agronomy

MDPI

Article

The Effect of Mycorrhizal Fungi and PGPR on Tree Nutritional Status and Growth in Organic Apple Production

Sebastian Przybyłko *, Wojciech Kowalczyk and Dariusz Wrona

Department of Pomology and Horticulture Economics, Institute of Horticultural Sciences SGGW, Nowoursynowska 159 Str., 02-787 Warsaw, Poland; wojciech_kowalczyk@sggw.edu.pl (W.K.); dariusz_wrona@sggw.edu.pl (D.W.)

* Correspondence: sebastian_przybylko@sggw.edu.pl

Abstract: The desire to reduce the negative impact of crops on the environment, as well as the growing concern for consumer health, is increasing interest in organic fruit production. In this context, the development of new environmentally friendly agrotechnical methods which allows for reducing the use of organic fertilizers by improving the nutrient use efficiency and consequently decreasing the leaching of them is a task of a great importance. The main purpose of this study was to evaluate the effect of mycorrhizal arbuscular fungi (AMF) combined with plant-growth-promoting rhizobacteria (PGPR) on growth and nutritional status of apple trees cultivated on a silty-loam, rich in clay minerals and humus soil under organic farming conditions. Thus, a trial was established in an experimental orchard in Wilanów in Central Poland with three cultivars ('Topaz', 'Odra', and 'Chopin') and a promising clone, U 8869. Trees were or were not inoculated with AMF + PGPR within a split-block experimental design with four replicates. According to the results, mycorrhizal frequency obtained in the inoculated tree roots was on average two-fold higher than in the roots of the control plants. After four years of AMF + PGPR inoculation, 24% higher trunk cross-section area (TCSA) was observed, with the nitrogen and magnesium concentrations in leaves increasing, on average, by 7.8% and 64.2%, and phosphorus and potassium content decreasing by 37.2% and 46.5%, respectively. This study shows that using AMF + PGPR inoculum supports tree roots colonization by AMF. As a result, better nitrogen nutrition status is observed that promote vigorous growth of trees and more efficient uptake of magnesium from the bulk soil. On the other hand, lower phosphorus content in inoculated tree leaves might be explained by a dilution effect, and potassium decrease could occur as a result of fungus–plant competition in conditions of this element deficiency in soil.

Keywords: organic; sustainability; arbuscular mycorrhiza; apple nutrition

Citation: Przybyłko, S.; Kowalczyk, W.; Wrona, D. The Effect of Mycorrhizal Fungi and PGPR on Tree Nutritional Status and Growth in Organic Apple Production. *Agronomy* **2021**, *11*, 1402. https://doi.org/10.3390/agronomy11071402

Academic Editor: Martine Dorais

Received: 17 May 2021
Accepted: 10 July 2021
Published: 13 July 2021

1. Introduction

A clear tendency of limiting the negative environmental impact of fruit crops has led to growing interest in organic fruit production in recent years [1]. Organic farming prohibits the use of synthetic inputs such as pesticides and fertilizers. As a result, the system is very demanding in terms of disease and pest control as well as plants' mineral nutrition, which are key factors in good and balanced growth and yield of fruit trees [2]. On the other hand, studies reported that low-input practices used in organic management systems and lower nutrient supply can improve activities of soil biota, in which essential components are arbuscular mycorrhizal fungi (AMF) [3,4]. The fungi are responsible for a characteristic type of symbiosis that occurs with the majority of higher plants called endomycorrhiza. Arbuscular mycorrhizae (endomicorrhyzae, AM) are established by widely occurring in nature biotrophic fungi with low specificity belonging to the phylum Glomeromycota [5]. Its structures in plant roots are very distinctive and easy to identify under the microscope after staining with certain types of dyes [6]. Many authors have shown that mycorrhizal fungi can improve the mineral nutrition status of plants, but the

reverse action is also possible [7]. The plants participating in the symbiosis that establish contact with beneficial mycorrhizal fungi have the ability to obtain inorganic nutrients via two pathways: a direct pathway via the root hairs and root epidermis directly from the soil solution, and an indirect pathway (mycorrhizal pathway) involving a fungal partner. Mycorrhizal-assisted uptake requires gathering minerals via extramatrical hyphae from the soil and transporting them to the arbuscules, where they are transferred to the plant [8]. The host-plant cost of establishing mycorrhizal contact is the carbohydrates produced during photosynthesis [9], which are the only source of energy for the fungus [10,11] and are estimated to account for up to 20% of total photosynthetic production, depending on plant–fungus factors as well as environmental conditions [12,13].

The main nutrient related to mycorrhiza is phosphorus [14]. There are many reports with clear evidence that mycorrhiza supports phosphorus uptake, specifically in soils low in this nutrient [15–17]. In light of previous studies, mechanisms of bilateral exchange of assimilates for phosphate ions Pi (H_2PO^- and $HPO_4{}^{2-}$) as well as nitrate ($NO_3{}^-$) and ammonium ($NH_4{}^+$) ions have been particularly well recognized both from physiological and molecular points of view. Studies conducted by Lopez-Pedros et al. [18] have identified the presence of both ammonium (AMT) and nitrate (NT) transporters as well as transport molecules in the form of specific amino acid permeases (AAPs) in *Glomus mossae* external hyphae. Therefore, in addition to the inorganic forms, $NH_4{}^+$ and $NO_3{}^-$ nitrogen can also be obtained by AMF in the form of amino acids from the decomposition of organic matter [19]. Strong correlation between phosphorus and potassium, especially in fungi hyphae and vesicles reported by Ollson et al. [20], implies that arbuscular mycorrhiza could also improve the potassium uptake. So far, little mycorrhiza research has been dedicated to the phenomenon of potassium uptake and exchange between mycorrhizal partners [21]. Due to the significant role of this nutrient in plant life and the often-occurring low content of available forms, especially in soils with high sorption properties, the importance of the mycorrhizal phenomenon may also be quite significant for this nutrient. According to Pallon et al. [22], potassium is accumulated in significant amounts in AMF spores. The results of Ollson et al. [23] showed that fungal hyphae and vesicles are also elements with high potassium content, which in the case of vesicles as storage organs seems to be particularly interesting. The mechanism of uptake and distribution of this component in mycorrhizal associations is not yet well understood. Despite the lack of detailed data on this subject, there are studies indicating a positive effect of the mycorrhization treatment in relation to plant potassium nutrition.

The cultural practices and inputs of organic fruit production differs from the more commonly used Integrated Production (IP) because of the limited list of approved pesticides and fertilizers. As a result of growing interest in undertaking this type of production, developing new agrotechnical methods resulting in better plant mineral nutrition, yield, and fruit quality is a task of a great importance. The main goal of our study was to determine the effects of AMF and plants-growth-promoting rhizobacteria (PGPR) on growth and nutritional status of different apple tree cultivars cultivated under organic farming, which can increase their nutrient use efficiency and productivity. We hypothesized that using AMF + PGPR inoculum increases the presence of mycorrhizal structures within roots and positively affects nutrient uptake, resulting in a better nutritional status of apple trees in field conditions. Results from this study can provide valuable information for practitioners interested in organic apple production.

2. Materials and Methods

The trial was located in an experimental orchard in Warsaw, Wilanów in Central Poland (N 52°9′36.1″, E 21°5′58.2″). The weather data for the experimental location are presented in Figure 1. They were collected using the Davis Vantage Pro 7 field weather station installed in the orchard. The material consisted of control plants (non-inoculated trees) and inoculated trees of cultivars ‘Topaz’, ‘Odra’, and ‘Chopin’ and a bred clone U 8869 in organic fruit growing conditions. The knip trees on M.9 rootstock were planted

in spring 2011 with 3.5 × 2 m spacing on a silty-loam alluvial soil within a split-block experimental design with 4 replications, and 3 trees per plot with 6 buffer trees between non-inoculated and inoculated plant blocks. Trees were trained in a spindle bush training system. Floor management in the orchard involved mowing the grass of the alleyways 3 to 5 times per season (depends on weather conditions) and mechanical tillage kept in rows of trees using a tractor-mounted rototiller-type tool equipped with a hydraulic system, enabling access to the area between trees, and the soil was tilled up to 5 cm deep twice in a growing season in April and October.

