

Article

Subcellular Element Distribution in Shoots of Wheat Grown in an Acidic Soil with Native AMF Extraradical Mycelium

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Abstract: Soil acidity can reduce crop growth by increasing bioavailable soil Al, Fe, and/or Mn to toxic levels. The presence of an intact extraradical mycelium (ERM) of arbuscular mycorrhizal fungi (AMF), developed by the native *Ornithopus compressus* in the acidic soil, can increase wheat growth and prevent symptoms of Mn toxicity. To understand the protective effect of the intact ERM of this native plant on wheat element balance and distribution, in the present study, shoot Al, Fe, K, Zn, Na, and Si levels and their subcellular partitioning were determined by inductively coupled plasma mass spectrometry (ICP-MS), for the first time, for this system. In undisturbed soil, where an intact ERM structure is maintained, wheat shoot growth was promoted, probably due to faster root mycorrhizal colonization. The levels of potentially toxic Al and Fe were reduced, the proportions of the macronutrient K and micronutrient Zn were higher in the symplast, and the Na proportion increased in the vacuole, while Si increased in the apoplast. Overall, the undisturbed soil from *O. compressus* treatment appeared to influence the uptake and distribution of essential and beneficial elements, as a strategy to reduce the negative effect of soil acidity on wheat growth. Understanding the dynamics of element distribution influenced by stress-adapted AMF on wheat growth can provide more sustainable approaches to intensive agriculture.

Keywords: acid soil; ICP-MS; manganese; metal toxicity; mycorrhiza; *Ornithopus compressus*; soil health; sustainable farming; wheat



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1. Introduction

Acidic soils are prone to accumulate high levels of bioavailable Al, Fe, and Mn. As the soil pH decreases, these metals are released from their soil-bound forms into the bioavailable soil solution and can reach toxic levels in plants. The occurrence of toxicity generally depends on the physico-chemical properties of the soil, the dynamics of the plant metal uptake, and plant tolerance [1]. The symptoms of toxicity vary greatly according to the metal(s) causing toxicity and the affected plants, but they include reduced plant growth, structural injury to the shoots and roots, and extensive oxidative damage. These can result directly from the toxic levels of metals, but also from an imbalance in nutrient homeostasis, due to the increased competition of some metals for transporters of nutrients with a similar ionic radius [2–4].

In soils of the Montado ecosystem, in south-east Portugal, acidity causes excessive bioavailable Mn. The symptoms of Mn toxicity have been detected in several crops, including wheat and subterranean clover [5–7]. In wheat, toxic levels of Mn reduce growth and productivity by inducing stunting and chlorosis in older leaves. In previous works,

concentrations above 200 mg Mn/kg of wheat shoot dry weight (considered excessive for cereal species) were found in this system and were linked to lower growth, chlorophyll levels, and photosynthetic performance [8,9]. In addition to the primary effects of excess Mn, the levels and subcellular distribution of other essential nutrients, namely Mg, Ca, and P, were also altered. In wheat root, Ca accumulated in the apoplast, while Mg and P were preferentially translocated to the shoot and increased their proportion in the vacuole, along with Mn [10]. Although Mn toxicity seemed to be the sole driver for these nutrient imbalances, high levels of bioavailable Al and Fe were also detected for this system and could influence wheat nutrient uptake [11].

The most common agricultural practice used to counteract soil acidity, as well as its resulting metal toxicities, is the application of soil correctives, namely, pulverized calcitic lime (Ca carbonates and oxides) or dolomitic lime (Ca and Mg carbonates). These act by increasing the soil pH along with the bioavailable levels of Ca and/or Mg, which effectively increase competition for root transmembrane transporters, and result in a lower uptake of toxic metals and higher internal nutrient/metal ratios [5,12]. Although effective, soil correctives alter soil biochemistry and can become an expensive strategy, if their application is inadequately performed [13].

Recent research has focused on screening alternative strategies that are capable of reducing farming costs and safeguarding soil health and biodiversity. The use of arbuscular mycorrhizal fungi (AMF) has shown notable results as a potential sustainable agricultural practice to counteract soil acidity. AMF are obligate symbionts of almost 80% of land plants. Colonization of plant roots leads to many advantages, with the most reported being increased access to water and nutrients with low soil mobility, e.g., phosphorus [14]. Besides these benefits, AMF are also believed to enhance plant tolerance to abiotic and biotic stress by influencing the host's internal biochemical mechanisms, its metabolome, and element homeostasis [15]. Under Mn toxicity, the AMF developed in symbiosis with stress-adapted native plants can colonize wheat and promote higher shoot weights, change shoot internal levels of important elements, and alter the antioxidant enzyme activity and chlorophyll content [8,9]. Mycorrhization of wheat, grown in acidic soil with Mn toxicity, through the extraradical mycelium (ERM) of AMF associated with *Ornithopus compressus*, a native Montado plant, leads to higher shoot P and S contents and lower Ca, Mg, and Mn levels [16–19]. This implies that the AMF consortium established in the acidic soil exerts a heavy influence on the nutritional composition of wheat. However, in this system, this influence has not been analyzed on other nutrients or non-essential beneficial elements with relevant roles in counteracting Mn toxicity.

