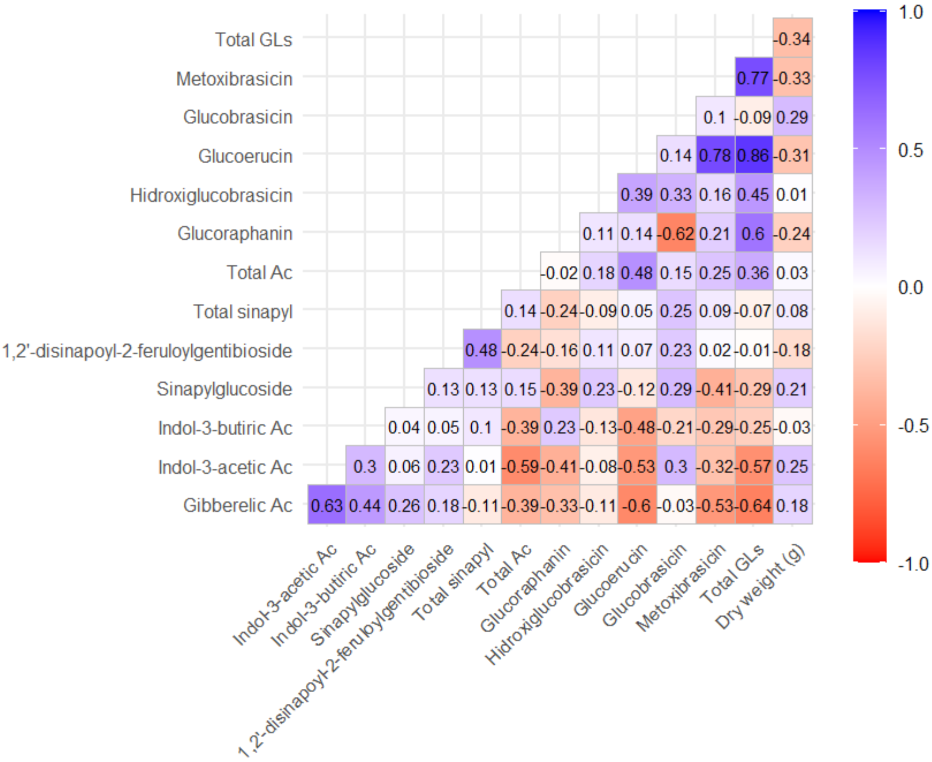
WP: without plastic; PS: with polystyrene; PE: with polyethylene. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test. Isorhmn-glc: Isorhamnetin-3-sophoroside-7-glucoside; Kae-pC-SG: Kaempferol-3-O-(p-coumaroyl)-sophoroside-7-O-glucoside; SG: Sinapylglucoside; DSG: 1,2-Disinapoylgentiobioside; SFG: 1-Sinapoyl-2-feruloylgentiobioside; TSG: 1,2,2′-Trisinapoylgentiobioside; DSFG: 1,2′-Disinapoyl-2-feruloylgentiobioside; UNK-1: Unknown compound 1 (sinapate derivative); UNK-2: Unknown compound 2 (sinapate derivative).

3.5. Antioxidant Capacity (ORAC) from broccoli sprouts exposed to micro/nanoplastics

Total antioxidant capacity (ORAC; Fig. S4) was primarily influenced by temperature and its interaction with CO_2 and polymer identity. At 500 ppm CO_2 / 20 °C, treatments were statistically indistinguishable. At 1000 ppm CO_2 / 20 °C, PE < PS (WP intermediate), whereas at 500 ppm CO_2 / 28 °C, WP < PE = PS. At 1000 ppm CO_2 / 28 °C, PE exceeded PS (WP intermediate). Thus, warming produced context-dependent shifts in ORAC: under elevated CO_2, PE and WP showed recovery or enhancement, while PS exhibited relative suppression. Because ORAC integrates the phenolic pool, these outcomes align with reports that temperature reprograms phenylpropanoid allocation in Brassica, typically redistributing between hydroxycinnamates and flavonoids rather than uniformly altering anthocyanins [62–64]. Elevated CO_2 can also enhance antioxidant capacity and bioactive metabolites in broccoli sprouts, though in a context-dependent fashion, consistent with the PE and WP increases observed at 1000 ppm / 28 °C [28]. Finally, modulation by microplastics is consistent with studies reporting MP-induced oxidative stress and variable antioxidant responses in Brassicaceae, with net effects often small or absent under low-to-moderate stress, depending on polymer type, dose, and environment [31,48].

3.6. Multivariate analysis and predictive models

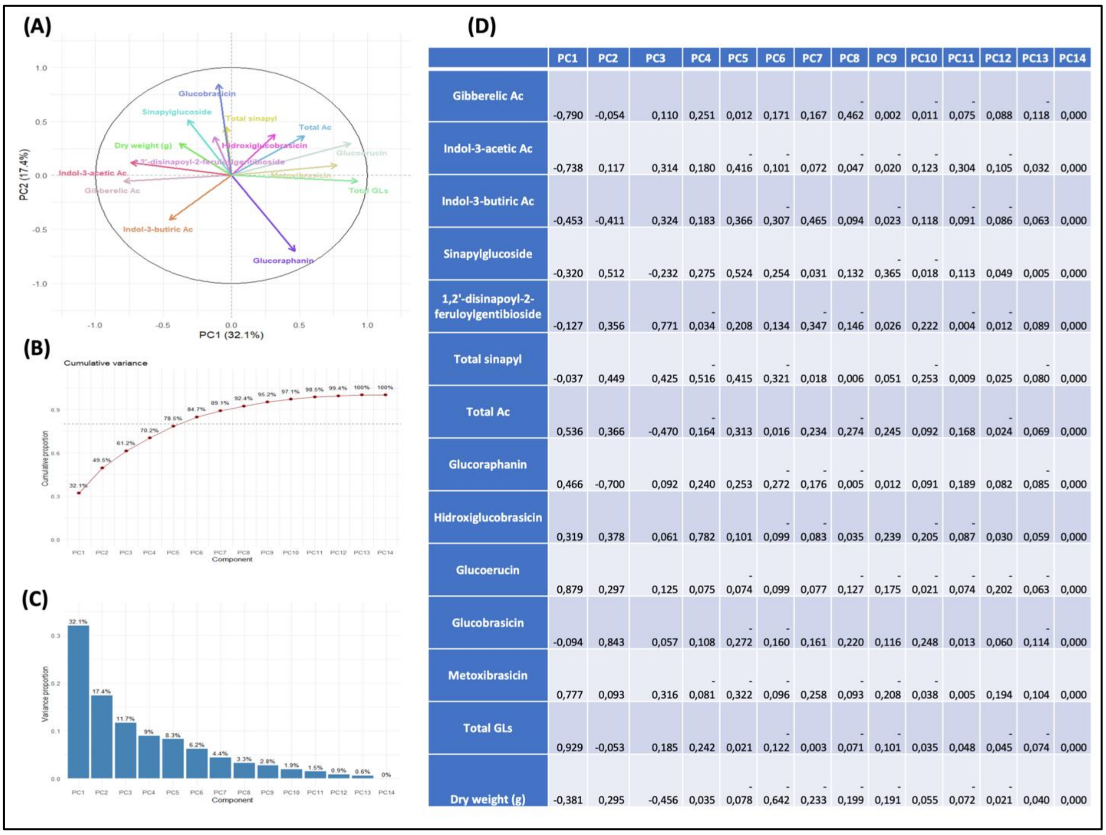
Correlation analyses and PCA revealed a dominant axis (PC1) that opposed glucosinolates (Total GLs, glucoraphanin, and methoxyglucobrassicin) to growth hormones (GA_3 and IAA), with loadings of opposite sign and a negative association with dry weight (GL–DW correlations between −0.30 and −0.34) (Fig. 3). PC1 explained 32.1 % and PC2 17.4 % of the total variance, together capturing nearly half (49.5 %) of the multivariate structure. PC1 was dominated by total glucosinolates (loading = 0.93) and methoxyglucobrassicin (0.77) on the positive side, in contrast to GA_3 (−0.79) and IAA (−0.74) on the negative side, confirming a clear antagonism between defense and growth metabolism. This pattern supports the interpretation that biomass decreases as chemical defense intensifies, consistent with the negative association of these hormones with dry weight (−0.34). Altogether, these results indicate a growth–defense trade-off, where allocation to defensive metabolites is favored at the expense of growth markers and biomass [66,67].