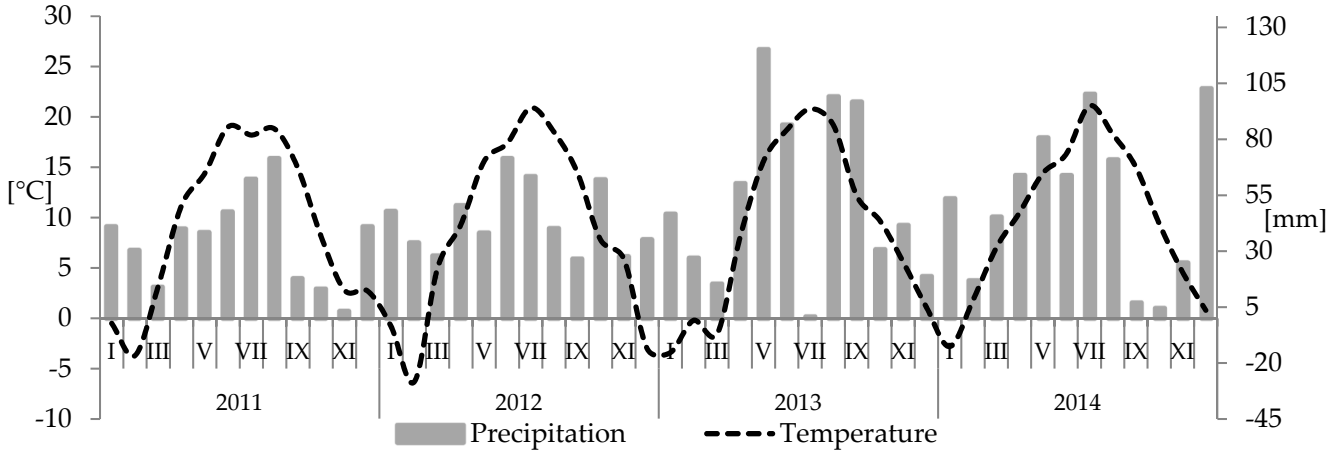

Figure 1. Weather conditions in the experimental orchard during 2011–2014.

Samples of soil, each of 1500–2000 g made of 15 subsamples collected before tree planting in the spring of 2011 using gouge auger set for stepwise sampling (Eijkelklamp, Giesbeek, The Netherlands), represented three soil layers (0–20, 21–40, and 41–60 cm) and were dried at room temperature. The soil pH values were measured in 1M KCl solution extract with Elmetron CPC-505 pH meter (Elmetron, Zabrze, Poland). Soil granulometric analysis was conducted using the Casagrande method with some modifications [24]. Macroelement content in 10 g soil samples was measured according to Egner–Riehm for P and K and Schachtschabel for Mg methods as described by Stafecka and Komosa [25]. Organic matter content was determined according to Tiurin's method described by Ładkiewicz et al. [26].

Analysis of soil taken before preparing the site for tree planting showed that the pH value of each layer varied (Table 1). In the 0–20 cm layer, pH was slightly acidic and reached 6.23. The deeper the soil samples were taken, the higher the pH values noted. Both in the 21–40 and 41–60 cm layers, the soil was characterized by alkali pH values of 7.3 and 7.5, respectively. Taking into account the soil layer and share of soil particles with diameters lower than 0.02 mm according to threshold levels developed by Sadowski et al., [27] the content of plant-available forms of phosphorus and potassium soil was low, while a high content of available magnesium was noted. This had a direct effect on the very low value of the K/Mg ratio. Moreover, the soil in the 0–20 cm layer showed content of organic matter exceeding 2.5%, and its share gradually decreased with the depth of sampling.

Table 1. Physicochemical soil parameters of the experimental site measured in spring 2011 before tree planting.

Depth	pH_{KCl}	Available Macroelements in Soil mg·100 g^{-1}			K/Mg Ratio	Organic Matter %	Share of Soil Particles with Diameter < 0.02
		P	K	Mg			
0–20	6.2	1.7	5.8	17.0	0.34	2.52	37.6
21–40	7.3	1.3	2.9	11.4	0.25	0.99	25.1
41–60	7.5	1.0	2.5	10.5	0.24	0.50	17.9

All agrotechnical treatments were performed in line with organic farming requirements, i.e., no synthetic agents were used during the course of the trial. During the whole period of the experiment neither fertilization nor irrigation were used. Apple scab was controlled using copper agents and lime sulphur. In 2013 predatory mites (*Typhlodromus pyri*) to control spider-mite population and mating disruption methods for codling-moth (*Cydia pomonella*) control were introduced.

Tree inoculation was performed in field conditions. Commercially available microbial inoculum Micosat F (CCS Aosta, Quart, Italy) with a total concentration of 10^6 CFU·g^{-1}, containing ground and shredded roots of host plants with spores and mycelium of arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* GP11, *G. viscosum* GC41, *G. intraradices* GB67, and plant-growth-promoting rhizobacteria (PGPR) *Bacillus subtilis* BA41 and *Streptomyces* spp. SB19 formulated as a powder, were applied to the 30 cm depth soil pits during tree planting at 10 g·$tree^{-1}$ dose, and then every year of the experiment 2 g·m^{-1} dose of the inoculum was applied next to the tree, three times each season at 3-week intervals with the first dose applied at the beginning of May.

For evaluating the nutritional status of the trees, 50 leaves per plot were harvested after the vegetative phase growth had finished at the end of July in each year of the study. Collected leaves were taken from the middle part of one-year-old shoots, dried at 70 °C for 24 h, and ground into powder, which was used for determining the N, P, K, and Mg contents. The nitrogen content was measured according to the Kjeldahl method [28]. The elements P, K, and Mg were marked with the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method [29] using a Thermo Scientific iCAP 6500 Duo spectrometer (Thermo Fischer Scientific, Waltham, MA, USA), with argon at 99.9% purity as a carrier gas after the samples were burnt in the oven and digested in a 0.5 M solution of HCl.

Root samples were taken from the field each year at the end of June. Tree roots were collected from each tree in a plot from a depth of up to 30 cm using a field spade and transferred to the lab where they were cleaned under tap water. Samples containing at least 20 g of cleared roots per plot were stained according to the method described by Derkowska et al. [6] using carbol fuchsin as a dying agent. Parameters of mycorrhizal frequency (F—share of root fragments in which mycorrhizal structures have formed), relative mycorrhizal intensity (RMI—share of colonized root fragments area in which mycorrhizal structures have developed), and the absolute intensity of mycorrhiza (AMI—share of all root fragments area in which mycorrhizal structures have developed) were assessed in specimens [30] using light microscope Leica DM1000 (Leica, Wetzlar, Germany). Each specimen represented one plot and contained thirty 1-cm-long parts of roots mounted in glycerin on a microscope slide that were crushed with cover glass. All mycorrhizal parameter values were calculated using MYCOCALC (INRA, Dijon, France) software and are given in percentage.

Leaf area was measured for 50 leaves per plot using a Li-3100 Area Meter (Li-Cor, Lincoln, NE, USA). For this purpose, leaf samples were taken with the same procedure as described for evaluating the nutritional status of plants. Tree size and growth are expressed on the basis of trunk cross-sectional area (TCSA), which is given in cm^2. The values of this parameter were calculated from the tree trunk diameter measured at a 30 cm height. Trunk

diameter was measured on each tree in a plot directly after tree planting and then in the October of every year of the experiment.

Data were analyzed using the analysis of variance (ANOVA) in the Statistica 13 software package (StatSoft, Cracow, Poland). Means were separated by Newman–Keuls post-hoc tests with a significance level of $p \leq 0.05$. The regression analysis was performed with the Weka open-source software (University of Waikato, Waikato, New Zealand) and is presented as figures graphed in Microsoft Excel.

3. Results

3.1. Mycorrhizal Parameters

Mycorrhizal parameters depend on factors used in the experiment as well as their interactions (Table 2). The inoculation of trees significantly increased the mycorrhizal frequency (F), absolute mycorrhizal intensity (AMI), and relative mycorrhizal intensity (RMI) in the tested roots in each year of the trial. Under the conditions of the non-inoculation treatment, excluding the last year of the study, a relatively low frequency was characteristic for the clone U 8869, in contrast to 'Chopin', which showed relatively high values of this parameter. These findings related to the clone U 8869 were also confirmed in the combination where arbuscular mycorrhizal fungi (AMF) + plants-growth-promoting rhizobacteria (PGPR) inoculum was applied in the first and third year of study to 'Odra' and 'Topaz', respectively.

