

## Article

# Mycorrhizal Inoculation Improves Mineral Content of Organic Potatoes Grown under Calcareous Soil

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**Abstract:** Soil mycorrhization can play an important role for the qualitative improvement of organically grown “early” potato tubers especially in low fertility soils (such as calcareous ones), by ameliorating plant uptake of limiting mineral nutrients in the soil. Hence, the objective of the present research was to elucidate the impact of soil mycorrhization on the tuber minerals content of three potato cultivars organically grown in two locations with different soil characteristics. Our data revealed the keyrole of soil mycorrhization on the tuber accumulation of Na, Cu, Mn, and P and on reducing the Na/K ratio, although the effects of soil mycorrhization were cultivar- and location-dependent. Accordingly, soil mycorrhization was able to enhance the levels of K and Ca in ‘Arizona’ and that of Mn in ‘Universa’, while it increased the Zn amount in all the cultivars under study. Additionally, soil mycorrhization significantly improved the levels of Cu and Mn in tubers in the location characterized by an initial higher soil level of these micro-minerals. This work highlighted the possibility to fortify organic early potato tubers, in terms of macro- and micro-mineral elements, by applying an eco-sustainable tool such as soil mycorrhization, provided that specific consideration is given to cultivar choice and soil characteristics.

**Keywords:** “early” potato; organic farming; arbuscular mycorrhizal fungi; calcareous soils; tuber mineral composition; sustainability



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## 1. Introduction

In the last decades, the combined concerns for environmental protection, food safety, and security and human and animal welfare significantly increased consumers’ demand for foods sustainably produced [1,2]. This has implicated an increasing development of organic farming, which avoids or widely excludes synthetic agrochemicals in crop management systems [3]. The leading areas are Oceania (35.9 Mha, representing over half of the global organic agricultural land) and Europe (14.6 Mha, which represents 21% of the world’s organic area). Within the European Union (EU), the countries with the widest organic farming areas are Spain (2.1 Mha), Italy (1.9 Mha), and France (1.7 Mha) [4].

Potato (*Solanum tuberosum* L.) is successfully produced under organic farming management both in the main cycle [5,6] and the off-season ones [7–9]. The latter are chiefly realized in the Mediterranean Basin (particularly in the North African countries, Cyprus, Turkey, and southern Italy), where the high air temperatures and abundant request for irrigation water do not allow the usual main cycle (spring–summer), leading for potato production under the winter–spring cycle (“early” potato crop) and summer–autumn ones (“late” potato crop) [10]. Although organic early potato can be sold 200–250 € per ton in Italy [9], it remains a relatively small segment of the market in most EU countries [11]. Indeed, restrictions upon the fertilizers permitted under organic farming generally caused

a reduced soil N availability, which may negatively affect potato plant growth, tuberization, and product quality [12–14]. This situation is exacerbated in the seaside farming areas of the Mediterranean countries, where the early crop potato is usually cropped under calcareous soils. These are characterized by high concentrations (over than 15%) of  $\text{CaCO}_3$  and  $\text{HCO}_3$  in the soil solution, along with a neutral-alkaline pH (always <8.5) [15]. One of the major problems of calcareous soils is their nutrients deficiency due to a high degree of P-fixation and Fe-precipitation, as well as a low N, Mg, and Zn availability [15]. Under these limiting production conditions, a proper soil fertilization management [16] and an improved nutrient use efficiency [17] are necessary for enhancing the productive and qualitative traits of organic early potato. Recently, the application of arbuscular mycorrhizal fungi (AMF), a group of obligate symbionts with the majority of land plants [18], has been considered for the several benefits to the host plant, such as drought and salinity tolerance, disease resistance, alleviation against heavy metal toxicity, and improved nutrient and water uptake [18–20]. AMF are ubiquitous in most agricultural ecosystems, and there have been studies undertaken to explore the impact of agricultural settings on AMF spore density, root colonization, and abundance using a taxon-based approach [21,22]. Moreover, in agricultural settings, fertilization [23,24], tillage [21,25] and cover cropping [26,27] can alter the indigenous AMF communities and diversity in roots and soil. However, several studies reviewed by Gosling et al. [28] demonstrated that the AMF diversity and abundance diminished under high input management practices. Therefore, inoculum of commercial AMF into the soil has been proposed for complementing the resident AMF community, with a view of enhancing also the benefits to host plants. Wu et al. [29] reviewed the scenario of beneficial microorganisms applied in potato cultivation for sustainable agriculture. In this framework, the application of selected AMF can overcome nutrient limitation, typical of organic farming, by improving soil nutrient availability and plant's uptake and assimilation [29]. This is of particular relevance for potato, which is the world's fourth most grown crop, after rice, wheat, and maize, and a richer source of minerals (e.g., K, Fe, and Zn) than grains and beans [30]. In terms of contribution to the US dietary reference intake (DRI), a medium-sized potato portion (about 200 g fresh weight) can meet the need of ~26% of the RDI of Cu; 17 to 18% of the DRI of K, P, and Fe; and between 5 and 13% of the DRI of Zn, Mg, and Mn [31]. Therefore, the basic goal of this study was to highlight if potato tubers could be fortified by improving their micro- and macro-minerals content, through the application of mycorrhizae, which favors the plant absorption of limiting mineral nutrients (e.g., P) and some micro-elements. Moreover, the enrichment of minerals content is an important objective given that the tubers are characterized by a potentially high number of minerals [31].

