agronomy

MDPI

Article

Inoculation of *Triticum Aestivum* L. (Poaceae) with Plant-Growth-Promoting Fungi Alleviates Plant Oxidative Stress and Enhances Phenanthrene Dissipation in Soil

Claudio Lagos [1], John Larsen [2], Alejandra Fuentes [1], Hector Herrera [1], Inmaculada García-Romera [3], Reinaldo Campos-Vargas [4] and Cesar Arriagada [1,*]

1 Laboratorio de Biorremediación, Departamento de Ciencias Forestales, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco 4811230, Chile; c.lagos05@ufromail.cl (C.L.); alejandra.fuentes@ufrontera.cl (A.F.); hector.herrera@ufrontera.cl (H.H.)
2 Laboratorio de Agroecología, Instituto de Investigación en Ecosistemas y Sustentabilidad UNAM Campus Morelia, Antigua Carretera a Pátzcuaro 8701, Morelia 58190, Michoacán, Mexico; jlarsen@cieco.unam.mx
3 Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), E-18008 Granada, Spain; inmaculada.garcia@eez.csic.es
4 Centro de Estudio Postcosecha–CEPOC, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago 1058, Chile; reinaldocampos@uchile.cl
* Correspondence: cesar.arriagada@ufrontera.cl; Tel.: +56-045-232-5635; Fax: +56-045-234-1467

Abstract: Polycyclic aromatic hydrocarbons (PAHs) are strong toxic compounds mainly released to the environment during combustion of fossil fuels, and have strong toxic effects on living organisms, with soil being one of their main reservoirs. High PAH levels in soils can interfere with plant growth and biomass production, causing several losses of diversity. In this study, we evaluated the effects of the co-inoculation of *Trichoderma viride* and *Funneliformis mosseae* on PAH dissipation and alleviation of oxidative stress in *Triticum aestivum* L. (wheat) plants growing in a phenanthrene-spiked soil. We determined the effect of single and dual fungal inoculation on phenanthrene dissipation rates, soil enzyme activities, dry biomass, antioxidant enzymes, lipid peroxidation, and organic acid exudation of plants growing in a soil spiked with phenanthrene at 500 and 1000 mg kg^{-1} soil. The co-inoculation with *T. viride* and *F. mosseae* resulted in a high phenanthrene dissipation from the soil. Also, dry biomass, soil enzymes, antioxidant response, organic acid exudation and phenanthrene content in roots were increased by the dual inoculation treatments, whereas lipid peroxidation and phenanthrene content in shoots were reduced. Our results show that the co-inoculation with these two soil fungi significantly promotes phenanthrene dissipation from soil and contributes to alleviating oxidative damage in wheat plants exposed to high levels of phenanthrene.

Keywords: antioxidant enzymes; arbuscular mycorrhizal fungi; polycyclic aromatic hydrocarbons; saprotrophic fungi; soil bioremediation

Citation: Lagos, C.; Larsen, J.; Fuentes, A.; Herrera, H.; García-Romera, I.; Campos-Vargas, R.; Arriagada, C. Inoculation of *Triticum Aestivum* L. (Poaceae) with Plant-Growth-Promoting Fungi Alleviates Plant Oxidative Stress and Enhances Phenanthrene Dissipation in Soil. *Agronomy* **2021**, *11*, 411. https://doi.org/10.3390/agronomy11030411

Academic Editor: Naeem Khan

Received: 24 December 2020
Accepted: 23 January 2021
Published: 24 February 2021

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic compounds that adversely affect the environment, with negative impacts on all living organisms [1,2]. According to Conejo-Saucedo et al. [3], PAHs can be present in the environment in carbon-containing organic materials such as charcoal; can be released by incomplete combustion of fossil fuels, forest fires, or volcanic eruptions; and can be synthetized during degradation of organic matter. In the soil, these compounds are mainly present after atmospheric deposition or wastewater irrigation, affecting microbial communities and plant diversity [4,5]. Soil microorganisms and plants have contributed to PAH dissipation, but also have caused a bioaccumulation of PAHs in plant tissues, leading to food chain contamination [6–8]. Therefore, it is necessary to understand the role of microorganisms in PAH dissipation from the plant rhizosphere (the soil in the close vicinity of the plant roots) in order to develop

efficient and environmentally friendly bioremediation strategies. Additionally, the use of microorganisms with plant growth promoting capabilities can significantly contribute to the recovery of PAHs-polluted soils, limiting the negative effects on diversity and richness of plant communities.

Soil microorganisms can contribute to the degradation of organic pollutants because they can sequester organic and inorganic compounds, produce plant-growth promoting metabolites, and contribute to increase nutrient availability, among others [9–11]. Recent studies have demonstrated that soil fungi can improve PAH dissipation rates from different soils [12–14]. In addition, some plants can play crucial roles in PAH dissipation by favoring the proliferation of specific soil microorganisms [15]. However, the establishment of a plant in PAH-polluted soils depends on its sensitivity and interactions with microorganisms [16–18]. The most negative effects of PAHs on plants are related to oxidative stress through reactive oxygen species (ROS), which can lead to oxidative damage to proteins, DNA, and lipids [19–21]. Thus, in order to cope with these ROS, plant cells have enzymatic and non-enzymatic antioxidant systems [22–24]. PAHs produce oxidative stress in *Arabidopsis thaliana* plants, reducing root and shoot growth, producing chlorosis and late flowering, and forming necrotic lesions [25]. Similarly, Yun, et al. [26] have reported that oxidative stress caused by PAHs can inhibit seed germination and initial plantlet development in *Hordeum vulgare.* Therefore, alternatives are needed to improve PAH dissipation rates and limit the detrimental effects on plant development.

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs from Glomeromycota that form mutualistic associations with most plant roots, which, through colonization of the soil with external mycelia, extend the nutrient and water absorption area of their hosts [27,28]. Alleviating plant stress caused by biotic and abiotic factors is another important trait of AMF [29,30]. These fungi are also known to play an important role in the phytoremediation of PAH-contaminated soils, contributing to their dissipation from soil through strategies such as accumulating in the roots and reducing the content in leaves [31,32]. Furthermore, indirect effects of AMF in relation to PAH dissipation through degradation have been suggested via proliferation of mycorrhizae-associated microorganisms [33]. Saprotrophic fungi such as *Trichoderma* spp. associated with roots are also able to alleviate oxidative stress and contribute to plant growth [34,35]. Given their broad enzyme profile, these fungi also exhibit traits for the bioremediation of recalcitrant contaminants like PAHs [36]. Li et al. [37] and Urana et al. [38] showed that different rhizobacteria isolated from plants growing in contaminated soils can be effective at both plant growth promotion and biodegradation of PAHs. Similarly, recent studies have shown that soil-borne fungi can contribute to PAHs' dissipation from soil, some of which can significantly enhance the stress tolerance and PAHs' removal from contaminated soils, contributing to soil remediation, change of microbial communities involved in PAHs-dissipation, and plant growth promotion [30,39,40]. The co-inoculation of mycorrhizal and saprotrophic fungi has been reported as promising for the degradation of several compounds from soil [9,41]. However, information on the combined effects of AMF and saprophytic fungi in relation to PAH soil bioremediation is still limited [42].

Triticum aestivum L. (Poaceae), commonly known as wheat, is one of the most important plant families used in phytoremediation, showing tolerance to a wide range of pollutants such as trace elements and PAHs [43–45]. Additionally, wheat is an important cereal crop worldwide and can grow in a broad range of environments, making it a good plant for phytoremediation studies. We hypothesized that the co-inoculation of saprotrophic and arbuscular mycorrhizal fungi can enhance PAH dissipation rates and improve the growth of *T. aestivum* plants grown in a contaminated soil. Therefore, the objective of this study was to investigate the effect of the co-inoculation of the AMF *Funneliformis mosseae* and the saprotrophic fungus *Trichoderma viride* on the dissipation of phenanthrene and alleviation of stress in *T. aestivum* plants grown in a contaminated soil.

2. Materials and Methods

2.1. Soil

The polluted soil was collected in the Puchuncaví Valley in central Chile, in a zone located 1.5 km southeast of the Ventanas copper smelter (32°46′30″ S; 71°28′17″ W). The soil had a pH of 5.54 (1:10, H_2O), 2.41% organic matter, total N 29 mg kg^{-1}, total P 40 mg kg^{-1}, and K 210 mg kg^{-1}, and available Cu content of 482 mg kg^{-1}. The soil samples were collected at a depth down to 20 cm.

2.2. Microorganisms

The saprotrophic fungus *T. viride* was isolated from the rhizosphere of *Baccharis linearis* (Ruiz et Pav.) Pers (Asteraceae), a plant that can colonize the polluted soil. This fungal strain was molecularly identified using a standard identification protocol based on 18S ribosomal RNA gene sequences [46], and showed 99% similarity with the strain wxm58 (Accession number HM037962). The saprophytic fungal strain was conserved in potato dextrose agar at 4 °C and periodically subcultured. The inoculum of the AMF *Funneliformis mosseae* was obtained from culture pots performed in the Bioremediation Laboratory at the Universidad de La Frontera, Temuco, Chile. This inoculum was a root and soil mixture consisting of rhizospheric soil containing spores and colonized root fragments of *Sorghum bicolor* (L.) Moench (Poaceae).