Several elements are known to be influenced by high Mn levels or to influence the symptoms of excessive Mn in many agricultural species. For example, in soybean, excessive Mn leads to changes in root or shoot P, K, B, or S contents [20]. In rice [21] and barley [22], the application of K under Mn toxic conditions lessens the damage caused by excessive Mn. Supplementation with K leads to changes in Ca and Mg uptake, but also to the internal concentrations of Fe, P, Zn, or Cu. Additionally, the protective properties provided by the supplementation of Si, a very abundant element that is not considered an essential nutrient for plants, have also been ascertained for plant growth under Mn toxicity [23] and other stresses, e.g., metal, drought or salt (NaCl) [24–26]. Si was found to enhance the uptake of S, Mg, Ca, B, Fe, and Mn, and to reduce that of N, Cu, Zn, and K, in hydroponically grown maize, lettuce, pea, carrot, and wheat [25]. In wheat under salt stress, Si acted by improving many biochemical parameters and altering the subcellular distribution of Na, by reducing its excess in the symplast and favoring accumulation in the apoplast [26].

In the present study, the influence of the ERM of AMF associated with *O. compressus* was investigated by considering the elemental composition of shoots of wheat grown in acidic soil with Mn toxicity. Given that Mn toxicity has already been established for wheat growing in this acidic soil, the potential toxicants Al and Fe, which generally occur in acidic soils; the essential nutrients, K (macronutrient) and Zn (micronutrient); and the beneficial elements Na and Si were quantified in the shoots of wheat grown in disturbed

or undisturbed acidic soil, in order to determine the importance of the intact mycorrhizal ERM structure on the host elemental balance. This work proposes that wheat grown in undisturbed soil avoids Mn toxicity by altering element uptake and distribution through the influence of an intact ERM. Understanding the mechanisms that govern AMF-induced nutritional homeostasis in wheat contributes to devising more sustainable strategies to manage Mn toxicity in acidic soils.

2. Materials and Methods

2.1. Characterization of Soil Fertility

The acidic soil was sampled from an experimental farm complex at Évora University, Portugal (38°32' N; 08°00' W), frequently used to study the impacts of Mn toxicity in crops [10,16,27]. Soil fertility assessment was performed at the certified laboratory of Laboratório Químico Rebelo da Silva, of Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, Portugal. The air-dried and 2 mm sieved sandy loam soil contained the extractable nutrients B at 0.2 mg/kg (hot water/UV–VIS molecular absorption spectrophotometry), Cu at 0.3 mg/kg (Lakanen/Flame atomic absorption spectroscopy (AAS)), Fe at 78 mg/kg (Lakanen/Flame AAS), K at 67 mg/kg (Egner-Rhiem/Flame atomic emission spectroscopy), Mg at 112 mg/kg (1 M ammonium acetate, pH 7/AAS), Mn at 41 mg/kg (Lakanen/Flame AAS), N-NO₃ at 0.4 mg/kg [16], P at 26 mg/kg (Egner-Rhiem/UV–VIS molecular absorption spectrophotometry), Zn at 0.8 mg/kg (Lakanen/Flame AAS), soil organic matter (SOM) at 11 g/kg (chromic acid wet oxidation), a cation exchange capacity (CEC) of 4.5 centimoles of charge per kg (cmol(+)/kg), a base saturation of 60%, and a pH of 5.6 (soil:water = 1:2.5 (*w/v*)) [10,11].