Considering all these aspects, the aim of the present work was to estimate the effect of soil mycorrhization on the tuber minerals content of three early potato cultivars organically produced under open-field conditions. Indeed, cultivar choice is essential in determining the yield and quality of the early potato under organic farming [32,33]. In this view, key traits for potato cultivars to be adaptable for organic production are a reliably high yield under low input production system, high efficiency in nutrient uptake, good level of resistance/tolerance to the common biotic and abiotic stresses and a high suitability to low temperatures of storage. In addition, it is recognized that AMF isolates may have a host genotype-specificity also depending on soil characteristics [34]. The research was therefore conducted in two different locations, in order to explore possible changes in the benefits of soil mycorrhization, in terms of tuber minerals accumulation, due to soil characteristics.

## 2. Materials and Methods

### 2.1. Site, Soil, and Climate

According to Johnstone et al. [35], the field trials were replicated in space in order to evaluate the influence of the soil type and the between-site variability. Field-experiments were carried out during the 2017 growing season in two different locations (hereafter indicated as location I and II) sited on the coastal plain of Siracusa (36°49' N, 14°57' E,

130 m a.s.l.), one of the most representative areas for early potato production in southern Italy. In both locations, the soils (moderately deep) were Calcixerollic Xerochrepts according to the USDA Soil Taxonomy Classification [36], but presented different physical and chemical characteristics (Table 1).

**Table 1.** Soil physical and chemical characteristics (from  $-0.05$  to  $-0.30$  m depth) of the two locations under study.

Soil Characteristic	Location I	Location II
Sand (%)	54.1	51.8
Silt (%)	24.8	22.0
Clay (%)	21.1	26.2
Total limestone (%)	44.2	65.6
Active limestone (%)	15.5	18.0
Organic matter (%)	1.7	2.6
C/N ratio	7.5	7.5
pH	7.8	7.5
Total N ( $\text{g kg}^{-1}$ )	1.3	2.0
Assimilable $\text{P}_2\text{O}_5$ ( $\text{mg kg}^{-1}$ )	66	135
Exchangeable $\text{K}_2\text{O}$ ( $\text{mg kg}^{-1}$ )	455	612
Fe ( $\text{mg kg}^{-1}$ )	5.58	10.45
Zn ( $\text{mg kg}^{-1}$ )	0.99	2.03
Mn ( $\text{mg kg}^{-1}$ )	11.48	25.11
Cu ( $\text{mg kg}^{-1}$ )	2.21	3.92
Electrical conductivity ( $\text{dS m}^{-1}$ )	1.32	1.14
Cation exchange capacity ( $\text{meq } 100 \text{ g}^{-1}$ )	22.8	26.0
Ca (%)	83.34	83.27
Mg (%)	9.95	10.67
K (%)	4.23	5.17
Na (%)	2.48	0.90

The latter were determined on a 0.25 m thick (from  $-0.05$  to  $-0.30$  m) soil layer, where  $\sim 90\%$  of active roots were present, using the analytical protocols proposed by the Italian Society of Soil Science [37]. The soil samples were collected along the diagonals of each  $100 \text{ m}^2$  sampling area ( $25 \text{ m length} \times 4 \text{ m width}$ ). The distance between sampling sites was 2.5 m. Results, reported in Table 1, highlighted that both locations had a high active limestone level, a medium organic matter content and a high organic matter mineralization as indicated by the low C/N ratio. While Ca and Mg soil levels were similar at both locations, Na was higher in location I while all the micro-minerals were higher in location II. The latter also reported higher values of total N, assimilable  $\text{P}_2\text{O}_5$  and exchangeable  $\text{K}_2\text{O}$  than location I (Table 1).

The studied locations (4–5 km apart) are situated in an area characterized by a semi-arid Mediterranean climate. Detailed information about the meteorological conditions recorded during the growing season (January–May) were previously reported [38]. Particularly, it emerged as the 2017 growing season was the driest of the 30-year period, with a total rainfall of 115 vs. 179 mm (data not shown). The rainfall was mainly concentrated in February (45% of the total), and quite absent in April (only 2 mm). By contrast, the average maximum temperature ( $18.8 \text{ }^\circ\text{C}$ ) and the average minimum temperature ( $10.4 \text{ }^\circ\text{C}$ ) were comparable to the long-term data ( $18.7$  and  $9.8 \text{ }^\circ\text{C}$ , respectively). Minima temperatures never fell below  $7.8 \text{ }^\circ\text{C}$  during the growing season, while the mean maximum temperature was above  $16.4 \text{ }^\circ\text{C}$  at the plants' emergence (February) and  $20.6 \text{ }^\circ\text{C}$  at the tuberification stage (April).

## 2.2. Field Experimental Design, Plant Material and Management Practices

A randomized split-plot design with three replicates including three cultivars (i.e., 'Arizona', 'Mondial', and 'Universa') commonly used for early potato production, as

the main plots, and two soil mycorrhization treatments (mycorrhizal inoculated vs. not mycorrhizal inoculated-control, hereafter indicated as AMF+ and AMF−, respectively), as the sub-plots, was adopted in both the studied locations.

All the plots received an optimal fertilization (consisting of 120, 80, and 130 kg ha<sup>−1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O), formulated following the indications from Research Institute of Organic Agriculture (FiBL, Frick, Switzerland) [39] and Sicily Department of Agriculture ([www.regionesicilia.it](http://www.regionesicilia.it); 5 December 2016), while considering both the NPK uptake by potato crops in Sicily with a target yield of 20 t ha<sup>−1</sup> and the average NPK availability of experimental soils. Both the adopted fertilization management and mycorrhizal treatments were summarized in Table 2. Particularly, in the AMF+ plots, a commercial product with propagules of the genus *Glomus* spp. and *Gigaspora* spp. (Xedaopen<sup>®</sup>, XEDA Italia s.r.l., Forlì, Italy) was manually applied by placing the microgranules of 1.5 mm directly beneath the tuber seed at sowing.