Table 2. Mycorrhizal frequency, absolute mycorrhizal intensity, and relative mycorrhizal intensity of tested apple trees during 2012–2014 with varying mycorrhizal inoculum and cultivar. NI: non-inoculated; I; inoculated. Data are the average of $n = 3$.

Year	Treatment	Cultivar	Mycorrhizal Parameters (%)		
			Mycorrhizal Frequency	Absolute Mycorrhizal Intensity	Relative Mycorrhizal Intensity
2012	NI	Topaz	A 20.7 ± 4.73 ab	A 1.33 ± 0.59 a	A 7.25 ± 4.17 a
		Odra	A 24.8 ± 6.38 ab	A 0.66 ± 0.45 a	A 2.98 ± 2.50 a
		U 8869	A 16.2 ± 7.20 a	A 1.36 ± 1.60 a	A 7.34 ± 6.47 a
		Chopin	A 37.3 ± 11.01 b	A 2.32 ± 1.54 a	A 6.05 ± 3.54 a
	I	Topaz	B 72.9 ± 9.57 a	B 19.15 ± 3.86 a	B 26.43 ± 4.37 a
		Odra	B 91.3 ± 4.19 b	B 36.41 ± 9.76 b	B 40.23 ± 11.29 b
		U 8869	B 69.6 ± 11.35 a	B 39.77 ± 4.81 b	B 57.85 ± 2.98 c
		Chopin	B 83.0 ± 7.39 ab	B 39.22 ± 4.58 b	B 47.60 ± 4.85 bc
2013	NI	Topaz	A 32.1 ± 11.01 ab	A 3.20 ± 1.76 a	A 9.84 ± 3.96 a
		Odra	A 28.8 ± 8.77 ab	A 1.60 ± 1.61 a	A 5.68 ± 5.16 a
		U 8869	A 24.6 ± 8.82 a	A 2.31 ± 1.16 a	A 9.13 ± 3.26 a
		Chopin	A 44.2 ± 4.19 b	A 6.28 ± 3.09 a	A 13.97 ± 5.74 a
	I	Topaz	B 72.6 ± 4.20 a	B 47.58 ± 9.65 ab	B 65.32 ± 11.12 a
		Odra	B 67.7 ± 7.39 a	B 52.45 ± 12.44 b	B 77.08 ± 12.63 a
		U 8869	B 55.9 ± 5.69 a	B 38.08 ± 6.35 a	B 68.17 ± 8.90 a
		Chopin	B 62.6 ± 16.68 a	B 56.87 ± 10.85 ab	B 65.22 ± 11.30 a
2014	NI	Topaz	A 37.3 ± 11.34 a	A 10.56 ± 2.34 a	A 23.73 ± 8.67 a
		Odra	A 33.9 ± 9.95 a	A 5.03 ± 1.82 a	A 16.67 ± 9.31 a
		U 8869	A 39.6 ± 15.64 a	A 9.63 ± 5.58 a	A 22.89 ± 6.02 a
		Chopin	A 31.0 ± 5.67 a	A 6.20 ± 3.77 a	A 19.32 ± 9.26 a
	I	Topaz	B 80.3 ± 12.15 b	B 50.68 ± 7.46 a	B 64.15 ± 5.74 a
		Odra	B 73.8 ± 9.81 ab	B 47.65 ± 3.42 a	B 65.50 ± 6.21 a
		U 8869	B 60.1 ± 9.18 a	B 38.45 ± 12.11 a	B 63.53 ± 13.13 a
		Chopin	B 61.8 ± 9.62 a	B 40.85 ± 1.83 a	B 67.16 ± 8.04 a

Table 2. *Cont.*

Year	Treatment	Cultivar	Mycorrhizal Parameters (%)		
			Mycorrhizal Frequency	Absolute Mycorrhizal Intensity	Relative Mycorrhizal Intensity
Year			0.0882	**<0.0001**	**<0.0001**
Treatment			**<0.0001**	**<0.0001**	**<0.0001**
Cultivar			**0.0062**	0.1259	0.0982
Year × Treatment			**<0.0001**	**0.0001**	**<0.0001**
Year × Cultivar			**0.0061**	**0.0001**	**0.0444**
Treatment × Cultivar			**0.0015**	**0.0204**	**0.0130**
Year × Treatment × Cultivar			0.1927	**0.0056**	**0.0239**

Note: Upper-case letters next to means indicate significant differences between treatments within cultivar in year, and lower-case letters indicate significant differences between cultivars within treatment in year (at $p \leq 0.05$ according to the Newman–Keuls test). NI, non-inoculated; and I, inoculated. Bold format intends to highlight significance.

Tree inoculation in field conditions had a significant effect on absolute as well as relative mycorrhizal intensity. Higher values of these parameters were noted for roots taken from trees treated with tested inoculum in every year of the trial. Genotype of cultivar also affected AMI in 2012 and 2013 and RMI in 2012, but only in the combination where inoculum was applied. In the roots of 'Topaz' trees in 2012, significantly lower RMI and AMI were observed in comparison to the other cultivars used in the experiment. In terms of RMI in 2013, higher relative mycorrhizal intensity in roots compared to clone U 8869 was observed in the 'Odra' cultivar.

3.2. Nutritional Status of Trees

According to data presented in Table 3, the nutritional status of trees was affected by main factors (Mg) or by their double (K and Mg) or triple (N and P) interactions. Higher leaf nitrogen content as the result of inoculation was observed within all tested cultivars excluding 'Topaz' in 2012 and 2013, while in 2014 there was no effect regardless of the treatment or variety. Cultivar genotype affected nitrogen concentration in leaves in 2012 and 2013, except for the 2013 inoculated trees. 'Topaz' and 'Chopin' showed higher concentrations of nitrogen in leaves than other cultivars, but this finding was only significant for trees that were not treated with microbial inoculum, except for 'Chopin' in 2012.

Table 3. Content of macronutrients in leaves of tested apple trees during 2012–2014 with varying mycorrhizal +PGPR inoculum and cultivar. NI: non-inoculated; I; inoculated. Data are the average of $n = 3$.

Year	Treatment	Cultivar	Macronutrient (% d.m.)			
			N	P	K	Mg
2012	NI	Topaz	A 1.98 ± 0.02 c	A 0.32 ± 0.04 a	B 1.13 ± 0.09 b	A 0.24 ± 0.02 a
		Odra	A 1.78 ± 0.05 b	B 0.42 ± 0.08 b	B 1.18 ± 0.14 b	A 0.18 ± 0.01 a
		U 8869	A 1.65 ± 0.11 a	B 0.34 ± 0.03 a	B 1.12 ± 0.24 b	A 0.17 ± 0.04 a
		Chopin	A 1.95 ± 0.08 c	B 0.48 ± 0.06 b	B 0.75 ± 0.12 a	A 0.21 ± 0.13 a
	I	Topaz	A 1.97 ± 0.04 a	A 0.36 ± 0.01 c	A 0.74 ± 0.04 ab	B 0.34 ± 0.03 ab
		Odra	B 1.94 ± 0.05 a	A 0.25 ± 0.04 b	A 0.50 ± 0.05 a	B 0.39 ± 0.05 ab
		U 8869	B 1.88 ± 0.06 a	A 0.15 ± 0.01 a	A 0.79 ± 0.05 b	B 0.30 ± 0.01 a
		Chopin	B 2.11 ± 0.09 b	A 0.21 ± 0.03 ab	A 0.37 ± 0.04 a	B 0.45 ± 0.01 b

