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Research Article

Contribution of arbuscular mycorrhizal fungi to nitrogen and phosphorus uptake efficiency and productivity of faba bean crop on contrasting cropping systems

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Abstract

The present study was focused on evaluating the effect of AMF (Arbuscular Mycorrhizal Fungi) inoculation on nitrogen and phosphorus uptake efficiency and productivity of faba bean (*Vicia faba* L.) crop, under different fertilization levels on organic or conventional cropping systems. The 2-year field experiment was conducted in central Greece and laid out in a split-plot design, with three replications, two main plots (AMF inoculation treatments) and five sub-plots (fertilization treatments). The results demonstrated that plants of AMF inoculated plots exhibited greater plant height, leaf area index (LAI), leading to higher biomass, and consequently higher final seed yields. Regarding the quality parameters, including nutrients (nitrogen and phosphorus) uptake and their utilization indices, similar results to those of the productivity results were found with the AMF inoculated plants presented the higher values. Finally, all the parameters of the root system, including AMF root colonization and weighted mycorrhizal dependency (WMD), were negatively affected by fertilization level, particularly in an inorganic form. As a conclusion, the current study confirmed that replacement of inorganic inputs by organic in combination with AMF inoculation, should be seriously considered as a sustainable practice of faba bean crop cultivation under Mediterranean conditions.

Keywords: leaf area index (LAI); nitrogen harvest index (NHI); nitrogen uptake; phosphorus utilization efficiency (PUtE); seed yield; *Vicia faba* L.; weighted mycorrhizal dependency (WMD)

Introduction

Arbuscular Mycorrhizal Fungi (AMF) constitute crucial and fundamental soil microbes, affecting plant and soil microorganism growth (Miransari, 2011; Sbrana *et al.*, 2014). Since AM fungi can form symbiotic relationships with the roots of most terrestrial plants, the symbiotic relationship between mycorrhizal fungi and plant roots is frequent in the natural environment (Feddermann *et al.*, 2010). There are several types of

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fungi that create these relationships, but for agriculture, the AMF of the phylum Glomeromycota score the most important capabilities for forming asymbiotic associations, with more than 80% of land plants in both natural and agricultural ecosystems (Schussler *et al.*, 2001; Begum *et al.*, 2019).

Crop management encompasses a variety of practices that can have an effect on the AM association, both directly by damaging or killing AMF and indirectly by producing conditions that are either favorable or unfavorable to AMF. Generally, agricultural practices have a detrimental impact on the AM relationship, and agricultural soils are AMF depleted, particularly in terms of species number (Menéndez *et al.*, 2001; Manoharan *et al.*, 2017; Begum *et al.*, 2019). In areas of intensive agricultural production, for instance, phosphorus (P) fertilizer use has far exceeded crop requirements, resulting in a buildup of total and, in some circumstances, freely available P in the soil (Kogelmann *et al.*, 2004). As a result, crops rely less on the AM association and have lower AM colonization and propagule density (Zhu *et al.*, 2016).

In contrast, the exclusion of soluble mineral fertilizers and the really limited utilization of biocides in organic agricultural systems means that it is heavily reliant on biological processes for nutrient supply, and AMF are commonly thought to play a pivotal role since it is assumed that they can compensate for the reduced use of P fertilizers (Galvez *et al.*, 2001; Zhu *et al.*, 2016). Nevertheless, the actual contribution of AMF to the operation of organic agroecosystems, particularly crop performance, is still unclear. Because the AM symbiosis can enhance plant growth and health, there is growing interest in determining its effectiveness in specific plant-producing systems and, as a result, controlling them so that they can be included in production procedures when feasible. Evidence is mounting that indigenous and/or introduced AM fungi can improve annual crops, and especially legumes which are often highly dependent on AMF (Yang *et al.*, 2015). The investigation of the ecological factors of AM inoculation and the selection of appropriate AM fungi are essential concerns for the application of mycorrhizal technology in agriculture (Estaún *et al.*, 2002; Vosatka and Dodd, 2002; Jin *et al.*, 2017).

Inoculation experiments have revealed that different AMF species cause a wide variety of growth responses in the host plant, ranging from significantly favorable to significantly negative (Kahiluoto *et al.*, 2009; Jin *et al.*, 2017; Begum *et al.*, 2019). The issue of selecting species for AMF inoculum is compounded by the fact that the most successful AMF species differ between host plants and may depend on whether the primary goal is nutrient uptake, increased disease resistance, or improved water relations. Failure to select the suitable AMF/host/inoculation method may describe why several inoculants utilized thus far have failed to have a positive effect, despite a high degree of colonization. Whenever an efficient AMF/host/inoculation combination is discovered, the problem of competing with the natural soil AMF remains (Gosling *et al.*, 2006; Yang *et al.*, 2015).

In this study, we investigated the contribution of AMF inoculation as well as the contribution of field AMF communities to crop growth and productivity of legumes such as faba bean and enlighten the underlying mechanisms in conventional and organic cropping systems. In addition, the study aimed to determine whether these cropping systems can influence AM root colonization by either indigenous or inoculum-introduced mycorrhizal populations.

Materials and Methods

Site description and experimental design

A faba bean (*Vicia faba* L. cv. 'Tanagra') crop was established in the experimental field of the Institute of Industrial and Forage Crops (IIFC) in Larissa, Central Greece (Latitude: 39°30′N, Longitude: 22°42′E, Elevation: 77 m above sea level) over a period of two agricultural seasons (2015-2016). The soil was classified as typical Vertisol (USDA, 2010), while the texture was clay with average soil particle size distribution: 51% clay, 23% silt and 26% sand, pH (1:1 H_2O) = 7.2, and organic matter content 1.6%. Total available nitrogen in the soil was moderate ($N_{Kjeldhal}$: 0.09 g 100g^{-1}) as well as available potassium (K_{exch}: 1.7 cmol kg^{-1}), while phosphorus availability was low (P_{Olsen}: 6.7 mg kg^{-1}). The meteorological data (mean temperature and precipitations) throughout the growing seasons were obtained from the Network of Agro-meteorological Stations Horta srl. and are presented in Figure 1. In general, the study area (Larissa, Central Greece) is characterized by a typical Mediterranean climate with hot, dry summer periods and cool, humid winters. During the crop cycle period (from January to June), the mean temperature was 12.7 °C and 14.6 °C for 2015 and for 2016, while the total precipitation was 316.2 mm and 266.2 mm for the respective years.

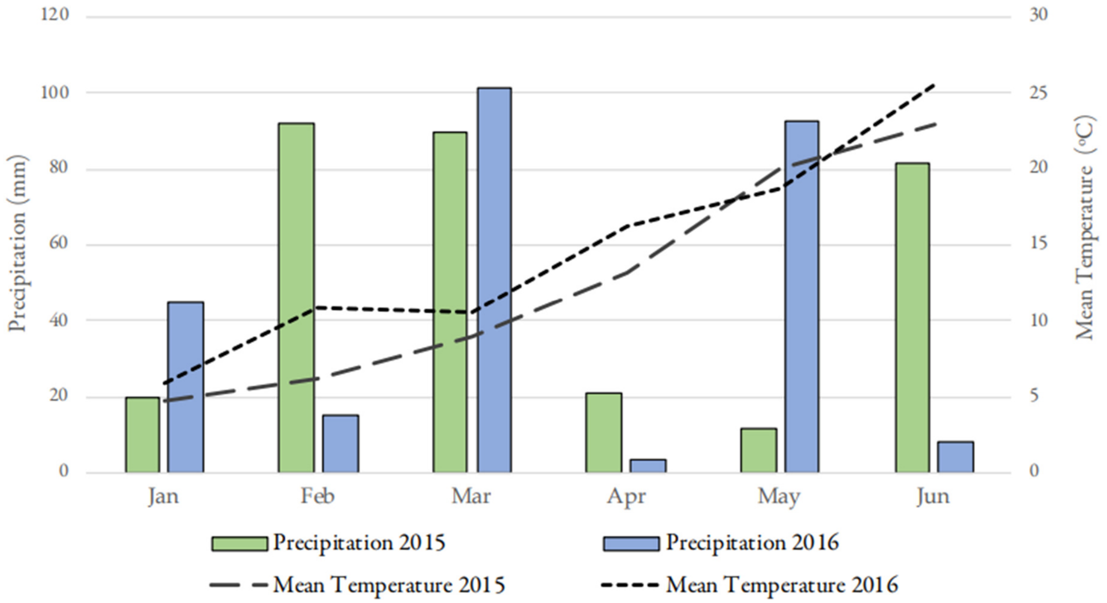


Figure 1. Weather data (mean monthly temperature and precipitation) for the experimental site during the crop cycle periods (January-June, 2015 and 2016)