2.2. Plants and Experimental Design

For the establishment of mycorrhizal ERM in this system, previously established and reported protocols were used [6,16,28,29]. *Ornithopus compressus* L. (ORN) and *Triticum aestivum* L. (cv. Ardila) seeds were acquired from local certified sellers and were germinated through sowing on hydrated filter paper and covered with a transparent film to avoid desiccation. Dark plastic pots (8 L) were packed with the soil described above, and five ORN seedlings were planted on equally distanced positions. Plants were kept in fully randomized pots in a greenhouse and freely developed AMF symbiosis with the natural microbiological communities of the soil [28]. Any germinated wild weed species were manually removed. The pots were weighed every day and kept hydrated to ca. 70% of the maximum water holding capacity, by weight. Air temperature (maximum and minimum) was recorded daily and kept below 30 °C by greenhouse cooling. After 7 weeks, ORN plants were eliminated with herbicide, by spraying 6 mL of Roundup® containing 1.3 g/L of glyphosate [16]. In half of the pots, the ERM structure was disrupted by disturbing the soil [30]. The shoots of ORN plants were excised and the soil was removed from each pot through two 0.2 m depth layers and were passed separately through a 4 mm sieve. The root material separated on the sieve was cut into 2 cm long segments and mixed into the soil of the respective layer. The soil was repacked in the pots and arranged in the same two layers. The shoot material was left intact on the soil surface. The pots with undisturbed soil were maintained in the same conditions and ORN shoots were similarly cut and kept in the soil surface. The pots were then kept for one week in the conditions described above for soil stabilization. Following this, six wheat seedlings were planted and grown for three weeks in each soil treatment (four replicate pots). The only fertilization performed was the application of N, ca. 15 mL NH₄NO₃ (1 M)/100 mL of distilled water, to a total concentration of 75 mg of N/kg of dry soil, in order to rule out any possible effects of *O. compressus* (a legume) or soil disturbance on N availability to wheat. The water content of the pots was kept at ca. 70% of the maximum water holding capacity, by weight, to ensure that temporary waterlogging, and consecutive poor aeration, did not occur and enhance Mn levels in the soil solution. Undisturbed soil would be more prone to this condition, which would influence Mn availability and possibly impact the benefits of an intact ERM

for wheat. At the end of this growth period, wheat plants (four replicates) were collected, and the shoots were weighed, immediately frozen in liquid nitrogen, and stored at -80°C until analysis. The steps involving the application of herbicide, soil disturbance, or soil N fertilization were also included in the methodology to more accurately simulate the regional farming practices, even though herbicide use and soil fertilization are known antagonists of AMF development [31,32]. Root colonization with AMF was previously determined to be 74% of the root length for the native ORN plants, and 14% and 56% in the roots of wheat grown in disturbed and undisturbed acidic soil, respectively [16].

2.3. Subcellular Partitioning

Flash frozen wheat shoots (100 mg) were ground to a fine powder, in liquid nitrogen, added to 2.5 mL of ice cold buffer solution (250 mM sucrose, 1.0 mM dithioerythritol, and 50 mM Tris-HCl, pH 7.5), and fractionated through differential centrifugation [11]. The cell wall fraction (CWF) was obtained in the pellet of a 15 min centrifugation of this homogenate, at $2500\times g$ and 4°C , in a Hermle Z 206 compact centrifuge (Hermle labortechnik, Wehingen, Germany). Following this, the supernatant was centrifuged at $100,000\times g$ for 60 min, at 4°C , in a Beckman Coulter Optima L-100K Ultracentrifuge (Beckman Coulter, Brea, CA, USA) in order to obtain the organelle fraction (OF) in the pellet and the vacuole fraction (VF) contained in the supernatant. All fractions were kept at -80°C until analysis.

2.4. Sample Digestion and Element Quantification

Ground flash frozen wheat shoots (50 mg) and the fractions obtained in the previous step were freeze dried on a Telstar LyoQuest lyophilizer (Telstar, Terrassa, Spain). The samples were then digested as described in [10]. The levels of Al, Fe, K, Na, Si, and Zn were quantified on the fully digested samples through inductively coupled plasma mass spectrometry (ICP-MS), in an Agilent 8800 Triple Quadrupole ICP-MS (Agilent, Santa Clara, CA, USA), equipped with a Micromist nebulizer, as described in [17]. The triple quadrupole mass spectrometer collision/reaction cell was set to “no-gas mode” for the quantification of Al, Na, and K; “ O_2 mode” for the quantification of Si; “ NH_3 mode” for the quantification of Fe; and “He mode” for the quantification of Zn.

2.5. Statistical Treatment

The statistical analysis was performed with SPSS version 27 statistics software (IBM, Armonk, NY, USA) [10]. Statistical significance of the data was determined with Student's *t* test for a means comparison at a 95% significance level ($p < 0.05$). The results were presented as the average and standard error of the replicates.

3. Results

3.1. Wheat Growth

Wheat growth was assessed by measuring the shoot dry weight (DW). In undisturbed soil from ORN, the average wheat shoot weight reached 1.2 ± 0.2 g/plant DW (Figure 1). After the disruption of the soil structure (disturbed treatment), the average shoot weight was significantly ($p < 0.05$) lower at 0.5 ± 0.1 g/plant DW.