2.3. Greenhouse Experiment

A greenhouse pot experiment was performed with a fully factorial design with three factors: phenanthrene (three levels: 0, 500 and 1000 mg kg^{-1} soil), *F. mosseae* (two levels: without and with), and *T. viride* (two levels: without and with). Each of the resulting 12 treatments had 12 replicates, yielding a total of 144 experimental units.

Wheat seeds were superficially disinfected by washing and shaking with 10% NaClO for 15 min and five times with sterile distilled water. Then, seeds were sown in vermiculite and were left to germinate for one week. The soil was spiked with phenanthrene 48 h before transplanting by first diluting with acetone, and then a portion of the soil (10%) was contaminated and mixed with the remaining soil [47]. Soil without phenanthrene was also supplied with acetone in order to discard an effect of this organic solvent on *T. aestivum*. Then, acetone was left to evaporate for 48 h. Then, 250 g of soil was transferred to 250 cm^3 pots. Treatments with saprophytic fungus were inoculated with 5 g of *T. viride* inoculum (previously inoculated on sterile wheat seeds), whereas treatments involving AMF were inoculated with 8 g of *F. mosseae.* Finally, plants were transplanted into the pots. Each pot was watered with 25 mL of distilled water every 48 h.

After eight weeks, the plants were harvested and the dry biomass of the shoots and roots were determined. The shoots and roots were stored at −80 °C to determine antioxidant enzyme activities and the phenanthrene content.

2.4. Mycorrhizal Colonization Parameters

After the harvest, samples of fresh roots were randomly taken and subsequently cleared in KOH and stained with Trypan blue in lactic acid [48]. The percentage of mycorrhizal root colonization was evaluated using 30 root fragments of 1 cm on each slide (3 per treatment) in a Leica CME 1349522x light microscope. Mycorrhizal frequency (F%), mycorrhizal intensity (I%), and arbuscule abundance (A%) were performed using the Mycocalc program [49]. The number of vesicles was counted for each root placed on the respective slides. This determination was made because vesicles are mainly composed of lipids; therefore, given the lipophilic nature of PAHs, vesicles could play an important role in the sequestration of PAHs in roots.

2.5. Analysis of Phenanthrene in Soil

Phenanthrene was extracted from 10 g of soil in 50 mL falcon tubes, adding 10 mL toluene and 10 mL sodium pyrophosphate (0.05 M) to each tube and then shaking for

16 h [50]. Toluene extracts were evaporated to dryness with N_2. Finally, the residue was re-suspended in acetonitrile and analyzed by high-performance liquid chromatography (HPLC) in a Shimadzu Prominence UFLC XR (Kyoto, Japan). A SUPELCOSIL ™ LC-18 HPLC column (Sigma-Aldrich, St Louis, MO, USA) was used to determine the phenanthrene concentration in the samples. A calibration curve was prepared using different concentrations of phenanthrene standard ranging from 0.5 to 1000 ppm (P11409; Sigma-Aldrich). The mobile phase consisted of acetonitrile–water (70:30 v/v and flow rate was 0.7 mL/min^{-1}. The analysis was performed at room temperature (25 ± 2 °C). The wavelength was 254 nm for phenanthrene detection. The volume used for injection was 20 µL, and the elution made was isocratic. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as previously reported [51] (LOD = 2.07 ppm and LOQ = 6.29 ppm).

Phenanthrene dissipation percentages were calculated by dividing the residual phenanthrene concentration in the soil samples by the initial dose of phenanthrene spiked into the soil. Then, in order to explain this dissipation, phenanthrene accumulation in plant tissues (shoot and root) and undetectable phenanthrene were calculated and expressed in percentages. The undetectable percentage of phenanthrene was calculated by the difference in initial concentration of both phenanthrene doses (100%) and the sum of percentages of root and shoot accumulation and residual phenanthrene in the soil. Therefore, undetectable phenanthrene was considered to be the quantity of phenanthrene dissipated in soil by means other than accumulation in plant tissues.

2.6. Phenanthrene in Plant Tissues and Translocation Factor (TF)

The determination of phenanthrene in plant tissues was performed according to the method described by Zitka et al. [52] with minor modifications. Root and shoot samples (0.1 g) were homogenized in a ceramic mortar using a 2 mL mixture of benzene and ethanol in a 3:1 (v:v) ratio. Then, this mixture of solvents was evaporated and 1 mL of the solvent mixture was transferred to 1.5 mL Eppendorf tubes and shaken for 30 min at 100 rpm. Next, samples were sonicated at 4 °C and 40 W for 30 min. Subsequently, the tubes were centrifuged at 15,000 g for 30 min. Finally, the supernatant was completely evaporated using a vacuum rotary evaporator (Buchi, Flawil, Suiza) and dissolved in 1 mL of acetonitrile. The samples in microtubes were stored at −20 °C for analysis. The determination was made using an HPLC as described before. The translocation factor was determined by dividing the phenanthrene concentration in shoots by the phenanthrene concentration in roots.

2.7. Soil Enzyme Activities

2.7.1. Dehydrogenase Activity

The dehydrogenase activity (DHA) was established as described by Casida et al. [53], with minor modifications. Soil samples (10 g) were placed in 50 mL conical tubes and mixed with 2.5 mL of phosphate buffer, 0.2 g $CaCO_3$, and 1 mL of Triphenyl tetrazolium chloride. After that, the mixture was incubated for 24 h and centrifuged at 3000 rpm for 10 min. The reduction of 2, 3, 5-triphenil tetrazolium to triphenyl formazan by dehydrogenase was measured by spectrophotometry at 485 nm in a BK-UV1800 spectrophotometer (Biobase, Jinan, China).

2.7.2. Total Microbial Activity

Total microbial activity was measured by monitoring fluorescein diacetate (FDA) hydrolysis according to Adam and Duncan [50]. Briefly, 2 g of soil samples were placed in 50 mL conical tubes and mixed with 15 mL of 60 mM KH_2PO_4 (pH: 7.6) and incubated for 10 min. After that, 0.2 mL of fluorescein diacetate (1 mg/mL acetone) was added and incubated in an orbital shaker for 1 h at 30 ° C at 100 rpm. Then, 15 mL of acetone was added to stop the reaction, and the samples were centrifuged at 10,000 rpm for 15 min. Finally, 2 mL of the supernatant was measured by spectrophotometry at 490 nm in a BK-UV1800 spectrophotometer (Biobase).

2.7.3. Manganese Peroxidase

Manganese peroxidase (MnP) activity was measured by mixing 1 g of soil, 2.0 mL of sodium tartrate (0.1 M pH 5.0), 2.0 mL of H_2O_2 (0.1 mM), and 2.0 mL of $MnSO_4$ (0.1 mM). Samples were incubated at 25 °C for 30 min and measured by spectrophotometry at an absorbance of 420 nm [10] in a BK-UV1800 spectrophotometer (Biobase).

2.7.4. Laccase

Laccase was extracted according to Elgueta et al. [54], and the activity was measured by mixing 0.5 mL of the extracted sample, 0.25 mL of 100 mM glycine (pH 3.0), and 0.25 mL of 4 mM ABTS, and incubating at 25 °C for 5 min before the measurement. Then, 1 mL of the sample was used for Laccase determination by spectrophotometry at 405 nm in a BK-UV1800 spectrophotometer (Biobase).

2.8. Organic Acid Extraction and Determination

Organic acids derived from root exudates were extracted according to Herrera et al. [55], with minor modifications. Roots were carefully washed with deionized water and then placed in 50 mL flasks with 25 mL of 0.25 mM KH_2PO_4 (pH = 5.5) under constant aeration for 1 h. After that, the collected samples were sonicated for 30 min and filtered (0.22 µm). Collected root-exudate samples were freeze-dried and suspended in ultrapure water for organic acid determination by HPLC. Calibration curves were prepared using an organic acids kit (47264, Supelco, Bellefonte, PA, USA). The analysis of organic acids was carried out in an HPLC (Shimadzu CTA-20AC, Kyoto, Japan) equipped with a UV-visible detector. Separation of acids was done in a C-18 reverse phase column (MultoHigh100 RP-18, 5 mm particle size, CS-GmbH, Langerwehe, Germany). The mobile phase was 93% (v/v) 25 mM KH_2PO_4 at pH 2.5 and 7% (v/v) methanol with a flow rate of 1 mL min^{-1}. The LOD and LOQ for citric acid were 0.46 and 1.4 ppm, respectively; whereas for malic acid they were 0.38 and 1.15 ppm, respectively.

2.9. Antioxidant Activities

The plant samples were ground with liquid nitrogen using a ceramic mortar. Then, 0.1 g of plant tissue was weighed and 2 mL of 0.1 M potassium phosphate buffer (pH 7.0) was added. The ground tissues were transferred to 1.5 mL Eppendorf tubes and centrifuged at 17,000 g for 15 min at 4 °C. The supernatant was used to measure the enzymes superoxide dismutase (SOD) and guaiacol peroxidase (GPX).

Total SOD activity was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium based on the method described by Donahue et al. [56]. GPX activity was determined according to Pinhero et al. [57] following the change in absorbance at 470 nm as a consequence of guaiacol oxidation.