**Table 2.** Fertilization and mycorrhizal management of potato crop organically grown.

Phenological Stage of Application	Commercial Product	Applications (n.)	Dose Per Application	Active Ingredient	Source	Manufacturer
At sowing	Ricin-Xed <sup>®</sup>	1	1.2 t ha <sup>−1</sup>	4% of N	Castor seeds	XEDA Italia s.r.l., Forlì, Italy
At sowing	Xedaneem Pel <sup>®</sup>	1	1.2 t ha <sup>−1</sup>	3% of N	Neem seeds after oil extraction	“
At sowing	Kalisop <sup>®</sup>	1	0.6 t ha <sup>−1</sup>	50% of K <sub>2</sub> O; 45% of SO <sub>3</sub>	Commercial granular product	K+S KALI GmbH, Verona, Italy
At sowing	Fosfonature 26 <sup>®</sup>	1	0.4 t ha <sup>−1</sup>	26% of P <sub>2</sub> O <sub>5</sub> ; 41% of CaO	‘Pheoflore’ algal origin	Fosfonature 26 <sup>®</sup> , TIMAC Agro, Milan, Italy
At sowing	Xedaopen <sup>®</sup> a		40 kg ha <sup>−1</sup>	7 active propagules g <sup>−1</sup> of the genus <i>Glomus</i> spp. and <i>Gigaspora</i> spp.	Commercial inoculant	XEDA Italia s.r.l., Forlì, Italy
After emergence	Biosin <sup>®</sup>	3	150 cc hL	7.7% of N	Commercial liquid product	“

<sup>a</sup> applied only in the mycorrhizal inoculated plots.

The trials were included in a potato–lettuce–carrot rotation, as commonly done in the cultivation area. In both locations, tillage consisted of a 30 cm depth ploughing followed by harrowing in October. Disease-free, whole and no-pre-sprouted “seed” tubers, from a single seed lot, were manually planted on 6 January 2017 by adopting a planting density of 5.55 plants m<sup>−2</sup> (0.24 m intervals in rows and 0.75 m apart). Each sub-plot (4.2 × 4.2 m) consisted of six rows, of which the two middle ones per plot were selected for harvesting the tubers at the commercial stage (121 days after planting). Origin, main biological, physiological, and tuber sensory traits of the studied cultivars are reported in Table 3. During the crop cycle, ~180 mm water was furnished by drip irrigation. Weed and pest control was carried out according to EU regulations (Regulation CE 834/2007, 889/2008, 967/2008, 1235/2008 and 1254/2008) for organic farming.

**Table 3.** Tuber seed origin, main biological, physiological, and tuber sensory traits of the studied potato cultivars.

Cultivar	Origin	Tuber Maturity	Plant Vigor	Skin Colour	Flesh Colour	Cooking Type <sup>a</sup>
Mondial (Spunta × VE66-295)	Dutch	late	medium-high	yellow	yellow	B
Arizona (UK 150-19D22 × Mascotte)	Dutch	medium-late	high	“	“	AB
Universa (Agata × 88F164.1)	French	early-medium	medium	“	“	AB

<sup>a</sup> Based on the E.A.P.R. (European Association for Potato Research) cooking type scale: A, firm texture (suitable for steaming, microwaving and boiling); B, mealy texture (multi-purpose cooking).

### 2.3. Sampling and Determination of Tuber Minerals Content

At each location, 3 replicates (each made of at least 20 marketable tubers (Ø 35–70 mm), of uniform size and disease-free) per each cultivar and soil mycorrhization treatment were used for the chemical analyses ( $n = 36$ ). Such sample is representative due to the low variability intra-cultivar. The tubers were washed with tap water, dried with tissue paper, diced and immediately oven-dried at 65 °C (Binder, Milan, Italy), until a constant weight was reached, in order to determine the tuber dry matter (DM) content. The obtained dehydrated material was ground and passed through a 1-mm sieve and used for the determination of ten mineral elements (i.e., N, P, K, Mg, Ca, Na, Zn, Fe, Mn, and Cu). N determination was performed by using Kjeldhal method. The determination of P content was carried out by colorimetry with the vanadate/molybdate method described by Lombardo et al. [32], reading the absorbance with a Shimadzu 1601 UV-Visible spectrometer (Shimadzu Corp., Tokyo, Japan). For the determination of the other minerals (i.e., Ca, Mg, K, Na, Cu, Fe, Zn, and Mn) it was followed the AOAC official method [40]. In brief, each mineral was separately analyzed with a Perkin Elmer (Norwalk, USA) AAnalyst 200 atomic absorption spectrometer. The quantification of Ca, Mg, K, Na, Cu, Fe, Zn, and Mn in the samples was performed by their calibration curves [41]. The content of each mineral element was expressed as mg kg<sup>-1</sup> of DM. All the chemical products adopted for analyses were purchased from Sigma-Aldrich (Milan, Italy) and were of analytical grade.