The experiment was set up on an area of 588 m^2, according to the split-plot design with three replications, two main plots for AMF inoculation (AMF+ = with inoculation, AMF- = without inoculation) and five sub-plots for fertilization. Concerning AMF inoculation, before sowing, furrows 0.25 m apart were opened to a depth of approximately 5-7 cm and AM fungi inoculum was evenly distributed along the bottom of the furrows of the AMF+ plots. The inoculum used was the commercial root inoculant product Symbivit (Symbiom Ltd., Lanškroun, Czech Republic) containing six AMF species of the phylum Glomeromycota, *Claroideoglomus etunicatum*, *Rhizoglomus microaggregatum*, *Rhizophagus intraradices*, *Claroideoglomus claroideum*, *Funneliformis mosseae*, and *Funneliformis geosporum*, in a form of AM colonized root pieces and spores and hyphae in fritted clay. The inoculation dosage was 65 kg of inocula per ha according to the recommendations of the manufacturer of the inoculant product. The five fertilization treatments included the control (untreated), 100% of recommended dose in organic form (100% Org), 60% of recommended dose in organic form (60% Org), 100% of recommended dose in inorganic form (100% Inorg) and 60% of recommended dose in inorganic form (60% Inorg). Fertilization was applied in two doses. The first was applied at sowing as basal dressing with 35 kg N ha^{-1}, 90 kg P ha^{-1} and 15 kg K ha^{-1} in all plots for the 100% dose and the second dose (equal amount of N) was applied on the onset vegetative phase. The main plot and sub-plot, sizes were 75 m^2 (15 × 5 m) and 15 m^2 (5 × 3 m). There was a space of 2 m between replications and 2 m between main plots. Two days prior to the sowing, the soil was prepared by mouldboard ploughing at the depth 0.25 m. Faba bean seeds were broadcasted by hand at a depth of 3-5 cm with row and intra-row spacing of 25 and 10 cm, respectively. The sowing rate was 170 kg ha^{-1}, and seed sowing was performed on 22nd January 2015 and 26th January 2016. Throughout the experimental periods, no irrigation was applied and crops were left rain-fed. In addition, there was no incidence of macro-nutrient deficit, water stress, or disease on the faba bean crops. Weeds were controlled by hand-hoeing when it was needed and before canopy closure.

Sampling, measurements, and methods

Plant height and leaf area index (LAI) were measured using twenty randomly selected plants from each sub-plot at 100 days after sowing (DAS). For the determination of LAI, the leaves of each plant sample were placed on a high-resolution scanner (Epson Perfection V330 Photo; Seiko Epson Corp., Nagano-ken, Japan) using ImageJ Ops software (Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA) (Rueden *et al.*, 2017). As a result, the plant-based measurements were converted into a LAI by dividing the readings by the average crop density of each plot. Moreover, twenty plant samples were randomly collected from each sub-plot at 130 DAS. The plants were separated into stems, fruits, seeds, green and yellow leaves, and weighted before being oven-dried for 48 hours at 70°C. The total nitrogen (N) content of the aerial biomass and the seeds was determined by grinding the dried samples to a fine powder and applying them to the Kjeldahl procedure using a Kjeltec 8400 autoanalyzer (Foss Tecator AB, Höganas, Sweden). For the measurement of phosphorus (P) content, twenty plants per sub-plot were also harvested at 130 DAS, separated into aerial biomass and seeds, dissolved in an $HNO_3H_2O_2$ solution, and heated under pressure in a CEM MDS 2000 microwave (CEM Ltd., Buckingham, UK). The extract was then analyzed using an iCAP 6500 DUO ICP-emission spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Total plant nitrogen and phosphorus uptake was determined as N and P absorption, respectively, in the total above-ground (aerial biomass + seeds) dry matter at the time of maturity (130 DAS). N and P harvest indices (NHI and PHI) were calculated as the ratio between the seed nutrient uptake and the total plant nutrient uptake, while the N and P utilization efficiencies (NUtE and PUtE) were estimated as the ratio between seed yield and the total plant nutrient uptake (Fageria and Baligar, 2005; Ye *et al.*, 2007).

Root samples were also collected from the 0–35 cm layer by using a cylindrical auger (25 cm length, 10 cm diameter) at the midpoint between successive plants within a row. Specifically, three samples per plot were analyzed at 130 DAS. For each sample, roots were separated from the soil by soaking the samples overnight in 30 ml of a 0.5% solution of sodium hexametaphosphate. Afterwards, the samples were stirred for 5 min and washed over a 5 mm mesh-sieve. The roots thus held on the sieves were cleaned and stained with lactic acid/fuchsin, according to the method described by Kormanik and McGraw (1982). The percentage of root length colonized by AM fungi was determined microscopically with the gridline-intersection method at a magnification of × 30- 40 (Giovannetti and Mosse, 1980). Weighted mycorrhizal dependency (WMD) was calculated according to a modified equation of Plenchette et al. (1983) as follows:

$$\text{WMD}\ (\%) = \frac{(\text{dry weight of inoculated plant} \times \%\ \text{colonization}) - (\text{dry weight of non-inoculated plant} \times \%\ \text{colonization})}{(\text{dry weight of inoculated plant} \times \%\ \text{colonization})} \times 100 \qquad (1)$$

The modification incorporates the effect of indigenous AMF populations on crop production, since control plots (not inoculated) were not subjected to inoculation and therefore "hosted" native AMF populations. Indigenous AMF population was expected to colonize faba bean crops of control plots, suggesting that plants of these plots were only non-inoculated and not non-mycorrhizal.

Finally, the plants were harvested at full seed maturity (seed moisture 13%) on 8th June 2015 (137 DAS) and 10th June 2016 (136 DAS). The seed yield was determined by plants derived from the middle sub-plot area (2 m^2). Harvest index (HI) was calculated as the ratio between seed yield and above-ground biomass yield (whole weight of plants derived for seed yield).

Statistical analysis

The experimental data were subjected to statistical analysis using the SPSS 22 statistical software (IBM Corp., Armonk, NY, USA). The trait data generated by AMF inoculation and fertilization treatments in the two years were assessed using 2 × 2 × 5 factorial design (two years, two AMF inoculation treatments and five fertilization treatments) laid out in split-plot design with three replications. A mixed model was used for the analysis of variance (ANOVA), with years and replications as random effects and AMF inoculation and fertilization as fixed effects. As test criterion for detecting differences between means, the $LSD_{0.05}$ was used (Steel *et al.*, 1980), as well as Tukey's honestly significant difference (Tukey's HSD) test at two levels, with significance ($p = 0.05$) and remarkable significance ($p = 0.01$) (Keselman and Rogan, 1977). Finally, in order to estimate the levels of correlation between the variables studied, a simple regression analysis was performed at the 5% level of significance ($p = 0.05$).

Results and Discussion

Plant height

The findings of the effects of arbuscular mycorrhizal fungi (AMF) inoculation and fertilization on the plant height of faba bean are presented in Table 1. Combined analysis of variance (Table 4) generally revealed that plant height was significantly affected by both AMF inoculation and different fertilization treatments. Specifically, the results showed that the (AMF+) plants had the highest plant height compared to the (AMF-) plants (+10.35% and +10.74% in 2015 and 2016, respectively) (Table 1). This could be due to the response of AMF application, which can increase the uptake of water and nutrients used in the metabolic process in the plant body, stimulating plant height growth (Koide and Mosse, 2004; Cho *et al.*, 2009; Begum *et al.*, 2019). For both AMF treatments, plants that received full N and P fertilization in organic or inorganic form, reached greater final heights (Table 1), while plants without fertilization achieved minor final heights, probably because of higher growth rate during the vegetative phase that was significantly affected by nitrogen (N) fertilization (Beslemes *et al.*, 2016). The increased height of faba bean plants with the increased nitrogen level was mainly due to the role of nitrogen in stimulating metabolic activity, which contributed to the increase of metabolites amount and therefore led to the internodes' elongation and increased plant height by increasing available nitrogen levels to the plant (Amoanimaa-Dede *et al.*, 2022). Since the relatively considerable height of the plant, a high concentration of photosynthetic products was found and a significant and positive correlation among plant height and biomass yield was recorded ($r = 0.636, p < 0.001$; Table 3).

