SCIENTIFIC NOTE

Intercropping wheat with ancestral non-mycorrhizal crops in a volcanic soil at early growth stage

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ABSTRACT

Intercropping is especially relevant for low-income farmers when crop production is developed in soils under the new scenario produced by climatic change, mainly water shortage and low availability of nutrients especially P. An example of this would happen in volcanic soils of Southern Chile with high P fixing capacity and where most cereals are cropped. The aim of this study was to compare the benefits obtained on soil biochemical properties and on wheat (*Triticum aestivum* L.) growth when sowed under monoculture or intercropped with non-mycorrhizal plants such as quinoa (*Chenopodium quinoa* Willd.), buckwheat (*Fagopyrum esculentum* Moench), canola (*Brassica napus* L.) and white lupin (*Lupinus albus* L.) Wheat plants parameters such as shoot growth and morphological root traits together with some soil biological and chemical characteristics were measured after 30 and 44 d of plant growing in pots under controlled conditions. Results showed nonsignificant differences on growth parameters (i.e., height and shoot/root biomass) and P acquisition between monocrop and intercropped wheat. Conversely, wheat root morphological traits namely total length, root area, and forks were greatly reduced (around 50%) regardless of plant species. Intercropping tended to increase P availability and significantly reduced rhizosphere soil pH, with the lowest levels observed for wheat-canola combination (from 7.5 to 5.6). Intercropping produced a slight reduction in mycorrhizal colonization but increased over 100% viable spores' number and exerted a variable effect on the microbial C-biomass, with greater values observed in wheat-canola combination (1.49 mg g^{-1}). These results encourage us to deepen the use of some new plant combinations in family farming carried out in volcanic soils.

Key words: Companion plants, P nutrition, quinoa, root traits, smallholders.

INTRODUCTION

At present, Chile leaders the bread consumption in Latin America, where wheat production satisfies only about 70% of the demand in recent years (ODEPA, 2018). Nonetheless, wheat is mainly produced in the Central-southern area of the country dominated by soils of volcanic origin with characteristics that make them an unusual soil group such as possessing variable surface charge, low pH, high P adsorption capacity, and high levels of soil organic matter highly humified (Borie et al., 2019). The high reactivity of Andisols explains the accumulation of humic materials, P retention, and the high activities of H^+ and Al^{3+} observed in some places (Seguel et al., 2013; Aguilera et al., 2018). Then, to overcome the global problem of decreasing the use of synthetic P fertilizers together to minimize environmental problems related to eutrophication of natural water pools it is urgent to apply novel agricultural practices bearing in mind a higher sustainability of the agroecosystems specially those employed by smallholders.

One of the most attractive approaches to enface the acute global problem of food scarcity could be to maximize the use of agricultural soils by enhancing of its productivity per area of usable land. An interesting way for attain such a goal is by using intercropping, an agricultural management practice involving two or more different crop species growing simultaneously in a field during a growing season (Mousavi and Scandari, 2011), producing an overlapping of their respective rhizospheres maximizing the efficiency in the use of growth resources like water, nutrients, and sunlight and minimizing the negative effects produced by weeds, diseases, and pests (Altieri et al., 2012). Additionally, some chemical, biological and physical soil properties are also remarkably affected by intercropping, giving net benefits to the following crops in a rotation (Liebman and Dyck, 1993).

In this regard, it is widely known that as a result of the highly heterogeneous soil nutrient bioavailability, both in space and time, plants have developed a vast array of root strategies (Morgan and Connolly, 2013) to cope with such limitations, being root morphological adaptations like increased root length and longer root hairs (Kumar et al., 2019) as well as physiological traits (protons and phosphatase root exudation) (Lambers et al., 2018) the most important ones. Another major mechanism for nutrient acquisition is root association with mycorrhizal fungi, forming a symbiosis that produces a lot of benefits to the plant host (Brundrett, 2009). In this context, such strategies could be advantageous to companion crop plants in the intercropping as well as to both partners taking into account that cereals and legumes are the most widely used crops (Matusso et al., 2013).