2.10. Lipid Peroxidation

Lipid peroxidation was determined as described by Du and Bramlage [58], with minor modifications, based on the doses of malondialdehyde (MDA), which are determined by the reaction given by thiobarbituric acid. Briefly, 200 mg of fresh tissue was added to 1 mL of 20% v/v trichloroacetic acid and heated to 95 °C for 30 min, cooled on ice, and centrifuged at 18,000× g for 10 min. The MDA that formed was determined in a spectrophotometer at absorbance of 532 nm (a correction was made by deducting absorbance at 600 nm for turbidity) using an extinction coefficient of 155 mM^{-1} cm^{-1} in a BK-UV1800 spectrophotometer (Biobase).

2.11. Statistical Analysis

The data were analyzed using a factorial analysis of variance with *F. mosseae*, *T. viride*, and phenanthrene as the main factors. Statistical procedures were performed with the software SPSS v.11.0 (SPSS Inc., Chicago, IL, USA, 1989–2001). Statistical significance was determined at $p < 0.05$. All variables were tested for normality and variance homogeneity

(Kolmogorov–Smirnov test and Cochran's C test, respectively), and log transformation was applied on the data when needed. Values expressed in percentage were arcsine transformed for subsequent analyses.

3. Results

3.1. Dry Biomass

Results observed by ANOVA table (Table S1) suggested that shoot and root dry weight were reduced significantly under phenanthrene exposure, but increased significantly in the treatments with fungal inoculation when they were compared with the control. Figure 1 shows that the shoot and root biomass of wheat plants decreased at 1000 mg kg^{-1} of phenanthrene, but improved when *T. viride*, *F. mosseae*, and both fungi were inoculated. Additionally, at all phenanthrene concentrations (500 and 1000 mg kg^{-1}), *F. mosseae* and its interaction with *T. viride* resulted in a higher biomass than for non-inoculated plants (control) or plants inoculated only with *T. viride* or *F. mosseae* (Figure 1).

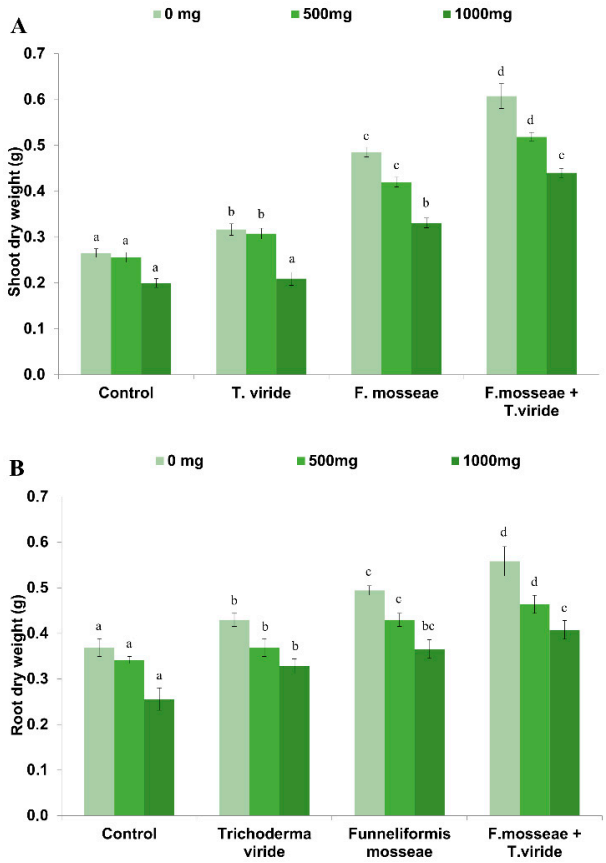

Figure 1. Shoot (**A**) and root (**B**) dry weight of wheat plants inoculated with *F. mosseae* and/or *T. viride* grown in soil spiked with phenanthrene. The data are the means ± standard error (n = 5; $p > 0.05$). Different letters indicate significant differences between inoculation treatments (control, *T. viride*, *F. mosseae*, or *F. mosseae* + *T. viride*) and their respective doses (0, 500, and 1000 mg kg^{-1}), according to the Tukey's multiple-range test ($p < 0.05$).

3.2. Arbuscular Mycorrhizal Colonization Parameters

The phenanthrene doses significantly reduced the parameters of AMF colonization in roots (frequency, intensity, and abundance), while inoculation with *T. viride* significantly increased these parameters (Table S2). However, *T. viride* significantly increased the AMF root-colonization parameters, reaching the highest colonization level in combination with phenanthrene. Vesicles were increased by both phenanthrene doses, and reached their highest number at 1000 mg kg^{-1} of phenanthrene combined with *T. viride* or not (Table 1;

Figure S1).

Table 1. Frequency (F%) and intensity (I%) of arbuscular mycorrhizal colonization, abundance of arbuscules (A%) and vesicles in roots of *T. aestivum* grown in soil spiked with phenanthrene (0, 500 and 1000 mg kg^{-1}). The data are the means ± standard deviation with $n = 5$. Different letters within the same column indicate significant differences between different phenanthrene doses according to the Tukey's multiple-range test ($p < 0.05$).

Parameter.	Phenanthrene Dose (mg kg^{-1})	*F. Mosseae*	*F. Mosseae* + *T. Viride*
F%	0	73.1 ± 8.5 c	80.3 ± 6.3 c
	500	51.3 ± 4.9 b	60.5 ± 3.8 b
	1000	39.8 ± 4.7 a	49.4 ± 4.4 a
I%	0	46.1 ± 8.6 b	51.2 ± 5.5 c
	500	36.1 ± 4.1 b	42.8 ± 4.9 b
	1000	28.5 ± 2.1 a	34.9 ± 1.4 a
A%	0	53.3 ± 3.9 c	56.5 ± 3.1 c
	500	36.8 ± 5.3 b	44.1 ± 6.7 b
	1000	27.0 ± 1.6 a	32.6 ± 2.1 a
Vesicles per 100 cm root	0	18.0 ± 1.9 a	28.4 ± 3.2 a
	500	52.0 ± 2.5 b	60.2 ± 1.6 b
	1000	165.2 ± 14.1 c	178.6 ± 11.7 c

3.3. Phenanthrene in Soil and Plant Tissues

The residual phenanthrene content in the soil was significantly decreased by dual inoculation with *T. viride* and *F. mosseae* (Table S3a), resulting in the lowest residual phenanthrene in soil in both treatments (phenanthrene at 500 and 1000 mg kg^{-1}) (Table 2). At both phenanthrene doses, the content of phenanthrene in shoots was significantly reduced by *F. mosseae*, whereas the content in roots was significantly increased also in combination with *T. viride* (Table 2). In shoots, the highest phenanthrene content was observed in plants without fungal inoculation (control) at 1000 mg kg^{-1}, whereas the lowest phenanthrene content in shoots was observed after dual inoculation with *F. mosseae* and *T. viride.* In contrast, the highest phenanthrene content in roots was obtained with dual fungal inoculation (Table 2). These results were also confirmed by the reduced translocation factor (TF) of phenanthrene after inoculation with *F. mosseae*. The lowest TF was observed after dual fungal inoculation (Table 2).

Table 2. Residual phenanthrene in soil and phenanthrene content in plant tissues. Values are means ± standard deviation with $n = 5$. Different letters within the same row indicate significant differences between different inoculation treatments according to the Tukey's multiple-range test ($p > 0.05$).

Parameter	Phenanthrene Dose (mg kg^{-1})	Control	*T. Viride*	*F. Mosseae*	*F. Mosseae* + *T. Viride*
Phenanthrene residual in soil (ppm)	500	422.8 ± 37.9 d	351.7 ± 28.9 c	211.8 ± 21.6 b	164.3 ± 22.7 a
	1000	753.1 ± 55.6 c	696.3 ± 66.8 c	426.6 ± 38.4 b	339.6 ± 29.4 a
Phenanthrene content in shoots (ppm)	500	11.1 ± 2.8 b	10.4 ± 2.5 b	5.0 ± 1.7 a	4.7 ± 1.1 a
	1000	19.1 ± 5.7 c	17.4 ± 5.4 c	9.3 ± 2.7 b	3.7 ± 0.9 a
Phenanthrene content in roots (ppm)	500	18.8 ± 4.9 ab	12.8 ± 6.1 a	23.1 ± 2.9 b	26.3 ± 3.9 b
	1000	25.2 ± 2.7 a	28.2 ± 3.9 a	34.0 ± 1.8 b	40.4 ± 3.8 c
Translocation factor	500	0.81 ± 0.17 c	0.49 ± 0.11 b	0.21 ± 0.05 a	0.18 ± 0.06 a
	1000	0.76 ± 0.14 d	0.61 ± 0.05 c	0.27 ± 0.03 b	0.09 ± 0.03 a

3.4. Phenanthrene Dissipation

Phenanthrene dissipation was significantly increased by dual inoculation (Table S3b). The highest phenanthrene dissipation in soil was reached by the dual inoculation for the 500 and 1000 mg kg^{-1} doses (74.5% and 66%, respectively) (Table 3). Accumulation in roots or shoots only accounted for a small portion of the phenanthrene dissipation, whereas undetectable phenanthrene accounted for the highest amount in treatments with dual fungal inoculation for the 500 and 1000 mg kg^{-1} doses (68.3% and 61.6%, respectively) (Table 3).

