

Proceeding Paper

Wheat Shoot Al, Fe, Mn and Zn Levels Are Influenced by Arbuscular Mycorrhiza Extraradical Mycelium Associated to *Ornithopus compressus* in Acidic Soils [†]

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[†] Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: <https://iecps2021.sciforum.net/>.

Abstract: In the present study, the levels of Al, Fe, Mn and Zn in wheat shoots were quantified, by inductively coupled plasma mass spectrometry (ICP-MS). Wheat was grown in an acidic soil with toxic levels of Mn and with intact or disrupted extraradical mycelium (ERM) as the arbuscular mycorrhizal fungi (AMF) inoculum source, resulting from the previous growth of *O. compressus*. In the presence of an intact ERM, toxic Mn levels were reduced, and the concentrations of Al, Fe and Zn decreased 2.3, 1.5 and 2.3-fold, respectively. Disruption of ERM, that leads to a later and slower AM colonization, induced higher wheat shoot Mn and Zn levels (55% and 28%, respectively), but not Al and Fe. Under Mn toxicity, the colonization of wheat by intact ERM of AMF associated to *O. compressus*, in an acidic soil, appears to influence the uptake of Al, Fe and Zn, and positively influence plant growth.

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Biol. Life Sci. Forum* **2021**, *1*, x. <https://doi.org/10.3390/xxxxx>

Keywords: acidic soil; arbuscular mycorrhizal fungi; extraradical mycelium; manganese; sustainable farming; wheat

Academic Editor: Dimitris Bouranis

Published: 2 December 2021

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) are the fungal partners of the oldest and most spread mutualistic association between plants and soil microbes (more than 80% of plants species). The plant hosts involved in the symbiotic partnership benefit from an increased uptake of water and soil nutrients, particularly the less mobile, e.g., phosphorous, (due to a larger surface area used for uptake), while the fungus receives photosynthetic carbon and other essential compounds from the plant host [1]. Nevertheless, several other advantages have been reported to arise from these associations, namely, the shaping of soil texture and fertility (particularly soil organic matter) and an overall protection against abiotic and biotic stress [2]. In acidic soils, where Al, Fe and Mn can increase their bioavailability to toxic levels, stress adapted microorganisms provide protection and promote plant growth. For example, wheat grown in acidic soils from the Montado ecosystem, in southeast Portugal, shows the characteristic symptoms of Mn toxicity, namely, impaired growth associated to leaf chlorosis and tip burn, that progresses from older to younger leaves [3–5]. Manganese toxicity causes severe damages to internal

structures and biochemical imbalances that culminate in intense oxidative stress [6–8]. Additionally, acidity in these soils, was recently seen to also lead to high levels of other metals, e.g., Al and Fe, in wheat tissues, which suggests a multi-metal toxicity, in varying degrees [8].

The growth of stress adapted native plants, previous to wheat planting, promotes the establishment of the extraradical mycelium (ERM) from beneficial AMF, that when kept intact, timely colonizes young crop seedlings and leads to lower contents of toxic Mn and increased growth of wheat plants [9]. The diversity of the AMF consortium that colonizes wheat depends mainly on the choice of the previous cultivated plant [10]. Highly mycotrophic plants provide the best protection and are seen to differentially influence wheat metabolism [11–13]. The ERM developed by AMF associated to *Ornithopus compressus*, a native Leguminosae, provides the highest benefits [9,12–15], yet the chemical and biochemical mechanisms of stress evasion are still being discovered for this system. In fact, the outcome of applying this system on wheat shoot levels of metals other than Mn has not yet been performed.

In the present work, the levels of Al, Fe, Mn and Zn were quantified in shoots of wheat grown in acidic soil without and with the previous development of ERM from AMF associated to *O. compressus*. Profiling the beneficial properties of this system contributes to the improvement of current soil management strategies, towards a more sustainable agriculture with lower fertilizer and pesticide inputs as well as safeguarding natural ecosystem functioning.

2. Materials and Methods

2.1. Plant Material and Experimental Protocol

The acidic soil used was a granitic Eutric Cambisol previously described [6,14]. The plants and experimental setup used were previously described [13]. Briefly, 8 L pots filled with the acidic Cambisol were used to grow *O. compressus*. A negative control (NOP, without the previous growth of *O. compressus*) was also included. After seven weeks, plants were eliminated leaving the soil undisturbed (intact ERM). For a disturbed soil treatment, *O. compressus* plants were eliminated, after the growth period, and the soil was disturbed to disrupt ERM, according to [16]. After seven days, ten wheat (*Triticum aestivum* L. cv. Ardila) seedlings were planted, and after 21 days of growth, shoots were excised, weighted, immediately frozen in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