Table 3. *Cont.*

Year	Treatment	Cultivar	Macronutrient (% d.m.)			
			N	**P**	**K**	**Mg**
2013	NI	Topaz	A 2.00 ± 0.02 b	B 0.47 ± 0.03 b	B 1.22 ± 0.12 b	A 0.31 ± 0.04 a
		Odra	A 1.84 ± 0.05 a	B 0.54 ± 0.03 b	B 1.03 ± 0.06 ab	A 0.28 ± 0.01 a
		U 8869	A 1.72 ± 0.08 a	B 0.32 ± 0.05 a	B 1.01 ± 0.24 ab	A 0.29 ± 0.03 a
		Chopin	A 1.99 ± 0.07 b	B 0.39 ± 0.08 ab	B 0.80 ± 0.17 a	A 0.32 ± 0.02 a
	I	Topaz	A 2.07 ± 0.06 a	A 0.40 ± 0.07 c	A 0.71 ± 0.31 b	A 0.32 ± 0.17 a
		Odra	B 2.09 ± 0.09 a	A 0.31 ± 0.06 b	A 0.45 ± 0.07 ab	B 0.47 ± 0.04 b
		U 8869	B 2.17 ± 0.08 a	A 0.19 ± 0.02 a	A 0.35 ± 0.03 a	B 0.43 ± 0.03 ab
		Chopin	B 2.14 ± 0.07 a	A 0.21 ± 0.02 a	A 0.32 ± 0.02 a	B 0.57 ± 0.05 b
2014	NI	Topaz	A 2.10 ± 0.07 a	A 0.27 ± 0.06 a	B 1.27 ± 0.07 b	A 0.26 ± 0.16 a
		Odra	A 2.04 ± 0.07 a	B 0.37 ± 0.05 ab	B 0.90 ± 0.02 a	A 0.25 ± 0.02 a
		U 8869	A 1.98 ± 0.08 a	B 0.30 ± 0.02 a	B 1.01 ± 0.13 ab	A 0.22 ± 0.04 a
		Chopin	A 2.11 ± 0.05 a	B 0.42 ± 0.11 b	B 0.81 ± 0.12 a	A 0.30 ± 0.02 a
	I	Topaz	A 2.07 ± 0.07 a	A 0.30 ± 0.04 a	A 0.80 ± 0.11 b	B 0.36 ± 0.03 a
		Odra	A 2.04 ± 0.03 a	A 0.27 ± 0.04 a	A 0.52 ± 0.13 a	B 0.39 ± 0.01 a
		U 8869	A 2.01 ± 0.11 a	A 0.27 ± 0.02 a	A 0.57 ± 0.11 ab	B 0.40 ± 0.08 a
		Chopin	A 2.09 ± 0.07 a	A 0.30 ± 0.04 b	A 0.56 ± 0.22 ab	B 0.47 ± 0.05 b
Year			**<0.0001**	**<0.0001**	**0.0246**	**<0.0001**
Treatment			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Cultivar			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Year × Treatment			**<0.0001**	**0.0107**	**0.0289**	0.6460
Year × Cultivar			**0.0312**	**<0.0001**	**0.0451**	0.2806
Treatment × Cultivar			**<0.0001**	**0.0002**	0.2699	**0.0006**
Year × Treatment × Cultivar			**0.0358**	**<0.0001**	0.4473	0.3523

Note: Upper-case letters next to means indicate significant differences between treatments within cultivar in year, and lower-case letters indicate significant differences between cultivars within treatment in year (at $p \leq 0.05$ according to the Newman–Keuls test). NI, non-inoculated; and I, inoculated. Bold format intends to highlight significance.

Lower phosphorus and potassium content in leaves were observed for the inoculated trees in comparison with the control ones. The exceptions were the 'Topaz' cultivar in 2012 and 2014, which did not respond to inoculation in terms of phosphorus concentration. Phosphorus and potassium nutritional status were also affected by scion wood genotype, in interaction with the inoculation treatment and year (P), and with the year (K). Using mycorrhizal + PGPR inoculum strongly affected leaf concentration of magnesium. Except for 'Topaz' in 2013, higher magnesium contents in leaves were observed for inoculated trees.

Regression analysis showed that there were varying relationships between mycorrhizal parameters and leaf mineral content (Figure 2). Their direction was similar; namely, higher F, AMI, and RMI values were positively correlated with higher leaf N and Mg contents and, simultaneously, lower phosphorus and potassium contents. The significance of these correlations depends on the parameter, and stronger correlations were recorded for RMI and AMI compared to F, as shown in Figure 3. AMI and RMI relationship with magnesium and potassium content in leaves was almost two-fold higher than the same parameter calculated for F.

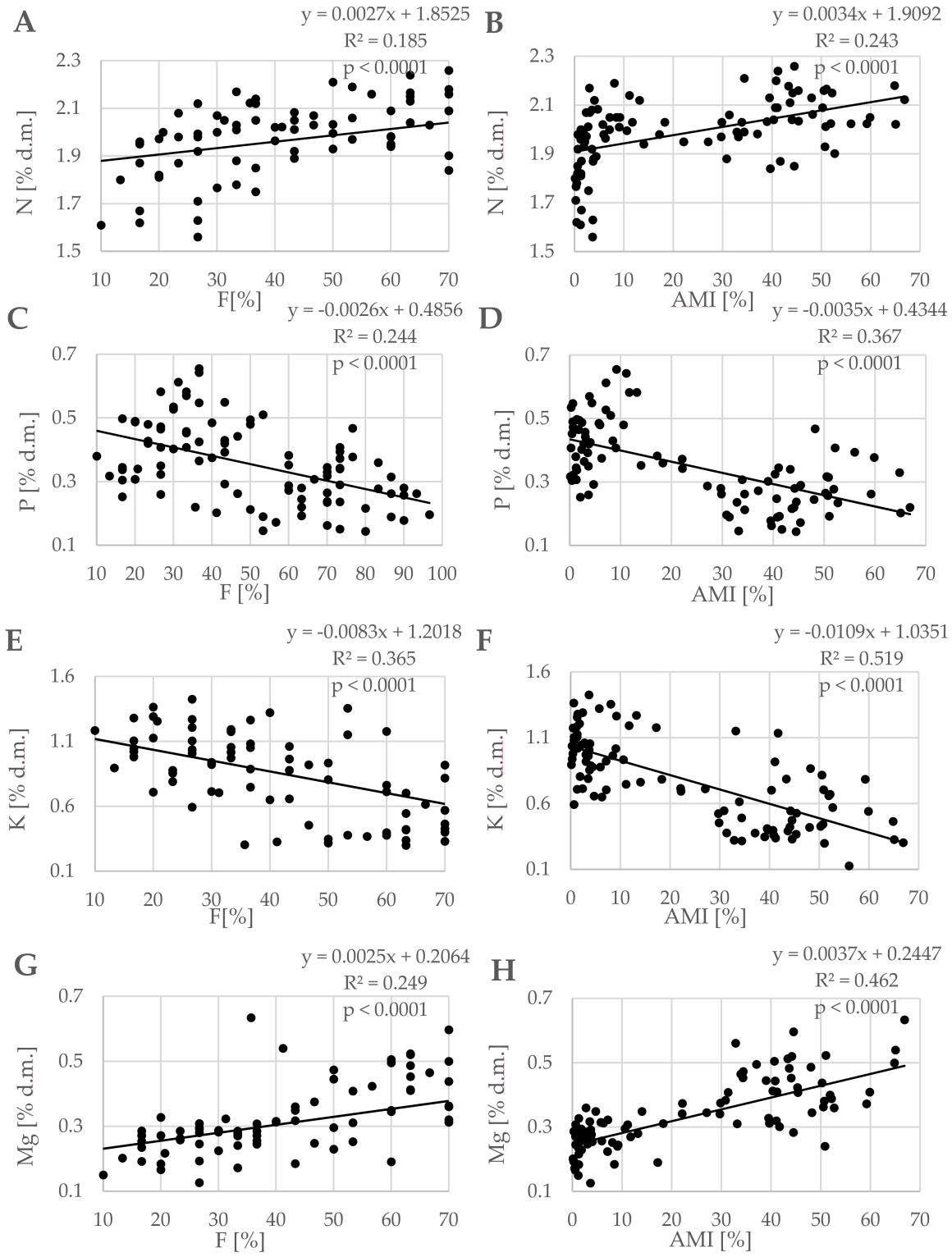

Figure 2. Linear relationships between mycorrhizal parameters (F—mycorrhizal frequency and AMI-absolute mycorrhizal intensity) and macroelement concentration (N-nitrogen, P-phosphorus, K-potassium, and Mg-magnesium) in tested apple tree leaves during 2012–2014 (**A**–**H**).