### 2.4. Soil Sampling, DNA Extraction, and Real-Time Quantitative PCR Assay

In soil the quantification of both *Glomus* spp. and *Gigaspora* spp. was carried out by quantitative PCR using SYBR Green I dye [38]. Briefly, three soil samples, about 500 g for each sub-plot and location, were collected from the top layer (about 20 cm) without taking weeds. Soil samples were sieved (2 mm) and DNA was extracted following Scavo et al. [42], then quantified spectrophotometrically (all with 260:280 ratios above 1.7). Prior to quantitative PCR, DNA extracts were kept at -20 °C. qRT-PCR was performed using an iCycler iQTM5 (BIORAD) and an absolute quantification method according to Lombardo et al. [38]. As well, PCR conditions and two primers set, such as Glofor (5'-GAAGTCAGTCATACCAACGGGAA-3') and Glorev (5'-CTCGCGAATCCGAAGGC-3'), Gigfor (5'-CTTTGAAAAGAGAGTTAAATAG-3') and Gigrev (5'-GTCCATAACCCAACACC-3') for *Glomus* and *Gigaspora* spp., respectively, were used according to Lombardo et al. [38].

### 2.5. Statistical Analysis

Bartlett's test was adopted to test for homoscedasticity, and then the data were subjected to a three-way analysis of variance (ANOVA), based on a factorial combination of “soil mycorrhization treatment (2) × cultivar (3) × location (2)”. Means were separated by Least Significant Difference (LSD) test, when the F-test was significant. Statistical analysis was performed using the default options within CoStat<sup>®</sup> computer package version 6.003 (CoHort Software, Monterey, CA, USA).

### 3. Results and Discussion

Based on the ANOVA outcomes soil mycorrhization, location, cultivar, and their interactions significantly influenced the levels of all the considered macro- and micro-mineral elements, except for N (Table 4).

**Table 4.** Mean square as percentage of total of effects resulting from analysis of variance.

Source of Variation	Df	Mineral Element										Na/K
		K	P	Mg	N	Na	Ca	Fe	Mn	Cu	Zn	
Soil mycorrhization (M)	1	0.0 <sup>NS</sup>	49.6 <sup>***</sup>	11.5 <sup>**</sup>	0.0 <sup>NS</sup>	39.1 <sup>**</sup>	10.7 <sup>NS</sup>	0.0 <sup>NS</sup>	40.1 <sup>***</sup>	50.3 <sup>***</sup>	1.6 <sup>NS</sup>	27.6 <sup>**</sup>
Location (L)	1	0.5 <sup>NS</sup>	22.5 <sup>***</sup>	60.4 <sup>***</sup>	41.1 <sup>NS</sup>	26.7 <sup>**</sup>	4.0 <sup>NS</sup>	59.2 <sup>***</sup>	13.6 <sup>***</sup>	27.1 <sup>***</sup>	92.3 <sup>***</sup>	10.3 <sup>*</sup>
Cultivar (C)	2	5.7 <sup>NS</sup>	22.2 <sup>***</sup>	20.6 <sup>NS</sup>	0.1 <sup>NS</sup>	1.1 <sup>NS</sup>	12.8 <sup>NS</sup>	0.6 <sup>NS</sup>	12.8 <sup>***</sup>	4.8 <sup>NS</sup>	1.2 <sup>NS</sup>	3.4 <sup>NS</sup>
(M) × (L)	1	2.7 <sup>NS</sup>	1.1 <sup>NS</sup>	1.2 <sup>NS</sup>	0.1 <sup>NS</sup>	8.7 <sup>NS</sup>	18.7 <sup>NS</sup>	0.5 <sup>NS</sup>	14.6 <sup>***</sup>	13.6 <sup>***</sup>	1.5 <sup>NS</sup>	13.8 <sup>NS</sup>
(M) × (C)	2	40.7 <sup>***</sup>	2.8 <sup>NS</sup>	3.7 <sup>NS</sup>	3.5 <sup>NS</sup>	18.4 <sup>**</sup>	30.7 <sup>*</sup>	13.8 <sup>*</sup>	8.6 <sup>***</sup>	1.6 <sup>NS</sup>	2.9 <sup>**</sup>	10.3 <sup>**</sup>
(L) × (C)	2	6.8 <sup>NS</sup>	1.6 <sup>NS</sup>	0.6 <sup>NS</sup>	41.5 <sup>NS</sup>	0.5 <sup>NS</sup>	7.1 <sup>NS</sup>	2.7 <sup>NS</sup>	5.0 <sup>NS</sup>	1.6 <sup>NS</sup>	0.1 <sup>NS</sup>	6.9 <sup>NS</sup>
Total mean square	-	2,804,890	2,165,154	39,276	1523	2073	142	28	5.01	1.25	4.11	0.003

\*\*\*, \*\* and \* indicate significant at  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , and NS, not significant.

Soil mycorrhization of potato plants resulted in higher tuber amounts of P, Cu, Mn, and Zn (+25%, +15, +23, and +26%, respectively) in the AMF+ samples compared to AMF– ones (Table 5). Indeed, it is known that AMF soil inoculation may contribute to plant uptake of relatively immobile nutrients, such as Cu, Mn, and Zn, and, particularly P, an essential macronutrient required for plant growth [43]. In particular, our results confirmed the soil AMF application as an efficient tool to increase the P level in the organic potato tubers, given that the low P uptake efficiency of this crop [44] may limit the root P uptake and therefore the accumulation of this macro-mineral in the tuber.

**Table 5.** Mineral elements (mg kg<sup>−1</sup> of DM) and Na/K ratio as affected by main factors under study. Different letters within the same parameter and main factor show significant differences among means (LSD test,  $p \leq 0.05$ ).