2.2. Element Quantification in Wheat Shoots

The quantification of toxic Mn and of Al, Fe and Zn in wheat shoots was performed by inductively coupled plasma mass spectrometry (ICP-MS) according to [8]. Briefly, the samples (50 mg) were firstly ground and lyophilized in a Telstar® LyoQuest lyophilizer for 3 days. Prior to the acidic digestion, samples were submitted to a pre-digestion step, by adding 2 mL of HNO_3 (Suprapur, 67–69%, Fisher Chemicals) to the freeze-dried powder and maintaining overnight at room temperature. Pre-digestion prevented overpressure inside the Teflon beakers during the acidic digestion at elevated temperatures. The complete digestion of the lyophilized samples took place in closed beakers at ca. $120\text{ }^{\circ}\text{C}$ for 24 h, followed by a quenching step at ca. $80\text{ }^{\circ}\text{C}$ with 0.5 mL of H_2O_2 (Suprapur, 30%, Merck). After a clear solution was obtained, samples were finally dried on a hotplate, the solid residue was resolubilized in 50 mL of a 2% HNO_3 solution and stored at $4\text{ }^{\circ}\text{C}$ until analysis. For method validation, namely the assessment of accuracy and precision, and the determination of the limits of detection (LOD) and quantification (LOQ), the certified reference materials NIST SRM 1573a (tomato leaves) and NCS ZC73030 (wheat) and a digestion blank were simultaneously submitted to the digestion procedure described above. Trace element analysis was performed using an ICP-MS from Agilent Technologies (8800 QQQ) operating with MS/MS scan type, which

was tuned for sensitivity optimization and the reduction of double charged species and oxides, by external calibration with a set of multi-elemental solutions from High-Purity Standards (Charleston, SC, USA), according to [8]. Instrumental drift and matrix effects were monitored using the internal standards ruthenium (Ru), rhodium (Rh) and iridium (Ir). The collision/reaction cell was set to “no-gas mode” for the quantification of Al and Mn, “He mode” for the quantification of Zn, and “NH₃ mode” for the quantification of Fe.

3. Results and Discussion

Wheat growth was assessed by measuring shoot dry weight (DW). In wheat grown in control soil (NOP, without the previous growth of *O. compressus*), shoot weight was substantially lower (about 66%) than when grown in soil with the previous growth of *O. compressus* (Figure 1). Disruption of ERM structure by soil disturbance was detrimental to wheat growth, leading to shoot weights similar to those of wheat grown in NOP soil. A clear influence of an intact ERM as AMF inoculum source could be seen in wheat growth.

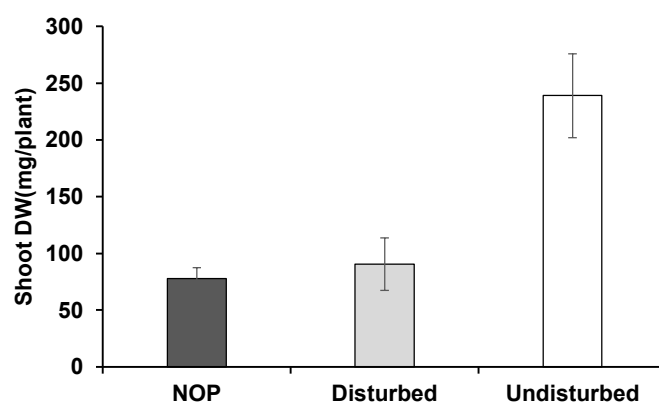


Figure 1. Shoot dry weight (DW, average and standard error) of wheat grown in soil without (NOP, dark grey columns) and with previously grown *Ornithopus compressus*, in disturbed (light grey columns) or undisturbed (white columns) conditions.

Wheat shoot Mn, Al, Fe and Zn levels were also characterized to determine the influence of an intact ERM, as main inoculum source, on plant element homeostasis. The levels of Mn were higher in shoots of wheat grown in acidic soil without the previous growth of *O. compressus* (Figure 2a). Soil with intact ERM led to considerably lower wheat shoot Mn levels (from 247 to 63 mg Mn/kg shoot DW), to levels considered below a toxicity threshold in cereals [9,17]. The disruption of the ERM structure (in disturbed soil) influenced Mn accumulation in the shoots (lower Mn levels), but not as extensively as in the presence of an intact ERM (Figure 2a). Besides the early AMF colonization of wheat, promoted by the intact ERM previously developed in the soil by *O. compressus*, the microbiological environment created in the soil may have promoted the establishment of other plant beneficial microorganisms with the ability to influence wheat Mn uptake or internal partition. For Al and Fe, the presence of an intact ERM in the soil led to a lower element concentration in the shoot, but no substantial influence was detected after soil disturbance (ERM disruption) (Figure 2b,c). For Zn, a similar tendency to Mn was observed (Figure 2d). Shoot Zn levels were higher when wheat was grown in NOP soil but lowered considerably in soil with an intact ERM. Soil disturbance led to intermediate Zn levels in wheat shoots (Figure 2d). These preliminary results suggest that the intact ERM formed by AMF associated to *O. compressus* deeply influence wheat shoot Mn, Al, Fe and Zn levels. Additionally, the rhizospheric environment created by the previous growth of *O. compressus* also influences Mn and Zn, but not Al and Fe.

