The growth and mycorrhization of young *Berberis Microphylla* G. Forst. plants are differently affected by organic and inorganic fertilizers, depending on the substrate

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Abstract

Information regarding the relationship between fertilization, mycorrhizas, and plant growth is scattered for non-conventional productive plant species. We evaluated the effect of different substrates and fertilization treatments on growth and colonization by arbuscular mycorrhizas of young *Berberis microphylla* plants, a native Patagonian shrub with edible fruits. We conducted a greenhouse experiment based on two factors: substrate (conventional or native soil) and fertilization (no fertilization, organic fertilization, or inorganic fertilization). When plants were grown in conventional substrate, both fertilizers promoted growth, having the inorganic fertilizer a greater effect. The effect of both fertilizers was similar when plants were cultivated in native soil, and lesser than in conventional substrate. Plants grown in native soil were larger than those in conventional substrate when organic fertilizer or no fertilizer was applied, but this was reversed when inorganic fertilizer was applied. There was no mycorrhization on plants grown in conventional substrate. In native soil, mycorrhization was highest for non-fertilized plants (60.1%), followed by those with organic fertilization (40.4%), and lowest when inorganic fertilizer was applied (29.9%). The relative abundances of both vesicles and arbuscules showed the opposite tendency, having both their highest values in treatments with inorganic fertilizer. Mycorrhization was positively correlated with plant size, but only when fertilizers were applied. Based on our results, we hypothesized that fertilization reduce mycorrhization but select more beneficial mycorrhizal fungi. We concluded that organic fertilizers have a comparable effect to inorganic fertilizers in terms of promoting plant growth, accompanied by a lesser reduction of mycorrhization.

Keywords Plant production · Arbuscular mycorrhizas · Fertilizer · Cultivation substrate · Sustainable practices

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1 Introduction

In recent decades, there has been a growing recognition of the need to improve plant production, while simultaneously mitigating the negative environmental impacts associated with conventional agricultural practices (Vitousek et al. 2009; Gregory and George 2011). In the pursuit of a more sustainable and environmentally friendly production, it has been suggested that a change in soil fertility management is crucial (Verma et al. 2020). The standard practice of relying on the use of inorganic fertilizers to improve soil fertility has shown significant short-term benefits, such as enhanced crop yields through increased nutrient availability. However, this practice has also been associated with unintended consequences, including soil and water pollution, greenhouse gas emissions, and biodiversity loss (Gundersen et al. 2006; Vitousek et al. 2009; Sharma 2017). Organic fertilizers

(such as manure and compost) represent a potential alternative to inorganic fertilizers (Gregory and George 2011). Organic fertilizers usually require to be applied in higher doses to provide the same amount of nutrients as inorganic ones, and have a slower nutrient release rate. However, they can provide several benefits, such as improving soil texture, and water retention capacity, and organic matter content, while also promoting soil microorganisms communities beneficial to plants (Masood et al. 2014; Sharma 2017; Liu et al. 2021).

Soil microorganisms play a crucial role in sustainable plant production. For example, there are many soil fungi that form mutualistic symbiotic associations with plant roots. These relationships are known as mycorrhizas and they usually enhance plant's capacity to absorb nutrients and water, as well as to tolerate various types of stress, in exchange for photosynthates required by the fungi as carbon source (Smith and Read 2008; Thirkell et al. 2017). However, under certain circumstances, mycorrhizas might not provide any benefits, or even occasionally present a parasitic behavior (Johnson et al. 1997; Purin and Rillig 2008; Hoeksema et al. 2010; Rúa et al. 2016). The negative impact of the application of inorganic fertilizer on these symbiotic associations has been proven multiple times (Treseder 2004; Han et al. 2020; Trejo et al. 2020; Alsharmani et al. 2023). Increases in nutrients such as N and P not only decrease mycorrhizal colonization and diversity (Treseder 2004; Ma et al. 2021), but also affect negatively the benefits mycorrhizal fungi provide, such as the mitigation of soil N and P loss (Qiu et al. 2022) as well as soil carbon storage and structure (Yang et al. 2018). In contrast, organic fertilizers have shown lesser effect on them, and even a positive impact on mycorrhizal colonization, propagules and diversity (Gryndler et al. 2006; Qin et al. 2015; Jiang et al. 2021).

The relationship between plant production, the application of different types of fertilizers and mycorrhizal dynamics has been previously explored for different crops (Qin et al. 2015; Bilalis et al. 2015). However, this type of information in relation to native species of emerging economic and ecological importance is limited, despite being essential for the implementation of sustainable production practices. The aim of this study was to evaluate in a greenhouse experiment the effect of different fertilization treatments (application of organic fertilizer, inorganic fertilizer, or no fertilization) on *Berberis microphylla* G. Forst. (Berberidaceae) young plants growth and association with arbuscular mycorrhizas (AM) when cultivated in different substrates (conventional cultivation substrate or native soil). We used this species as a model since it is a native shrub of great ecological importance in Patagonian woodlands that produces high quality nutritional fruits and there is a strong and emerging interest in the market for their national and international commercialization (Bustamante et al. 2020; Cofré Guerrero and Jofre Vásquez 2021). We hypothesized that: (1) The application of fertilizers negatively affects the colonization by AM because these associations are mainly involved in plant nutrition, needs that are mostly met by fertilizers applications. This effect is particularly strong where nutrient availability is higher (inorganic fertilizer), and the availability of natural AM inoculum is lower (conventional substrate). (2) The application of inorganic fertilizer, by providing a more readily available nutrients input to plants, has a greater effect on plant growth and mycorrhization than organic fertilizer. (3) Plants in treatments with higher percentages of colonization by AM are sturdier, as these symbiotic associations promote not only plant growth but also their sturdiness.