	Indol-3-acetic Ac	Indol-3-butiric Ac	Sinapylglucoside	1,2'-disinapoyl-2-feruloylgentibioside	Total sinapyl	Total Ac	Glucoraphanin	Hidroxiglucobrasicin	Glucoerucin	Glucobrasicin	Metoxibrasicin	Total GLs	Dry weight (g)
Total GLs													-0.34
Metoxibrasicin												0.77	-0.33
Glucobrasicin											0.1	-0.09	0.29
Glucoerucin										0.14	0.78	0.86	-0.31
Hidroxiglucobrasicin									0.39	0.33	0.16	0.45	0.01
Glucoraphanin								0.11	0.14	-0.62	0.21	0.6	-0.24
Total Ac							-0.02	0.18	0.48	0.15	0.25	0.36	0.03
Total sinapyl						0.14	-0.24	-0.09	0.05	0.25	0.09	-0.07	0.08
1,2'-disinapoyl-2-feruloylgentibioside					0.48	-0.24	-0.16	0.11	0.07	0.23	0.02	-0.01	-0.18
Sinapylglucoside				0.13	0.13	0.15	-0.39	0.23	-0.12	0.29	-0.41	-0.29	0.21
Indol-3-butiric Ac			0.04	0.05	0.1	-0.39	0.23	-0.13	-0.48	-0.21	-0.29	-0.25	-0.03
Indol-3-acetic Ac		0.3	0.06	0.23	0.01	-0.59	-0.41	-0.08	-0.53	0.3	-0.32	-0.57	0.25
Gibberelic Ac	0.63	0.44	0.26	0.18	-0.11	-0.39	-0.33	-0.11	-0.6	-0.03	-0.53	-0.64	0.18

Figure 3. Correlation matrix of glucosinolates, phytohormones, and biomass traits.

This multivariate pattern aligns with our univariate results, in which glucosinolates increased with temperature, whereas biomass and GA_3 tended to be depressed or decoupled from the defense profile, with evidence for cross-regulation between aliphatic/indolic MYBs and growth hormones in Brassicaceae [55,56]. Samples exposed to higher temperatures clustered toward the positive side of PC1—associated with defensive metabolism—while control or low-temperature plants were aligned on the negative side, characterized by higher GA_3, IAA, and biomass. A strong opposition between hormonal (GA_3, IAA) and defensive (total GLs, methoxyglucobrassicin) vectors was evident in the biplot (Fig. 4A), further illustrating the trade-off between growth and defense processes. PC2 separates glucobrassicin and sinapics (e.g., sinapyl glucoside, 1,2′-disinapoyl-2-feruloylgentiobioside) positively from glucoraphanin, reflecting a reorganization between indolic and aliphatic/sinapics pools (Fig 4).



	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Gibberelic Ac	-0,790	-0,054	0,110	0,251	0,012	0,171	0,167	-0,462	0,002	-0,011	-0,075	0,088	-0,118	0,000
Indol-3-acetic Ac	-0,738	0,117	0,314	0,180	-0,416	-0,101	0,072	-0,047	-0,020	-0,123	0,304	-0,105	0,032	0,000
Indol-3-butiric Ac	-0,453	-0,411	0,324	0,183	0,366	-0,307	0,465	0,094	-0,023	0,118	-0,091	-0,086	0,063	0,000
Sinapylglucoside	-0,320	0,512	-0,232	0,275	0,524	0,254	0,031	0,132	-0,365	-0,018	0,113	0,049	0,005	0,000
1,2'-disinapoyl-2-feruloylgentibioside	-0,127	0,356	0,771	-0,034	0,208	0,134	-0,347	-0,146	0,026	0,222	-0,004	-0,012	0,089	0,000
Total sinapyl	-0,037	0,449	0,425	-0,516	0,415	-0,321	0,018	0,006	0,051	-0,253	0,009	0,025	-0,080	0,000
Total Ac	0,536	0,366	-0,470	-0,164	0,313	0,016	0,234	-0,274	0,245	0,092	0,168	-0,024	0,069	0,000
Glucoraphanin	0,466	-0,700	0,092	0,240	0,253	-0,272	-0,176	-0,005	0,012	0,091	0,189	0,082	-0,085	0,000
Hidroxiglucobrasicin	0,319	0,378	0,061	0,782	0,101	-0,099	-0,083	0,035	0,239	-0,205	-0,087	0,030	0,059	0,000
Glucoerucin	0,879	0,297	0,125	0,075	-0,074	0,099	0,077	-0,127	-0,175	-0,021	-0,074	-0,202	-0,063	0,000
Glucobrasicin	-0,094	0,843	0,057	0,108	-0,272	-0,160	0,161	0,220	0,116	0,248	0,013	0,060	-0,114	0,000
Metoxibrasicin	0,777	0,093	0,316	-0,081	-0,322	-0,096	0,258	-0,093	-0,208	-0,038	0,005	0,194	0,104	0,000
Total GLs	0,929	-0,053	0,185	0,242	0,021	-0,122	0,003	-0,071	-0,101	0,035	0,048	-0,045	-0,074	0,000
Dry weight (g)	-0,381	0,295	-0,456	0,035	-0,078	-0,642	-0,233	-0,199	-0,191	0,055	-0,072	-0,021	0,040	0,000