3.2. Al and Fe Shoot Concentrations and Subcellular Distribution

The levels of Al and Fe were determined in the shoots of wheat grown in disturbed and undisturbed acidic soil, so as to assess the influence of intact ERM on countering high metal concentrations. Lower metal levels were observed in the shoots of wheat grown in undisturbed soil, 62% less for Al and 40% less for Fe, when compared with the disturbed soil (Figure 2a,b). For Al, the disruption of the soil structure led to a higher proportion in the cell wall fraction (CWF) and lower proportion in the organelles (OF) and vacuole fractions (VF) (Figure 2c). For Fe, the wheat shoots showed a lower proportion in the CWF and a higher proportion in the VF in the soil with intact ERM (Figure 2d).

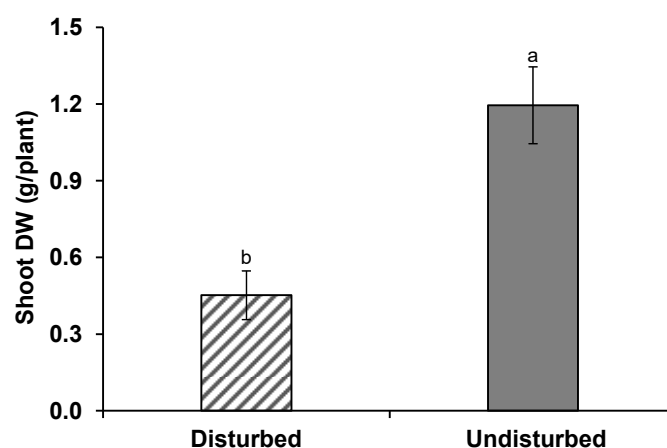


Figure 1. Dry weight (DW) of the shoots of wheat plants grown in disturbed (striped columns) or undisturbed (grey columns) acidic soil, after the growth of *Ornithopus compressus*. Data are presented as means $\pm$ standard error of four independent biological replicates. Different letters indicate statistically significant differences ($p < 0.05$).