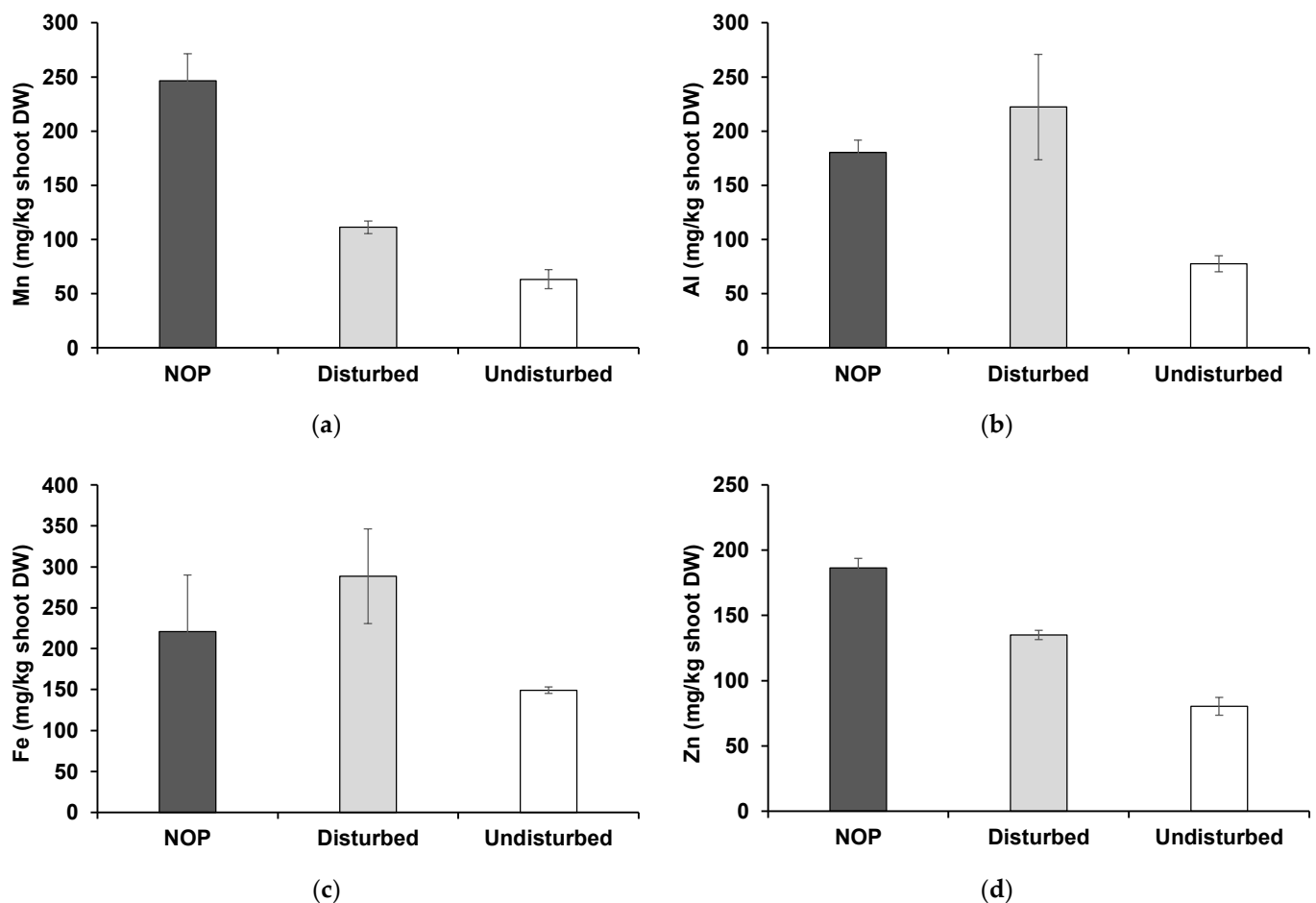


Figure 2. Concentrations (average and standard error) of manganese (a), aluminium (b), iron (c) and zinc (d) (mg element/kg of shoot dry weight (DW) in shoots of wheat plants grown in soil without (NOP, dark grey columns) and with previously grown *Ornithopus compressus*, in disturbed (light grey columns) or undisturbed (white columns) conditions.

Although Al has no known function in plant tissues, in some plants its presence under nontoxic concentrations appears to be beneficial to growth. The mechanisms behind this beneficial effect are believed to be related to the influence of Al assimilation on the uptake dynamics of plant essential nutrients, and in the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) [18]. Fe and Zn are important microelements with essential functions in photosynthesis and plant energy metabolism. Further studies will determine the importance of the tight control of metal elements being exerted by the intact ERM of AMF associated to *O. compressus* on wheat.

Funding: This study was partially funded by Fundo Europeu de Desenvolvimento Regional (FEDER), Programa Operacional Regional Alentejo 2020 under research contract ALT20-03-0145-FEDER-000039.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the findings of this study are available from the corresponding author (Jorge M. S. Faria) upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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