Figure 4. Principal component analysis (PCA) of glucosinolates, phytohormones, and plant biomass, (a) PCA biplot showing the distribution of traits across the first two principal components (PC1 and PC2), with arrows indicating the contribution of each variable, (b) Scree plot of the proportion of variance explained by each component, (c) Cumulative variance explained by successive PCs, showing that the first components account for most of the variation, (d) Loadings of each variable on the principal components, highlighting those with the strongest associations.

This is consistent with reports that warming and other stressors modulate phenylpropanoid and GL metabolism in a class-specific manner—with sinapates relatively dampened or redirected, and differential changes between indolic and aliphatic GLs [53,54,63,64]. Thus, the PC1–PC2 plane captures two interpretable biological dimensions, defense vs. growth and internal partitioning among GL/sinapate families. MANOVA (Table S2) confirms that PC1, the broadest physiological dimension, is sensitive to all three factors (MPs, CO_2, temperature), whereas PC2 and PC5 respond primarily to CO_2, and PC6 to micro/nanoplastics, validating at a latent scale what is observed at the trait level, temperature as a dominant modulator of the defense–growth axis (increased GLs, hormonal adjustment, and biomass changes), elevated CO_2 with light, context-dependent effects on specific pathways (phenylpropanoids and GL subclasses), and micro/nanoplastics with detectable but bounded impacts, especially on the component loading dry weight and IBA, consistent with mild physico-chemical microstress and fine, tuned growth adjustments [37,39,68,69]. The negative loadings of GA_3 (−0.79) and IAA (−0.74) on PC1 and their vectorial opposition to GLs in the biplot support the interpretation that gibberellins and auxins antagonize GL accumulation under the evaluated scenarios. The literature shows that heat tends to attenuate GA signaling and readjust auxin homeostasis, favoring tolerance traits over vegetative expansion [44,45], consistent with the greater defense load concentrated by PC1 under warm conditions.

A neural-network model was trained using temperature, CO_2 concentration, and micro/nanoplastic exposure as input variables, and the first six principal components (PC1–PC6) as outputs (Fig. 5). This framework allowed us to evaluate how environmental and pollutant factors jointly predict the major axes of physiological and biochemical variation in broccoli sprouts. Model performance was highest for the leading components—PC1 to PC3 (R^2 = 94.4 %, 92.1 %, and 86.7 %; RMSE = 0.69, 0.60, and 0.63, respectively)—and declined for PC4–PC6 (R^2 = 59.4–83.7 %; RMSE = 0.58–0.89). These results indicate that most of the variability induced by temperature, CO_2, and MNPs is structured and predictable, yet exhibits nonlinear relationships that cannot be fully captured by traditional linear models. Importantly, the model demonstrates that environmental conditions—particularly temperature—can independently drive the main functional transitions in plants, such as the shift between growth and defense-oriented metabolism. Overall, the neural-network approach supports the use of machine-learning tools to anticipate integrated plant responses under complex global-change scenarios, highlighting that the leading latent axes (PC1–PC3) contain the most robust and biologically meaningful predictive signals [68–70] (Fig 5).

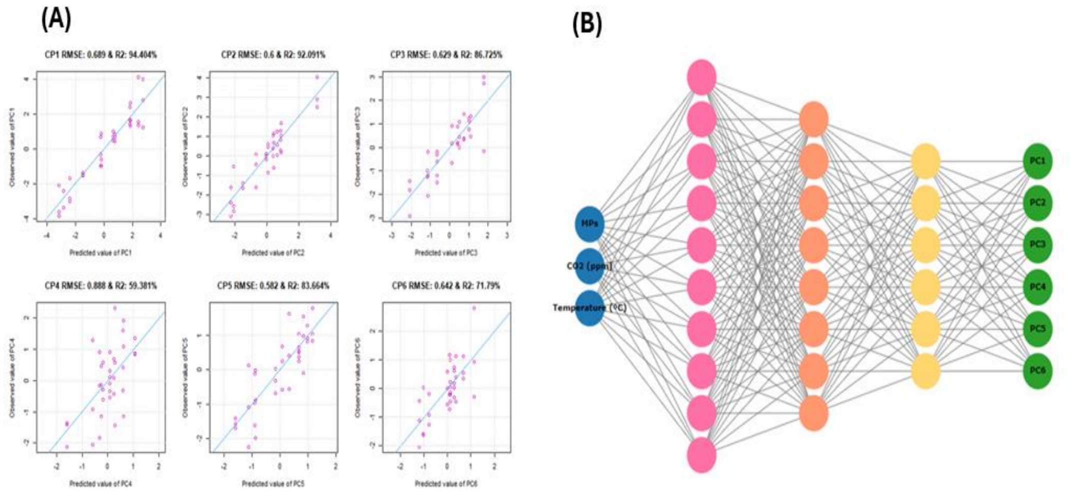


Figure 5. Predictive modeling of PCA components (a) using a neural network (b): architecture and model performance.

In synthesis, the integration of correlations, PCA, MANOVA, and neural-network modeling converges on the view that warming reallocates resources from hormone-mediated growth toward sulfur-rich defenses and phenylpropanoids. Elevated CO_2 modulates subsets of pathways (notably PC2 and PC5) with effects dependent on nutritional context and tissue state, whereas microplastics induce measurable but secondary adjustments along the axis associated with biomass and IBA. Importantly, both multivariate and machine-learning models reveal that the balance between growth and defense in Brassicaceae does not occur randomly but rather emerges from complex yet predictable interactions between plant hormones and environmental cues. This multivariate reading, consistent with our prior univariate analyses, provides a mechanistic framework to interpret how climate-change factors and pollutants jointly reconfigure growth–defense homeostasis in Brassicaceae.