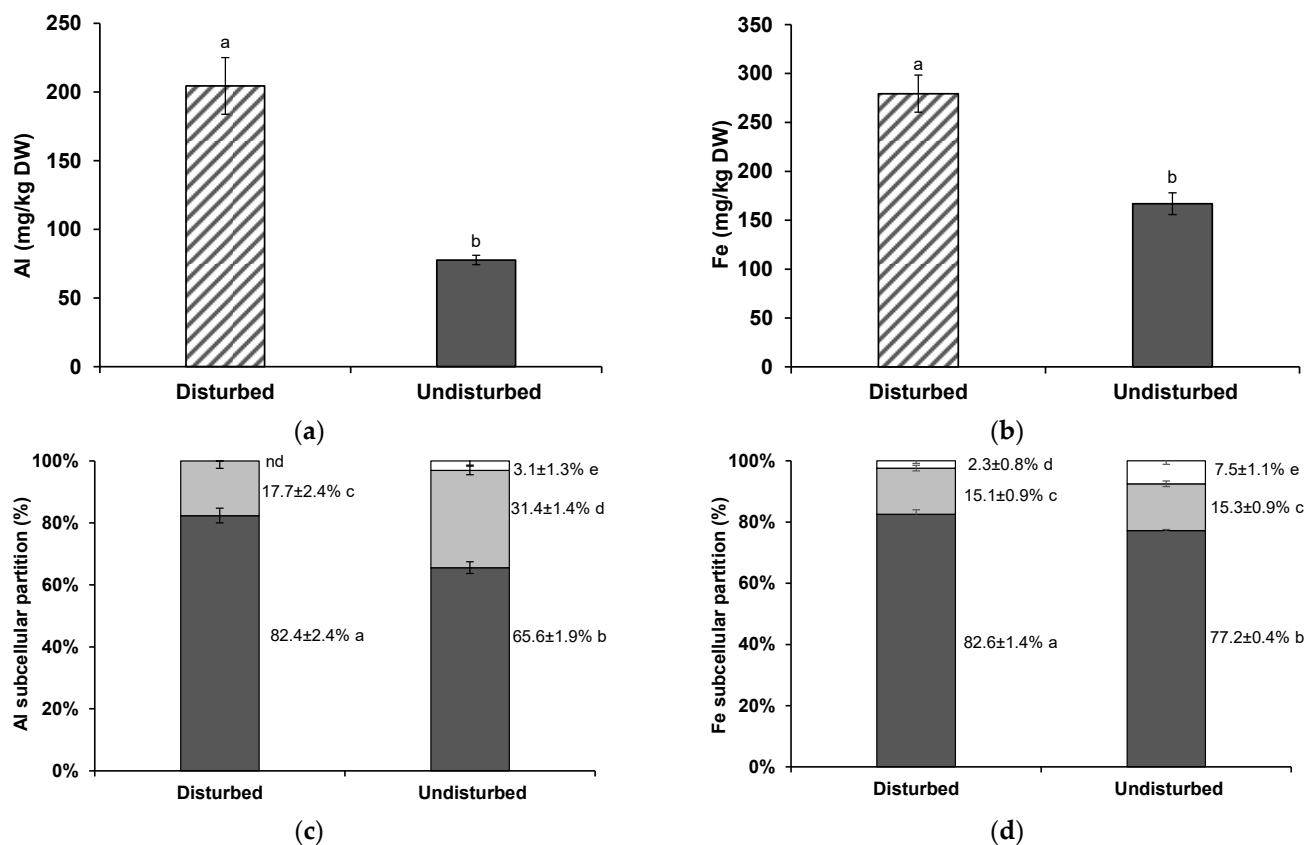


Figure 2. Concentration (average and standard error) of Al (a) and Fe (b) in the shoots of wheat plants grown in disturbed (striped columns) or undisturbed (grey columns) acidic soil, after *Ornithopus compressus* growth. The proportions (average and standard error) in the cell wall fraction (dark grey columns), organelle fraction (light grey columns), and vacuole contents fraction (white columns) were also determined for Al (c) and Fe (d). Values next to bars are the element percentages for each fraction. nd = Below detection limits. Different letters indicate statistically significant differences ($p < 0.05$).

3.3. K and Zn Shoot Concentrations and Subcellular Distribution

The concentrations of the essential macronutrient K and micronutrient Zn were 9% and 35% lower, respectively, in the shoots of wheat grown in undisturbed acidic soil (Figure 3a,b). When compared with disturbed soil, the K proportions were lower in the CWF and OF and higher in VF (Figure 3c), while the Zn proportions were lower in the CWF and higher in OF and VF (Figure 3d).