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2 Materials and methods

2.1 Experimental design and soil collection site

We grew *B. microphylla* plants in 3-liter pots over a complete cultivation cycle from November 2021 (spring) to June 2022 (winter) at the experimental greenhouse of the Universidad Nacional de Río Negro, in San Carlos de Bariloche (Argentina). The seedlings were germinated from fruits harvested during 2021–2022 summer in San Carlos de Bariloche (Argentina). At the end of November 2021, when the seedlings were three months old, we transplanted them into their corresponding pots. At that point, seedlings shoot length was 3.5 cm on average, and although all of them already had true leaves, some of them still retained their cotyledons.

Our study tested two factors: (1) the type of substrate, with two levels, based on either native shrubland soil (hereinafter referred to as "native soil") or based on commercial products (referred to as "conventional substrate"); and (2) the type of fertilizer applied, with three levels including no fertilizer, organic fertilizer, and inorganic fertilizer. A completely randomized factorial design was implemented, combining the levels of all factors (six treatments in total), and fifteen replicates ($n = 15$) for each of them, resulting in a total of 90 pots.

The native soil was collected from "Conciencia" property (a project that focuses on biodiversity conservation, biodiversity education, and provides space for the development of scientific projects in collaboration with universities, public agencies, and NGOs) in El Foyel, Río Negro (Argentina). Soils are young Andisols classified as Hapludands (Soil Survey Staff 2022), which usually retain P and are limited by N. Mycorrhizal plants growing in this type of soil are mostly limited by N (Diehl et al. 2008). The climate

is temperate-cold, with an average temperature of approximately 9 °C, and an annual rainfall of 920 mm (Reque et al. 2007). The site is a native mixed shrubland, dominated by species such as *Nothofagus antarctica* (G. Forst.), *Lomatia hirsuta* (Lam.) Diels subsp. obliqua (Ruiz & Pav.) R.T. Penn, *B. microphylla*, and *Fabiana imbricata* (Ruiz & Pav.). Numerous studies have been conducted in the property before, including an analysis of mycorrhization of adult *B. microphylla* plants where it was observed that they are naturally colonized by AM (approximately 20% of root length colonized; Fioroni et al. submitted). This is the reason why we decided to use this soil, since we already knew that there was available AM inoculum that successfully colonized *B. microphylla* plants. Furthermore, during plant production in local nurseries, it is common practice to use soil from the surrounding area, which naturally contains AM inoculum.

The "native soil" substrate consisted of three parts of soil obtained from a native mixed shrubland and one part of perlite, while the "conventional substrate" was composed of four parts of *Sphagnum* sp. peat, two parts of perlite, and one part of vermiculite. Chemical characteristics of both substrates are summarized in Table 1. The pots were completely filled with each of the substrates and, two weeks after seedlings´ transplantation, when plants were already well established, we applied the fertilization treatment. For organic fertilization we added Guanito from Italpollina (containing NPK, 4 N – 4 P_2O_5 – 4 K_2) at a dose of 5 g per plant, and for inorganic fertilization we used Basacote Plus 6 M 16-8-12(+2+TE) at a dose of 1.25 g per plant. The doses have been calculated so that both fertilizers deliver the same amount of nitrogen (0.2 g per plant). Fertilization was repeated in early February 2022. During the experiment, the plants were irrigated three times a day through aspersion for 8 min each time.

2.2 Morphological measurements of the cultivated plants

After the active growth period, the plants were transported to the laboratory, where we carefully removed them from their containers. We washed their roots with tap water to remove any adhered substrate without damaging the root system. We recorded the number of axes, collar diameter, and length of the longest axis for each plant (hereinafter referred to as "shoot length"). We considered length as related to the photosynthetic capacity and the competitive advantage against weeds, while the diameter is regarded as the best predictor

of growth and survival. We also calculated the shoot length: diameter ratio, which is considered an indicator of plants´ sturdiness and survival capacity (Haase 2008).

For biomass estimation, we separated the aerial fraction of each plant from the roots. Prior to drying the plant material, we preserved a small fraction of the root system (approximately 20% of the fresh fine roots) to analyze AM colonization, which was conserved in alcohol 70% until staining. We recorded the fresh weight of the complete root system and the weight of the remaining roots after separating a fraction for AM analysis. Next, we dried each fraction of the plant at 60 °C until constant weight, and measured the dry weight of each part separately (aerial and roots dry biomass). The roots dry fraction was used to estimate the dry weight of the complete root system based on the following formula (Rydlová and Püschel 2020):

$$\frac{(CR_F \times FR_D)}{FR_F} \approx CR_D$$

where, CR: weight of the complete root system, FR: fraction of the root system not used for AM analyses, F and D: fresh and dry samples, respectively. The measurement of these variables was considered important as aerial biomass is an indicator of photosynthetic capacity and growth potential, while radical biomass is an indicator of survival capacity. The shoot: root dry weight ratio was also calculated as an indicator of the quality of the plants (Haase 2008). Finally, we calculated the Dickson Quality Index (DQI), since it is a comprehensive quality index based on an integration of some of the quality indicators previously mentioned, and it's calculated as follows (Dickson et al. 1960; Lin et al. 2018):

$$DQI = \frac{Total\ plant\ dry\ weight}{(Shoot\ length:diameter + Shoot:root\ dry\ weight)}$$

2.3 Mycorrhizal colonization

To analyze root colonization by AM, the technique described by Phillips and Hayman (1970) was used for staining the fungal structures present inside the roots. The technique was modified for this study and involved the following steps: (a) Clarification: the roots were removed from the alcohol and rinsed with tap water, then immersed in a 10% w/v KOH solution and boiled for 30 min. After boiling, the KOH

Table 1 Chemical characteristics of the native soil and the conventional substrates used for the greenhouse experiment