In spite of biodegradable plastics (e.g., PBAT, PLA, PHA) were not evaluated in this study it is important to consider them. The available evidence indicates that these materials can elicit earlier [71] and more pronounced plant–soil responses than polyethylene or polystyrene [72], owing to the release of assimilable carbon and reactive degradation intermediates capable of restructuring the soil microbiome and modulating nitrogen availability [73–76]. Accordingly, our inferences should be regarded as conservative with respect to bioplastics. Future investigations should employ orthogonal experimental designs—crossing polymer chemistry with particle size/shape and climatic factors—to rigorously contrast the rapid bioactivity characteristic of biopolymers with the predominantly substrate-physical, short-term responses documented for PE/PS.

Conclusions

Temperature emerged as the dominant driver of broccoli sprout responses, enhancing glucosinolate biosynthesis while constraining biomass production. Elevated CO_2 exerted moderate and context-dependent effects, stimulating metabolic and hormonal activity under moderate conditions but losing its influence under heat stress. In contrast, micro- and nanoplastics acted as secondary modulators, subtly altering biomass and phenolic composition depending on the surrounding environment.

Glucosinolates behaved as early-defense markers, showing activation under moderate stress and attenuation under combined or severe conditions, reflecting a resource reallocation from defense to maintenance. Hormonal profiling revealed a CO_2- and temperature-dependent reconfiguration of gibberellin and auxin signaling consistent with the growth–defense balance. Together, these coordinated shifts across metabolic and hormonal networks underline the integrative nature of plant stress responses.

The joint action of CO_2 enrichment, elevated temperature, and microplastics resulted in predominantly antagonistic effects, where the stimulatory influence of CO_2 was offset by thermal and pollutant stress. Multivariate and neural-network analyses validated this pattern, confirming a temperature-centered hierarchy of regulation and highlighting the predictive power of machine-learning tools for disentangling complex environmental interactions.

This study advances a mechanistic understanding of how plants integrate multiple concurrent stressors in a climate-contaminant framework. Future research should couple physiological and omics-based approaches to elucidate how plastic particles intersect with hormonal and redox regulation, improving predictions of crop resilience under emerging global-change scenarios.

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DISCUSIÓN GENERAL Y CONCLUSIONES

Esta Tesis Doctoral integró, por primera vez en un marco coherente, la evidencia conceptual y experimental sobre cómo los microplásticos (MPs) interactúan con estreses abióticos típicos del cambio climático, particularmente CO_2 elevado y altas temperaturas, para reconfigurar la fisiología y la bioquímica de cultivos de interés alimentario. El Capítulo II estableció que los MPs constituyen un modulador transversal de procesos vegetales (germinación, desarrollo radicular, fotosíntesis, metabolismo secundario e interacciones rizosféricas), y destacó que su co-ocurrencia con estreses ambientales genera respuestas mayormente no aditivas, con implicancias para productividad, calidad e inocuidad de alimentos. En paralelo, se sintetizaron avances metodológicos clave para su detección y caracterización (FTIR, Raman, pirolisis-GC/MS e imágenes hiperespectrales), al tiempo que se argumentó la necesidad de marcos estandarizados y de enfoques ómicos y de aprendizaje automático para proponer biomarcadores y mejorar la capacidad predictiva en escenarios complejos.

El Capítulo III aportó evidencia experimental bajo un diseño factorial en brotes de brócoli que combinó dos niveles de CO_2, dos regímenes de temperatura y tres condiciones de plástico (sin plástico, poliestireno y polietileno). Los resultados permiten concluir, que la temperatura es el determinante primario de las respuestas fisiológicas y bioquímicas. A 28 °C se intensificó la acumulación de glucosinolatos y se reprogramó el metabolismo fenólico, mientras que a 20 °C predominó un perfil más defensivo asociado a actividad enzimática antioxidante. El CO_2 elevado actuó como modulador secundario, con efectos contextuales y consistentes sobre el balance crecimiento–defensa. El tipo de plástico (polietileno o poliestireno) explicó variación adicional solo en escenarios específicos, confirmando un papel terciario y dependiente del ambiente. Estas conclusiones derivan de patrones convergentes observados en hormonas de crecimiento, enzimas antioxidantes y metabolitos especializados, y de contrastes significativos en el contenido total de glucosinolatos dominados por la señal térmica.

El análisis multivariante reveló como la planta responde a esta combinación de estreses, un eje fisiológico dominante que contrapone metabolitos defensivos frente a hormonas de crecimiento y biomasa, y un segundo eje que separa compuestos indólicos/sinapatos de alifáticos. Esta arquitectura confirma un trade-off crecimiento–defensa como principio organizador bajo estrés combinado, y explica por qué los efectos de los MPs no se comprenden plenamente desde variables aisladas ni bajo condiciones ambientales únicas. En síntesis, los MPs funcionan como moduladores que amplifican o reconfiguran respuestas inducidas por el ambiente, pero cuyo efecto aislado queda eclipsado por la dominancia térmica.

Desde la perspectiva de seguridad alimentaria y sustentabilidad, el trabajo muestra que la exposición simultánea a MPs y a condiciones climáticas extremas puede afectar rasgos de desempeño con impacto directo en la calidad y el rendimiento, además de facilitar la transferencia de contaminantes asociados a la cadena alimentaria. En el plano ecosistémico, la interacción CO_2–temperatura–MPs complejiza los flujos de carbono y la dinámica microbiana del suelo, lo que exige enfoques integrados de manejo y control para evitar externalidades no previstas. En este sentido, el Capítulo II identifica rutas plausibles de riesgo y la brecha de conocimiento que persiste cuando se extrapolan resultados de estreses individuales a escenarios multiestrés.

En términos metodológicos y de gestión del conocimiento, esta Tesis Doctoral nos deja cuatro mensajes o conclusiones principales:

Primera, la estandarización de métodos para muestreo, extracción, identificación y cuantificación de MPs en matrices agrícolas es condición necesaria para comparabilidad y meta-análisis de alto poder inferencial.

Segunda, la integración de ómicas y modelos predictivos (incluyendo aprendizaje automático) es promisoria para construir paneles de biomarcadores, explorar relaciones no lineales, y anticipar puntos de quiebre fisiológicos bajo multiestrés.

Tercera, la incorporación de diseños factoriales con control riguroso de variables ambientales es crucial para estimar efectos principales e interacciones y evitar conclusiones sesgadas por confusiones en la interpretación de los datos experimentales.

Cuarta, la lectura multivariante de la fisiología, que priorice ejes latentes por sobre marcadores aislados, aumenta la capacidad explicativa y la transferibilidad de hallazgos entre especies y ambientes.