Main Factor	Mineral Element										Na/K	
	K	P	Mg	N	Na	Ca	Fe	Mn	Cu	Zn		
Soil mycorrhization												
AMF+	3776 ± 50	2116 ± 55 a	180 ± 3 b	122 ± 2	104 ± 4 b	100 ± 3	20.8 ± 0.5	2.48 ± 0.17 a	2.11 ± 0.10 a	1.26 ± 0.12 a	0.028 b	
AMF–	3769 ± 33	1693 ± 70 b	207 ± 6 a	122 ± 2	115 ± 2 a	98 ± 1	20.9 ± 0.7	1.90 ± 0.11 b	1.79 ± 0.20 b	0.93 ± 0.10 b	0.031 a	
Cultivar												
Arizona	3612 ± 137	1760 ± 31 b	174 ± 3 b	123 ± 3	108 ± 3	98 ± 2	20.7 ± 0.1	2.32 ± 0.21 a	1.91 ± 0.20	1.16 ± 0.10	0.031	
Mondial	3874 ± 101	2188 ± 60 a	177 ± 2 b	122 ± 1	110 ± 5	101 ± 3	20.9 ± 0.3	1.87 ± 0.71 b	2.05 ± 0.10	1.01 ± 0.10	0.028	
Universa	3833 ± 132	1766 ± 26 b	230 ± 6 a	122 ± 2	112 ± 2	99 ± 2	21.0 ± 0.4	2.39 ± 0.10 a	1.89 ± 0.10	1.11 ± 0.10	0.029	
Location												
I	3749 ± 39	1762 ± 81 b	225 ± 10 a	117 ± 9	115 ± 1 a	99 ± 2	21.7 ± 0.5 a	2.36 ± 0.11 a	2.07 ± 0.50 a	0.69 ± 0.31 b	0.031 a	
II	3797 ± 61	2074 ± 43 a	162 ± 9 b	127 ± 8	105 ± 4 b	100 ± 2	20.0 ± 0.7 b	2.02 ± 0.13 b	1.83 ± 0.30 b	1.49 ± 0.43 a	0.028 b	

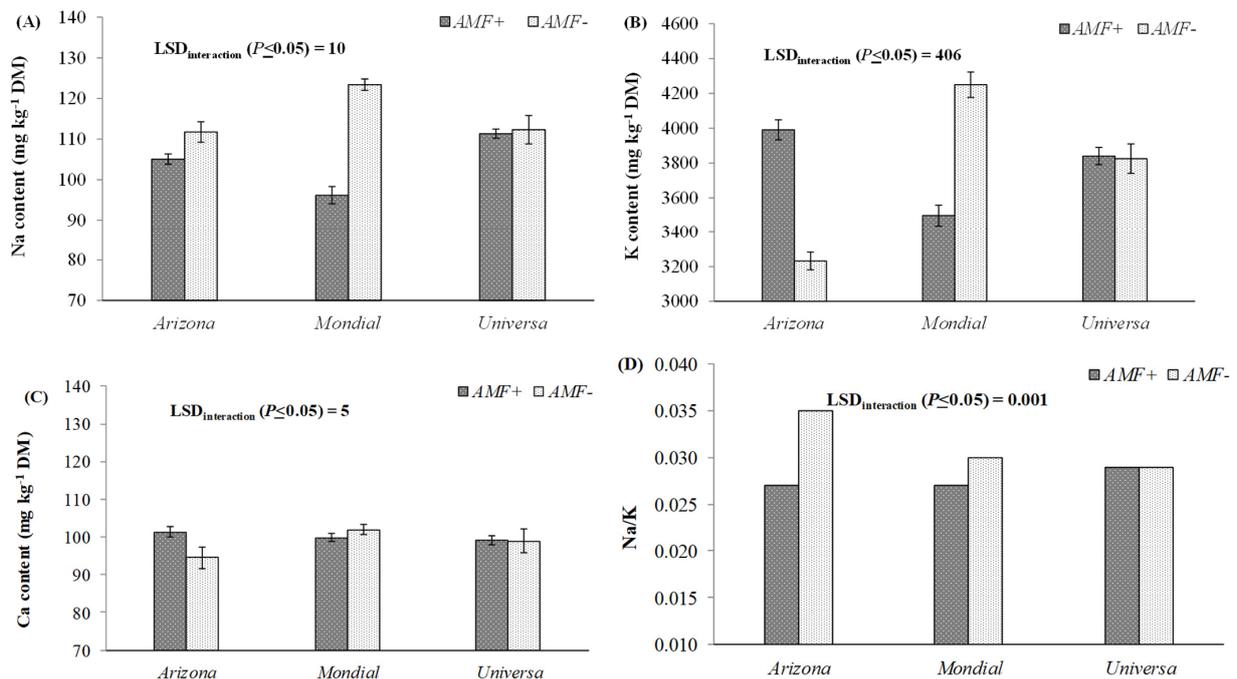
AMF+: mycorrhizal inoculated plots; AMF–: non-mycorrhizal inoculated plots. Data are presented as mean ± standard deviation.

The lack of statistical differences in Fe concentration between AMF+ and AMF– potato tubers was quite unexpected (Table 5), but coincided with our findings on tuber P level. Indeed, the competition Fe-P might have limited the accumulation of Fe in AMF+ potato tubers, that accumulated more P than AMF– ones. On the other hand, the low Fe availability in calcareous soils and its high immobility in plants is well established and this may have hardly influenced the positive effect of AMF application on the Fe uptake, usually found in literature [43,45]. In addition, the highest tuber accumulation of Zn in the AMF+ tubers is a relevant finding considering that Zn deficiency is the main micronutrient problem in the Mediterranean-type alkaline-calcareous soils [46], characterized by free CaCO<sub>3</sub> and high pH, as in our experimental fields. Therefore, the highlighted fortification of organic potato tubers in terms of P and Zn accumulation deserves attention given their vital roles in human health and nutrition [47]. Additionally, previous works also reported a significant yield increase in potato plants inoculated with AMF and grown under

calcareous soils [38,48]. In our previous work [38], an increase of marketable tuber yield (+25%) was recorded, as such as the number of tubers plant<sup>-1</sup> (+21%) and the average tuber weight (+10%). In addition, the mycorrhization with AMF improved the potato tolerance to limestone stress by ameliorating photosynthesis rate and stomatal conductance.