Table 3. Phenanthrene dissipation from soil, dissipation by accumulation in plant tissues of *T. aestivum* (roots and shoots), and not-detected phenanthrene (ND%). Values are means ± standard deviation with $n = 5$. Different letters within the same row indicate significant differences between different phenanthrene doses according to the Tukey's multiple-range test ($p > 0.05$).

Parameter	Phenanthrene Dose (mg kg^{-1})	Control	*T. Viride*	*F. Mosseae*	*F. Mosseae + T. Viride*
Soil (%)	500	15.4 ± 4.4 a	29.7 ± 3.8 b	57.6 ± 5.8 c	74.5 ± 3.6 d
	1000	24.7 ± 5.4 a	30.3 ± 8.8 a	56.3 ± 5.2 b	66.0 ± 4.1 c
Shoots (%)	500	2.2 ± 0.3 b	2.1 ± 0.6 b	1.0 ± 0.2 a	0.9 ± 0.2 a
	1000	1.9 ± 0.6 b	1.7 ± 0.6 b	0.9 ± 0.7 a	0.4 ± 0.3 a
Roots (%)	500	4.6 ± 0.8 b	2.6 ± 0.5 a	4.6 ± 1.1 b	5.3 ± 1.5 b
	1000	2.5 ± 0.7 a	2.8 ± 0.6 a	3.4 ± 1.0 ab	4.0 ± 1.1 b
ND (%)	500	8.6 ± 1.2 a	25.0 ± 2.8 b	52.0 ± 6.2 c	68.3 ± 10.1 d
	1000	20.3 ± 3.5 a	25.8 ± 7.7 a	53.0 ± 7.1 b	61.6 ± 12.5 b

3.5. Soil Enzyme Activities

The highest DHA and FDA activities for each phenanthrene level were observed after dual inoculation with *F. mosseae* and *T. viride* (Figure 2A,B). In general, DHA and FDA increased in all treatments with 500 mg kg^{-1} of phenanthrene, but decreased at 1000 mg kg^{-1}, mainly in treatments without *F. mosseae* inoculation. However, both single and dual fungal inoculation enhanced these activities in combination with 1000 mg kg^{-1} phenanthrene compared with the non-inoculated control treatment (Figure 2A,B). Enzymes related to PAH degradation such as laccase and manganese peroxidase (MnP) also reached their highest levels with dual fungal inoculation. In the case of MnP, the highest level was obtained at 1000 mg kg^{-1} of phenanthrene, whereas laccase reached its highest activity by dual fungal inoculation without phenanthrene and decreased under increasing phenanthrene doses (Figure 2C,D).

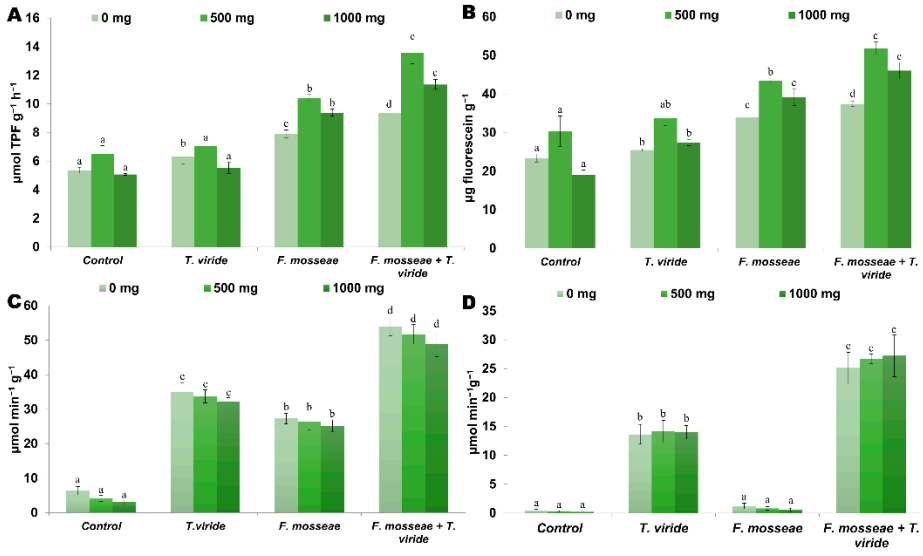

Figure 2. Biological activities in soil of wheat plants inoculated with *F. mosseae* and *T. viride* grown in soil spiked with phenanthrene (0, 500, and 1000 mg kg^{-1}). (**A**) Dehydrogenase activity (µmol TPF g^{-1} h^{-1}). (**B**) Fluorescein diacetate activity (µg fluorescein g^{-1}). (**C**) Laccase activity (µmol min^{-1} g^{-1}). (**D**) Manganese peroxidase activity (µmol min^{-1} g^{-1}). The data are the means ± standard error. Different letters indicate significant differences between different inoculation treatments and their respective doses, according to the Tukey's multiple-range test ($p > 0.05$).

3.6. Organic Acids

The main organic acids determined in root exudates were citric and malic acid. The interaction of the three factors significantly increased malic and organic acid exudation

(Table S1). The highest malic and citric acid exudation was observed in the interaction of *T. viride*, *F. mosseae*, and phenanthrene at 1000 mg kg^{-1} (Figure 3A,B).

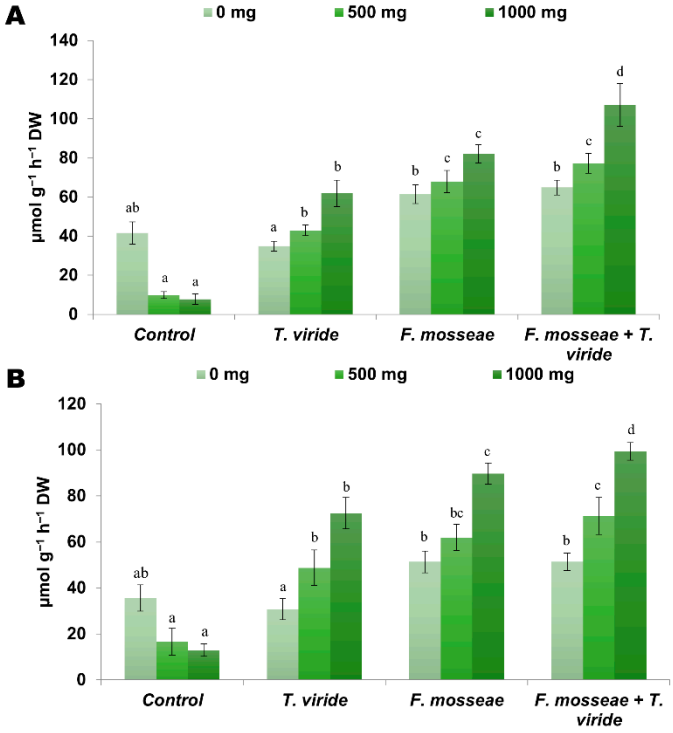

Figure 3. Organic acids collected from root exudates of wheat plants inoculated with *F. mosseae* and/or *T. viride* grown in soil spiked with phenanthrene (0, 500, and 1000 mg kg^{-1}). (**A**) Malic acid (µmol g^{-1} h^{-1} DW). (**B**) Citric acid (µmol g^{-1} h^{-1} DW). The data are the means ± standard error with $n = 5$. Different letters indicate significant differences between different inoculation treatments and their respective doses, according to the Tukey's multiple-range test ($p > 0.05$).

3.7. Antioxidant Enzymes and Lipid Peroxidation

Antioxidant activities in wheat plants increased along with the phenanthrene doses applied, as well as with *F. mosseae* inoculation (Figure 4A,B). SOD and GPX activities increased significantly with dual fungal inoculation in combination with phenanthrene, although the main factor in this response was attributed to phenanthrene (Table S1). In the case of the significant increase in GPX activity, *F. mosseae* was the main factor involved in this response, followed by phenanthrene and *T. viride*. In terms of the overall antioxidant response, the results for SOD and GPX show that the highest activities were produced by dual fungal inoculation at 1000 mg kg^{-1} of phenanthrene applied to the soil (Figure 4A,B). Lipid peroxidation in wheat plants in terms of MDA production increased significantly after phenanthrene application, whereas a single inoculation of *F. mosseae* and *T. viride* significantly reduced lipid peroxidation. MDA production was highest in non-inoculated plants at 1000 mg kg^{-1} of phenanthrene in soil, whereas dual fungal inoculation resulted in the lowest MDA production at this phenanthrene concentration (Figure 4C).