Considering that wheat (*Triticum aestivum* L.) is the most important cereal around the world and is most suitable for cultivating with other plants, it is crucial to deep in wheat-based intercropping with other grain crops not forming symbiosis with mycorrhizal fungus, like canola. Accordingly, there are many of less known crops with great potential to be used in small-scale farms, be in intercropping or in rotation like quinoa (*Chenopodium quinoa* Willd.) which is used by its exceptional adaptation to diverse stresses (Jakobsen, 2003); white lupin (*Lupinus albus* L.), which besides growing in soils subjected to environmental stresses possess a very high capacity to solubilize/hydrolyze phosphate in soils with scarce available P (Lambers et al., 2015). Buckwheat (*Fagopyrum esculentum* Moench) is another crop having outstanding nutritional properties (Martinez-Villaluenga et al., 2020) but with few studies for its use in intercropping designs despite its reported role as P scavenger (Hallama et al., 2019).

Consequently, taking into account that plant-plant interactions can occur at different spaces (mixed cropping and intercropping) and time (crop rotation), the present study reports the effects produced on some soil characteristics and wheat growth and its root morphological traits when this cereal is intercropped with some non-mycorrhizal Andean crops like quinoa, buckwheat, and white lupin in an Andisol with low available P. Canola (*Brassica napus* L.) also a non-mycorrhizal crop was included in this study because it is habitually used by local farmers as pre crops in wheat rotation.

MATERIALS AND METHODS

Experimental soil and crops

Pot trial was carried out in a greenhouse of Universidad Católica de Temuco in 2019, Temuco (38º39’ S, 72º27’ W), Central-Southern Chile. The soil used was an Andisol, air-dried, ground, and sieved to pass through a 5 mm mesh with the following main characteristics: pH-H_2O 5.7, soil organic matter 17.0% (Walkley-Black), available Olsen P 9.0 mg kg^{-1}, effective cation exchange capacity (ECEC) 12.87 cmol kg^{-1}. Wheat (*Triticum aestivum* L.) ‘Invento’, and quinoa (*Chenopodium quinoa* Willd.) ‘Regalona’, buckwheat (*Fagopyrum esculentum* Moench) ‘Mankan’, white lupin (*Lupinus albus* L.) ‘Alboroto’, and canola (*Brassica napus* L.) ‘Inminent’ as non-mycorrhizal companion crops were used in this experiment. All seeds were sterilized with sodium hypochlorite 1% for 10 min, thoroughly washed with distilled water, and germinated in Petri dishes in the dark for 48 h at 20 °C before transplanting.

Experimental design

The trial was conducted with six planting treatments in 1.5 L pots containing a mixture of soil:sand at 70:30 as substrate, fertilized with the equivalent to 50 kg N ha^{-1} (as $NaNO_3$), 30 kg P ha^{-1} (as TSP), 40 kg K ha^{-1} (as KCl). The design consisted of six wheat plants as monocropping and three wheat plants plus three other ones of white lupin, buckwheat, quinoa, or canola as companion crops, respectively in intercropping with a total of 40 pots in a randomized block design. Plants were grown under a glasshouse with appropriate light and temperature conditions and maintaining moisture at

70% field capacity with four replicates. Plants harvesting was made at 30 and 44 d after sowing (DAS) corresponding to Zadoks scale Z1.4 and Z1.2, Z2.2 for wheat, respectively. At harvesting, plants and their roots and shoots were carefully separated, thoroughly washed and dried with absorbent paper, and prepared for measurements of the different parameters. Soil was refrigerated at 4 °C for further analysis.