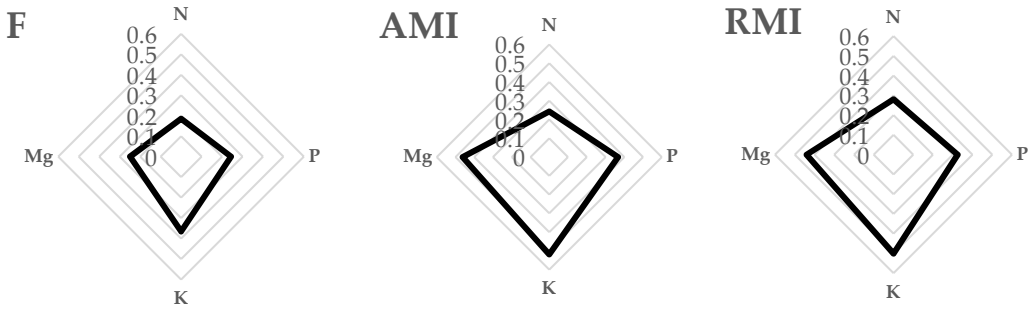

Figure 3. Coefficients of determination calculated from linear relationships between F (mycorrhiza frequency), AMI (absolute mycorrhizal intensity), and RMI (relative mycorrhizal intensity) and N, P, K, and Mg concentrations measured in leaves during 2012–2014.

3.3. Growth of Trees

Application of mycorrhizal + PGPR inoculum and cultivar genotypes affected tree growth in the next years of the trial, as shown by the significant interactions (Year × Treatment and/or Year × Cultivar) presented in Table 4. Measurements taken just after planting the trees in spring 2011 showed that the planting material was uniform in size, and using mycorrhizal + PGPR inoculum significantly increased the trunk cross-sectional area (TCSA) as shown in Figure 4. Higher values of this parameter were recorded after each completed growing season for inoculated plants when compared to the non-inoculated control. The influence of the cultivar genotype was already proved at the experimental setup stage in 2011 and is expressed by the trunk cross-sectional area (TCSA) where U 8869 and 'Topaz' trees had higher values of TCSA than 'Odra' and 'Chopin'. While this higher growth effect was maintained for these two cultivars compared with 'Odra', 'Chopin' had a similar TCSA than 'Topaz' and U 8869 in 2012 to 2014 (Figure 5).

Table 4. TCSA, its increment, and leaf area of tested apple trees during the trial depends on year, microbial inoculation and cultivar. NI: non-inoculated; I; inoculated.

Treatment	Cultivar	TCSA (cm^2)				TCSA Increment 2011–2014 (cm)	Leaf Area (cm^2)		
		2011	2012	2013	2014		2012	2013	2014
NI	Topaz	2.10 ± 0.08	3.46 ± 0.38	9.06 ± 1.41	13.9 ± 1.5	11.8 ± 1.5	20.4 ± 2.1	20.5 ± 3.1	19.6 ± 1.2
	Odra	1.79 ± 0.25	2.39 ± 0.31	5.39 ± 0.62	9.50 ± 1.2	7.80 ± 1.1	25.0 ± 3.7	25.5 ± 3.0	31.6 ± 3.1
	U 8869	2.64 ± 0.21	4.37 ± 0.43	11.2 ± 0.72	17.5 ± 1.7	14.8 ± 1.6	31.7 ± 2.7	32.1 ± 3.6	27.3 ± 0.6
	Chopin	1.85 ± 0.09	3.43 ± 0.60	7.99 ± 1.68	13.7 ± 2.4	11.9 ± 2.5	33.7 ± 5.1	30.7 ± 2.3	30.7 ± 1.5
Mean value for NI		6.89 ± 5.0				11.6 ± 3.0		27.4 ± 5.6	
I	Topaz	2.46 ± 0.15	4.11 ± 0.35	10.9 ± 2.03	16.4 ± 1.4	14.0 ± 1.4	25.2 ± 5.1	25.4 ± 5.2	25.1 ± 0.9
	Odra	1.87 ± 0.49	2.83 ± 0.68	6.62 ± 0.97	11.3 ± 2.7	9.50 ± 2.3	30.3 ± 1.2	31.1 ± 1.7	35.0 ± 3.1
	U 8869	2.37 ± 0.32	5.02 ± 0.08	12.9 ± 1.47	20.1 ± 0.3	17.7 ± 0.5	32.9 ± 6.1	31.7 ± 4.7	28.4 ± 0.6
	Chopin	2.05 ± 0.18	4.51 ± 0.96	11.2 ± 4.25	18.1 ± 3.9	16.0 ± 3.8	36.4 ± 4.7	38.9 ± 2.2	33.0 ± 1.0
Mean value for I		8.29 ± 6.2				14.3 ± 3.8		31.1 ± 5.4	
Year		**<0.0001**				-		0.9337	
Treatment		**<0.0001**				**0.0009**		**<0.0001**	
Cultivar		**<0.0001**				**<0.0001**		**<0.0001**	
Year × Treatment		**0.0162**				-		0.9559	
Year × Cultivar		**<0.0001**				-		**0.0020**	
Treatment × Cultivar		0.1282				0.6907		0.0904	
Year × Treatment × Cultivar		0.9439				-		0.9759	

Note: bold format intends to highlight statistical significance.

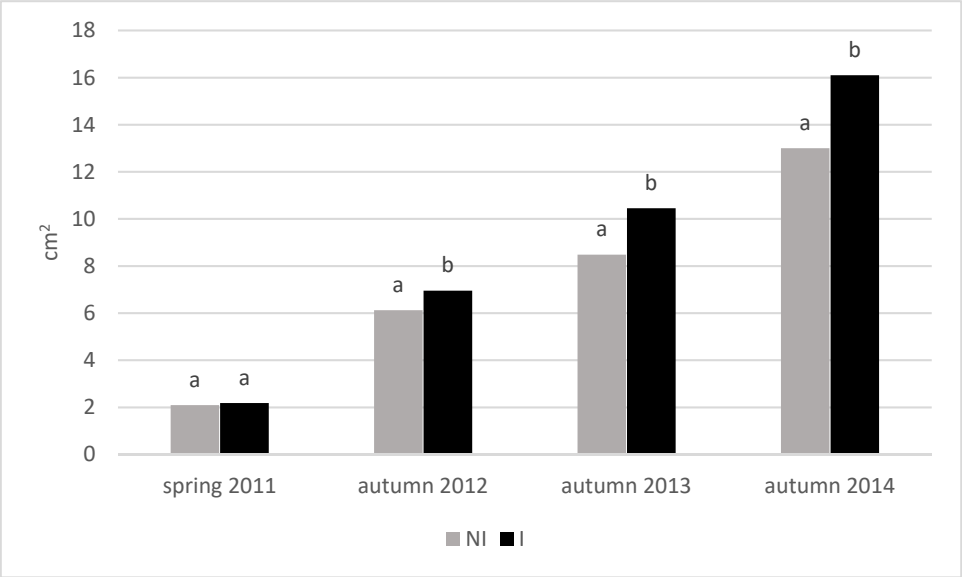

Figure 4. Trunk sross-sectional area TCSA of apple tree depends on inoculation with AMF + PGPR during the course of trial; mean for cultivars (means marked with the same letter within term do not significantly differ at $p \leq 0.05$ according to the Newman–Keuls test). NI: non-inoculated; I; inoculated.

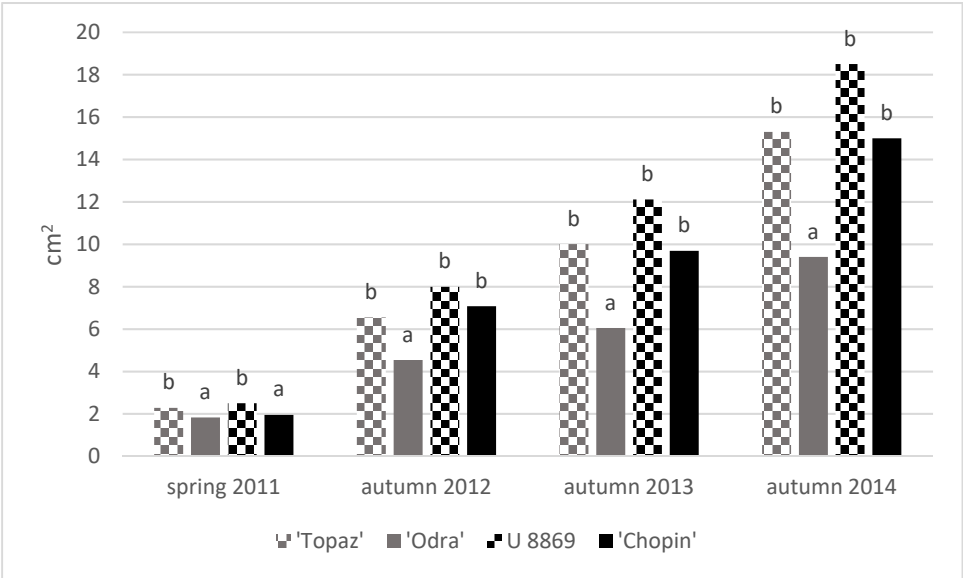

Figure 5. TCSA of apple tree depends on cultivar during the course of trial; mean for treatments (means marked with the same letter within term do not significantly differ at $p \leq 0.05$ according to Newman–Keuls test).