	C_T	C_O	N_T	N_{NO3}	P_A	pH	Conductivity
Native soil	4.24	3.19	0.23	85.89	3.43	5.75	159.33
Conventional substrate	8.78	5.99	0.96	31.99	12.37	5.95	228

C_T Total organic carbon (%), C_O Oxidizable organic carbon (%), N_T Total nitrogen (%), N_{NO3} Nitrates (ppm), P_A Available phosphorus (ppm)

solution was removed, and the samples were washed three times with tap water. The samples were left in water for three days to ensure the thorough removal of excess KOH and cellular content before the next step; (b) Acidification: roots were immersed in a 1% v/v HCl solution for 4–5 min. After that time, the acid was discarded, without rinsing the roots; (c) Staining: the samples were submerged in a 0.05% Trypan-Blue solution (0.05% w/v Trypan-Blue, 31% v/v glycerol, 31% v/v lactic acid) until fully covered and then left for 10 min in boiling water. Then, the dye was removed and the roots were rinsed carefully under running water to remove any excess of dye.

To estimate the percentage of roots colonized by AM, eight portions of stained roots, approximately 3.5 cm long each, were randomly chosen and arranged parallel to each other on a slide. Two slides per sample were prepared, and were observed under an optical microscope (Olympus BX40). At least 150 fields were examined in each slide at 200X magnification (approximately 300 fields per sample). The percentage of AM root colonization was estimated by counting the number of fields in which structures corresponding to AM fungi were observed and comparing them with the total number of quantified fields (McGonigle et al. 1990). The structures corresponding to AM were discriminated into hyphae, arbuscules, and vesicles to evaluate the relative abundance of each of these structures. Therefore, the evaluated variables were: total AM colonization, relative abundance of vesicles and relative abundance of arbuscules. The structures observed were photographically documented

Table 2 Analysis of deviance to assess the impact of different substrates (conventional substrate and native soil) and fertilization treatments (no fertilization, organic fertilization and inorganic fertilization) on morphological measurements and mycorrhizal colonization of *Berberis microphylla* plants

	Fertilizer	Substrate	Fertilizer: Substrate
SL	24.83***	57.22***	18.85***
CD	95.18***	32.49***	50.01***
SL: CD	7.19**	23.57***	12.58***
NAx	45.99***	3.10°	16.11***
SDW	130.54***	0	73.12***
RDW	71.00***	25.69***	52.21***
SDW: RDW	21.23***	26.26***	19.46***
DQI	81.59***	1.08	14.89***
AM%	19.32***	-	-
A	8.31*	-	-
V	8.33*	-	-

SL Shoot length, *CD* Collar diameter, *SL: CD* Shoot length: diameter ratio, *NAx* Number of axes, *SDW* Shoot dry weight, *RDW* Root dry weight, *SDW: RDW* Shoot: root dry weight ratio, *DQI* Dickson Quality Index, *AM%* Arbuscular mycorrhizal colonization, *A* Arbuscule relative abundance, *V* Vesicle relative abundance

Values indicate F statistic for SL, SL: CD and SDW: RDW, and X^2 statistic for CD, NA, SDW, RDW, DQI, MC, A and V. Asterisks indicate the p-value as it follows: 0 '***' 0.001 '**' 0.01 '*' 0.05 '°' 0.1

as brightfield images using a Sony ExwaveHAD camera and the TCapture software for Windows (v. 4.3.0.605, Tucsen Photonics Co., Ltd. 2019).

2.4 Data analysis

To evaluate the effect of substrate type and fertilization, we performed linear models for shoot length, shoot length: diameter ratio, and shoot: root dry weight ratio; generalized linear models (GLM) for collar diameter, number of axes, shoot dry weight, root dry weight, and DQI; and generalized linear mixed-effects models (GLMM) for total mycorrhizal colonization, relative abundance of arbuscules and relative abundance of vesicles. Substrate type, fertilization, and their interaction were included as explanatory variables in the models for morphological variables, and only fertilization was included in mycorrhizal colonization models, since we found AM colonization only in one of the evaluated substrates. For mycorrhizal colonization variables we decided to utilize the cbind function (as follows: cbind(number of positive fields, total number of fields - number of positive fields), to ensure that the model adequately represent the nature of the data. Plant ID was included as a random effect for mycorrhizal colonization to account for the fact that the fields counted within the same plant are not independent of each other, as they are with respect to those from another plant. All GLMs were fitted using a Gamma distribution, and a Binomial distribution for GLMMs. We performed Tukey's post hoc pairwise comparisons. Finally, we carried out a correlation analysis between morphological variables and AM colonization, to provide insights into the relationship between plants' growth and their symbiosis. All statistical analyses were conducted in R software version 4.2.2 (R Core Team 2022). We used the *lme4* package version 1.1–31 (Bates et al. 2015) to estimate model parameters, the *DHARMa* package version 0.4.6 (Hartig 2020) to verify the models' assumptions, the *emmeans* package version 1.8.4-1 for post hoc comparisons (Lenth et al. 2018), and the *corrplot* package version 0.92 (Wei and Simko 2021) to carry out the correlation analysis.