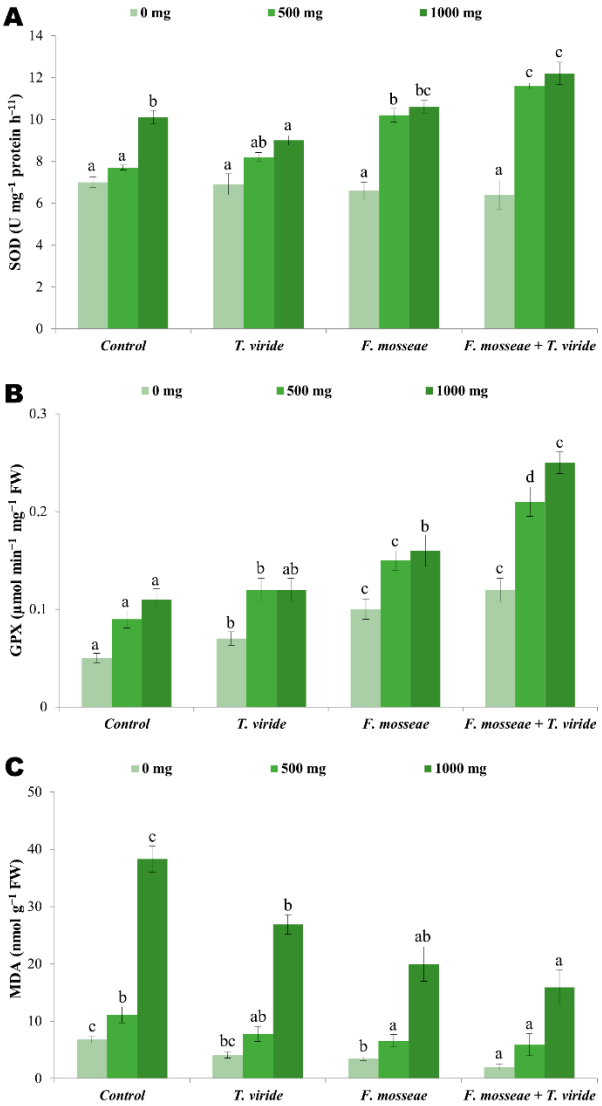

Figure 4. Antioxidant enzymes activities and lipid peroxidation in leaves of *T. aestivum* inoculated with *F. mosseae* and *T. viride* grown in soil spiked with phenanthrene (0, 500, and 1000 mg kg^{-1}). (**A**) Superoxide dismutase activity (U mg^{-1} protein h^{-1}). (**B**) Guaiacol peroxidase activity (µmol min^{-1} mg^{-1} FW). (**C**) Lipid peroxidation (MDA nmol g^{-1} FW). The data are the means ± standard errors for $n = 5$. Different letters indicate significant differences between different inoculation treatments and their respective doses, according to the Tukey's multiple-range test ($p > 0.05$).

4. Discussion

The adverse effects of PAHs on plants have been widely reported [59,60]. Results from the present study showed that the negative effects of phenanthrene on wheat plants were alleviated by single and dual inoculation with *F. mosseae* and/or *T. viride*. Additionally, plant growth promotion was observed after inoculation with *F. mosseae*, even in phenanthrene-spiked soil. AMF can increase P uptake and protect their host from abiotic stress [27,42]. However, our results detected a decrease in the colonization parameters in plants grown in the phenanthrene-spiked soil. Such reduced colonization has been previously reported and linked to the inhibition of spore germination and hyphal growth in soil [12,61,62]. Similarly, Gaspar et al. [63] reported that phenanthrene negatively affects the mycorrhizal association of the AMF *Glomus geosporum* in symbiosis with maize.

Our study also detected positive effects of *T. viride* on the growth of the inoculated plants, which seems to be related to both phytohormone production and plant stress

alleviation [34,64,65]. Additionally, plants inoculated with *F. mosseae* presented the highest colonization parameters when co-inoculated with *T. viride*. Previous studies have found that some saprophytic fungal strains can influence spore germination or development of mycorrhizal structures that often can improve the plant growth [66–68]. A similar mode of interaction may occur between *T. viride* and *F. mosseae*. We also detected the highest number of vesicles in roots from the treatment with *T. viride* and phenanthrene at 1000 mg kg^{-1}, but the number of arbuscules decreased. Such changes in colonization parameters can be a stress response of AMF to the contamination with hydrocarbons and other abiotic or biotic stress conditions [69,70].

Phenanthrene dissipation from the soil was at least twofold higher in plants inoculated with *F. mosseae* than in non-inoculated plants, whereas a significant enhancement in dissipation was observed with the interaction of *F. mosseae* and *T. viride*, which agrees with recent studies analyzing the role of AMF in PAH dissipation [31,61]. Increased activity of soil microorganisms has been proposed as the main mechanism through which AMF increase PAH dissipation in the soil due to an enhancement in enzymatic activities induced by microbial exudation [33]. Enzymes such as peroxidases and polyphenol oxidase have been well documented as being enriched by AMF in PAH-polluted soils, and can significantly contribute to the biodegradation of PAHs [14,71]. In relation to *T. viride*, PAH dissipation by the genus *Trichoderma* has also been reported [64]. Hence, the enhanced phenanthrene removal observed in our study could be linked to the combined soil enzymes that both fungi can induce in PAH-contaminated soil.

Direct accumulation of PAHs in plant tissues is another mechanism that can contribute to phenanthrene dissipation in soil [72]. In our study, we noted that phenanthrene content in shoots was higher in non-inoculated plants, whereas plants with dual inoculation exhibited lower phenanthrene content. In contrast, phenanthrene accumulation was higher in roots with the dual inoculation. Additionally, the TF showed that dual inoculation reduced this parameter significantly, thus reflecting the higher accumulation in roots and lower translocation into the shoots, especially at the 1000 mg kg^{-1} dose. Similar studies have reported that PAHs are mainly accumulated in the roots of plants colonized by AMF, which restrict PAH translocation to vital tissues [31,33]. According to our results, a relation between a higher root biomass and accumulation can be established, given that the treatment that produced the highest root biomass under phenanthrene exposure was the dual fungal inoculation. PAHs are lipophilic compounds that accumulate in lipids [42]. In this sense, AMF structures like vesicles and mycelia have been described as lipid structures [27,73]. In particular, the number of vesicles in our study was higher under phenanthrene exposure, which coincided with the higher phenanthrene accumulation in roots, thereby suggesting that phenanthrene may be accumulated in vesicles. Nevertheless, more studies are needed to clarify this effect.

Our study also showed an enhancement of soil enzymes in the treatments with phenanthrene, which reflects a positive effect of the dual inoculation, improving both the phenanthrene dissipation and microbial activity. Laccase and MnP have been reported as being among the main enzymes responsible for phenanthrene degradation [50]. In our results, laccase and MnP enzymes were stimulated mainly by *T. viride*, which catalyzes the oxidation of various hydrocarbons into intermediate products [74]. In this sense, the improved soil biological activities in terms of DHA and FDA in phenanthrene-spiked soil in combination with dual fungal inoculation coincided with the highest phenanthrene dissipation. The FDA activity has been used as an indicator of microbial activity to evaluate the sensitivity of microorganisms in a broad range of stress conditions in soils [32,75]. Similarly, DHA activity has been suggested as an important parameter involved in organic matter oxidation as a result of biological degradation by soil microorganisms, as well as an indicator of their activity and biomass [76,77]. Hence, a higher DHA in the soil with the co-inoculation treatment could be related to a higher microbial biomass in the soil, promoting phenanthrene dissipation from soil. In our results, the root and shoot dry weight and the improved plant growth as a result of dual fungal inoculation could increase the production

of root exudates in the rhizosphere, thereby stimulating microbial activity, which in turn helps to dissipate PAHs from soil. Indeed, increased DHA has previously been linked to PAH dissipation in soil grown with *T. aestivum*, *Solanum melongena*, and *Vigna radiate* associated with *F. mosseae* [78].

The dual inoculation also affects the root exudates, which maybe directly related with improved plant growth [55]. In our study, malic and citric acids were observed as the main root exudates from plants under PAH stress, which agrees with recent studies analyzing the concentration of root exudates of native plants subjected to different abiotic stresses [79,80]. Furthermore, malic and citric acid can mobilize inorganic P in soil, promoting plant growth [81]. Therefore, under phenanthrene exposure, the increase of organic acid exudation can be considered a mechanism by which *F. mosseae* and *T. viride* enhance nutrient availability to cope with adverse environmental conditions. Similarly, organic acids can act as a carbon source and energy for microorganisms, and also improve hydrocarbon degradation in the rhizosphere by stimulating the population able to degrade PAHs [33], which is consistent with the improved soil enzyme activities induced by the dual inoculation and the corresponding enhancement in the phenanthrene dissipation. In addition, a higher organic acid exudation by plants promotes the bioavailability of PAHs in soils, increasing their desorption and increasing their susceptibility to biodegradation [82], a process that could play a relevant role in the phenanthrene dissipation rates detected in our study.

Inconsistent results have been reported regarding the activities of antioxidant enzymes in plants exposed to PAHs. Song et al. [83] observed that SOD and other antioxidant enzymes were increased by PAHs in *Kandelia candel* leaves and roots. In contrast, Wei et al. [84] found that phenanthrene tended to decrease antioxidant activities. In our study, we observed the highest production of antioxidant enzymes under phenanthrene exposure with the dual fungal inoculation treatment. Several studies suggest that plants in better conditions produce higher antioxidant enzymes than stressed plants or those that have suffered several types of damage by an external factor such as abiotic or biotic stress [85,86]. According to Chang et al. [87], this higher activity helps to remove the ROS generated by stress factors like PAHs efficiently. This is closely related to the results of lipid peroxidation that was reduced by the dual inoculation due to these higher antioxidant activities that minimized the impact of phenanthrene compared to non-inoculated treatments. On the other hand, the reduced translocation of phenanthrene from the soil to the shoot in mycorrhizal wheat plants may allow a higher production of antioxidant enzymes against PAHs, as well as contribute to reducing lipid peroxidation. These results suggest that wheat plants co-inoculated with *F. mosseae* and *T. viride* mitigate the negative effects of PAH contamination by increasing the production of antioxidant enzymes to alleviate stress.