The application of mycorrhizal + PGPR inoculum significantly affected the increment of TCSA calculated for the time period covered by the trial. Trees treated with microbial inoculum showed 23% higher TCSA increment calculated for a whole period of the trial (Table 4), and higher leaf area when compared to the non-inoculated control regardless of the cultivars and years. However, a significant interaction between year and cultivars was observed. The smallest leaves were observed in 'Topaz', and the highest leaf area except for 2014 was noted for 'Chopin' (Figure 6).

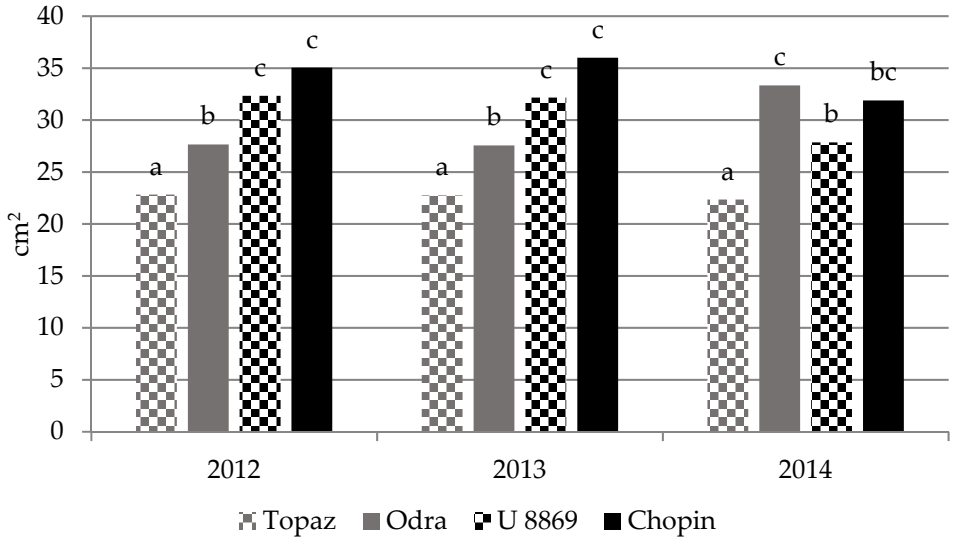

Figure 6. Leaf area of apple tree depends on cultivar during the course of trial; mean for treatments (means marked with the same letter within term do not significantly differ at $p \leq 0.05$ according to Newman–Keuls test).

4. Discussion

Organic farming has constantly been growing in European countries since 1991 when the first organic production regulations in EU were established [31]. We might expect that the trend will be continued while organic farming principles are strictly in line with the current EU Green Deal development strategy, which, in particular, promotes the sustainable use of resources, including soil, atmosphere, and water [32]. Limiting the negative impact of fruit production on the natural environment can be achieved by reduction of the use of fertilizers and improved nutrient use efficiency which also results in the reduction of nutrient leaching and more balanced plant nutrition. This forces producers to look for methods that use naturally occurring processes, contributing to plants in a positive way.

Our goal in this study was to determine the effect of arbuscular mycorrhizal fungi (AMF) + plants-growth-promoting rhizobacteria (PGPR) inoculation on organic apple growing and to determine its effect on the nutritional status and growth of trees. During the research we proved that using microbiological inoculum at the early stage of orchard development resulted in boosting mycorrhiza interaction and that the method of inoculum application of arbuscular mycorrhizal fungi described in our work was very effective in terms of root colonization by AMF. At the end of the experiment period, the values of mycorrhizal frequency obtained for inoculated plants were on average 94.7%, higher than in control plots, and variations for other mycorrhizal parameters were even higher. It should be pointed out that there was also an indigenous colonization of the control plant root system (non-inoculated) by beneficial fungi naturally occurring in soil. Similar results to the field experiment were also obtained and previously shown by other authors [33–38]. This indicates the widespread presence of mycorrhiza in nature and proves that, under field conditions, it is almost impossible to prevent interaction between the plant and AMF [15], especially when low content of phosphates and other nutrients does not restrict plant–AMF interaction [8,39–41].

Relatively high rates of AMF structures in the root system also affected plant nutrition status, but the effect seems to not always be direct and also depends on the mineral element considered. In the case of nitrogen in 2012 and 2013, excluding the 'Topaz' cultivar, we found that inoculated trees showed higher concentration of this element in leaves. The lack of variation in 2014 may result from a dilution effect which is very often observed due to a better nitrogen nutritional status, which was also the case in our study. Higher nitrogen uptake had a significant impact on the growth of trees, expressed by the trunk cross-sectional area (TCSA), and increased the overall vigor of trees. After a four-year

observation period, trees treated with AMF inoculum showed 24% higher TCSA than control plants. Similar results were previously obtained in apple cultivation [33] and in stone-fruit production [42] in Polish conditions. In our experiment we proved that using AMF + PGPR inoculum affected not only tree size but also resulted in higher leaf area. This finding was proven in all years of the trial and might be the reason that phosphorus and potassium content in leaves decreased after microbial inoculation as a consequence of a dilution effect, which was also observed by other authors [43–45]. It is also possible that the presence of well-distributed and active AM structures within roots results in the suppression of the direct phosphorus root pathway and promotes the mycorrhizal tract for uptake of nutritional elements from soil, as well as nutrient partitioning within plant organs in plant roots extensively colonized by arbuscular fungi. This mechanism reported previously by other authors [43,44,46] could even enhance the observed dilution effect, but there is no clear evidence for supporting this statement in our research.

In the case of potassium which was reported by Ollson et al. [20] to have a strong positive correlation with phosphorus in mycorrhizal structures, its decreased content in leaves affected by inoculation treatment seems to have had a similar background. Furthermore, soil sample analysis made before the trial establishment showed that potassium level was low and share of clay particles was up to 37.2%. In the experimental site, clay minerals in soil are represented mostly by the montmorillonite, kaolinite, and illite group—well known for their tendency for strong potassium fixation in the interpack spaces [47], which occasionally leads to visible potassium deficiencies observed on leaves in neighboring orchard plots [48]. In such conditions, deficiency of potassium in soil might play a trigger role in shifting the plant–AMF relation to competition in the case of this nutrient uptake.

The obtained higher magnesium content determined in the leaves of inoculated apple trees indicates a beneficial effect of mycorrhiza on the uptake of this component from the soil, which was also confirmed in other research papers [36,49–52]. Some authors claimed that a better plant supply of magnesium is an indirect effect related to the one of the mechanisms of transport and absorption of these element by the plant [44]. The mechanism that could be responsible for increased uptake of Mg by inoculated trees in our trial is the better water uptake which improved mass flow through the soil to tree roots. This finding can be supported by high magnesium content observed in soil, which does not restrict its uptake, and better nitrogen nutrition, which is associated with higher transpiration rates [53].

Presence of PGPR in the microbial cocktail we used in our experiment also seemed to affect gathered results. Regarding the regression analysis that showed some correlations between mycorrhizal parameters and mineral content in leaves, it must be pointed out that the r^2 values of the regression model were no higher than 0.5. This suggests that improving AMF colonization was not the only factor that decided on varying the nutritional status of non-inoculated and inoculated trees in the experiment, but that PGPR could also have played a role. This statement can be supported by other authors studies proved PGPR effect on growth, yield, crop quality as well as macro- and micro-nutrient uptake [54–56].

The results on phosphorus and nitrogen content in leaves may also suggest that the 'Topaz' cultivar, when compared to the other apple varieties used, does not naturally show such a strong mycorrhizal growth response. There is a lack of reports on this phenomenon in fruit plants, and the mycorrhizal growth response is discussed mainly at the level of individual plant species [57–59], although, according to Tawaraya [60], it may also refer to specific varieties, which was also confirmed by Berdeni et al. [35].