Effects of soil mycorrhization in the uptake of K, Ca, and Mg are scarcely available in literature, and often the findings are contradictory [49–51]. Our results highlighted a higher Mg concentration in AMF– samples (208 mg kg<sup>-1</sup> of DM), while no statistical differences between AMF– and AMF+ tubers were observed for K, Ca and N levels (Table 5). As reported for Mg concentration, the Na level appeared lowest in the AMF+ potato tubers (104 mg kg<sup>-1</sup> of DM) (Table 5) and, as consequence, a lower Na/K ratio was recorded in the AMF+ samples (Table 5). This could have an important consequences for human health since high values of Na/K ratio are correlated to a higher incidence of elevated blood pressure and cardiovascular disease [52].

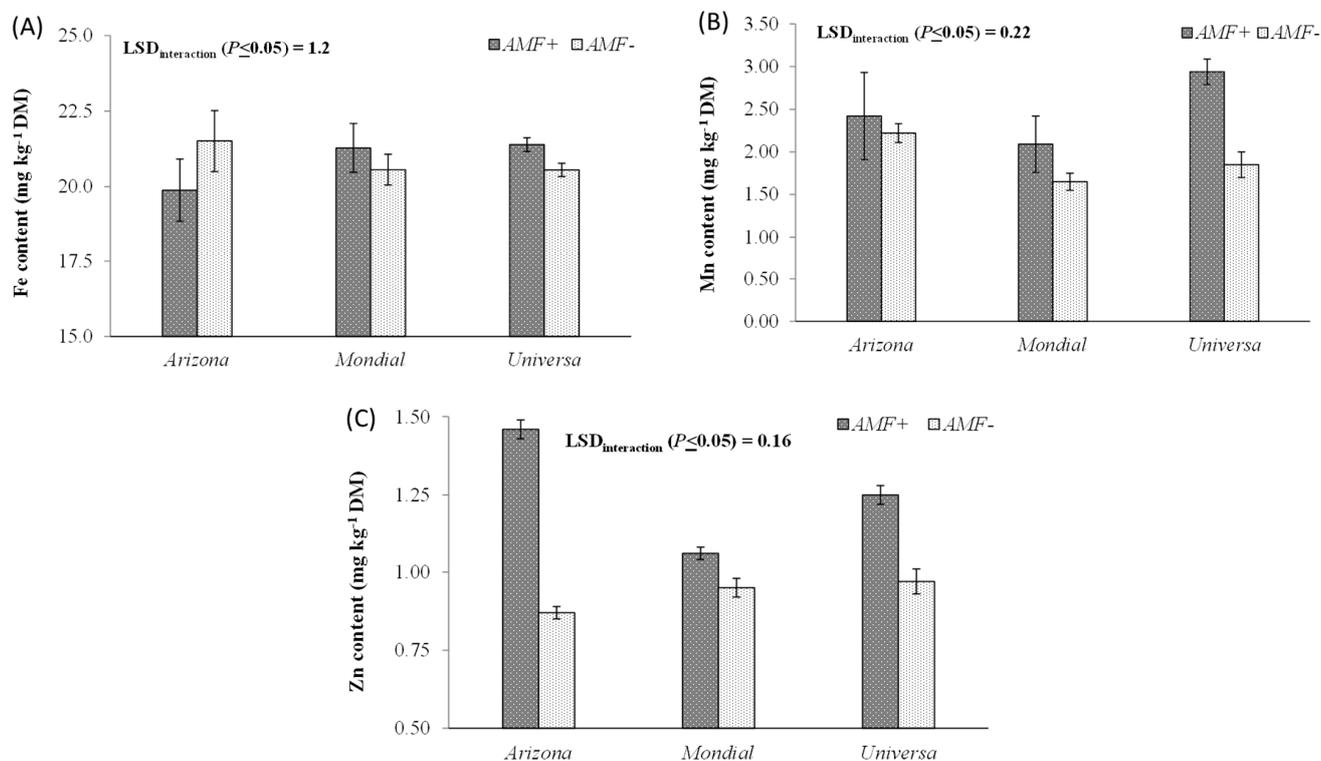
Our results also demonstrated that soil mycorrhization effects on tuber minerals accumulation depended upon cultivar and location (Table 4). Actually, there is some evidence of a different cultivar response to field management practices in terms of mineral accumulation in several crops, including potato, globe artichoke, and durum wheat [32,53]. Here, the “soil mycorrhization × cultivar” interaction appeared to be the predominant factor influencing the tuber K and Ca content, displaying 40.7 and 30.7% of total variation, respectively (Table 4). In particular, soil mycorrhization was able to enhance the levels of K and Ca in ‘Arizona’ and that of Mn in ‘Universa’, while it increased the Zn amount in all the cultivars under study (Figure 1).



**Figure 1.** Effect of “soil mycorrhization × cultivar” interaction on the tuber levels of Na (A), K (B), Ca (C), and Na/K (D). Data are presented as mean ± standard deviation. The ± standard deviation of ratio Na/K is ≤0.001. AMF+: mycorrhizal inoculated plots; AMF–: non-mycorrhizal inoculated plots.