To the best of our knowledge, this is the first report on plant stress alleviation with saprotrophic and mycorrhizal fungi in PAH-polluted soils, which should be further explored when developing phytoremediation strategies of soils contaminated with PAHs.

5. Conclusions

Our study showed that the association of wheat roots with the plant-growth-promoting fungi *F. mosseae* and *T. viride* improved phenanthrene soil dissipation and alleviated oxidative stress in wheat. These improved dissipation rates are related to the enhancement of soil enzymes involved in PAH removal and organic acid exudation. Additionally, according to the undetectable phenanthrene results, the main mode of phenanthrene dissipation in soil could be attributed to its degradation.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/3/411/s1, Table S1: Significance of the main treatment effects based on factorial ANOVA, Table S2: Significance of the main treatment effects based on factorial ANOVA on arbuscular mycorrhizal colonization parameters, Table S3: Significance of the main treatment effects based on factorial ANOVA on phenanthrene quantity in soil and vegetal tissues and phenanthrene dissipation,

Figure S1: Intraradical colonization of *Triticum aestivum* inoculated with *F. mosseae* growing in a soil with phenanthrene at 0 and 1000 mg/kg^{-1}.

Author Contributions: Conceptualization, C.L. and C.A.; methodology, C.L. and A.F.; validation, C.L. and J.L.; formal analysis, C.L., R.C.-V. and H.H.; investigation, C.L.; data curation, C.L.; writing—original draft preparation, C.L.; writing—review and editing, C.L., I.G.-R., H.H. and C.A.; funding acquisition, C.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fondo Nacional de Desarrollo Científico y Tecnológico, grant numbers 1170931 to C.A. and 3200134 to H.H.; Comisión Nacional de Investigación Científica y Tecnológica, grant number 21120476 to C.L.; and Universidad de la Frontera-MECE Program 2, grant number FRO 1204 to C.A.

Data Availability Statement: Data is contained within the article or Supplementary Material.

Acknowledgments: We would like to thank Javier Ortiz and Javiera Soto for their technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rajendran, P.; Jayakumar, T.; Nishigaki, I.; Ekambaram, G.; Nishigaki, Y.; Vetriselvi, J.; Sakthisekaran, D. Immunomodulatory effect of mangiferin in experimental animals with benzo (a) pyrene-induced lung carcinogenesis. *Int. J. Biomed. Sci.* **2013**, *9*, 68. [PubMed]
2. Sogbanmu, T.O.; Nagy, E.; Phillips, D.H.; Arlt, V.M.; Otitoloju, A.A.; Bury, N.R. Lagos lagoon sediment organic extracts and polycyclic aromatic hydrocarbons induce embryotoxic, teratogenic and genotoxic effects in Danio rerio (zebrafish) embryos. *Environ. Sci. Pollut. Res.* **2016**, *23*, 14489–14501. [CrossRef] [PubMed]
3. Conejo-Saucedo, U.; Olicón-Hernández, D.R.; Robledo-Mahón, T.; Stein, H.P.; Calvo, C.; Aranda, E. Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs) Contaminated Soil Through Fungal Communities. In *Recent Advancement in White Biotechnology through Fungi*; Yadav, N., Mishra, S., Singh, S., Gupta, A., Eds.; Springer: Cham, Switzerland, 2019; pp. 217–236.
4. Li, Y.; Li, Y.; Yang, H.; Chen, L.; Cao, Y. Sequestration Specificity of Single or Co-existing Benzene, 1, 3, 5-Trimethylbenzene, and Naphthalene in Soil. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 299–304. [CrossRef]
5. Zhang, S.; Yao, H.; Lu, Y.; Yu, X.; Wang, J.; Sun, S.; Liu, M.; Li, D.; Li, Y.-F.; Zhang, D. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. *Sci. Rep.* **2017**, *7*, 1–11. [CrossRef]
6. Li, Z.; Sandau, C.D.; Romanoff, L.C.; Caudill, S.P.; Sjodin, A.; Needham, L.L.; Patterson, D.G., Jr. Concentration and profile of 22 urinary polycyclic aromatic hydrocarbon metabolites in the US population. *Environ. Res.* **2008**, *107*, 320–331. [CrossRef]
7. Cheema, S.A.; Khan, M.I.; Shen, C.; Tang, X.; Farooq, M.; Chen, L.; Zhang, C.; Chen, Y. Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. *J. Hazard. Mater.* **2010**, *177*, 384–389. [CrossRef]
8. Wild, E.; Dent, J.; Thomas, G.O.; Jones, K.C. Direct observation of organic contaminant uptake, storage, and metabolism within plant roots. *Environ. Sci. Technol.* **2005**, *39*, 3695–3702. [CrossRef]
9. Arriagada, C.; Aranda, E.; Sampedro, I.; Garcia-Romera, I.; Ocampo, J. Interactions of Trametes versicolor, Coriolopsis rigida and the arbuscular mycorrhizal fungus Glomus deserticola on the copper tolerance of Eucalyptus globulus. *Chemosphere* **2009**, *77*, 273–278. [CrossRef]
10. Herrera, H.; Palma, G.; Almonacid, L.; Campos, R.; Fuentes, A.; Garcia-Romera, I.; Arriagada, C. Improving Soil Simazine Dissipation Through an Organic Amendment Inoculated with Trametes versicolor. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 262–269.
11. Raimondo, E.E.; Aparicio, J.D.; Briceño, G.E.; Fuentes, M.S.; Benimeli, C.S. Lindane Bioremediation in Soils of Different Textural Classes by an Actinobacteria Consortium. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 29–41. [CrossRef]
12. Aranda, E.; Scervino, J.M.; Godoy, P.; Reina, R.; Ocampo, J.A.; Wittich, R.-M.; García-Romera, I. Role of arbuscular mycorrhizal fungus Rhizophagus custos in the dissipation of PAHs under root-organ culture conditions. *Environ. Pollut.* **2013**, *181*, 182–189. [CrossRef] [PubMed]
13. Atagana, H.I. Biodegradation of PAHs by fungi in contaminated-soil containing cadmium and nickel ions. *Afr. J. Biotechnol.* **2009**, *8*, 5780–5789.
14. Yu, X.; Wu, S.; Wu, F.; Wong, M.H. Enhanced dissipation of PAHs from soil using mycorrhizal ryegrass and PAH-degrading bacteria. *J. Hazard. Mater.* **2011**, *186*, 1206–1217. [CrossRef] [PubMed]
15. Yi, H.; Crowley, D.E. Biostimulation of PAH degradation with plants containing high concentrations of linoleic acid. *Environ. Sci. Technol.* **2007**, *41*, 4382–4388. [CrossRef] [PubMed]
16. Liao, X.; Wu, Z.; Li, Y.; Cao, H.; Su, C. Effect of various chemical oxidation reagents on soil indigenous microbial diversity in remediation of soil contaminated by PAHs. *Chemosphere* **2019**, *226*, 483–491. [CrossRef]
17. Mao, Y.; Zhang, L.; Wang, Y.; Yang, L.; Yin, Y.; Su, X.; Liu, Y.; Pang, H.; Xu, J.; Hu, Y. Effects of polycyclic aromatic hydrocarbons (PAHs) from different sources on soil enzymes and microorganisms of Malus prunifolia var. Ringo. *Arch. Agron. Soil Sci.* **2020**. [CrossRef]