5. Conclusions

Most of the time it is not obvious to observe any beneficial effect of PGPR and AMF under organic farming, because some organic amendments already provide several microbial communities or may promote indigenous microbial communities. During this four-year trial, we confirmed that mycorrhiza is a widespread phenomenon, and apple tree root colonization by indigenous AMF in organic conditions occurs naturally regardless

of scion wood used. The strategy of artificial tree inoculation with AMF increases the presence of mycorrhizal structures within roots and provides more vigorous growth and better magnesium uptake as a result of improved tree nutrition with nitrogen. Results of our study may also suggest that in practice AMF + PGPR inoculation should be used mainly for improving nutritional status of trees in terms of nitrogen and magnesium, but only on soils where deficit of potassium is not observed. The next step of our research will be to determine the impact of AMF + PGPR inoculation of apple trees on the yield and quality as well as storage potential of fruit produced in organic farming conditions.

Author Contributions: Conceptualization, S.P. and D.W.; methodology, S.P., W.K. and D.W.; formal analysis, S.P.; investigation, S.P., W.K.; writing—original draft preparation, S.P.; writing—review and editing, S.P., D.W. and W.K.; visualization, S.P. and W.K.; supervision, D.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Polish Ministry of Science and Higher Education with funds from the Institute of Horticultural Sciences.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: Authors would like to thank Karolina Molska-Kawulok and Jacek Marszał (Department of Pomology, Institute of Horticultural Sciences, Warsaw University of Life Sciences—WULS) for their participation in the experiment.

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

References

1. Barański, M.; Rempelos, L.; Iversen, P.O.; Leifert, C. Effects of organic food consumption on human health; the jury is still out! *Food Nutr. Res.* **2017**, *61*, 1287333. [CrossRef]
2. Kowalczyk, W.; Wrona, D.; Przybyłko, S. Content of minerals in soil, apple tree leaves and fruits depending on nitrogen fertilization. *J. Elem.* **2012**, *22*, 67–77. [CrossRef]
3. Sas-Paszt, L.; Głuszek, S. Rola korzeni oraz ryzosfery we wzroście i plonowaniu roślin sadowniczych. *Postęp. Nauk Roln.* **2007**, *6*, 27–39.
4. Parniske, M. Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nat. Rev. Microbiol.* **2008**, *6*, 763–775. [CrossRef]
5. Schüβler, A.; Schwarzott, D.; Walker, C. A new fungal phylum, the Glomeromycota: Phylogeny and evolution. *Mycol. Res.* **2001**, *105*, 1413–1421. [CrossRef]
6. Derkowska, E.; Paszt, L.S.; Dyki, B.; Sumorok, B. Assessment of Mycorrhizal Frequency in the Roots of Fruit Plants Using Different Dyes. *Adv. Microbiol.* **2015**, *5*, 54–64. [CrossRef]
7. Smith, S.E.; Jakobsen, I.; Grønlund, M.; Smith, F.A. Roles of Arbuscular Mycorrhizas in Plant Phosphorus Nutrition: Interactions between Pathways of Phosphorus Uptake in Arbuscular Mycorrhizal Roots Have Important Implications for Understanding and Manipulating Plant Phosphorus Acquisition. *Plant Physiol.* **2011**, *156*, 1050–1057. [CrossRef] [PubMed]
8. Smith, S.E.; Smith, F.A. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* **2012**, *104*, 1–13. [CrossRef]
9. Smith, S.E.; Smith, F.A.; Jakobsen, I. Mycorrhizal Fungi Can Dominate Phosphate Supply to Plants Irrespective of Growth Responses. *Plant Physiol.* **2003**, *133*, 16–20. [CrossRef] [PubMed]
10. Ho, I.; Trappe, J.M. Translocation of 14C from Festuca Plants to their Endomycorrhizal Fungi. *Nat. New Biol.* **1973**, *244*, 30–31. [CrossRef]
11. Pfeffer, P.E.; Douds, D.D.; Bécard, G.; Shachar-Hill, Y. Carbon Uptake and the Metabolism and Transport of Lipids in an Arbuscular Mycorrhiza1. *Plant Physiol.* **1999**, *120*, 587–598. [CrossRef]
12. Jakobsen, I.; Rosendahl, L. Carbon Flow into Soil and External Hyphae of Vesicular–Arbuscular Mycorrhizae in Carbon and Mineral Nutrition. In *Mycorrhizal Ecology*; van der Heijden, G.A., Sanders, I.R., Eds.; Springer: Berlin, Germany, 1990; pp. 75–92.
13. Bryla, D.R.; Eissenstat, D.M. Respiratory Costs of Mycorrhizal Associations. In *Plant Respiration*, 1st ed.; Lambers, H., Ribas Carbo, M., Eds.; Springer: Dordrecht, The Netherlands, 2005; Volume 18, pp. 207–224. [CrossRef]
14. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: Cambridge, UK, 2007.
15. Smith, S.E.; Smith, F.A. Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. *Annu. Rev. Plant Biol.* **2011**, *62*, 227–250. [CrossRef]
16. Drew, E.; Murray, R.; Smith, S.; Jakobsen, I. Beyond the rhizosphere: Growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant Soil* **2003**, *251*, 105–114. [CrossRef]