3 Results

3.1 Morphological variables

We observed an interactive effect between substrate type and fertilization on all the evaluated morphological variables ($p < 0.001$ in all cases; Table 2; Fig. 1, illustrated and summarized in Supp. Fig. S1). Regarding the effect of the fertilizers for each cultivation substrate, we observed that when plants were cultivated in a conventional substrate, the application

Fig. 1 Morphometric measures of *Berberis microphylla* plants. **a** Stem length (SL), **b** Collar diameter (CD), **c** Stem length: diameter ratio (SL: CD), **d** Number of axes (NAx), **e** Shoot dry weight (SDW), **f** Root dry weight (RDW), **g** Shoot: root dry weight ratio (SDW: RDW), **h** Dickson Quality Index (DQI). F-: no fertilization, OF: organic fertilization, IF: inorganic fertilization. Means and standard error are represented by the bars
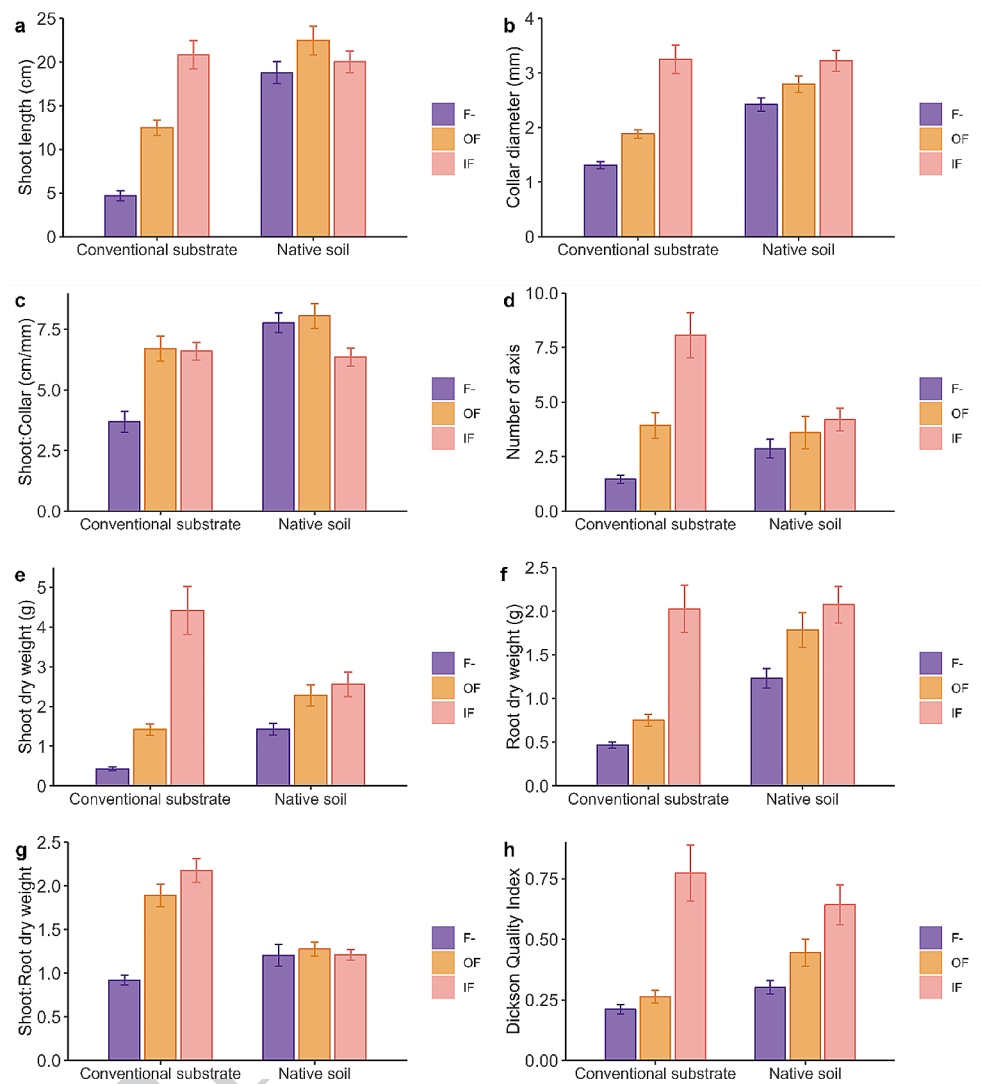
of fertilizers improved plant growth, leading to higher values in all the morphological variables evaluated (shoot length −256.5% higher-, collar diameter −96.4% higher-, number of axes −308.9% higher-, as well as shoot and root dry weight −583.3% and 198.1% higher respectively-; Fig. 1). Besides, the effect of the inorganic fertilizer was more pronounced than that of the organic fertilizer (67.1% higher for shoot length, 72.7% higher for collar diameter, 105.2% higher for number of axes, and 212.1% and 169.9% higher for shoot and root dry weight, respectively; $p < 0.01$ in all cases; Fig. 1; Table 3). Additionally, both fertilizers resulted in equally higher shoot length: diameter (80.3%) and shoot: root dry weight (121.2%) ratios with respect to the no fertilized treatment ($p < 0.001$ in all cases). For plants grown in conventional substrate, the addition of inorganic fertilizer increased the DQI by a 225.9% in comparison with the remaining two fertilization treatments ($p < 0.001$ in both cases), while plants treated with organic fertilizer did not differ significantly with non-fertilized plants ($p = 0.388$). On

the other hand, when grown in native soil both organic and inorganic fertilizers enhanced only shoot (69.5% higher) and root (56.8% higher) dry weight of *B. microphylla* young plants ($p < 0.05$ in all cases), while only the inorganic fertilizer significantly increased collar diameter (33.3% higher, Fig. 1b). No significant differences were observed between the plants treated with one fertilizer in comparison with the other. When the plants were cultivated in the native soil, fertilization had no significant impact on shoot length, number of axes, and the shoot: root dry weight ratio, but the addition of the inorganic fertilizer decreased the stem length: diameter ratio by a 18.2% ($p = 0.019$). Finally, the DQI was higher for plants when inorganic fertilizer was added in comparison with non-fertilized plants by a 113.3% ($p < 0.001$; Fig. 1h), having intermediate values the treatment with organic fertilizer.