Las limitaciones detectadas a lo largo de estas investigaciones abren líneas claras de trabajo futuro. Falta densidad de estudios en condiciones de campo que representen gradientes realistas de contaminación y clima, y que integren períodos críticos del cultivo más allá de la etapa de brotes. Se requiere resolver incertidumbres sobre dosis–respuesta en rangos ambientalmente plausibles, efectos del tamaño y envejecimiento de partículas y la identidad del plástico, y su interacción con propiedades del suelo. Igualmente, urge conectar respuestas de corto plazo con consecuencias funcionales a escala de ciclo y poscosecha (estabilidad de fitoquímicos, calidad nutricional y tecnológica), incorporando métricas de desempeño agronómico y de inocuidad en un mismo marco experimental.

A la luz de los resultados, se recomiendan tres lineamientos operativos: (i) Adoptar enfoques de gestión multiestrés que prioricen la mitigación térmica (por ejemplo, manejo microclimático, fechas de siembra, selección varietal y sombreados), dado su peso determinante sobre la fisiología; (ii) incorporar vigilancia de MPs y de co-contaminantes en suelos y sustratos, alineada con protocolos estandarizados y con capacidades analíticas validadas; (iii) desplegar analítica avanzada (ómicas y modelos predictivos) integrada a redes de monitoreo para anticipar riesgos y orientar decisiones de manejo y política pública en tiempo oportuno. Estas acciones permitirán resguardar productividad e inocuidad en un contexto de intensificación climática y de contaminación emergente.

A modo de recapitulación, la Tesis establece un paradigma integrador, la temperatura gobierna la arquitectura de respuesta de los cultivos, mientras que el CO_2 y la identidad del plástico actúan como moduladores secundarios y dependientes del ambiente; los MPs, más que agentes con efectos uniformes, son amplificadores contextuales de sensibilidades preexistentes. Este marco ayuda a reconciliar discrepancias de la literatura, orienta el diseño experimental hacia factores de mayor varianza explicada y sugiere trayectorias tecnológicas y de gestión para transitar desde la detección y descripción del problema hacia su predicción y mitigación efectiva en sistemas agroalimentarios reales.

GENERAL DISCUSSION AND CONCLUSIONS

This Doctoral Thesis integrates, within a single coherent framework, conceptual and experimental evidence on how microplastics (MPs) interact with abiotic stressors characteristic of climate change—particularly elevated CO_2 and high temperatures—to reconfigure plant physiology and biochemistry in food-relevant crops. Chapter I establishes that MPs act as cross-cutting modulators of plant processes (germination, root development, photosynthesis, secondary metabolism, and rhizosphere interactions) and emphasizes that their co-occurrence with environmental stressors elicits predominantly non-additive responses, with implications for productivity, quality, and food safety. In parallel, the chapter synthesizes key methodological advances for MP detection and characterization (FTIR, Raman spectroscopy, pyrolysis–GC/MS, and hyperspectral imaging), while arguing for standardized frameworks and the integration of omics and machine-learning approaches to propose biomarkers and improve predictive capacity under complex scenarios.

Chapter II provides experimental evidence from a factorial design in broccoli sprouts combining two CO_2 levels, two temperature regimes, and three polymer conditions (no plastic, polystyrene, and polyethylene). The results support, robustly, that temperature is the primary determinant of physiological and biochemical responses: at 28 °C, glucosinolate accumulation intensified and phenolic metabolism was reprogrammed, whereas at 20 °C a more defensive profile associated with antioxidant enzymatic activity prevailed. Elevated CO_2 acted as a secondary modulator, exerting context-dependent yet consistent effects on the growth–defense balance. Polymer identity explained additional variation only in specific scenarios, confirming a tertiary, environment-dependent role. These conclusions derive from convergent patterns across growth hormones, antioxidant enzymes, and specialized metabolites, and from significant contrasts in total glucosinolate content dominated by the thermal signal.

Multivariate analyses revealed the latent structure of the response: a dominant physiological axis opposing defensive metabolites to growth hormones and biomass, and a second axis separating indolic/sinapate derivatives from aliphatic compounds. This architecture confirms a growth–defense trade-off as an organizing principle under combined stress and clarifies why MP effects cannot be fully understood from isolated variables or single environmental conditions. In brief, MPs function as contextual modulators that amplify or reconfigure environment-induced responses, while their isolated effect is overshadowed by thermal dominance.

From the perspectives of food safety and sustainability, the work shows that simultaneous exposure to MPs and extreme climatic conditions can affect performance traits with direct impacts on quality and yield, in addition to facilitating the transfer of associated contaminants along the food chain. At the ecosystem level, CO_2–temperature–MP interactions complicate carbon fluxes and soil microbial dynamics, demanding integrated management and surveillance to avoid unforeseen externalities. In this regard, Chapter I identifies plausible risk pathways and the persistent knowledge gap that arises when extrapolating single-stressor results to multi-stressor scenarios.

Methodologically and in terms of knowledge management, the thesis advances four central messages or general conclusions:

First, the standardization of sampling, extraction, identification, and quantification methods for MPs in agricultural matrices is a necessary condition for comparability and meta-analyses with high inferential power.

Second, the integration of omics and predictive modeling (including machine learning) is promising for constructing biomarker panels, exploring non-linear relationships, and anticipating physiological tipping points under multi-stressor conditions.

Third, the adoption of factorial designs with rigorous control of environmental variables is crucial to estimate main effects and interactions and to avoid confounding-driven conclusions in the interpretation of the research results.

Fourth, a multivariate reading of plant physiology—prioritizing latent axes over isolated markers—enhances explanatory power and the transferability of findings across species and environments.

The identified limitations throughought these research activities delineate clear avenues for future research. There is a lack of field studies that represent realistic gradients of contamination and climate and that encompass critical crop periods beyond the sprout stage. Uncertainties remain regarding dose–response relationships within environmentally plausible ranges, the effects of particle size and aging, polymer identity, and their interaction with soil properties. Equally urgent is the connection between short-term responses and functional consequences at the whole-cycle and postharvest scales (stability of phytochemicals, nutritional and technological quality), incorporating agronomic performance and food-safety metrics within a single experimental framework.

In light of these results, three operational guidelines are recommended. (i) Adopt multi-stressor management approaches that prioritize thermal mitigation (e.g., microclimate management, planting dates, varietal selection, and shading), given its determinative influence on plant physiology; (ii) implement surveillance of MPs and co-contaminants in soils and substrates, aligned with standardized protocols and validated analytical capabilities; and (iii) deploy advanced analytics (omics and predictive models) integrated with monitoring networks to anticipate risks and inform management and policy decisions in a timely manner. These actions will help safeguard productivity and safety in a context of intensifying climate pressures and emerging contamination.