Table 1. Plant height, leaf area index (LAI), biomass yield, seed yield and harvest index of faba bean for each fertilization type and level and AMF inoculation treatment

Inoculation		Fertilization											
		2015						2016					
		100% Org.	60% Org.	100% Inorg.	60% Inorg.	Control	Mean	100% Org.	60% Org.	100% Inorg.	60% Inorg.	Control	Mean
Plant Height (cm)	AMF+	77.3^{b}	75.5^{b}	76.3^{b}	75.0^{b}	72.2^{a}	75.26^{B}	79.1^{c}	77.6^{b}	78.4^{c}	77.4^{b}	74.1^{a}	77.3^{B}
	AMF-	69.6^{a}	69.4^{a}	69.3^{a}	66.9^{a}	65.8^{a}	68.2^{A}	70.1^{a}	70.3^{a}	69.5^{a}	68.4^{a}	67.2^{a}	69.8^{A}
	LSD†	**	***	*	*	**		**	**	***	**	**	
LAI ($m^2 m^{-2}$)	AMF+	5.3^{c}	4.1^{a}	4.9^{c}	4.3^{ab}	4.5^{b}	4.6^{B}	6.2^{c}	5.2^{a}	5.6^{bc}	5.4^{ab}	5.5^{ab}	5.6^{B}
	AMF-	4.6^{c}	3.2^{a}	4.3^{c}	3.9^{b}	3.5^{ab}	3.1^{A}	5.8^{c}	4.1^{a}	5.7^{c}	4.7^{b}	4.1^{a}	4.9^{A}
	LSD†	*	*	*	ns	*		ns	*	ns	*	***	
Biomass Yield (tn ha^{-1})	AMF+	10.98^{b}	10.38^{b}	10.34^{b}	10.60^{b}	9.29^{a}	10.33^{B}	12.88^{b}	12.89^{b}	13.09^{b}	12.96^{b}	11.36^{a}	12.63^{B}
	AMF-	8.74^{b}	8.65^{b}	8.69^{b}	8.39^{ab}	7.89^{a}	8.47^{A}	10.81^{ab}	10.92^{b}	10.43^{ab}	10.69^{ab}	9.69^{a}	10.51^{A}
	LSD†	*	**	***	*	**		*	*	**	***	*	
Seed Yield (tn ha^{-1})	AMF+	0.57^{b}	0.53^{ab}	0.55^{b}	0.52^{ab}	0.50^{a}	0.53^{B}	0.72^{b}	0.69^{ab}	0.67^{ab}	0.68^{b}	0.63^{a}	0.68^{B}
	AMF-	0.46^{b}	0.42^{ab}	0.45^{ab}	0.45^{ab}	0.41^{a}	0.44^{A}	0.60^{b}	0.58^{b}	0.58^{b}	0.54^{ab}	0.49^{a}	0.56^{A}
	LSD†	*	**	**	***	**		*	**	*	***	***	
Harvest Index	AMF+	0.55^{c}	0.52^{ab}	0.53^{bc}	0.51^{a}	0.52^{ab}	0.52^{A}	0.56^{a}	0.53^{a}	0.51^{a}	0.53^{a}	0.56^{a}	0.54^{A}
	AMF-	0.53^{b}	0.52^{ab}	0.52^{ab}	0.48^{a}	0.52^{ab}	0.51^{A}	0.55^{a}	0.53^{a}	0.55^{a}	0.56^{a}	0.51^{a}	0.54^{A}
	LSD†	ns	ns	ns	*	ns		ns	ns	ns	ns	ns	

*Different letters indicate significant differences between means of fertilization treatments under the same AMF inoculation treatment at $p < 0.05$, according to Tukey's method, and LSD† indicates significant differences between AMF treatments within the same fertilization treatment.

Leaf Area Index (LAI)

According to the combined analysis (Table 4), the main effect of inoculation as well as of fertilization on leaf area index (LAI) was statistically significant. The results revealed that the (AMF+) plants presented the highest LAI value compared to the (AMF-) plants (+48.39% and 14.28% in 2015 and 2016, respectively) (Table 1). The beneficial effect of mycorrhizal fungi on plant growth and specifically on leaf area have been demonstrated in various plant species, by Feng et al. (2000) in maize, Al-Karaki et al. (2001) and Felföldi et al. (2022) in tomato, Beslemes *et al.* (2016) in barley, Gao et al. (2020) in upland cotton and Bi and Zhou (2021) in peanut plants. The effect of fertilization type and level was also significant with the highest values (5.3 and 6.2 m^2 m^{-2} in 2015 and 2016, respectively) were found in the case of fertilization with 100% of recommended dose in organic form in combination with AMF inoculation, confirming that replacement of inorganic inputs by organic in combination with AMF inoculation, would not increase the risk of an open canopy (which could result in a significantly greater loss of assimilation and productivity) and should be seriously considered as a sustainable practice (Beslemes *et al.*, 2016; Trisilawati *et al.*, 2019; Felföldi *et al.*, 2022).

Biomass yield

Concerning biomass yield, the combined analysis of variance (Table 4) revealed that the AMF inoculation was found to be statistically significant and the highest values (10.33 and 12.63 tn ha^{-1} in the first and second year, respectively) were achieved in the AMF inoculated plots (Table 1). Other researchers (Jadhav and Patil, 1996; Bi and Zhou, 2021) found that inoculating plants with AMF had the potential to increase shoot dry weight and crop grain yield while also improving nitrogen (N) and phosphorus (P) accumulation. The increased biomass accumulation was most likely caused by the AMF inoculation. Fertilization type (organic or inorganic) had also a significant effect, with both fertilization types appearing to be equally able to produce high levels of biomass in the case of 100% of recommended dose (Table 1). The improved growth development of the plant's aboveground parts, particularly the leaf area, combined with the higher nitrogen levels in the 100% Org. and 100% Inorg. treatments resulted in higher rates of photosynthesis and thus the highest biomass yields. The high correlation coefficient between biomass yield and the leaf area index of the faba bean crop ($r = 0.968$, $p < 0.001$; Table 3) supports this relationship. The relative soluble protein ratio, carboxylase activity, and chlorophyll and total nitrogen concentrations all contribute to an increase in photosynthesis rate and hence to the supply of photosynthetic products to the whole plant and seed (Evans, 1983; Kakabouki *et al.*, 2019).

Seed yield and Harvest Index (HI)

According to the combined analysis of variance (Table 4) and Table 1, seed yield was affected by both AMF inoculation and fertilization during the experimental periods. Concerning the effect of AMF inoculation, the seed yields recorded in AMF inoculated plots (0.53 and 0.68 tn ha^{-1} in 2015 and 2016, respectively) were significantly higher than in non-inoculated treatments (0.44 and 0.56 tn ha^{-1} in 2015 and 2016, respectively). It is well known that legumes benefit agricultural sustainability in both developed and developing countries due to symbiotic atmospheric nitrogen fixation. In this study, AMF stimulated the seed yield of faba bean in accordance with several previous studies (Liu *et al.*, 2018; Liang *et al.*, 2019). According to the global meta-analysis, AMF inoculation increased crop grain yield by about 20% (Lekberg and Koide, 2005, Pellegrino *et al.*, 2015). In response to fertilization effect, averaged over AMF treatments, plants that received full N and P fertilization in organic or inorganic form, reached greater seed yields with the averaged two-year values being 0.59 and 0.56 tn ha^{-1} for the cases of fertilization with 100% of recommended dose in organic and inorganic form, respectively, while plants without fertilization achieved minor seed yields (Table 1). The results of the current study confirmed that seed yield of faba bean crop was positively and linearly influenced by the increase in nitrogen rates (Karkanis *et al.*, 2018). Also, in the current study, leaf area index (LAI) was linearly correlated with seed yield ($r = 0.808$, $p < 0.001$; Table 3). This was attributable to increased photosynthesis, which resulted in an increase in photosynthetic products, improving both the biomass and seed yield of the crop (Bilalis *et al.*, 2012; Kakabouki *et al.*, 2019).