In terms of comparisons between substrates, we observed that when no fertilizer or organic fertilizer was applied, plants cultivated in the native soil exhibited significantly

Table 3 Effect of fertilization treatments obtained from an interaction analysis (Fertilization treatment × Substrate type) on morphological measurements and mycorrhizal colonization of *Berberis microphylla* plants

	Conventional substrate			Native soil		
	F- vs. OF	F- vs. IF	OF vs. IF	F- vs. OF	F- vs. IF	OF vs. IF
SL	-4.39***	-9.10***	-4.71***	-2.08	-0.7	1.39
CD	4.43***	9.79***	6.43***	1.79	3.52**	1.77
SL: CD	-4.89***	-4.72***	0.18	-0.47	2.30°	2.76*
NAx	3.91***	5.36***	3.07**	1.06	1.75	0.72
SDW	5.88***	7.91***	5.70***	2.79*	3.40**	0.73
RDW	3.14**	7.32***	5.76***	2.50*	3.42**	1.03
SDW: RDW	-6.63***	-8.59***	-1.96	-0.48	-0.04	0.45
DQI	1.32	6.03***	5.36***	2.31°	4.13***	2.16°
AM%	-	-	-	2.18°	4.4***	2.23°
A	-	-	-	-1.61	-2.88*	-1.31
V	-	-	-	-1.26	-2.88*	-1.64

SL Shoot length, *CD* Collar diameter, *SL: CD* Shoot length: diameter ratio, *NAx* Number of axes, *SDW* Shoot dry weight, *RDW* Root dry weight, *SDW: RDW* Shoot: root dry weight ratio, *DQI* Dickson Quality Index, *AM%* Mycorrhizal colonization, *A* Arbuscule relative abundance, *V* Vesicle relative abundance, *F-* Non-fertilized, *OF* Organic fertilizer, *IF* Inorganic fertilizer

Values indicate t ratio for SL, SL: CD, SDW: RDW and DQI, and z ratio for CD, NA, SDW, RDW, AM%, A and V. Asterisks indicate the p-value as it follows: 0 '***' 0.001 '**' 0.01 '*' 0.05 '°' 0.1

Table 4 Effect of different substrates obtained from an interaction analysis (Fertilization treatment × Substrate type) on morphological measurements and mycorrhizal colonization of *Berberis microphylla* plants

	F-	OF	IF
	Nat vs. Conv	Nat vs. Conv	Nat vs. Conv
SL	7.94***	5.63***	0.64
CD	-7.15***	-4.78***	0.1
SL: CD	6.62***	2.19*	-0.4
NA	-2.89**	0.42	2.83**
SDW	-5.90***	-2.82**	3.21**
RDW	-5.67***	-5.22***	-0.16
SDW: RDW	1.94°	-4.21***	-6.61***
DQI	-2.1*	-3.03**	1.11

SL Shoot length, *CD* Collar diameter, *SL: CD* Shoot length: diameter ratio, *NAx* Number of axes, *SDW* Shoot dry weight, *RDW* Root dry weight, *SDW: RDW* Shoot: root dry weight ratio, *DQI* Dickson Quality Index. *F-* Non-fertilized, *OF* Organic fertilizer, *IF* Inorganic fertilizer, *Nat* Native soil, *Conv* Conventional substrate

Values indicate t ratio for SL, SL: CD, SDW: RDW and DQI, and z ratio for CD, NA, SDW and RDW. Asterisks indicate the p-value as it follows: 0 '***' 0.001 '**' 0.01 '*' 0.05 '°' 0.1

greater shoot length (302.1% or 80.2% higher), collar diameter (85.3% or 48.6% higher), and shoot (233.4% or 60.5% higher) and root (163.8% or 137.9% higher) dry weight compared to those grown in a conventional substrate ($p < 0.001$ in all cases; Table 4). It is worth noting that, when compared with the conventional substrate, the application of inorganic fertilizer had a negative impact on the number of axes (47.9% less) and shoot dry weight (42.2% lower) when plants were cultivated in the native soil ($p < 0.01$ in all cases). The stem collar: diameter ratio was greater in plants grown in native soil when no fertilizer (110.7% higher) or organic fertilizer (20.2% higher) was applied, whereas the

shoot: root dry weight ratio was lower in native soil by a 38.9% when either fertilizer was used ($p < 0.01$ in all cases). Finally, the DQI was higher for plants in native soil than in conventional substrate by a 42.7% when no fertilizer and by a 69.4% when organic fertilizer was added ($p = 0.038$ and $p = 0.003$, respectively), but there were no differences between these substrates when inorganic fertilizer was added ($p = 0.27$).

3.2 Mycorrhizal colonization

We did not find any evidence of mycorrhizal colonization in the roots of plants grown in conventional substrate, while plants grown in native soil successfully established associations with AM fungi in all the treatments (Fig. 2). However, we observed a detrimental effect of inorganic fertilizer on mycorrhizal colonization, compared to non-fertilized plants ($p < 0.001$, Table 3; Fig. 3a). Plants treated with organic fertilizer exhibited higher colonization than those treated with inorganic fertilizers by a 48.3%, but lower than non-fertilized plants by a 26.2%, although the differences were only marginal ($p = 0.075$ and 0.066, respectively; Table 3; Fig. 3a). Interestingly, we observed the opposite tendency for both the relative abundance of arbuscules and the relative abundance of vesicles. That is, we found a lower abundance of both these structures in non-fertilized plants than in those treated with inorganic fertilizers (45.4% lower for vesicles and 59.3% lower for arbuscules; $p = 0.011$ in both cases; Table 3; Fig. 3b), while plants treated with organic fertilizer showed intermediate values (Table 3; Fig. 3b).