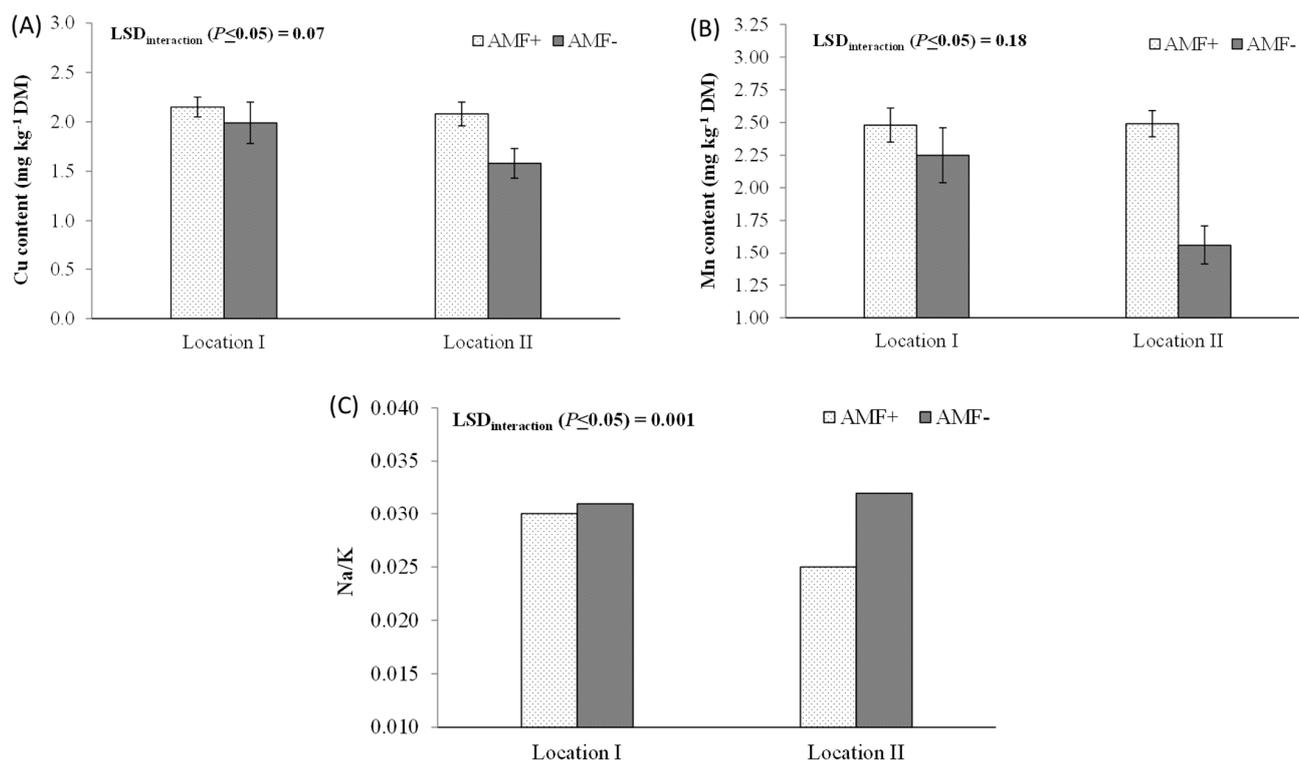
In the case of Fe, ‘Arizona’ reported the highest level of Fe in AMF– tubers compared to AMF+ ones, whereas no statistical difference was noted between AMF– and AMF+ tubers for both ‘Mondial’ and ‘Universa’ (Figure 2). Looking at the Na/K ratio ‘Mondial’ and ‘Arizona’ had the lowest value in the AMF+ tubers (0.027), whereas ‘Universa’ did not report any statistical difference between AMF– and AMF+ samples, as well as for the levels of Na, K, and Ca. This suggests that the specificity of AMF-host plant relationships,

which could have affected the different response to soil mycorrhization in terms of mineral nutrients accumulation in the potato cultivars under study. Indeed, we previously observed that ‘Arizona’ was mainly colonized by *Gigaspora* spp. and ‘Mondial’ by *Glomus* spp., whereas ‘Universa’ had a scanty mycorrhizal colonization [38]. Similar findings were also highlighted by Senés-Guerrero et al. [54].



**Figure 2.** Effect of “soil mycorrhization × cultivar” interaction on the tuber levels of Fe (A), Mn (B), and Zn (C). Data are presented as mean ± standard deviation. AMF+: mycorrhizal inoculated plots; AMF–: non-mycorrhizal inoculated plots.

As previously reported [38], in both locations, the quantitative real-time PCR (qRT-PCR) technique revealed that the AMF+ plots were efficiently colonized by both genera, displaying values of Ct (cycle threshold) about 20–25, more than not-inoculated plots (Ct = 30–40). Given that Ct values are inversely proportional to the amount of target nucleic acid in the sample, a good tolerance of *Glomus* spp. and *Gigaspora* spp. to high active limestone, soil alkaline reaction and P levels can be deduced. Soil mycorrhization enhanced the tuber Mn level to a greater extent in location II, which also presented a higher soil Mn content (Table 1). Similarly soil mycorrhization increased the Cu amount only in the tubers harvested in the location II, which also recorded the lowest value of Na/K ratio (0.025) (Figure 3), probably due to the low Na content found in such location (Table 5).