The results of the present study indicated that harvest index (HI) was not influence by AMF inoculation, but it was only affected by different fertilization regimes. In particular, the only significant difference among fertilization treatments was observed in the first year (2015) of the experiment, where plants fertilized with 100% of recommended dose in organic form had statistically higher value (0.54), while the lowest value (0.495) was obtained in plots fertilized with 60% of recommended dose in inorganic form. The results are line with those reported by several authors (Papastylianou *et al.*, 2021; Sellami *et al.*, 2021) investigated the performance of faba bean crop under Mediterranean conditions.

Table 2. Biomass nitrogen (N) uptake, seed N uptake, total plant N uptake, nitrogen harvest index (NHI), biomass phosphorus (P) uptake, seed P uptake, total plant P uptake and phosphorus harvest index (PHI) of faba bean for each fertilization type and level and AMF inoculation treatment.

Inoculation		**Fertilization**											
		2015						**2016**					
		100% Org.	**60% Org.**	**100% Inorg.**	**60% Inorg.**	**Control**	**Mean**	**100% Org.**	**60% Org.**	**100% Inorg.**	**60% Inorg.**	**Control**	**Mean**
Biomass N Uptake (kg N ha^{-1})	AMF+	4.62^{b}	4.67^{b}	5.13^{a}	3.66^{a}	3.58^{a}	4.3^{B}	7.24^{ab}	7.97^{ab}	9.06^{b}	6.49^{a}	5.90^{a}	7.3^{B}
	AMF-	2.58^{a}	2.76^{a}	3.97^{a}	2.43^{a}	3.49^{a}	3.1^{A}	4.86^{a}	5.67^{a}	7.24^{b}	5.44^{a}	6.54^{ab}	5.9^{A}
	LSD†	**	ns	*	**	ns		***	*	ns	*	ns	
Seed N Uptake (kg N ha^{-1})	AMF+	25.56^{b}	22.25^{a}	22.79^{a}	22.09^{a}	21.66^{a}	22.9^{B}	34.27^{a}	30.95^{a}	30.78^{a}	29.44^{a}	29.91^{a}	31.1^{B}
	AMF-	21.41^{b}	18.52^{ab}	19.02^{ab}	16.75^{a}	16.59^{a}	19.6^{A}	28.43^{a}	25.31^{a}	26.20^{a}	23.55^{a}	21.40^{a}	24.9^{A}
	LSD†	ns	**	**	***	**		ns	*	ns	**	**	
Total Plant N Uptake (kg N ha^{-1})	AMF+	30.19^{b}	26.92^{ab}	27.92^{ab}	25.75^{a}	25.24^{a}	27.2^{B}	41.52^{b}	38.93^{a}	38.51^{a}	37.28^{a}	35.82^{a}	38.4^{B}
	AMF-	23.99^{a}	21.29^{a}	22.99^{a}	19.19^{a}	20.08^{a}	21.5^{A}	33.29^{b}	30.97^{ab}	33.45^{b}	29.00^{a}	27.94^{a}	30.9^{A}
	LSD†	*	**	**	***	*		*	*	ns	**	*	
NHI (%)	AMF+	89.27^{a}	87.00^{a}	82.72^{a}	87.30^{a}	83.06^{a}	85.9^{A}	82.55^{b}	79.52^{ab}	76.44^{a}	82.59^{b}	83.42^{b}	80.9^{A}
	AMF-	84.65^{bc}	82.67^{b}	81.63^{a}	85.78^{c}	85.75^{c}	84.1^{A}	85.21^{b}	81.63^{ab}	78.51^{ab}	81.19^{ab}	76.67^{a}	80.6^{A}
	LSD†	**	*	ns	*	ns		ns	ns	ns	ns	*	
Biomass P Uptake (kg P ha^{-1})	AMF+	0.41^{c}	0.40^{bc}	0.35^{bc}	0.23^{a}	0.29^{ab}	0.33^{B}	0.70^{c}	0.61^{c}	0.55^{bc}	0.33^{a}	0.43^{ab}	0.59^{B}
	AMF-	0.16^{a}	0.24^{a}	0.25^{a}	0.17^{a}	0.21^{a}	0.20^{A}	0.32^{a}	0.37^{a}	0.23^{a}	0.37^{a}	0.25^{a}	0.31^{A}
	LSD†	***	**	*	ns	ns		***	**	*	ns	ns	
Seed P Uptake (kg P ha^{-1})	AMF+	3.64^{b}	3.52^{ab}	3.71^{b}	3.36^{ab}	3.05^{a}	3.5^{B}	4.32^{ab}	4.65^{b}	4.71^{b}	4.66^{b}	4.11^{a}	4.5^{B}
	AMF-	3.06^{b}	2.81^{a}	2.96^{a}	2.66^{a}	2.55^{a}	2.8^{A}	3.78^{a}	3.75^{a}	4.11^{a}	3.82^{a}	3.44^{a}	3.8^{A}
	LSD†	**	**	**	**	ns		ns	*	ns	**	*	
Total Plant P Uptake (kg P ha^{-1})	AMF+	4.04^{b}	3.92^{b}	4.06^{b}	3.59^{ab}	3.34^{a}	3.80^{B}	4.99^{ab}	5.53^{b}	5.01^{ab}	5.27^{b}	4.54^{a}	5.1^{B}
	AMF-	3.11^{a}	3.06^{a}	3.3^{a}	2.82^{a}	2.76^{a}	3.10^{A}	3.98^{a}	4.09^{ab}	4.4^{b}	4.07^{ab}	3.72^{a}	4.1^{A}
	LSD†	*	ns	*	**	ns		*		*	**	*	
PHI (%)	AMF+	95.05^{b}	92.40^{a}	92.61^{a}	94.11^{a}	92.56^{a}	93.3^{B}	93.64^{ab}	90.96^{a}	91.58^{ab}	94.26^{b}	91.50^{ab}	92.4^{B}
	AMF-	89.94^{a}	89.91^{a}	91.27^{ab}	93.7^{b}	91.42^{ab}	91.3^{A}	85.77^{a}	88.34^{ab}	89.31^{b}	93.27^{c}	90.47^{b}	89.4^{A}
	LSD†	***	**	ns	ns	ns		***	**	ns	*	ns	

*Different letters indicate significant differences between means of fertilization treatments under the same AMF inoculation treatment at $p < 0.05$, according to Tukey's method, and LSD† indicates significant differences between AMF treatments within the same fertilization treatment.

Biomass nitrogen (N) uptake

The effects of the AMF inoculation and fertilization on biomass nitrogen (N) uptake of faba bean are presented in Table 2. Combined analysis of variance (Table 4) revealed that biomass N uptake was influenced by both inoculation and different fertilization treatments during the two-year experiment. In particular, the results showed that the (AMF+) plants had the highest biomass N uptake compared to the (AMF-) plants (+38.71% and +23.73% in 2015 and 2016, respectively). In response to fertilization, for both AMF treatments, plants that received full N and P fertilization in inorganic form, reached greater biomass N uptake (Table 2). Absorbed nitrogen (N-uptake) is the product of multiplication biomass by the percentage of nitrogen in plant tissues and the variability of its values depends on the degree of correlation between these two factors. During plant flowering, under low nitrogen inflows, there is a strong negative correlation between productivity and the percentage of nitrogen in the tissues (solution effect), resulting in a reduction in the variability of absorbed nitrogen controlled by genetic factors (Gallais and Hirel, 2004). However, until flowering, in all typical forage plants, the above negative correlation between the production and the percentage of nitrogen in the tissues is observed to a lesser extent (Lemaire and Gastal, 1997), and then at maturity, the observed small variability for total absorbed nitrogen and for low nitrogen inflows, indicates that there is a limiting factor between the availability of nitrogen in the soil and the ability of the plant to absorb it. In contrast to low inputs, high nitrogen inputs have high variability in absorbed nitrogen, with no negative correlation between production and tissue nitrogen content (Gallais and Hirel, 2004).