Fig. 2 Mycorrhizal colonization in *Berberis micorphylla* roots. A: Arbuscule, H: Intracellular hyphae, V: Vesicle
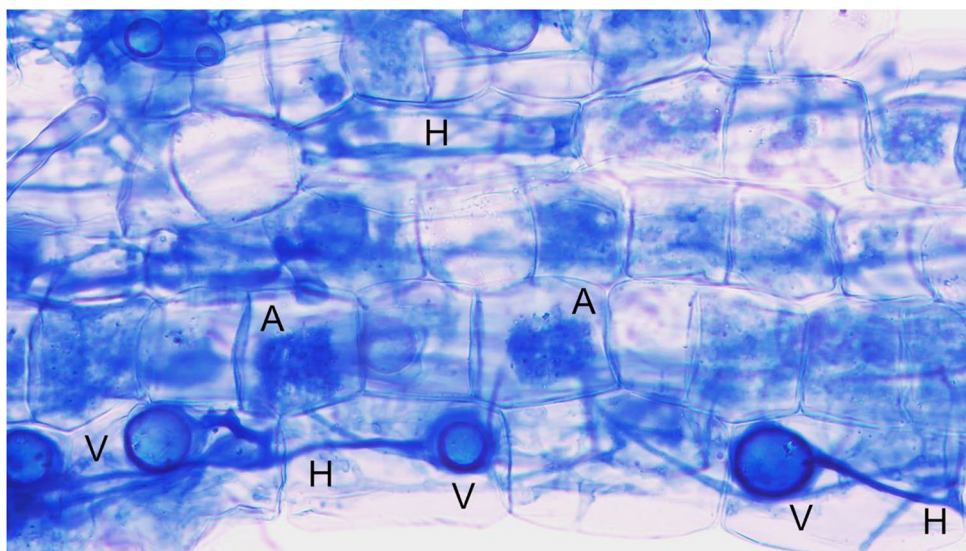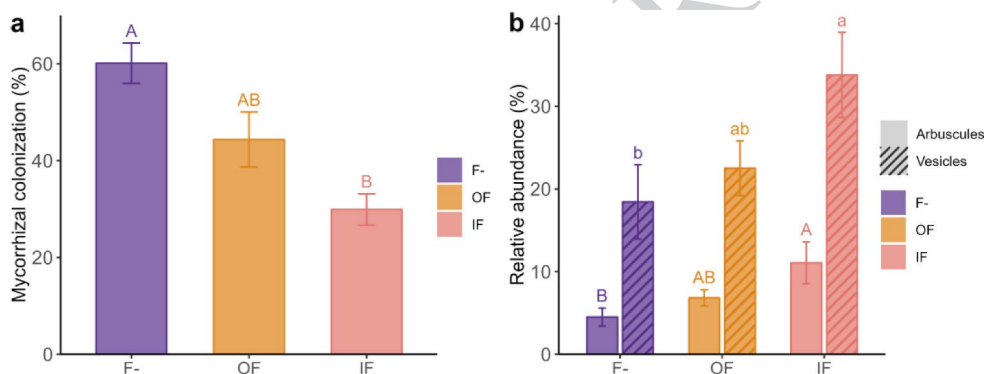

Fig. 3 Mycorrhization of *Berberis microphylla* plants. **a** Total mycorrhizal colonization. **b** Relative abundance of vesicles and arbuscules. F- indicates no fertilization, OF organic fertilization, and IF inorganic fertilization. Means and standard error are represented by the bars. Different letters indicate significant differences

3.3 Correlation between morphological characteristics and mycorrhization

In plants colonized by AM fungi, we did not find any correlation between morphological characteristics and mycorrhization when no fertilizer was added to the pots, but several correlations were found when fertilizers were applied (Fig. 4). When plants were treated with organic fertilizer, total mycorrhizal colonization and the relative abundance of vesicles showed a positive correlation with collar diameter, number of axes, and root and shoot dry weight. Additionally, the relative abundance of vesicles showed a positive correlation with shoot length (Fig. 4). The relative arbuscule abundance was negatively correlated with shoot length and the shoot length: diameter ratio under this fertilization treatment (Fig. 4).

Similarly, when plants were treated with inorganic fertilizer our results showed a positive correlation between total mycorrhization and collar diameter, number of axes, root and shoot dry weight, and shoot: root dry weight ratio. However, and in contrast to the tendencies observed for the organic fertilizer, in this case we did not find any correlation

between the relative abundance of arbuscules and morphometric measurements, and the relative abundance of vesicles was only positively correlated with the number of axes (Fig. 4).

For both fertilizers, a positive and significant correlation between total mycorrhization and DQI was registered (Fig. 4). In the case of the organic fertilizer, this was also true for the relative abundance of vesicles (Fig. 4).

4 Discussion

4.1 Substrate and fertilization type effect on morphological measurements

In general, plants that received either organic or inorganic fertilizers exhibited increased growth, a well-documented phenomenon supported by numerous previous studies, both in controlled greenhouse experiments and natural field conditions (Obsa 2017; Ji et al. 2017; Moe et al. 2019; Bergstrand 2022). The enhanced growth can be attributed to the nutrients supplied by fertilizers, which are essential for