Summarizing, the Thesis establishes an integrative paradigm: temperature governs the architecture of crop responses, whereas CO_2 and polymer identity act as secondary, environment-dependent modulators; MPs—rather than agents with uniform effects—are contextual amplifiers of pre-existing sensitivities. This framework helps reconcile discrepancies in the literature, orients experimental design toward factors with greater explained variance, and suggests technological and management trajectories to progress from mere detection and description of the problem toward its prediction and effective mitigation in real agro-food systems.

V. DIVULGACIÓN DE RESULTADOS

Proyectos

1. FONDECYT REGULAR: Interactions of Food Bioactives from Brassica Sprouts and Gut Microbiota (“Sprouts4healthyGut”). 1201950. Agencia Nacional de Investigación y Desarrollo (ANID), Chile.

2. FONDECYT REGULAR: Assessment Of Exposure Of Micro(nano)plastics On The Physiology And Metabolic Profiling Of Brassicas. 1240947. Agencia Nacional de Investigación y Desarrollo (ANID), Chile.

3. Proyecto de vinculación con el medio bidireccional (VRIM2504) - 2025 - Compromiso UdeC para la reducción de las pérdidas y el desperdicio de alimentos. Vicerrectoría de Vinculación con el Medio (UdeC), Chile

Artículos (S.C.I.)

1. Illanes M., MT Toro, M. Schoebitz, N. Zapata, DA. Moreno, MD. López-Belchí. Integrating microplastic research in sustainable agriculture: Challenges and future directions for food production, Current Plant Biology, Volume 42, (2025). https://doi.org/10.1016/j.cpb.2025.100458.
2. Illanes M. MT Toro, F. Noriega, M. Schoebitz, R. Fustos-Toribio, N. Zapata, DA. Moreno, MD. López-Belchí. Physiological and phytochemical responses of broccoli sprouts to micro-/nanoplastics, elevetaed CO_2, and heat stress, with predictive insights. 2025.
(Enviado a: Environmental Science & Technology - ACS Publications)

Participación en otras publicaciones

3. Maycotte, P., Illanes, M. & Moreno, D.A. Glucosinolates, isothiocyanates, and their role in the regulation of autophagy and cellular function. Phytochem Rev 24, 49–83 (2024). https://doi.org/10.1007/s11101-024-09944-w.
4. López-Belchí, M.D., Toro, M.T., Illanes, M. et al. Correction: Exploring strategies to grow wild turnip sprouts as healthy food. Chem. Biol. Technol. Agric. 11, 59 (2024). https://doi.org/10.1186/s40538-024-00585-x.
5. M.D. López, M.T. Toro, G. Riveros, M. Illanes, F. Noriega, M. Schoebitz, C. García-Viguera, D.A. Moreno, Brassica sprouts exposed to microplastics: Effects on phytochemical constituents, Science of The Total Environment, Volume 823, (2022). https://doi.org/10.1016/j.scitotenv.2022.153796.
6. Toro, M.T.; Ortiz, J.; Becerra, J.; Zapata, N.; Fierro, P.; Illanes, M.; López, M.D. Strategies of Elicitation to Enhance Bioactive Compound Content in Edible Plant Sprouts: A Bibliometric Study. Plants (2021), 10, 2759. https://doi.org/10.3390/plants10122759.

Congresos, seminarios y conferencias

1. 1st Interdisciplinary Congress with a Focus on Sustainable Agroindustry (Noviembre 2024). Presentación de póster: "Efectos de las Luces LED Monocromáticas y nanoplásticos en el crecimiento, composición nutricional y propiedades bioactivas de Mizuna (Brassica rapa var. nipposinica)". Illanes M., M.D. López y D. Moreno.
2. IV Workshop de Jóvenes Investigadores en Ciencias Agronómicas (9-10 de enero de 2024). Presentación oral: "Efectos de la combinación de Luz LED monocromática y el microplástico en la fisiología y los compuestos bioactivos de brotes de Mizuna". Primer lugar en trabajos orales.

3. 8th Jornadas Doctorales EIDUM-EINDOC-CMN, Universidad de Murcia, España (26-28 de junio de 2023). Presentación oral: "Efecto de alta temperatura y aumento de CO2 en el crecimiento de Brócoli (Brassica oleracea var. italica)".

4. III Workshop de Jóvenes Investigadores en Ciencias Agronómicas (10-11 de enero de 2023). Presentación oral: "Ácido Salicílico como elicitor para mitigar el estrés ambiental y mejorar la calidad de frutos en postcosecha".

5. II Congreso de Jóvenes Investigadores en Ciencias Agronómicas (11-12 de enero de 2022, Termas de Catillo, Parral). Presentación oral: "Efectos de microplástico sobre las propiedades nutricionales y la actividad antioxidante en brotes de brócoli".

VI. GLOSARIO

MPs, Microplásticos
NPs, Nanoplásticos
MNPs, Micro/nanoplásticos
eCO_2, CO_2 elevado
PAR, Radiación fotosintéticamente activa
ROS, Especies reactivas de oxígeno
UV, Radiación ultravioleta
DW, Peso seco
ppm, Partes por millón
WP, Sin plástico (control)
PS, Poliestireno
PE, Polietileno
HDPE, Polietileno de alta densidad
PBAT, Poli(butileno adipato-co-tereftalato)
PLA, Ácido poliláctico
PHA, Polihidroxialcanoatos
IAA, Ácido indol-3-acético
GA, Giberelinas
GA_3, Ácido giberélico (GA3)
IBA, Ácido indol-3-butírico
CAT, Catalasa
GLs, Glucosinolatos
GRA, Glucorafanina
GER, Glucoerucina
GBS, Glucobrassicina
HGB, 4-Hidroxiglucobrassicina
MGB, 4-Metoxiglucobrassicina
HPLC, Cromatografía líquida de alta eficiencia
DAD, Detector de arreglo de diodos
ESI, Ionización por electrospray
MS, Espectrometría de masas
LC-MS, Cromatografía líquida–espectrometría de masas
FTIR, Espectroscopía infrarroja por transformada de Fourier
ORAC-FL, Capacidad de absorción de radicales de oxígeno (fluoresceína)
H_2O_2, Peróxido de hidrógeno
ANOVA, Análisis de varianza
MANOVA, Análisis multivariante de la varianza
PCA, Análisis de componentes principales
MLP, Perceptrón multicapa
RMSE, Raíz del error cuadrático medio
R^2, Coeficiente de determinación

VII. ANEXOS

MATERIAL SUPLEMENTARIO

Tablas complementarias artículo 2

Table S1. Physicochemical characterization of the coconut fiber substrate with different suspension of micro/nanoplastics.