Seed nitrogen (N) uptake

Seed nitrogen (N) uptake was estimated by multiplying the N content in seeds with the seed yield. The results of current study indicated that N uptake in seeds was affected by the AMF inoculation and fertilization during the experimental periods. In regard to AMF inoculation, the highest values (22.9 and 31.1 kg N ha^{-1} in 2015 and 2016, respectively) obtained in AMF inoculated plants (Table 2). Averaged over years and AMF inoculation treatments, the highest seed N uptake value (27.42 kg N ha^{-1}) was achieved in the case of fertilization with 100% of recommended dose in organic form. With the development of more sustainable agricultural techniques, organic N fertilization is garnering more attention in both research and agriculture (Matson *et al.*, 1997). Inorganic N fertilization may lower agricultural soil mycorrhizal inoculum potential (Liu *et al.*, 2012), increase pathogen severity (Matson *et al.*, 1997; Jin *et al.*, 2017), and increase greenhouse gas fluxes from agricultural soils (McSwiney and Robertson, 2005). Combining organic N fertilization with AMF may be effective in addressing some of these issues and improving fertilizer N uptake into plants, which is currently limited to 40-60% in crop plants to which inorganic N is applied (Huber and Watson, 1974; Paustian *et al.*, 1995; Jin *et al.*, 2017).

Total plant nitrogen (N) uptake

Total plant nitrogen (N) uptake was estimated by multiplying the N content of total above-ground (aerial biomass + seeds) dry matter and the total above-ground dry matter yield at the time of maturity (130 DAS). Total plant N uptake was significantly affected by the different AMF inoculation and fertilization treatments. Averaged over years and fertilization treatments, the highest value (32.8 kg N ha^{-1}) was recorded when plants subjected to AMF inoculation (Table 2). In regard to fertilization, the highest total plant N uptake, averaged over AMF treatments, was achieved in the case of 100% of recommended dose in organic form treatment with the values being 27.09 (19.55 % higher than control) and 37.41 kg N ha^{-1} (17.33% higher than control) in 2015 and 2016, respectively. Although AMF have been shown to transmit N to their associated host (Hodge *et al.*, 2001; Barrett *et al.*, 2011), significant questions about the ecological ramifications of such an AMF-N uptake pathway remain (Smith and Smith, 2011). The exact mechanism of N transfer, as well as, more importantly, the amounts of N transmitted via the AMF in proportion to the plant's N requirements is still not specified (Smith and Smith, 2011). Despite the fact that data from root organ culture experiments reveal that up to 50% of root N can be received via the AMF pathway (Govindarajulu *et al.*, 2005), as useful as these systems are for dissecting mechanisms involved in nutrient exchange, inferring much about whole plant nutrient dynamics may be risky. Given the growth conditions used, source-sink interactions, for example, are probably unrealistic (Smith and Smith, 2011). In field experiments, which used whole plants and added N as organic matter patches, revealed that AMF can contribute up to 15-20% of plant N uptake (Leigh *et al.*, 2009; Barrett *et al.*, 2014). AMF also improves legumes' ability to fix N_2 and reduces the quantity of inorganic N that leaches (Veresoglou *et al.*, 2012). Because nitrogen is a component of chlorophyll, it is essential for photosynthesis. The transport of photosynthetic resources to the roots increases the activity of soil microorganisms such as AMF as well as plant growth promoting bacteria (PGPB) (Etesami *et al.*, 2021).

Nitrogen Harvest Index (NHI)

Nitrogen Harvest Index (NHI) is an essential index for determining the efficiency with which the absorbed nitrogen is transferred from plant vegetative parts to seeds. NHI did not show statistically significant differences for mycorrhiza inoculation, although in AMF inoculated plots a small increase from 84.9% to 85.87% and from 80.6% to 80.9% was observed in 2015 and in 2016 respectively. On the contrary, the application of fertilization affected the values of NHI, recording statistically significant differences, mainly between the different levels of applied fertilization and not fertilization type. As shown in Table 2, application with mycorrhizae increased the values of the Nitrogen Harvest Index at the low levels of applied inputs, while at the high levels of applied inputs in some cases it had no effect and, in some others, resulted in a lower value of the Index (these differences were not statistically significant). This indicator is extremely important for monitoring nitrogen distribution in cultivated plants, as it indicates how effectively absorbed nitrogen was utilized for seed production (Fageria and Baligar, 2005). High NHI values imply that nitrogen is distributed more evenly in seeds (Bulman and Smith, 1994). In addition, the effects of evaluated treatments on the NHI were proportional to that of the harvest index (HI). This is also confirmed by the positive and strong correlation among these evaluated traits ($r = 0.842, p < 0.001$; Table 3).

Biomass phosphorus (P) uptake

The effects of the AMF inoculation and fertilization on biomass phosphorus (P) uptake of faba bean are presented in Table 2. In the treatment with AMF inoculation, the values of biomass P uptake were substantially higher (0.33 and 0.59 kg P ha^{-1} in 2015 and 2016, respectively), than in the non-inoculated plants (0.20 and 0.31 kg P ha^{-1} for the respective years). Moreover, the mean values of biomass P uptake provided good evidence of the fertilization effect. The highest values (0.41 and 0.70 kg P ha^{-1} in 2015 and 2016, respectively) were found in the case of fertilization 100% of recommended dose in organic form and AMF inoculation. AMF inoculation raised P uptake in aerial biomass relative to control. In the same manner, Karandashov and Bucher (2005) observed that one of the most significant effects of AMF inoculation on the host plant is an increase in P, primarily as a result of mycorrhiza absorbing phosphate from the soil and transferring it to host roots. The fungal hyphae transfer phosphorus across long distances into the cortical cell of the root (Golubkina *et al.*, 2020). Clark and Zeto (2000) found that AMF increases phosphorus solubilization and nutrient absorption as P, N, and K. In general, it is evident that AMF have the capability to boost the uptake of inorganic nutrients in almost all plants, specifically phosphate (Nell *et al.*, 2010; Begum *et al.*, 2019). Furthermore, organic fertilizers may also have an indirect positive effect on mycorrhiza-mediated nutrient uptake because increased soil organic matter promotes the growth of extraradical hyphae (Joner and Jakobsen, 1995), and P uptake in mycorrhizal plants appears to be determined by extraradical hyphal lengths rather than fungus-specific P affinity (Schweiger and Jakobsen, 1999; Begum *et al.*, 2019).

Table 3. Correlation coefficients between evaluated properties

Property	**Coefficient of correlation (*r*)**															
	Plant Height	Leaf Area Index (LAI)	Biomass Yield	Seed Yield	Harvest Index (HI)	Biomass Nitrogen (N) Uptake	Seed N Uptake	Total Plant N Uptake	Nitrogen Harvest Index (NHI)	Biomass Phosphorus (P) Uptake	Seed P Uptake	Total Plant P Uptake	Phosphorus Harvest Index (PHI)	Nitrogen Utilization Efficiency (NUtE)	Phosphorus Utilization Efficiency (PUtE)	AMF Colonization
LAI	0.563***															
Biomass Yield	0.636***	0.968***														
Seed Yield	0.737***	0.808***	0.804***													
HI	0.283*	0.504***	0.525***	0.491***												
Biomass N uptake	0.473***	0.646***	0.574***	0.777***	0.138^{ns}											
Seed N Uptake	0.665***	0.814***	0.809***	0.964***	0.534***	0.720***										
Total Plant N Uptake	0.650***	0.815***	0.790***	0.969***	0.452***	0.844***	0.980***									
NHI	-0.159^{ns}	-0.297*	-0.195^{ns}	0.568**	0.842***	-0.848***	-0.270*	-0.453***								
Biomass P Uptake	0.095^{ns}	-0.414**	-0.329*	-0.433***	-0.122^{ns}	-0.484***	-0.461***	-0.495***	0.309*							
Seed P Uptake	0.674***	0.753***	0.735***	0.920***	0.311*	0.860***	0.878***	0.926***	-0.536***	-0.442***						
Total Plant P Uptake	0.705***	0.757***	0.737***	0.936***	0.328*	0.879***	0.889***	0.940***	-0.554***	-0.408**	0.992***					
PHI	-0.551***	-0.384**	-0.362**	-0.566***	-0.285*	-0.570***	-0.498***	-0.549***	0.429***	-0.063^{ns}	-0.438***	-0.548***				
NUtE	-0.350**	-0.658***	-0.589***	-0.696***	-0.294*	-0.813***	-0.788***	-0.843***	0.586***	0.488***	-0.750***	-0.749***	0.384**			
PUtE	-0.262*	-0.251^{ns}	-0.210^{ns}	-0.301*	0.152^{ns}	-0.617***	-0.255*	-0.375**	0.664***	0.106^{ns}	-0.623***	-0.608***	0.227^{ns}	0.476***		
AMF Colonization	0.379**	0.135^{ns}	0.106^{ns}	0.343**	0.252^{ns}	0.145^{ns}	0.358**	0.318*	0.051^{ns}	0.139^{ns}	0.182^{ns}	0.240^{ns}	-0.513***	-0.203^{ns}	0.125^{ns}	
Weighted Mycorrhizal Dependency (WMD)	-0.409*	0.102^{ns}	-0.015^{ns}	-0.291^{ns}	-0.290^{ns}	0.016^{ns}	-0.271^{ns}	-0.214^{ns}	-0.192^{ns}	-0.188^{ns}	-0.182^{ns}	-0.191^{ns}	0.140^{ns}	-0.029^{ns}	-0.089^{ns}	0.052^{ns}