Fig. 4 Correlogram of *Berberis microphylla* morphological measures and mycorrhization (overall colonization and relative abundance of vesicles and arbuscules). Non-significant correlations are denoted with an "X", numbers in white indicate correlation coefficient. Results are separated by the fertilization treatment
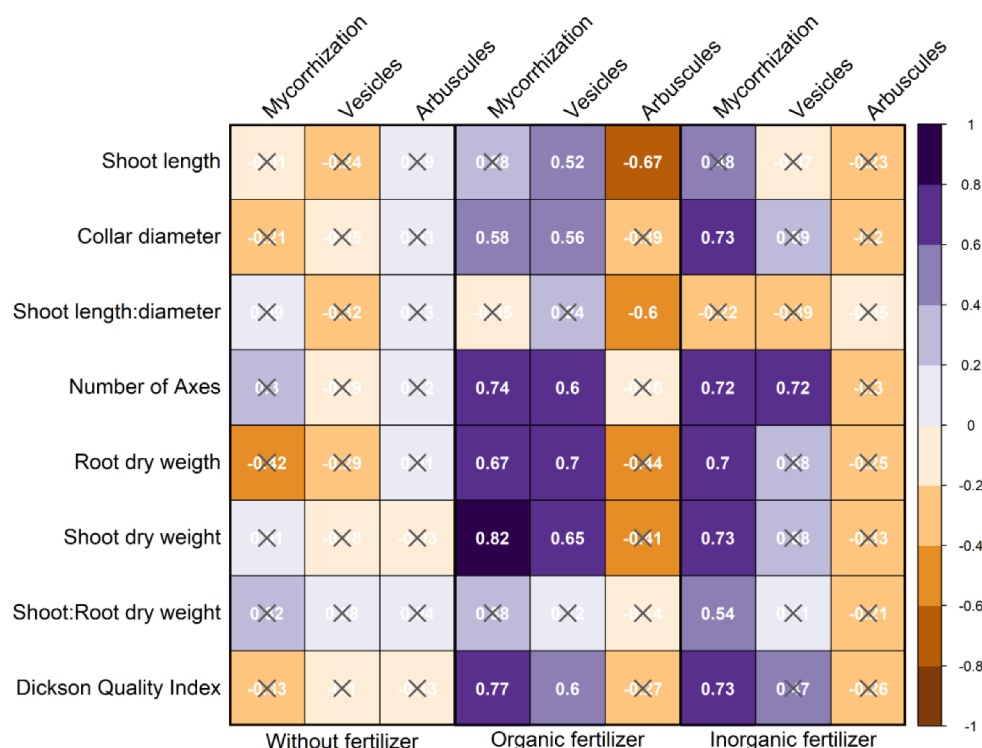
optimal plant development (Havlin et al. 2017; Ahmadi et al. 2020).

However, this fertilization effect diminished when we cultivated plants in native soil. In this substrate, differences in plant growth between the fertilization treatments were smaller, and in some cases, negligible. For example, the increase in aerial biomass for fertilized plants was 584.3% (average effect of organic and inorganic fertilizers) in the conventional substrate but only 69.5% in native soil, and we found no differences between organic and inorganic fertilizers in plants grown in native soil. There are several factors that might contribute to this differential effect of the fertilizers, depending on the cultivation substrate. On the one hand, non-fertilized plants grown in conventional substrate were already smaller than those grown in native soil, possibly due to the lower nutrient availability of the substrate, as forest soils generally have a higher nutrient content than non-fertilized inert substrates (Hussain et al. 2014). Even though conventional substrate had higher percentages of total nitrogen, since is mostly vegetal organic matter, the nitrogen available for plants (nitrates, among other forms of nitrogen) were higher for native soil. On the other hand, conventional growth substrates are optimal for retaining nutrients in a manner that makes them readily available for plant uptake (Wilkinson et al. 2014). This can be attributed to the inherent difficulty that plants encounter in acquiring nutrients from natural soils, as contrary to conventional substrates. For instance, phosphorus availability is severely limited in Andisols by the extremely high phosphorus retention

capacity of the andic materials (Brady and Weil 2008). Consequently, and depending on its proportion of sand and clay, the use of soil can lead to nutrient immobilization or the outright leaching of nutrients through irrigation (Wilkinson et al. 2014).

While analyzing these results as a whole, it can be observed that the disparities in plant size between fertilized and unfertilized plants are more pronounced in conventional substrate, with unfertilized plants being considerably smaller and fertilized ones notably larger. In contrast, in native soil, the differences are less dramatic, with unfertilized plants being moderately smaller (but still larger than their counterparts in conventional substrate) and fertilized plants reaching a larger size. For instance, when we added inorganic fertilizer, plants grown in conventional substrate had more axes in comparison with native soil, which translated to a greater shoot dry weight. This highlights the significant impact of substrate type on the fertilization effect, with conventional substrate accentuating differences and native soil moderating them.

In addition, we found that inorganic fertilizer resulted in greater plant growth compared to organic fertilizer when growing plants in conventional substrate. The greater effect of inorganic fertilizer may be due to its faster release and more readily attainable acquisition of its nutrients input by plants in the short term, when compared to organic fertilizer (Sharma 2017; Niedziński et al. 2021). However, we did not observe differences between both types of fertilizers in plants grown in native soil. It is possible that the

physical and chemical characteristics of native soil previously mentioned made it more difficult for plants to access the nutrients provided by inorganic fertilizers (Wilkinson et al. 2014). Instead, greater colonization by mycorrhizae (as seen in plants treated with organic fertilizer in comparison with those treated with inorganic fertilizer) could have facilitated the absorption of nutrients supplied by organic fertilizer (Hodge 2017). However, it is noteworthy that the higher nutritional quality and greater availability of mycorrhizal inoculum in the native soil than in conventional substrate, makes it difficult to discern the effect of each factor.

4.2 Effect of substrate and fertilization type on mycorrhization

We did not find any evidence of mycorrhizal colonization in plants grown in conventional substrate. This may be attributed to the absence of inoculum in this substrate, in contrast to the natural availability of spores and hyphae in the native soil, where plants successfully formed AM. In native soil, we found strong effects of fertilization on mycorrhization. When no fertilizer was applied, the mycorrhizal colonization in plants grown in native soil was found to be 60.1%. However, when we applied inorganic fertilizer, the colonization percentages were significantly lower (29.9%), whereas the application of organic fertilizer resulted in an intermediate level of mycorrhization (44.4%). These findings are consistent with similar studies conducted in other systems (El Kinany et al. 2019; Ikoyi et al. 2020; Liu et al. 2020; Yazici et al. 2021). It is known that plants establish mycorrhizal associations to obtain nutrients, among other reasons. The application of inorganic fertilizer, which provides a greater amount of available nutrients, could reduce the need for plants to associate with mycorrhizal fungi (Nagy et al. 2009; Balzergue et al. 2011; Amir et al. 2021). This could allow plants to allocate less carbon to maintain mycorrhizal associations and use it instead for vegetative growth (Konvalinková et al. 2017; Andrino et al. 2021; Salmeron-Santiago et al. 2021). On the other hand, nutrients from organic fertilizers are less accessible to plants, and so mycorrhizal fungi could still play an important role in nutrient uptake **AQ3** (Hodge 2017).