Coconut Fiber Substrate	
pH (1:5)	6.3
Electrical Conductivity (1:5) (dS m^{-1})	0.38
Organic Matter (%)	39.84
Organic Carbon (%)	22.14
Total Nitrogen (%)	0.46
C/N Ratio	48.1
Ammonium (N–NH4) (mg Kg^{-1})	18.2
P_2O_5 (%)	0.3
K_2O (%)	0.25
CaO (%)	1.01
MgO (%)	0.68
Iron (Fe) (mg Kg^{-1})	30800
Manganese (Mn) (mg Kg^{-1})	715
Zinc (Zn) (mg Kg^{-1})	47
Copper (Cu) (mg Kg^{-1})	25
Boron (B) (mg Kg^{-1})	62
Moisture (wet basis) (%)	46.9
Dry Matter (%)	53.1

Figure S1. Characterization by FTIR of particles of polyethylene (A) and polystyrene (B) and average size from Mastersizer for MP500 polyethylene (C) and Zetasizer for NP500 polystyrene (D)solutions, as well as SEM microparticles of polyethylene (E).

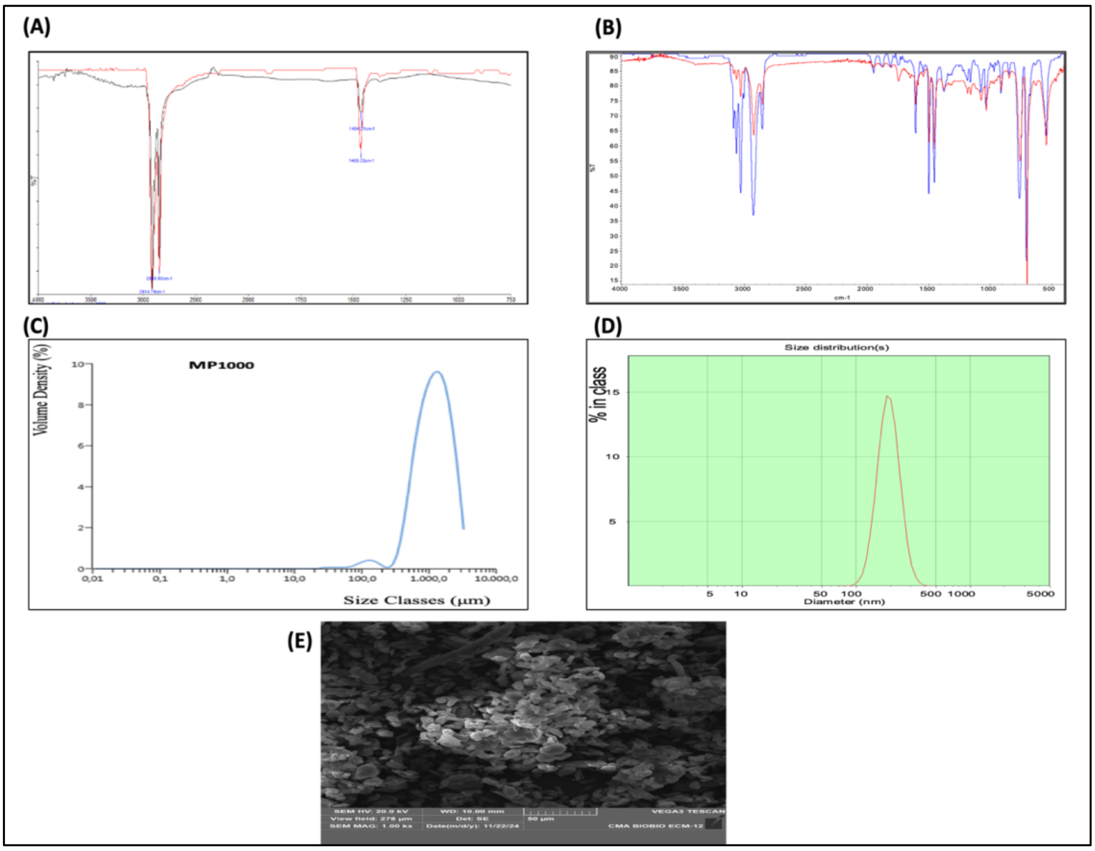
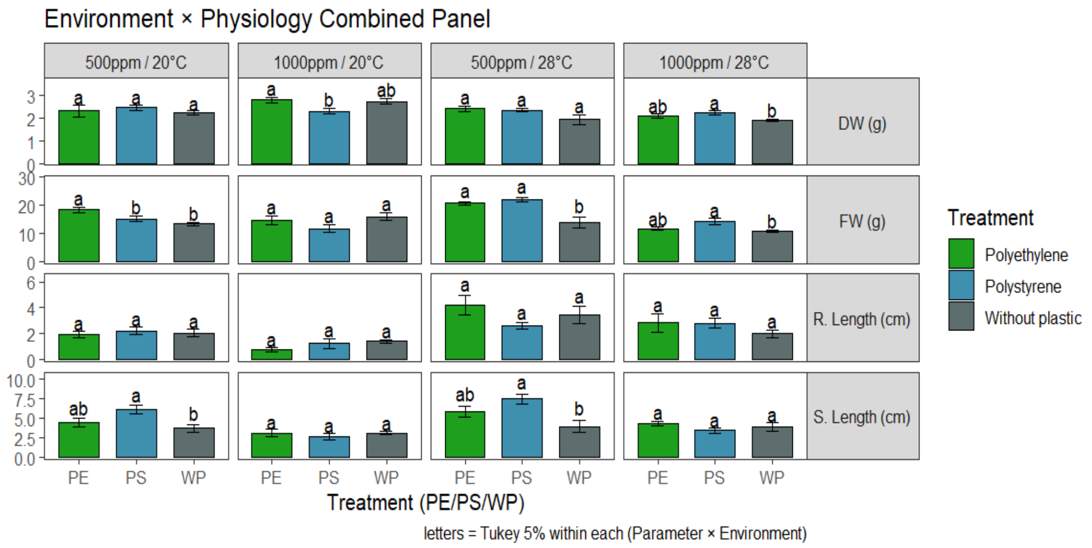
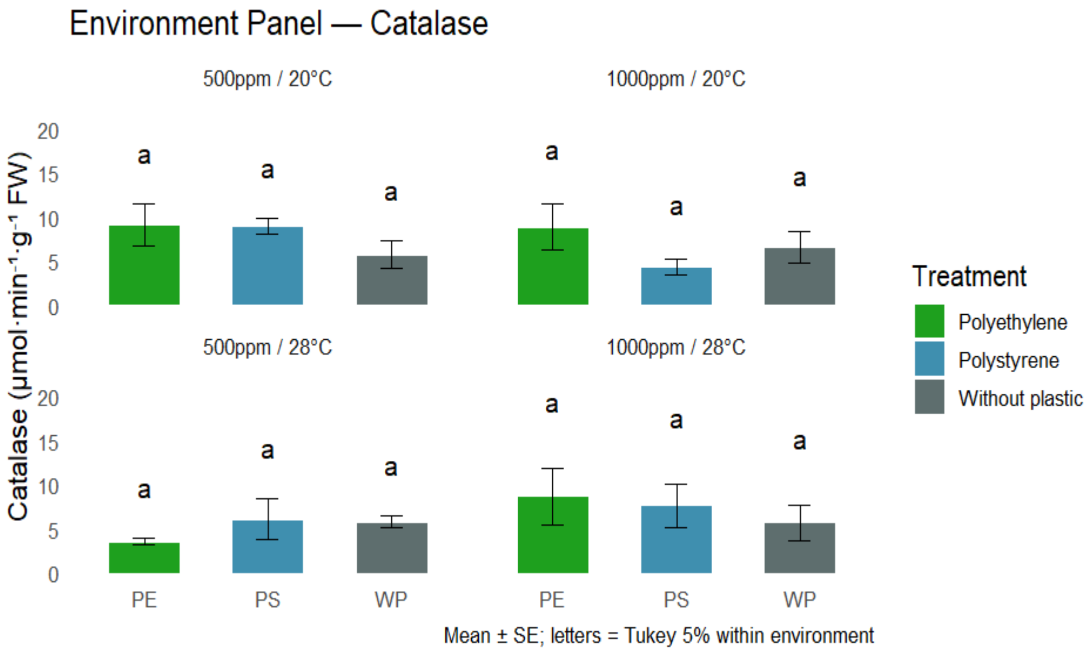