*, **, *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; ns, not significant ($p > 0.05$)

Seed phosphorus (P) uptake

Regarding seed phosphorus (P) uptake, the combined analysis of variance (Table 4) revealed that the AMF inoculation was found to be statistically significant and the highest values (3.05 and 4.50 kg P ha^{-1} in the first and second year, respectively) were achieved in the AMF inoculated plots (Table 2). In regard to fertilization, the highest total plant N uptake, averaged over AMF treatments, was achieved in the case of 100% of recommended dose in organic form treatment with the values being 3.34 and 4.41 kg P ha^{-1} in 2015 and 2016, respectively. The response trends for P uptake into seeds and total above-ground biomass at different AMF inoculation and fertilization treatments are similar to those of seed and biomass yield, respectively, as determined and described by Daur *et al.* (2011) and Qiao *et al.* (2015). In the current study, this is confirmed by the significant positive correlations of seed P uptake with seed yield (r = 0.920, p <0.001; Table 3) and biomass yield (r = 0.735, p <0.001; Table 3).

Table 4. Combined analysis of variance (*F* values) for the effects of AMF inoculation and fertilization on measured properties of faba bean during the 2-year experiment

Source of Variance	Df	**Plant Height**	**Leaf Area Index (LAI)**	**Biomass Yield**	**Seed Yield**	**Harvest Index (HI)**	**Biomass Nitrogen (N) Uptake**
Year (Y)	1	15.9***	389.2***	477.3***	421.1**	8.5**	196.4***
Inoculation (I)	1	224.9***	173.2***	342.1***	270.2***	2.0^{ns}	40.4***
Fertilization (F)	4	8.7***	87.2***	14.9***	17.4***	7.5***	9.2***
Y × I	1	0.9^{ns}	1.5^{ns}	4.6**	3.3^{ns}	0.6^{ns}	0.05^{ns}
Y × F	4	0.2^{ns}	1.4^{ns}	1.6^{ns}	1.2^{ns}	0.1^{ns}	0.8^{ns}
I × F	4	0.7^{ns}	12.4*	0.7^{ns}	0.7^{ns}	2.2^{ns}	4.5*
Y × I × F	4	0.8^{ns}	1.0^{ns}	0.7^{ns}	0.5^{ns}	3.4*	0.3^{ns}
Source of Variance	Df	**Seed N Uptake**	**Total Plant N Uptake**	**Nitrogen Harvest Index (NHI)**	**Biomass Phosphorus (P) Uptake**	**Seed P Uptake**	**Total Plant P Uptake**
Year (Y)	1	201.6***	307.3***	47.8***	1054.8***	3887.8***	4117.7***
Inoculation (I)	1	102.6***	125.5***	1.53^{ns}	122.1***	68.1***	90.6***
Fertilization (F)	4	10.7***	9.7***	9.6***	13.3***	6.5***	7.8***
Y × I	1	2.6^{ns}	2.3^{ns}	2.7^{ns}	62.1***	43.2***	55.4***
Y × F	4	0.3^{ns}	0.2^{ns}	0.2^{ns}	6.5***	4.8**	5.5**
I × F	4	1.3*	0.5^{ns}	6.1**	8.2***	0.2^{ns}	0.5^{ns}
Y × I × F	4	0.4^{ns}	0.1^{ns}	0.5^{ns}	3.8*	0.2^{ns}	0.4^{ns}
Source of Variance	Df	**Phosphorus Harvest Index (PHI)**	**Nitrogen Utilization Efficiency (NUtE)**	**Phosphorus Utilization Efficiency (PUtE)**	**AMF Colonization**	**Weighted Mycorrhizal Dependency (WMD)**	
Year (Y)	1	23.7***	76.6***	8.2**	0.6^{ns}	0.1^{ns}	
Inoculation (I)	1	80.7***	7.6**	2.8*	63.4***	-	
Fertilization (F)	4	18.4***	4.2**	3.2*	21.6***	2.9*	
Y × I	1	2.8^{ns}	0.6^{ns}	0.3^{ns}	0.4^{ns}	-	
Y × F	4	2.4^{ns}	0.6^{ns}	1.4^{ns}	0.5^{ns}	0.6^{ns}	
I × F	4	14.0***	0.7^{ns}	1.0^{ns}	4.4*	-	
Y × I × F	4	0.8^{ns}	0.2^{ns}	0.1^{ns}	0.6^{ns}	-	

* F-test ratios are from ANOVA. *, **, *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; ns, not significant ($p > 0.05$). Df: Degrees of freedom.

Total plant phosphorus (P) uptake

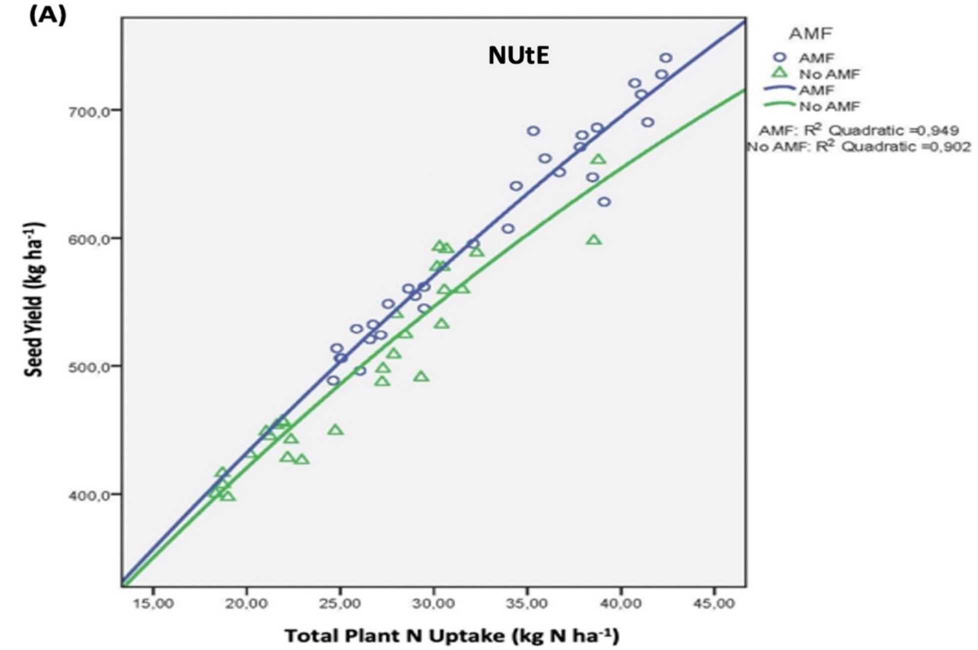
Regarding the absorption of phosphorus (P) in total above-ground dry matter (total plant P uptake), the combined analysis of variance (Table 4) showed that this evaluated trait was significantly influenced by AMF inoculation and fertilization during the two-year experiment. In particular, the results showed that the (AMF+) plants had the highest total plant P uptake compared to the (AMF-) plants (+22.58% and +24.39% in 2015 and 2016, respectively). In response to fertilization, for both AMF treatments, plants that received full N and P fertilization in inorganic form, reached greater total plant P uptake (Table 2). As in the case of seed P uptake, total plant P uptake trends are also in line with those of seed and biomass yield (Qiao *et al.*, 2015), and this confirmed by the strong and positive correlations of total plant P uptake with seed yield ($r = 0.936$, $p < 0.001$; Table 3) and biomass yield ($r = 0.737$, $p < 0.001$; Table 3).