Surprisingly, even though the overall percentage of mycorrhizal colonization decreased with fertilizers application, we observed the opposite tendency for both the relative abundance of arbuscules and the relative abundance of vesicles. We found that plants that were not fertilized had lower abundance of both these structures, while higher abundances were found when an inorganic fertilizer was used. Arbuscules are responsible for the exchange of water, nutrients, and sugars between the plant and the mycorrhizal fungus (Smith and Read 2008). When the abundance of

arbuscules is high, it generally means that there is a greater benefit to the plant (Gange and Ayres 1999). One possible explanation for our observations is that when fertilizers are applied to the soil, the plant's need to associate with mycorrhizal fungi decreases. Thus, plants growing in nutrient-rich soils are able to selectively associate with mycorrhizal fungi that offer higher benefits, unlike plants growing in environments where mycorrhizal associations are more crucial (i.e. nutrient-poor soils), which cannot afford to be as selective in their fungal partnerships. These results are surprising considering the vast amount of studies reporting the reduction in benefits for plants provided by mycorrhizal fungi after fertilization (Hoeksema et al. 2010) and, furthermore, that mycorrhizal fungi could turn parasitic when fertilizers are applied (Johnson 1993; Peng et al. 2023) These disparities could indicate that the role of fertilizer as "filter agent" is dependent on the context, including plant and fungal species, as shown by Li et al. (2016) meta-analysis. In addition, it has been suggested that plants could discriminate mycorrhizal fungi in terms of the benefits they provide, and allocate more carbon to their preferred fungal partners (Wang et al. 2017). This could explain the observed increase in the abundance of vesicles, which are considered to be storage structures of the fungi, and tend to be more abundant when plants allocate more carbon to the mycorrhiza (Smith and Read 2008). **AQ4**

4.3 Correlation between mycorrhization and morphological measurements

When analyzing the relationship between the levels of mycorrhization and plant growth responses, we found that mycorrhization had a positive correlation with morphological variables only in the fertilized treatments. These results were mainly observed in terms of total mycorrhization percentages and relative abundance of vesicles. The relative abundance of arbuscules had a negative correlation with shoot length and the shoot length: diameter ratio. Although this behavior could suggest that a higher abundance of arbuscules is associated with less plant growth, we hypothesize that this is not the case since no significant correlations were found with other morphological variables. On the contrary, we believe that this correlation could indicate greater plant sturdiness when there is a higher relative abundance of arbuscules (a decrease in shoot length but not in collar diameter translates to a lower shoot length: diameter ratio), as reported in several studies (Urgiles et al. 2014; Corsini et al. 2022; Tapwal et al. 2022). The correlations found in this study could also support the aforementioned selective symbiosis hypothesis, where the use of fertilizers could lead to the selection of more beneficial mycorrhizal fungi, as we only observed these tendencies in the fertilized treatments.

It is noteworthy to mention that this hypothesis remains untested and it should be addressed in future research.

4.4 Future perspectives

Our results indicate that growing plants on shrubland soil supplied with organic fertilizer could result in high quality plants, possibly with a greater capacity for survival upon transplanting for establishment in field production systems (Haase 2008). Even though plants growing on conventional substrate had a greater shoot dry weight (as a consequence of a higher number of axes), it is important to take into consideration that only plants grown in native soil presented associations with AM. Whether the benefits of having young plants with a higher number of axes (which normally are pruned to enhance fructification; Albert et al. 2010) surpasses or not those of AM colonization related to an increase in plants survival capacity during transplant, yield and fruit quality, is yet to be studied. Additionally, it is noteworthy that when we calculated the cost of the production of young plants of *B. microphylla* in our study, we found that cultivation with native soil and organic fertilization was 18.5% cheaper than the production with conventional substrate and inorganic fertilizer (Supp. Table S1). However, further research is necessary to evaluate field performance. It is also necessary to consider that the discussions in this study do not aim to promote the extraction of soils from native forests for massive greenhouse plant cultivation, since it could significantly harm natural systems. This approach could deplete the forest's soil resources, which would disrupt the delicate balance of these ecosystems. Nevertheless, it is important to note that when plants are produced using soil as part of the substrate, the application of organic fertilizers allows for a similar growth than inorganic fertilizers, while not severely impacting the association of plants with mycorrhizal fungi.

5 Conclusion

This study was the first one to analyze the role of mycorrhization on a Patagonian native fruit plant species growth, shedding light to the production of not only this but also other native and economic relevant fruiting shrubs. Our results revealed that the plants grown in conventional substrate and treated with inorganic fertilizer had a significant growth, but with a low colonization by mutualistic fungi. On the other hand, the plants grown in native soil and treated with organic fertilizer showed a similar growth, but higher plant quality and mycorrhization rates. These results provide support for our first two hypotheses. We also found that overall higher percentages of mycorrhization are correlated with plants sturdiness and quality, which supports our

third hypothesis. Additionally, we observed that fertilization may lead to a selective process of specific mycorrhizal fungi that provide more benefits for the plants, even though this is a hypothesis derived from our results and needs to be approached in future studies. While further research is necessary to provide cultivation recommendations that improve production and reduce environmental impacts, the results of this study demonstrate the potential for implementing alternatives that are more sustainable than conventional methods of production without compromising plant quality.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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