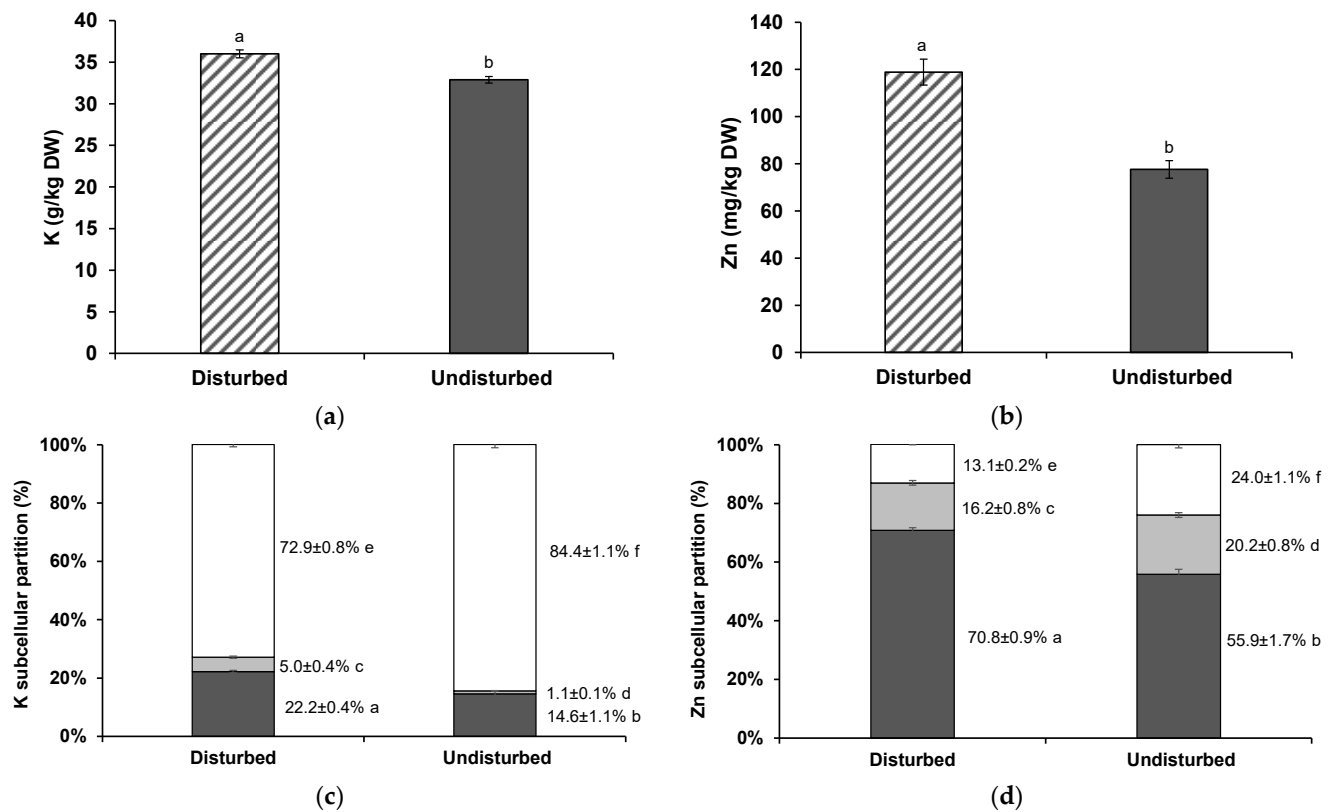


Figure 3. Concentration (average and standard error) of K (a) and Zn (b) in the shoots of wheat plants grown in disturbed (striped columns) or undisturbed (grey columns) acidic soil, after *Ornithopus compressus* growth. The proportions (average and standard error) in the cell wall fraction (dark grey columns), organelle fraction (light grey columns), and vacuole contents fraction (white columns) were also determined for K (c) and Zn (d). Values next to bars are the element percentages for each fraction. Different letters indicate statistically significant differences ($p < 0.05$).

3.4. Na and Si Shoot Concentrations and Subcellular Distribution

The disturbance of the acidic soil structure led to higher Na and Si wheat shoot concentrations (Figure 4a,b). In the presence of a fully formed intact ERM, Na showed lower proportions in the CWF and higher proportions in the OF and VF (Figure 4c). For Si, higher proportions were detected in the CWF and lower proportions were detected in the OF (Figure 4d).