Plant parameters

Fresh wheat shoots and roots were weighed and its height and main root measured, oven-dried at 60 °C for 48 h, ground up in a mill, ashed at 550 °C, and analyzed for P by spectrophotometry using the molybdenum blue method (Kalra and Maynard, 1991) in an aliquot which was previously ashed and acid solubilized. Mobilized P was expressed as mg plant^{-1} using P concentration in shoots and its dried weight. Additionally, parameters related to root architecture such as total root length, root area, and root bifurcations of wheat plants were determined by means of WinRhizo software (Regent Instruments, Quebec, Canada).

Soil parameters

Soil pH was measured with a pH meter in a soil:water suspension (1:2.5). Available P was determined using Olsen method ($NaHCO_3$ 0.5 M at pH 8.5), filtered and quantified spectrophotometrically at 700 nm as above. Soil microorganism biomass is an important soil quality indicator involved in nutrient cycling. Therefore, soil microbial-C biomass was determined by killing and lysing soil microbes by fumigation with chloroform according to Vance et al. (1987), extracted with potassium sulfate, and microbial-C was determined utilizing an automatic total organic C analyzer (TOC-L, Shimadzu, Kyoto, Japan), using a k_C 0.40 according to Kaiser et al. (1992).

Mycorrhizal parameters

Total glomalin was measured according to Wright and Upadhyaya (1996) by quantification carried out spectrophotometrically at 595 nm. Root mycorrhizal colonization was determined using Giovannetti and Mosse (1980) procedure. Mycorrhizal spores were quantified by using the wet sieving and decanting method in a sucrose suspension sieved under 500 to 50 µm mesh size and placed in a Doncaster dish for its enumeration.

Statistical procedures

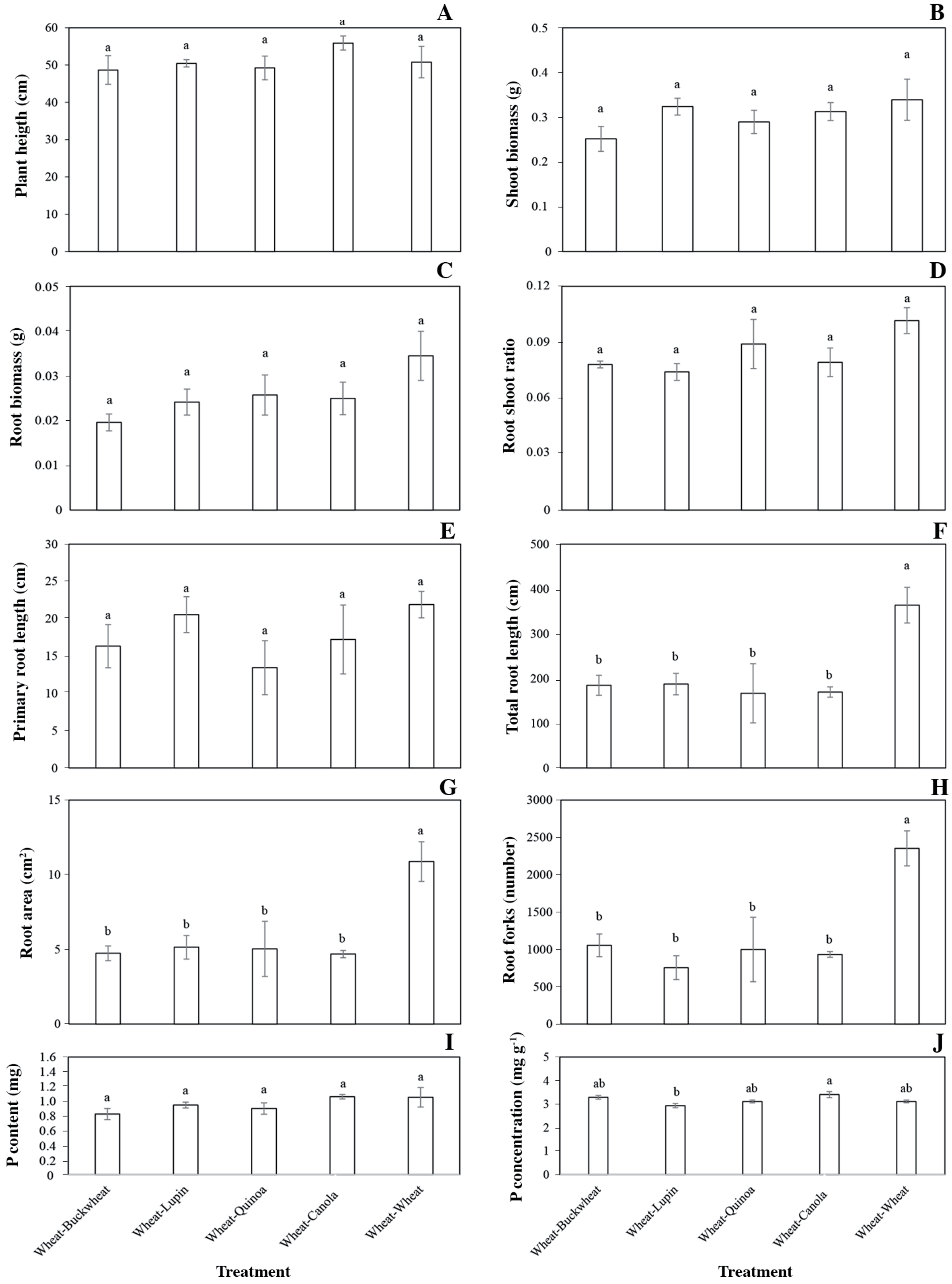
The effects of companion crops were tested by means of ANOVA. Before data analysis, the Shapiro-Wilk and Levene's tests were used to test the normality and homoscedasticity of the data, respectively. Significant differences between means were analyzed by the Tukey HSD test ($p \leq 0.05$). A principal component analysis (PCA) was performed to visualize and describe data patterns. All analyses were carried out using SPSS for Windows v.15.0 (IBM, Armonk, New York, USA), and graphs were constructed in RStudio software (RStudio, Boston, Massachusetts, USA).