17. Schnepf, A.; Jones, D.; Roose, T. Modelling Nutrient Uptake by Individual Hyphae of Arbuscular Mycorrhizal Fungi: Temporal and Spatial Scales for an Experimental Design. *Bull. Math. Biol.* **2011**, *73*, 2175–2200. [CrossRef]
18. López-Pedrosa, A.; Gonzalez-Guerrero, M.; Valderas, A.; Azcon, C.; Ferrol, N. GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of Glomus intraradices. *Fungal Genet. Biol.* **2006**, *43*, 102–110. [CrossRef]
19. Hodge, A.; Fitter, A.H. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13754–13759. [CrossRef]
20. Olsson, P.A.; Hammer, E.C.; Pallon, J.; van Aarle, I.M.; Wallander, H. Elemental composition in vesicles of an arbuscular mycorrhizal fungus, as revealed by PIXE analysis. *Fungal Biol.* **2011**, *115*, 643–648. [CrossRef]
21. Garcia, K.; Zimmermann, S.D. The role of mycorrhizal associations in plant potassium nutrition. *Front. Plant Sci.* **2014**, *5*, 337. [CrossRef] [PubMed]
22. Pallon, J.; Wallander, H.; Hammer, E.; Arteaga Marrero, N.; Auzelyte, V.; Elfman, M. Symbiotic fungi that are essential for plant nutrient uptake investigated with NMP. *Nucl. Instrum. Methods Phys. Res. B* **2007**, *260*, 149–152. [CrossRef]
23. Olsson, P.A.; Hammer, E.C.; Wallander, H.; Pallon, J. Phosphorus Availability Influences Elemental Uptake in the Mycorrhizal Fungus Glomus intraradices, as Revealed by Particle-Induced X-Ray Emission Analysis. *Appl. Environ. Microbiol.* **2008**, *74*, 4144–4148. [CrossRef] [PubMed]
24. Kondratowicz-Maciejewska, K.; Kobierski, M. Content of available magnesium, phosphorus and potassium forms in soil exposed to varied crop rotation and fertilisation. *J. Elem.* **2011**, *16*, 543–553. [CrossRef]
25. Stafecka, A.; Komosa, A. The effect of soil orchard samples drying on the phosphorus, potassium, and magnesium contents determined by the universal, Egner-Riehm and Schachtschabel methods. *Rocz. Akad. Rol. Pozn. CCCLVI* **2004**, 199–208.
26. Łądkiewicz, K.; Wszędyrówny-Nast, M.; Jaśkowiak, K. Comparison of different methods for determination of organic matter content. *Sci. Rev. Eng. Sci.* **2017**, *26*, 99–107. [CrossRef]
27. Sadowski, A.; Nurzyński, J.; Pacholak, E.; Smolarz, K. *Określanie Potrzeb Nawożenia Roślin Sadowniczych. Zasady, Liczby Graniczne i Dawki Nawożenia*, 1st ed.; SGGW-AR: Warszawa, Poland, 1990.
28. Bilbao, B.; Giraldo, D.; Hevia, P. Quantitative determination of nitrogen content in plant tissue by a colorimetric method. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 1997–2005. [CrossRef]
29. Galas, W.; Kita, A. Application of the ICP-AES method to the multielemental analysis in the reference samples of tobacco leaves. *Rocz. Państw. Zakł. Hig.* **1995**, *46*, 53–57.
30. Trouvelot, A.; Kough, J.L.; Gianinazzi-Pearson, V. Mesure du Taux de Mycorhization VA d'un Systemeradiculaire. Recherche de Methods D'estimation Avant une Signification Fonctionnelle. In *Physiological and Genetical Aspects of Mycorrhizae*, 1st ed.; Gia-ninazzi-Pearson, V., Gianinazzi, S., Eds.; INRA: Paris, France, 1986; pp. 217–221.
31. Katsoulas, N.; Løes, A.-K.; Andrivon, D.; Cirvilleri, G.; De Cara, M.; Kir, A.; Knebl, L.; Malińska, K.; Oudshoorn, F.W.; Willer, H.; et al. Current use of copper, mineral oils and sulphur for plant protection in organic horticultural crops across 10 European countries. *Org. Agric.* **2020**, *10*, 159–171. [CrossRef]
32. Diacono, M.; Montemurro, F.; Trinchera, A. An Overview on Agroecology and Organic Agriculture Strategies for Sustainable Crop Production. *Agronomy* **2021**, *11*, 223. [CrossRef]
33. Gąstoł, M.; Domagała-Świątkiewicz, I. Mycorrhizal inoculation of apple in replant soils–enhanced tree growth and mineral status. *Acta Sci. Pol. Hortorum Cultus* **2015**, *14*, 17–37. Available online: https://czasopisma.up.lublin.pl/index.php/asphc/article/view/2589 (accessed on 14 May 2021).
34. Derkowska, E.; Paszt, L.S.; Sumorok, B.; Dyki, B. Colonisation of apple and blackcurrant roots by arbuscular mycorrhizal fungi following mycorrhisation and the use of organic mulches. *Folia Hortic.* **2013**, *25*, 117–122. [CrossRef]
35. Berdeni, D.; Cotton, T.E.A.; Daniell, T.J.; Bidartondo, M.I.; Cameron, D.; Evans, K.L. The Effects of Arbuscular Mycorrhizal Fungal Colonisation on Nutrient Status, Growth, Productivity, and Canker Resistance of Apple (*Malus pumila*). *Front. Microbiol.* **2018**, *9*, 1461. [CrossRef] [PubMed]
36. Gnekow, M.A.; Marschner, H. Role of VA-mycorrhiza in growth and mineral nutrition of apple (*Malus pumila var. domestica*) rootstock cuttings. *Plant Soil* **1989**, *119*, 285–293. [CrossRef]
37. Derkowska, E.; Sas-Paszt, L.; Szwonek, E.; Głuszek, S. The influence of mycorrhization and mulches on myccorhizal frequency in apple and strawberry roots. *J. Fruit Ornam. Plant Res.* **2008**, *16*, 227–242.
38. Sumorok, B.; Sas-Paszt, L.; Głuszek, S.; Derkowska, A.; Żurawicz, E. The effect of mycorrhizal and mulching of apple trees 'Gold Milenium' and blackcurrant bushes 'Tiben' on the occurrence of arbuscular mycorrhizal fungi. *J. Fruit Ornam. Plant Res.* **2011**, *19*, 35–49.
39. Miller, R.L.; Jackson, L.E. Survey of vesicular–arbuscular mycorrhizae in lettuce production in relation to management and soil factors. *J. Agric. Sci.* **1998**, *130*, 173–182. [CrossRef]
40. Kahiluoto, H.; Ketoja, E.; Vestberg, M. Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 1. Bioassays in a growth chamber. *Plant Soil* **2000**, *227*, 191–206. [CrossRef]
41. Kahiluoto, H.; Ketiga, E.; Vestberg, M. Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization. 2. Field studies. *Plant Soil.* **2001**, *231*, 65–79. [CrossRef]
42. Świerczyński, S.; Stachowiak, A. The influence of mycorrhizal fungi on the growth and yielding of plum and sour cherry trees. *J. Fruit Ornam. Plant Res.* **2010**, *18*, 71–77.

43. Granger, R.L.; Plenchette, C.; Fortin, J.A. Effect of A Vesicular Arbuscular (Va) Endomycorrhizal Fungus (Glomus Epigaeum) on The Growth and Leaf Mineral Content of Two Apple Clones Propagated In Vitro. *Can. J. Plant Sci.* **1983**, *63*, 551–555. [CrossRef]
44. Pinochet, J.; Fernández, C.; Jaizme, M.D.C.; Tenoury, P. Micropropagated Banana Infected with Meloidogyne javanica Responds to Glomus intraradices and Phosphorus. *HortScience* **1997**, *32*, 101–103. [CrossRef]
45. Syvertsen, J.P.; Graham, J.H. Phosphorus supply and arbuscular mycorrhizas increase growth and net gas exchange responses of two *Citrus* spp. grown at elevated [CO_2]. *Plant Soil* **1999**, *208*, 209–219. [CrossRef]
46. Kothari, S.K.; Marschner, H.; Römheld, V. Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. *New Phytol.* **1990**, *116*, 637–645. [CrossRef]
47. Pietranek, A.; Jadczuk, E. Mineral status of 'Katja' apple trees depending on irrigation, fertilisation and rootstock. *Acta Sci. Pol. Hortorum Cultus* **2005**, *4*, 69–76.
48. Wrona, D.; Sadowski, A. Effect of nitrogen and soil management on soil mineral nitrogen in the apple orchard. *J. Fruit Ornam. Plant Res.* **2004**, *12*, 191–199.
49. Allen, M.F. *The Ecology of Micorrhizae*, 1st ed.; Cambridge University Press: Cambridge, UK, 1991.
50. Latef, A.A.H.A.; He, C. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. *Sci. Hortic.* **2011**, *127*, 228–233. [CrossRef]
51. Xiao, J.X.; Hu, C.Y.; Chen, Y.Y.; Yang, B.; Hua, J. Effects of low magnesium and an arbuscular mycorrhizal fungus on the growth, magnesium distribution and photosynthesis of two citrus cultivars. *Sci. Hortic.* **2014**, *177*, 14–20. [CrossRef]
52. Taylor, J.; Harrier, L.A. A comparison of development and mineral nutrition of micropropagated Fragaria×ananassa cv. Elvira (strawberry) when colonised by nine species of arbuscular mycorrhizal fungi. *Appl. Soil Ecol.* **2001**, *18*, 205–215. [CrossRef]
53. Xia, G.; Cheng, L.; Lakso, A.; Goffinet, M. Effects of Nitrogen Supply on Source-sink Balance and Fruit Size of 'Gala' Apple Trees. *J. Am. Soc. Hortic. Sci.* **2009**, *134*, 126–133. [CrossRef]
54. Bashan, Y.; De-Bashan, L.E. Bacteria/Plant Growth Promotion. In *Encyclopedia of Soils in the Environment*; Hillel, D., Ed.; Elsevier: Oxford, UK, 2005; pp. 103–115.
55. Estiken, A.; Karlidag, S.; Ercisli, M.T.; Sahin, F. The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). *Aust. J. Agric. Res.* **2003**, *54*, 377–380. [CrossRef]
56. Tenuta, M. Plant Growth Promoting Rhizobacteria: Prospects for Increasing Nutrient Acquisition and Disease Control. 2003. Available online: http://www.umanitoba.ca/faculties/afs/MAC_proceedings/2003/pdf/tenuta_rhizobacteria.pdf (accessed on 24 June 2021).
57. Plenchette, C.; Fortin, J.A.; Furlan, V. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. *Plant Soil* **1983**, *70*, 199–209. [CrossRef]
58. Saif, S.R. Growth responses of tropical forage plant species to vesicular-arbuscular mycorrhizae. *Plant Soil* **1987**, *97*, 25–35. [CrossRef]
59. Siqueira, J.O.; Saggin-Júnior, O.J. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species. *Mycorrhiza* **2001**, *11*, 245–255. [CrossRef]
60. Tawaraya, K. Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Sci. Plant Nutr.* **2003**, *49*, 655–668. [CrossRef]