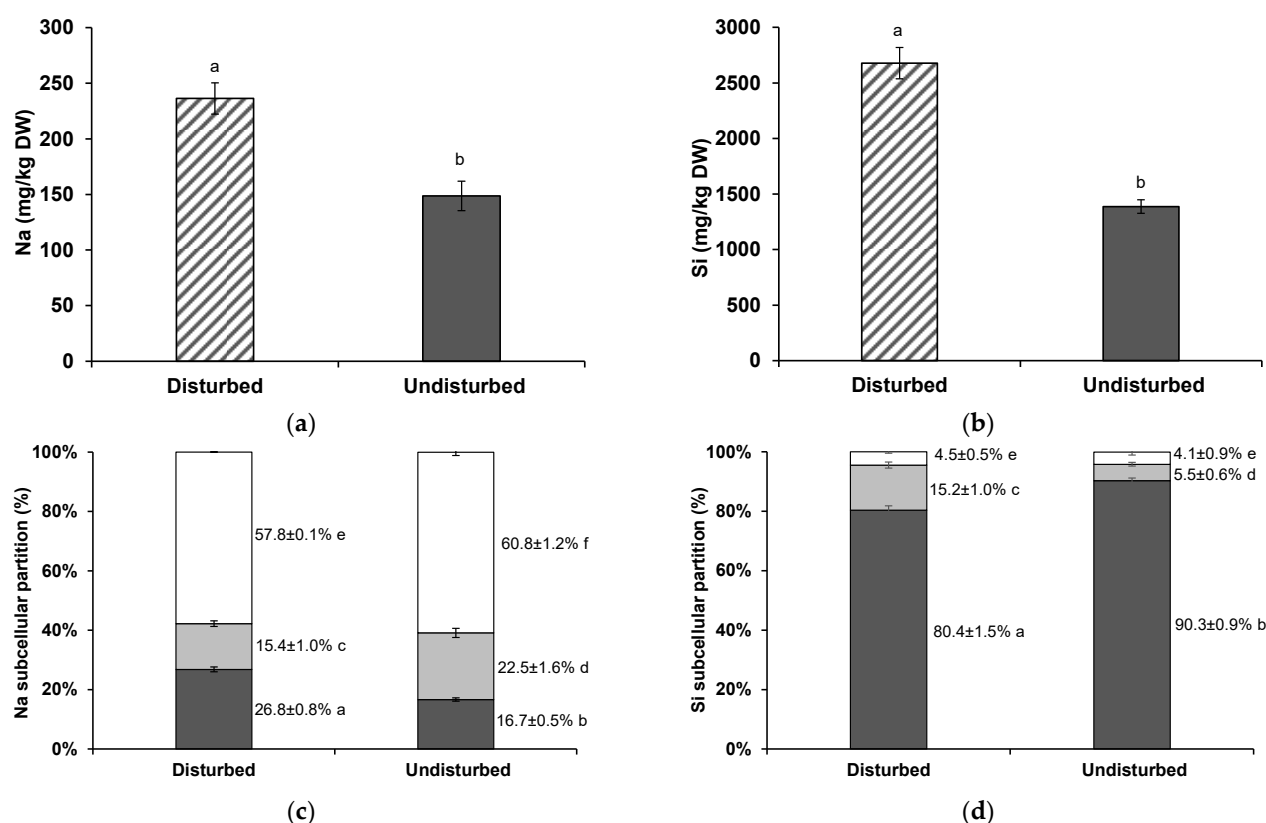


Figure 4. Concentration (average and standard error) of Na (a) and Si (b) in the shoots of wheat plants grown in disturbed (striped columns) or undisturbed (grey columns) acidic soil, after *Ornithopus compressus* growth. The proportions (average and standard error) in the cell wall fraction (dark grey columns), organelle fraction (light grey columns), and vacuole contents fraction (white columns) were also determined for Na (c) and Si (d). Values next to bars are the element percentages for each fraction. Different letters indicate statistically significant differences ($p < 0.05$).

4. Discussion

The beneficial properties of AMF, e.g., the plant growth promoting capacity and protection against biotic and abiotic stress, have been extensively studied for crop plants. However, using AMF as crop fertilizers/protectors is still a practice with very low frequency in modern agriculture. Research on the crop protection and growth stimulation properties of AMF has mainly been performed in controlled conditions and using one or a limited number of fungal species [33]. This condition does not reflect what occurs in the field, where a considerable diversity of locally adapted AMF colonize crop roots and influence plant growth and health. The advantages of using a fully developed ERM from AMF associated with native plants are a faster colonization and establishment of symbiosis with crop roots, and the possibility of selecting the most adequate AMF consortiums to promote higher crop productivities [28]. In acidic soils, when these advantages were provided by the previous growth of *O. compressus*, wheat counteracted Mn toxicity [17]. Some of the biochemical mechanisms involved promoted changes in the activity of plant antioxidant enzymatic machinery and in the levels and subcellular distribution of the toxic Mn and the macronutrients Ca, P, and Mg [17,19,34]. For wheat grown in acidic soils with an intact mycorrhizal ERM associated to *O. compressus*, previous studies determined that shoot Mn concentrations were reduced by 50%, from ca. 200 to under 100 mg Mn/kg shoot DW, below the levels considered toxic for cereals, and its proportion in the cell wall fraction was increased in over 10% when compared with the Mn proportion in the symplast [17].

In a previous study, acidic soils from the Montado ecosystem in the Alentejo region were found to exhibit toxic levels of Mn and high levels of Al and Fe, contributing to the toxicity imposed by this soil in wheat [11]. In the present study, the intact ERM

from *O. compressus* not only increased plant growth (higher shoot dry weight), but also decreased the Al and Fe shoot concentrations. These metals increased their proportion in the symplast, including the vacuole, where they were probably complexed to reduce potential toxic effects. Plants can generally form metal complexes for the detoxification and storage or elimination of toxic metals, resorting to complexation with peptide metal binding ligands, e.g., phytochelatins and metallothioneins, and chelation with organic acids, amino acids, and phosphate derivatives [35]. The beneficial effect of an intact ERM could be seen through the maintenance of the levels of these metals below concentrations considered to be high [11]. Under adequate concentrations, these metals can be responsible for fundamental mechanisms in plants. Fe is an essential plant nutrient involved in important physiological roles, such as chlorophyll synthesis and the overall maintenance of the chloroplast structure and function. Al is not considered an essential element; however, in non-toxic concentrations, it appears to provide beneficial functions in plants, e.g., the stimulation of root growth; changes in the uptake dynamics of plant essential nutrients; and increased antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), including in plants growing under soil acidity [36–38].