Phosphorus Harvest Index (PHI)

Another parameter of particular interest is the phosphorus harvest index (PHI), which is defined as the ratio of seed phosphorus to the total amount of phosphorus absorbed by the plant. PHI affects the nutritional quality of the seed, but studies that have attempted to determine the genetic basis of seed composition have concluded that the effects of cultivation techniques are inhibited by a seemingly inversely proportional relationship between yield and phosphate content of the seed. In contrast to nitrogen harvest index (NHI), significant effects ($p < 0.05$) of mycorrhizal inoculation and fertilization levels were found on the PHI values, which varied both between the type of fertilization applied, as well as between the level of fertilization (Table 2). For faba bean, these values were 93.3% and 91.3% with and without AMF respectively, for the first year and 92.4% and 89.4% with and without AMF respectively, for the second year. For PHI, the variation recorded shows that phosphorus accumulation and mobilization, although it seems to be under genotypic control, is also influenced by factors that may affect the range of movement of pre-flowering assimilated components and phosphorus to the seed. In addition, with a major effect of mycorrhiza inoculation on increased phosphorus uptake and confirmed by analyzes of increased phosphorus uptake on leaves and stems, the reduction in PHI is due to plant growth in no excess phosphorus environment, by reducing the production of total biomass, or total seed. The increases in the phosphorus recovery fraction were small probably due to the high mycorrhizal native population of the experimental field. Within the mycorrhizosphere, AMF interacts with beneficial rhizosphere microorganisms, including free-living N-fixing bacteria and generally plant growth promoting bacteria (PGPR) (Galleguillos *et al.*, 2000; Biro *et al.*, 2000). The symbiosis of legume-root bacteria is strongly influenced by AMF and there is ample evidence that legume nodules contain AMF communities that are quite different from those found in legume roots (Scheublin *et al.*, 2004). Coexistence with root bacteria is dependent on P concentrations and thus enhanced P nutrition resulting from AMF colonization can lead to increased nodule formation and N fixing (Ibibijen *et al.*, 1996; Vazquez *et al.*, 2002).

Nitrogen Utilization Efficiency (NUtE)

Nitrogen utilization efficiency (NUtE), expresses the seed yield produced per unit of available nitrogen, and is affected by the absorption, distribution, and redistribution of nitrogen between plant parts or cells, as determined specific metabolic processes at the cellular level (Engels and Marschner, 1995; Maxclaux *et al.*, 2001). In plant physiology, this ratio is expressed by the nitrogen utilization efficiency (N utilization efficiency, NUtE; kg kg $^{-1}$), as its ratio (production in N_x - production in N_o) to (N absorbed in N_x - N absorbed in N_o), and is shown diagrammatically by the slopes of the curves of Figure 2A. NUtE recorded an increase in mycorrhiza inoculated plots from 11.11 kg kg^{-1} to 14.28 kg kg^{-1}. Specifically, for mycorrhizal inoculated plants, a linear relationship is observed between absorbed nitrogen and seed production, a linearity that is associated with low nitrogen concentrations in plant tissues under conditions of deprivation, suggesting that maximum potential production was not achieved (Lopez-Bellido *et al.*, 2007). In contrast, for plots without mycorrhiza inoculation, productivity was close to maximum, as with increasing nitrogen application (organic or inorganic) NUtE deviated from linearity, signaling high nitrogen concentrations in plant tissues (luxurious growth). Nitrogen uptake in excess of the amount required to determine optimum seed yield results in lower NUtE values than the corresponding maximum value for the given production level and is related to the increased nitrogen concentration in the shoots and seed mentioned above (Muchow and Davis, 1998).



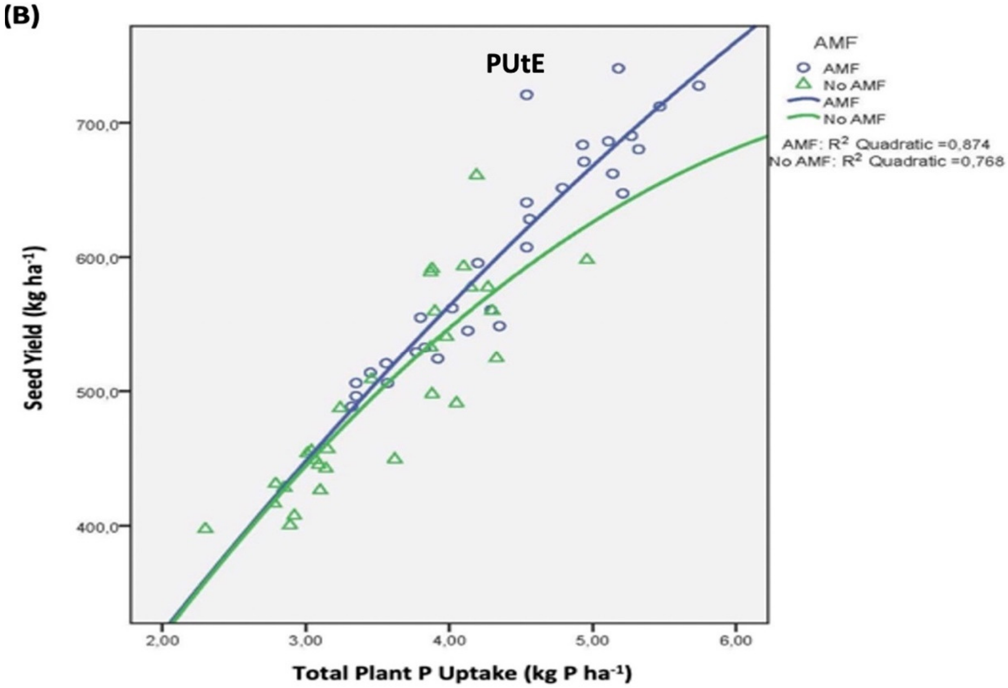


Figure 2. Effect of AMF inoculation on (A) nitrogen utilization efficiency (NUtE) and (B) phosphorus utilization efficiency (PUtE) of faba bean crop

Phosphorus Utilization Efficiency (PUtE)

Similar to NUtE, phosphorus utilization efficiency (PUtE; kg kg $^{-1}$), was expressed as its ratio (production at P_x - production at P_0) to (P absorbed at P_x - P absorbed at P_0), and is presented diagrammatically from the slopes of the curves of Figure 2B. The PUtE increased almost at double scores in mycorrhiza inoculation from 76.92 kg kg^{-1} to 142.85 kg kg^{-1} for inorganic fertilization, while remaining stable at approximately 112.35 kg kg^{-1} for organic fertilization, demonstrating the importance of slow-release fertilizers in their ability to utilize phosphorus. Otherwise (inorganic fertilizers) the applied phosphorus is rapidly converted to organic form and complexes that cannot be utilized by plants with a negative effect on PUtE. For both mycorrhizal and non-mycorrhizal embolization plants, a linear relationship was observed between absorbed phosphorus and seed production. This linearity is associated with low phosphorus concentrations in plant tissues under conditions of deprivation, suggesting that the maximum potential production was not achieved (Lopez-Bellido, 2001), while setting the minimum requirements for phosphorus and the maximum PUtE, for a specific seed production (Kahiluoto *et al*, 2001). In Figure 2B, we can observe the also high correlation that exists between the phosphorus absorbed by the total plant biomass of the crop and the final production of seeds by the crop plants. In this diagram, the superiority of the plants of the plots inoculated with mycorrhizae in terms of seed production is evident, as well as the exponential form of this relation which suggests that higher phosphorus absorption by faba bean plants implies higher production in seed, to the point beyond which the supply of phosphorus does not contribute to the production of extra seed, but to the luxurious growth of the cultivated plants.