18. Cristaldi, A.; Conti, G.O.; Jho, E.H.; Zuccarello, P.; Grasso, A.; Copat, C.; Ferrante, M. Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. *Environ. Technol. Innov.* **2017**, *8*, 309–326.
19. Wagner, D.; Przybyla, D.; op den Camp, R.; Kim, C.; Landgraf, F.; Lee, K.P.; Würsch, M.; Laloi, C.; Nater, M.; Hideg, E. The genetic basis of singlet oxygen–induced stress responses of Arabidopsis thaliana. *Science* **2004**, *306*, 1183–1185. [CrossRef]
20. Liu, H.; Weisman, D.; Tang, L.; Tan, L.; Zhang, W.-K.; Wang, Z.-H.; Huang, Y.-H.; Lin, W.-X.; Liu, X.-M.; Colón-Carmona, A. Stress signaling in response to polycyclic aromatic hydrocarbon exposure in Arabidopsis thaliana involves a nucleoside diphosphate kinase, NDPK-3. *Planta* **2015**, *241*, 95–107. [CrossRef]
21. Pašková, V.; Hilscherová, K.; Feldmannová, M.; Bláha, L. Toxic effects and oxidative stress in higher plants exposed to polycyclic aromatic hydrocarbons and their N-heterocyclic derivatives. *Environ. Toxicol. Chem.* **2006**, *25*, 3238–3245. [CrossRef]
22. Oenel, A.; Fekete, A.; Krischke, M.; Faul, S.C.; Gresser, G.; Havaux, M.; Mueller, M.J.; Berger, S. Enzymatic and non-enzymatic mechanisms contribute to lipid oxidation during seed aging. *Plant Cell Physiol.* **2017**, *58*, 925–933. [CrossRef] [PubMed]
23. Huang, H.; Ullah, F.; Zhou, D.-X.; Yi, M.; Zhao, Y. Mechanisms of ROS regulation of plant development and stress responses. *Front. Plant Sci.* **2019**, *10*, 800. [CrossRef] [PubMed]
24. Hashemi, S. Effect of Nanoparticles on Lipid Peroxidation in Plants. In *Advances in Lipid Metabolism*; Valenzuela, R., Ed.; IntechOpen: London, UK, 2019.
25. Alkio, M.; Tabuchi, T.M.; Wang, X.; Colon-Carmona, A. Stress responses to polycyclic aromatic hydrocarbons in Arabidopsis include growth inhibition and hypersensitive response-like symptoms. *J. Exp. Bot.* **2005**, *56*, 2983–2994. [CrossRef] [PubMed]
26. Yun, Y.; Liang, L.; Wei, Y.; Luo, Z.; Yuan, F.; Li, G.; Sang, N. Exposure to Nitro-PAHs interfere with germination and early growth of Hordeum vulgare via oxidative stress. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 756–761. [CrossRef] [PubMed]
27. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*; Academic Press: Cambridge, MA, USA, 2010.
28. Herrera, H.; García-Romera, I.; Meneses, C.; Pereira, G.; Arriagada, C. Orchid Mycorrhizal Interactions on the Pacific Side of the Andes from Chile. A Review. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 187–202. [CrossRef]
29. Wu, Q.-S.; Srivastava, A.K.; Zou, Y.-N. AMF-induced tolerance to drought stress in citrus: A review. *Sci. Hort.* **2013**, *164*, 77–87. [CrossRef]
30. Ren, C.-G.; Kong, C.-C.; Bian, B.; Liu, W.; Li, Y.; Luo, Y.-M.; Xie, Z.-H. Enhanced phytoremediation of soils contaminated with PAHs by arbuscular mycorrhiza and rhizobium. *Int. J. Phytoremediat.* **2017**, *19*, 789–797. [CrossRef]
31. Gao, Y.; Cheng, Z.; Ling, W.; Huang, J. Arbuscular mycorrhizal fungal hyphae contribute to the uptake of polycyclic aromatic hydrocarbons by plant roots. *Bioresour. Technol.* **2010**, *101*, 6895–6901. [CrossRef]
32. Fuentes, A.; Almonacid, L.; Ocampo, J.A.; Arriagada, C. Synergistic interactions between a saprophytic fungal consortium and Rhizophagus irregularis alleviate oxidative stress in plants grown in heavy metal contaminated soil. *Plant Soil* **2016**, *407*, 355–366. [CrossRef]
33. Gao, Y.; Li, Q.; Ling, W.; Zhu, X. Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene. *J. Hazard. Mater.* **2011**, *185*, 703–709. [CrossRef]
34. Contreras-Cornejo, H.A.; Macías-Rodríguez, L.; Cortés-Penagos, C.; López-Bucio, J. Trichoderma virens, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Plant Physiol.* **2009**, *149*, 1579–1592. [CrossRef] [PubMed]
35. Harman, G.E. Overview of mechanisms and uses of Trichoderma spp. *Phytopathology* **2006**, *96*, 190–194. [CrossRef] [PubMed]
36. Tripathi, P.; Singh, P.C.; Mishra, A.; Chauhan, P.S.; Dwivedi, S.; Bais, R.T.; Tripathi, R.D. Trichoderma: A potential bioremediator for environmental clean up. *Clean Technol. Environ. Policy* **2013**, *15*, 541–550. [CrossRef]
37. Li, X.; Peng, D.; Zhang, Y.; Ju, D.; Guan, C. Klebsiella sp. PD3, a phenanthrene (PHE)-degrading strain with plant growth promoting properties enhances the PHE degradation and stress tolerance in rice plants. *Ecotoxicol. Environ. Saf.* **2020**, *201*, 110804. [CrossRef]
38. Urana, R.; Dahiya, A.; Sharma, P.; Singh, N. Effects of Plant Growth Promoting Rhizobacteria on Phytoremediation of Phenanthrene Contaminated Sodic Soil. *Polycycl. Aromat. Compd.* **2019**. [CrossRef]
39. Li, X.; Wu, Y.; Lin, X.; Zhang, J.; Zeng, J. Dissipation of polycyclic aromatic hydrocarbons (PAHs) in soil microcosms amended with mushroom cultivation substrate. *Soil Biol. Biochem.* **2012**, *47*, 191–197. [CrossRef]
40. Zafra, G.; Cortés-Espinosa, D.V. Biodegradation of polycyclic aromatic hydrocarbons by Trichoderma species: A mini review. *Environ. Sci. Pollut. Res.* **2015**, *22*, 19426–19433. [CrossRef]
41. Arriagada, C.; Manquel, D.; Cornejo, P.; Soto, J.; Sampedro, I.; Ocampo, J. Effects of the co-inoculation with saprobe and mycorrhizal fungi on Vaccinium corymbosum growth and some soil enzymatic activities. *J. Soil Sci. Plant Nutr.* **2012**, *12*, 283–294. [CrossRef]
42. Lagos, C.; Larsen, J.; Correa, E.S.; Almonacid, L.; Herrera, H.; Fuentes, A.; Arriagada, C. Dual inoculation with mycorrhizal and saprotrophic fungi suppress the maize growth and development under phenanthrene exposure. *J. Soil Sci. Plant Nutr.* **2018**, *18*, 721–734. [CrossRef]
43. Bouranis, D.; Chorianopoulou, S.; Nocito, F.; Sacchi, G.; Serelis, K. The crucial role of sulfur in a phytoremediation process: Lessons from the Poaceae species as phytoremediants: A review. In *Proceedings of an International Conference; Protection and Restoration of the Environment XI*; Katsifarakis, K., Theodossiou, N., Christodoulatos, C., Koutsospyros, A., Mallios, Z., Eds.; Natural Treatment Systems: Thessaloniki, Greece; pp. 634–643.