Figure S2. Fresh, Dry weight, sprout and root length under varying CO_2 (500/1000 ppm) and temperature (20/28 °C) conditions.



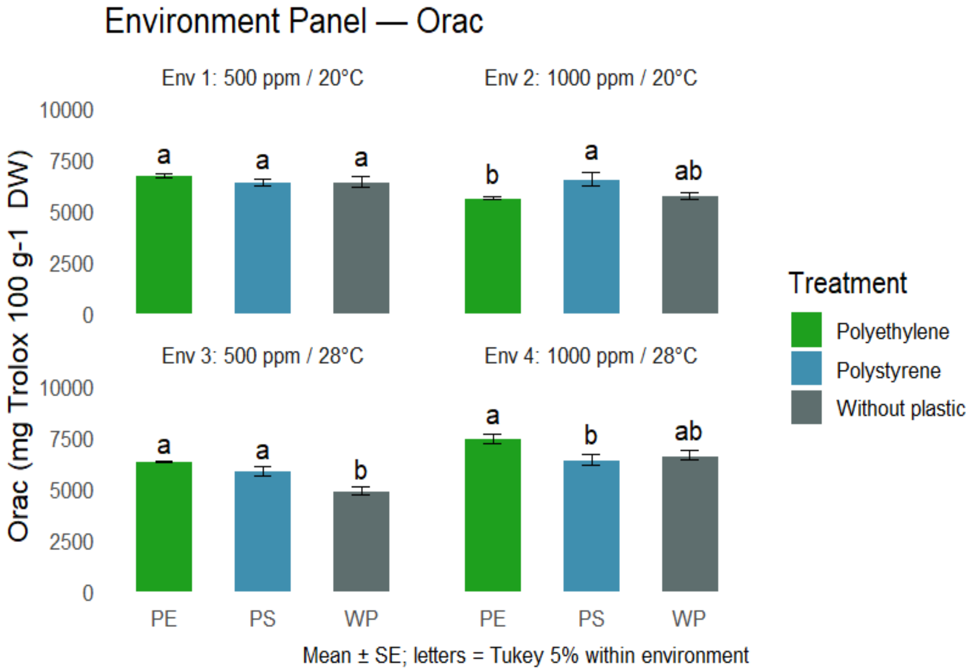
WP: without plastic; PS: with polystyrene; PE: with polyethylene. DW: Dry Weight; FW: Fresh Weight; R: Root; S: Shoot. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

Fig S3. Catalase enzyme under varying CO_2 (500/1000 ppm) and temperature (20/28 °C) conditions.



WP: without plastic; PS: with polystyrene; PE: with polyethylene. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

Fig S4. Antioxidant activity (ORAC) under varying CO_2 (500/1000 ppm) and temperature (20/28 °C) conditions.



WP: without plastic; PS: with polystyrene; PE: with polyethylene. Mean ± SE for six replications within each environment. Different letters mean significant differences at $p < 0.05$ in polymer treatments for broccoli sprouts analyzed within each environment according to Tukey test.

Tabla S2. **Multivariate analysis of variance (MANOVA) results by independent component**

Principal Component 1	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MPs	2	11.231	5.615	4.826	**0.015**
CO_2 (ppm)	1	5.406	5.406	4.646	**0.039**
Temperature° (°C)	1	104.519	104.519	89.828	**0.000**
Residuals	31	36.070	1.164		

Principal Component 2	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MPs	2	5.228	2.614	1.791	**0.184**
CO_2 (ppm)	1	30.983	30.983	21.222	**0.000**
Temperature° (°C)	1	3.901	3.901	2.672	**0.112**
Residuals	31	45.259	1.460		

Principal Component 3	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MPs	2	4.662	2.331	1.484	**0.242**
CO_2 (ppm)	1	2.783	2.783	1.772	**0.193**
Temperature° (°C)	1	1.263	1.263	0.804	**0.377**
Residuals	31	48.690	1.571		

Principal Component 4	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MPs	2	5.287	2.643	2.135	**0.135**
CO_2 (ppm)	1	0.068	0.068	0.055	**0.816**
Temperature° (°C)	1	0.114	0.114	0.092	**0.763**
Residuals	31	38.389	1.238		

Principal Component 5	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MPs	2	2.190	1.095	1.030	**0.369**
CO_2 (ppm)	1	4.793	4.793	4.508	**0.042**
Temperature° (°C)	1	0.627	0.627	0.590	**0.448**
Residuals	31	32.963	1.063		

Principal Component 6	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MPs	2	11.082	5.541	9.707	**0.001**
CO_2 (ppm)	1	0.682	0.682	1.194	**0.283**
Temperature° (°C)	1	1.161	1.161	2.034	**0.164**
Residuals	31	17.695	0.571		