AMF colonization and Weighted Mycorrhizal Dependency (WMD)

According to the results of the statistical analysis and as shown in the diagrams of the following figures, the effect of inoculation with AMF was statistically significant ($p < 0.05$) in all measured values of mycorrhizal colonization, both in the plots where inorganic fertilization was applied, as in the plots where organic fertilization was applied, while there were no mycorrhizal interactions × type of fertilization ($p > 0.05$).

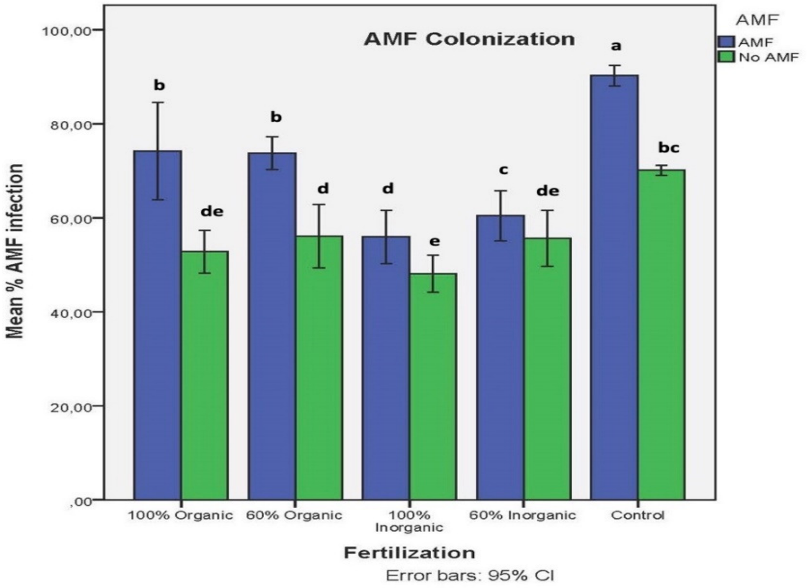


Figure 3. Effect of AMF inoculation and fertilization on AMF colonization of faba bean crop

Blue shaded bars represent the AMF infection (%) of inoculated plants and green bars represent the AMF infection of non-inoculated plants. Error bars represent ± S.E. Different letters above bars represent significant differences at $p < 0.05$ according to Tukey's method.

As shown in Figure 3, the mycorrhizal settlement was larger in the plots where AMF inoculation was applied, confirming on the one hand the success of the vaccination and on the other hand the positive effect that soil enrichment has on the population in terms of AMF number. Also, the high degree of mycorrhizal colonization of the plots in which no inoculation was applied can be observed, a fact that indicates the existence of a satisfactory, healthy, native mycorrhizal population in the soil of the experimental field, as a result of the good agricultural practices followed for many years, such as the limited use of agrochemicals and fertilizers, the application of crop rotation and the cultivation of legumes. Finally, a high negative correlation can be observed between the percentages of settlement and the amount of fertilizer inputs, especially for inorganic inputs. For organic fertilization, the negative correlation is smaller, possibly due to the slow release of nutrients into organic fertilizers, which promote the dependence of nutrient uptake on the cooperation of the fungus to a greater extent, and vice versa.

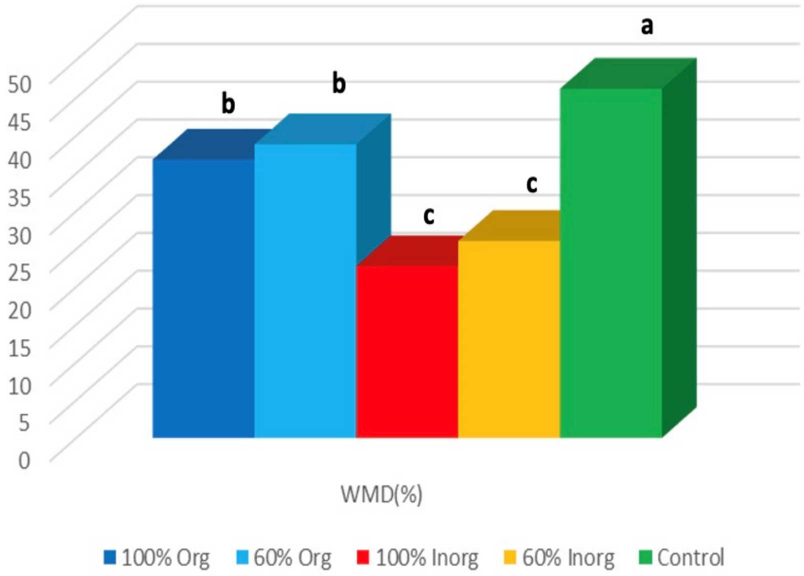


Figure 4. Effect of fertilization type and level on weighted mycorrhizal dependency (WMD) (%) of faba bean crop

Different letters above bars represent significant differences at $p < 0.05$ according to Tukey's method.

The negative correlation of mycorrhizal colonization and fertilization levels is also reflected in the degree of mycorrhizal dependence of the faba bean cultivation as shown in Figure 4, which shows the weighted mycorrhizal dependence (WMD) of the crop, meaning the real differences that arise when the inherent presence of mycorrhizae in the soil is taken into account. There is a decrease in mycorrhizal dependence for a corresponding increase in the level of fertilization, mainly in inorganic form, and a much smaller corresponding decrease in mycorrhizal dependence for organic fertilization.

AMF can play a very important role in P nutrition of plants, increasing the total absorption and in some cases the efficiency of P use (Koide *et al.*, 2000), which can have a positive effect on plant growth and development, as well as in final production (Ibibijen *et al.*, 1996; Koide *et al.,* 2000), When AMF colonization is disrupted, P absorption, growth and development, and in some cases productivity, may be significantly reduced. However, there are examples where the plants failed to respond to settlement by native AMF e.g., Ryan et al. (2002), mainly due to high concentrations of P available in plants in the soil (Sorensen *et al*., 2005). Under such conditions, AMF root colonization is often suppressed (Al-Karaki and Clark, 1999; Kahiluoto *et al.*, 2001), whereas if there is a strong settlement at high soil P concentrations, result in plant growth inhibition

(Gavito and Varela, 1995; Kahiluoto *et al.*, 2001). Plants may not respond to native AMF (Ryan *et al.*, 2002), or vaccinated with other AMFs (Sainz *et al.*, 1998) even in soils with low P availability, despite any increase in settlement, for reasons which have not yet been identified.

Conclusions

According to the results of the present research work and their evaluation, it was revealed that AMF inoculation can positively affect growth and productivity of faba bean crop, in both organic and conventional cropping systems. Results demonstrated that plants of AMF inoculated plots exhibited greater plant height, leaf area index (LAI), leading to higher biomass, and consequently higher final seed yields, even at lower N, P inputs. Regarding the quality parameters, including nutrients (nitrogen and phosphorus) uptake and their utilization indices, similar results to those of the productivity results were found with the AMF inoculated plants presented the higher values. Finally, all the parameters of the root system, including AMF root colonization and weighted mycorrhizal dependency (WMD), were negatively affected by fertilization level, particularly in an inorganic form. As a conclusion, the current study confirmed that replacement of inorganic inputs by organic, as well as reduction of inputs, in combination with AMF inoculation are both able to produce excellent results and thus should be seriously considered as sustainable practices of faba bean crop cultivation under Mediterranean conditions.

Authors' Contributions

Conceptualization: D.B., E.T. and D.V.; Data curation: D.B., E.T., I.R., I.K., A.M. and D.V.; Formal analysis: D.B., E.T., I.R., I.K., A.M. and D.V; Investigation: D.B., E.T., I.R., I.K., A.M. and D.V; Methodology: D.B., E.T., I.R., I.K., A.M. and D.V; Project administration; Resources: D.B., E.T., I.R., I.K., A.M. and D.V; Supervision: D.B. and D.V; Validation: D.B., E.T., I.R., I.K., A.M. and D.V; Visualization: D.B. and D.V; Writing - original draft: D.B., E.T., I.R., I.K., A.M. and D.V ; Writing - review and editing: D.B., E.T., I.R., I.K., A.M. and D.V. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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