44. Kuang, S.; Yu, W.; Song, Y.; Su, Y.; Wang, H.; Wang, L. Dissipation of polycyclic aromatic hydrocarbons in crop soils amended with oily sludge. *Acta Geochim.* **2016**, *35*, 437–444. [CrossRef]
45. Yu, W.; Kuang, S.; Zhao, L. Uptake, accumulation and translocation of polycyclic aromatic hydrocarbons by winter wheat cultured on oily sludge-amended soil. *Chin. J. Geochem.* **2013**, *32*, 295–302. [CrossRef]
46. Herrera, H.; Sanhueza, T.; Martiarena, R.; Valadares, R.; Fuentes, A.; Arriagada, C. Mycorrhizal Fungi Isolated from Native Terrestrial Orchids from Region of La Araucanía, Southern Chile. *Microorganisms* **2020**, *8*, 1120. [CrossRef] [PubMed]
47. Northcott, G.L.; Jones, K.C. Spiking hydrophobic organic compounds into soil and sediment: A review and critique of adopted procedures. *Environ. Toxicol. Chem. Int. J.* **2000**, *19*, 2418–2430. [CrossRef]
48. Phillips, J.M.; Hayman, D. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* **1970**, *55*, 158–161. [CrossRef]
49. Mycocalc. Available online: https://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html (accessed on 20 March 2020).
50. Acevedo, F.; Pizzul, L.; del Pilar, M.C.; Cuevas, R.; Diez, M.C. Degradation of polycyclic aromatic hydrocarbons by the Chilean white-rot fungus Anthracophyllum discolor. *J. Hazard. Mater.* **2011**, *185*, 212–219. [CrossRef]
51. Mannino, G.; Di Stefano, V.; Lauria, A.; Pitonzo, R.; Gentile, C. Vaccinium macrocarpon (Cranberry)-Based Dietary Supplements: Variation in Mass Uniformity, Proanthocyanidin Dosage and Anthocyanin Profile Demonstrates Quality Control Standard Needed. *Nutrients* **2020**, *12*, 992. [CrossRef]
52. Zitka, O.; Babula, P.; Sochor, J.; Kummerova, M.; Krystofova, O.; Adam, V.; Havel, L.; Beklova, M.; Hubalek, J.; Kizek, R. Determination of eight polycyclic aromatic hydrocarbons and in pea plants (Pisum sativum L.) extracts by high performance liquid chromatography with electrochemical detection. *Int. J. Electrochem. Sci.* **2012**, *7*, 908–927.
53. Casida, L.; Klein, D.; Santoro, T. Soil dehydrogenase activity. *Soil Sci.* **1964**, *98*, 371–376. [CrossRef]
54. Elgueta, S.; Santos, C.; Lima, N.; Diez, M. Immobilization of the white-rot fungus Anthracophyllum discolor to degrade the herbicide atrazine. *AMB Express* **2016**, *6*, 1–11. [CrossRef]
55. Herrera, H.; Valadares, R.; Oliveira, G.; Fuentes, A.; Almonacid, L.; do Nascimento, S.V.; Bashan, Y.; Arriagada, C. Adaptation and tolerance mechanisms developed by mycorrhizal Bipinnula fimbriata plantlets (Orchidaceae) in a heavy metal-polluted ecosystem. *Mycorrhiza* **2018**, *28*, 651–663.
56. Donahue, J.L.; Okpodu, C.M.; Cramer, C.L.; Grabau, E.A.; Alscher, R.G. Responses of antioxidants to paraquat in pea leaves (relationships to resistance). *Plant Physiol.* **1997**, *113*, 249–257. [CrossRef] [PubMed]
57. Pinhero, R.G.; Rao, M.V.; Paliyath, G.; Murr, D.P.; Fletcher, R.A. Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol-induced chilling tolerance of maize seedlings. *Plant Physiol.* **1997**, *114*, 695–704. [CrossRef] [PubMed]
58. Du, Z.; Bramlage, W.J. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. *J. Agric. Food Chem.* **1992**, *40*, 1566–1570. [CrossRef]
59. Jajoo, A.; Mekala, N.R.; Tomar, R.S.; Grieco, M.; Tikkanen, M.; Aro, E.-M. Inhibitory effects of polycyclic aromatic hydrocarbons (PAHs) on photosynthetic performance are not related to their aromaticity. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 151–155. [CrossRef] [PubMed]
60. Weisman, D.; Alkio, M.; Colón-Carmona, A. Transcriptional responses to polycyclic aromatic hydrocarbon-induced stress in Arabidopsis thalianareveal the involvement of hormone and defense signaling pathways. *BMC Plant Biol.* **2010**, *10*, 59. [CrossRef] [PubMed]
61. Nwoko, C.O. Effect of Arbuscular Mycorrhizal (AM) Fungi on the physiological performance of phaseolus vulgaris grown under crude oil contaminated soil. *J. Geosci. Environ. Prot.* **2014**, *2*, 9–14. [CrossRef]
62. Franco-Ramírez, A.; Ferrera-Cerrato, R.; Varela-Fregoso, L.; Pérez-Moreno, J.; Alarcón, A. Arbuscular mycorrhizal fungi in chronically petroleum-contaminated soils in Mexico and the effects of petroleum hydrocarbons on spore germination. *J. Basic Microbiol.* **2007**, *47*, 378–383. [CrossRef]
63. Gaspar, M.; Cabello, M.; Cazau, M.; Pollero, R. Effect of phenanthrene and Rhodotorula glutinis on arbuscular mycorrhizal fungus colonization of maize roots. *Mycorrhiza* **2002**, *12*, 55–59.
64. Zafra, G.; Moreno-Montaño, A.; Absalón, Á.E.; Cortés-Espinosa, D.V. Degradation of polycyclic aromatic hydrocarbons in soil by a tolerant strain of Trichoderma asperellum. *Environ. Sci. Pollut. Res.* **2015**, *22*, 1034–1042. [CrossRef]
65. Korolev, N.; David, D.R.; Elad, Y. The role of phytohormones in basal resistance and Trichoderma-induced systemic resistance to Botrytis cinerea in Arabidopsis thaliana. *BioControl* **2008**, *53*, 667–683. [CrossRef]
66. Fracchia, S.; Mujica, M.; García-Romera, I.; Garcia-Garrido, J.; Martin, J.; Ocampo, J.; Godeas, A. Interactions between Glomus mosseae and arbuscular mycorrhizal sporocarp-associated saprophytic fungi. *Plant Soil* **1998**, *200*, 131–137. [CrossRef]
67. Saldajeno, M.; Chandanie, W.; Kubota, M.; Hyakumachi, M. Effects of interactions of arbuscular mycorrhizal fungi and beneficial saprophytic mycoflora on plant growth and disease protection. In *Mycorrhizae: Sustainable Agriculture and Forestry*; Siddiqui, Z., Akhtar, M., Futai, K., Eds.; Springer: Dordrecht, The Netherlands, 2008; pp. 211–226.
68. Almonacid, L.; Fuentes, A.; Ortiz, J.; Salas, C.; Garcia-Romera, I.; Ocampo, J.; Arriagada, C. Effect of mixing soil saprophytic fungi with organic residues on the response of S olanum lycopersicum to arbuscular mycorrhizal fungi. *Soil Use Manag.* **2015**, *31*, 155–164. [CrossRef]
69. Hawkes, C.V.; Hartley, I.P.; Ineson, P.; Fitter, A.H. Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. *Glob. Chang. Biol.* **2008**, *14*, 1181–1190. [CrossRef]

70. Cabello, M.N. Hydrocarbon pollution: Its effect on native arbuscular mycorrhizal fungi (AMF). *FEMS Microbiol. Ecol.* **1997**, *22*, 233–236. [CrossRef]
71. Lu, Y.-F.; Lu, M. Remediation of PAH-contaminated soil by the combination of tall fescue, arbuscular mycorrhizal fungus and epigeic earthworms. *J. Hazard. Mater.* **2015**, *285*, 535–541. [CrossRef]
72. Wu, F.; Yu, X.; Wu, S.; Lin, X.; Wong, M.H. Phenanthrene and pyrene uptake by arbuscular mycorrhizal maize and their dissipation in soil. *J. Hazard. Mater.* **2011**, *187*, 341–347. [CrossRef]
73. Sadhana, B. Arbuscular Mycorrhizal Fungi (AMF) as a biofertilizer-a review. *Int. J. Curr. Microbiol. App. Sci* **2014**, *3*, 384–400.
74. Gerhardt, K.E.; Huang, X.-D.; Glick, B.R.; Greenberg, B.M. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Sci.* **2009**, *176*, 20–30. [CrossRef]
75. Li, Y.-T.; Rouland, C.; Benedetti, M.; Li, F.-B.; Pando, A.; Lavelle, P.; Dai, J. Microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. *Soil Biol. Biochem.* **2009**, *41*, 969–977. [CrossRef]
76. Kumar, S.; Chaudhuri, S.; Maiti, S. Soil dehydrogenase enzyme activity in natural and mine soil-a review. *Middle East J. Sci. Res.* **2013**, *13*, 898–906.
77. Zhang, N.; Xing-Dong, H.; Yu-Bao, G.; Yong-Hong, L.; Hai-Tao, W.; Di, M.; Zhang, R.; Yang, S. Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in Artemisia ordosica community. *Pedosphere* **2010**, *20*, 229–235. [CrossRef]
78. Rabie, G.H. Role of arbuscular mycorrhizal fungi in phytoremediation of soil rhizosphere spiked with poly aromatic hydrocarbons. *Mycobiology* **2005**, *33*, 41–50. [CrossRef] [PubMed]
79. Ortiz, J.; Soto, J.; Fuentes, A.; Herrera, H.; Meneses, C.; Arriagada, C. The endophytic fungus Chaetomium cupreum regulates expression of genes involved in the tolerance to metals and plant growth promotion in Eucalyptus globulus roots. *Microorganisms* **2019**, *7*, 490. [CrossRef]
80. Patel, J.S.; Singh, A.; Singh, H.B.; Sarma, B.K. Plant genotype, microbial recruitment and nutritional security. *Front. Plant Sci.* **2015**, *6*, 608. [CrossRef] [PubMed]
81. Wei, L.; Chen, C.; Xu, Z. Citric acid enhances the mobilization of organic phosphorus in subtropical and tropical forest soils. *Biol. Fertil. Soils* **2010**, *46*, 765–769. [CrossRef]
82. Ling, W.; Sun, R.; Gao, X.; Xu, R.; Li, H. Low-molecular-weight organic acids enhance desorption of polycyclic aromatic hydrocarbons from soil. *Eur. J. Soil Sci.* **2015**, *66*, 339–347. [CrossRef]
83. Song, H.; Wang, Y.-S.; Sun, C.-C.; Wu, M.-L.; Peng, Y.-L.; Deng, C.; Li, Q.P. Effects of polycyclic aromatic hydrocarbons exposure on antioxidant system activities and proline content in Kandelia candel. *Oceanol. Hydrobiol. Stud.* **2011**, *40*, 9. [CrossRef]
84. Wei, H.; Song, S.; Tian, H.; Liu, T. Effects of phenanthrene on seed germination and some physiological activities of wheat seedling. *C. R. Biol.* **2014**, *337*, 95–100. [CrossRef]
85. Carrasco-Ríos, L.; Pinto, M. Effect of salt stress on antioxidant enzymes and lipid peroxidation in leaves in two contrasting corn, 'Lluteno' and 'Jubilee'. *Chil. J. Agric. Res.* **2014**, *74*, 89–95. [CrossRef]
86. Kusvuran, S.; Kiran, S.; Ellialtioglu, S.S. Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. In *Abiotic and Biotic Stress in Plants-Recent Advances and Future Perspectives*; InTechOpen: London, UK, 2016; pp. 481–503.
87. Chang, W.; Sui, X.; Fan, X.-X.; Jia, T.-T.; Song, F.-Q. Arbuscular mycorrhizal symbiosis modulates antioxidant response and ion distribution in salt-stressed Elaeagnus angustifolia seedlings. *Front. Microbiol.* **2018**, *9*, 652. [CrossRef]