The concentrations of beneficial nutrients K and Zn were lower in the shoots of wheat grown in the presence of an intact ERM. Their subcellular distribution was also different under this condition when compared with soil with a disrupted ERM. With an intact ERM, K showed a higher proportion in the vacuole contents fraction, which is comprised of vacuole and cytosol contents, and a lower proportion in the cell wall fraction and organelles fraction. Zinc was detected in a higher proportion in the organelles and vacuole contents fraction and lower proportion in the cell wall fraction. Generally, colonization with AMF promotes an enhancement in plant nutrient levels [39], and the essential nutrients N, S, Ca, Mg, K, Zn, and Cu, as well as the beneficial Si, have been reported to increase in plants grown in acidic soil [40]. In carrot, *Daucus carota*, AMF inoculation improved the levels of carbohydrates and N, P, and K in storage roots, while in faba bean, *Vicia faba*, it increased the levels of C, N, P, and K on both the soil and plant tissues, under elevated carbon dioxide conditions [41,42]. In other works, the enhancement of mineral levels in the soil is generally believed to be partly due to glomalin, a protein generated by AMF with mineral sequestering functions. For example, in peanut, *Arachis hypogaea*, leaf K accumulation, tolerance to drought and overall pod yield was benefited when plants were grown in sterilized soil with a history of AM fungi inoculation [43], which were thus rich in glomalin. Additionally, root exudates of these plants promoted the dissociation of glomalin bound K, which indicates the importance of this AMF generated protein for plant nutritional status. The uptake of K via AMF was recently also linked to a high-affinity potassium transporter identified in colonized tomato, *Solanum lycopersicum*, (SIHAK10) [44], which improves K nutrition and the accumulation of carbohydrates in the host plant, facilitating its AMF colonization. For Zn, AMF colonization can promote two types of responses. Plant uptake appears to be induced under low soil Zn levels, while under high soil Zn, translocation to the shoot tissues is inhibited, although Zn nutrition can also be highly influenced by P levels [45,46]. The increase in plant biomass through an AMF mediation of Zn levels was linked to the regulation of different zinc transporters which protects plant tissues from toxicity or deficiency symptoms [47]. In wheat, the efficiency of AMF colonization on Zn nutrition was additionally reported to be dependent on wheat variety and age [48]. However, these studies were not performed with natural AMF consortiums, but with one or a limited number of species, commonly from *Glomus* or *Claroideoglomus* genera, which are not entirely representative of naturally developed AMF consortiums.

Sodium and silicon are not considered essential nutrients for plants, but are known to contribute to important physiological functions. For example, under low K supply, Na can replace K in some biophysical functions because of its hydrated forms being chemically and structurally very similar. Sodium can take up the function of K in maintaining ionic balance, regulating osmotic pressure, and providing partial K-substitution in protein synthesis, contributing to vacuolar functions and improving water balance via the regulation of stomatal

conductance. In some grasses, trace levels of Na are vital to drive the uptake of pyruvate into chloroplasts by a Na^+ -pyruvate co-transporter, in these cases being considered essential [49,50]. Silicon is the second most abundant element in the Earth's crust, and although it is not considered essential for plant development, a growing body of work points towards a great importance of this metalloid in the interaction of plants with their environment, particularly under stress. Silicon is known to fortify the plant cell wall (protecting against fungal attacks), but also influence the plant's ionome (regulating nutrient acquisition and transport) and metabolome (managing phytohormone levels), contributing to increased plant vigour and resistance to exogenous abiotic and biotic stresses [51].

In the present study, lower concentrations of the beneficial elements Si and Na were found on the shoots of wheat grown in soil with an intact ERM. Additionally, while Na proportions were lower in the apoplast than in the symplast (OF and VF), for Si, unlike the other studied elements, the cell wall fraction proportions of this metalloid were higher for shoots of wheat grown in undisturbed soil. In other studies, Si supplementation to plants growing under Mn toxicity was seen to heavily influence leaf element compartmentalization and biochemical responses. For example, in beans, Si prevented local accumulation of toxic Mn on leaf tissues by influencing its transport into the vacuoles [52]; in wheat, it was seen to increase the thickness of the epidermal layers, influencing Mn accumulation on non-photosynthetic tissues [23]; in rice, it restricted Mn uptake and translocation and influenced non-enzymatic antioxidants [53]; and in cucumber, Si promoted a stronger binding of Mn to the cell walls, lowered Mn concentrations within the symplast, and led to the formation of Si-polyphenol complexes [54,55].

Although the presence of an intact ERM and the faster colonization it provides to the wheat seedlings are known to promote growth and alter nutrient uptake and distribution in this system, undisturbed soil can also be rich in other associated microbiota that can contribute to these beneficial effects [9].

5. Conclusions

The use of natural resources adapted to the stressful conditions of acidic soils provides protection to cultivated crops. Wheat planted in acidic soil grows faster and greater in the presence of an intact ERM from the AMF of Montado native *Ornithopus compressus*. In wheat grown in this soil, the levels of toxic Mn are controlled, high levels of Al and Fe are reduced, the subcellular distribution of the essential nutrients K and Zn are altered, and the beneficial elements Na and Si are compartmentalized so as to reduce the stress imposed by the acidic soil on wheat growth. The biochemical mechanisms promoted in wheat by mycorrhizal colonization with intact ERM are still being researched, but the use of native plants as AMF developers in acidic soils seems to have the potential to contribute to the establishment of sustainable farming practices by obtaining a higher production with lower carbon emissions.

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