**Figure 3.** Effect of “soil mycorrhization × location” interaction on the tuber levels of Cu (A), Mn (B), and Na/K (C). Data are presented as mean ± standard deviation. The ± standard deviation of ratio Na/K is ≤0.001. AMF+: mycorrhizal inoculated plots; AMF–: non-mycorrhizal inoculated plots.

Regardless of both location and soil mycorrhization, the cultivar significantly affected the tuber Mg, P, and Mn level (Table 5). This disagreed with previous works, highlighting a systematic difference in macro- and micro-mineral elements among commercial potato cultivars grown under the same field conditions [32,41,55], but coincided with the findings of Andre et al. [56]. In the present study, ‘Universa’ reported a higher value of Mg content (230 mg kg<sup>-1</sup> of DM) than ‘Arizona’ and ‘Mondial’ (on average, 175 mg kg<sup>-1</sup> of DM) (Table 5). This result may have influenced the tuber P level recorded in the studied cultivars, since a relationship between yield and tuber mineral concentrations was previously documented for potato. In this view, some studies observed that higher-yielding potato cultivars have lower P concentrations than lower-yielding ones [57,58]. Accordingly ‘Mondial’, the lowest-yielding cultivar as previously reported by Lombardo et al. [38], recorded the highest content of P (2188 mg kg<sup>-1</sup> of DM) (Table 5). In addition, the higher tuber P amount reported for ‘Mondial’ may be also imputable to its lower tuber Mn level, since Sarkar et al. [59] reported an antagonism between P and Mn.

Location was the higher source of variation for several macro- and micro-mineral elements (Table 4). In particular, in the location I the highest levels of Mg, Na, and values of Na/K ratio were recorded (Table 5). These results reflected the soil chemical composition observed in both locations (Table 1). As known, the plant uptake of Mg depends not only on the content of available Mg in the soil, but also on its antagonism with other cations [60]. Particularly, an excessive soil K level reduces the bioavailability of Mg by affecting its movement towards the roots [61,62]. In addition, the absorption antagonism of K and Mg is also responsible for a reduced transportation of Mg from the root system to the aerial part [63]. Here, the lower exchangeable K<sub>2</sub>O content observed in location I compared to that in location II could explain the significant high level of Mg observed in the potato tubers grown in location I (Tables 1 and 5). Similar findings were reported in rice [64] and tomato [65]. On the other hand, Wekesa et al. [66] previously demonstrated that the minerals content of potato tubers was linked to soil physical and chemical characteristics

of the production site. In our study, it was observed a higher Na content in the tubers grown in location I, as a consequence of its higher level of exchangeable Na than location II (Table 1). The Na/K ratio was also affected, reporting the highest value in the location I compared to the location II (0.031 vs. 0.028) (Table 5). A direct relationship between soil and tuber levels of mineral elements was also observed for the P, so that the tuber P level was about 34% higher than that recorded for tubers grown in location I in agreement with the higher assimilable P recorded in the soil of location II (Table 5). In addition, it is known the negative interaction between P and Fe, since P anion competes with plant roots for Fe, reducing the absorption and transfer of Fe in the plant [67]. This could explain the highest tuber Fe value observed in the location I (21.7 mg kg<sup>-1</sup> of DM). In addition, the highest level of Fe and Mg, which are two mineral elements required by plants for the synthesis of chlorophyll, may have stimulated the pronounced Cu and Mn accumulation in the potato tubers grown in the location I respect to those cultivated in the location II (Table 5). The direct involvement of Mn content in the increase of the photosynthetic rate has been reported in literature [68,69], whereas the Cu appeared to form Cu-chlorophyll complexes in several photosynthesis pigments, even if its role is unclear to date [70]. Concerning the Zn, a higher content was registered in potato tubers grown in the location II (1.49 mg kg<sup>-1</sup> of DM), in line with the greater level observed in the soil from location II. When looking at the content of K, Ca, and N, once again a positive relationship was found between mineral levels in the soil and tuber (Table 5). This result is in agreement with previous multisite researches, which found changes in the tuber content for only specific minerals [71,72].

#### 4. Conclusions

The present work provides a better understanding on the potential benefits from the mutualistic association between AMF and organically grown potato plants, highlighting for the first time the possible quantitative fortification of potato tubers, in terms of macro- and micro-mineral elements content, through the soil mycorrhization. In addition to being a of major source of starch, potato crops could provide substantial amounts of minerals, essential for human health since their deficiencies are responsible for acute metabolic disorders and may compromise the health of the organism. Our results revealed that the content of P, Cu, Mn, and Zn in the tubers may be enhanced by soil AMF application, which ameliorated the uptake of important nutrients like P and relatively immobile micro-mineral nutrients. However, the effect of soil AMF application on the minerals accumulation in the tubers strictly depended upon cultivar choice. In this sense, the soil mycorrhization promoted the accumulation of K and Ca in 'Arizona', whereas it was able to enrich the tuber Mn level in both 'Mondial' and 'Universa'. Our findings also highlighted that the benefits from soil AMF application strongly depended on location and soil characteristics, since levels of Mn and Cu were enhanced in the tubers from inoculated plants grown in the location with higher soil levels of these minerals. In conclusion, our data show that it is possible to fortify organic potato tubers, in terms of macro- and micro-mineral elements content, through an eco-sustainable management tool such as soil mycorrhization. In addition, the soil mycorrhization could potentially replace chemical fertilizers, which are very limited in organic production systems and, often, are not totally effective in potato plant's nutrition. Further researches are still required with the aim of evaluating the effects of soil AMF application on other tuber quality parameters, also by studying other cultivars and locations, since, according our data, specific consideration is given to cultivar choice and soil characteristics.

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