RESULTS

Plant growth, root architecture and P nutrition

According to the methodology described above, plants were harvested at 30 and 44 DAS. Due to the different growth rates of all crops involved, no clear differentiation in wheat growth was observed at 30 DAS, especially regarding soil and root parameters. Therefore, only data obtained at 44 DAS is presented here. In general, intercropped wheat did not affect wheat height compared with monocropping. On the other hand, plant biomass production was slightly reduced in the presence of a non-wheat companion crop, especially regarding root biomass production, although not significantly. The root architecture of wheat plants was greatly affected by a non-wheat companion crop. Total root length, surface area, and number of branches were reduced by more than 50%, independently of the companion crop. Concretely, height and shoot biomass of wheat plants were not affected by companion crops (Figures 1A, and 1B), but slight differences in root biomass (Figure 1C) and primary root length began to be observed (Figure 1E). Detrimental effects of companion crops are observed in Figure 1C, which is also reflexed in root:shoot ratio (Figure 1D). Significant negative effects of about 50% on total root length (Figure 1F), root area (Figure 1G), and root forks (Figure 1H) were evidenced. Despite the severe reduction in some wheat root traits produced by companion crops, wheat P acquisition was not reduced as it was expected (Figures 1I and 1J).

Figure 1. Main plant parameters of wheat plants grown either in monoculture or intercropped with buckwheat, lupin, quinoa and canola plants in a low-P volcanic soil at 44 DAS. Graphics represent plant height (A), shoot biomass (B), root biomass (C), root:shoot ratio (D), primary root length (E), total root length (F), root area (G), root forks (H), plant P content (I), plant P concentration (J).



Data represent mean ± SE, n = 3. Means for each response followed by the same letter do not differ significantly by Tukey's HSD test ($p \leq 0.05$). DAS: Days after sowing.

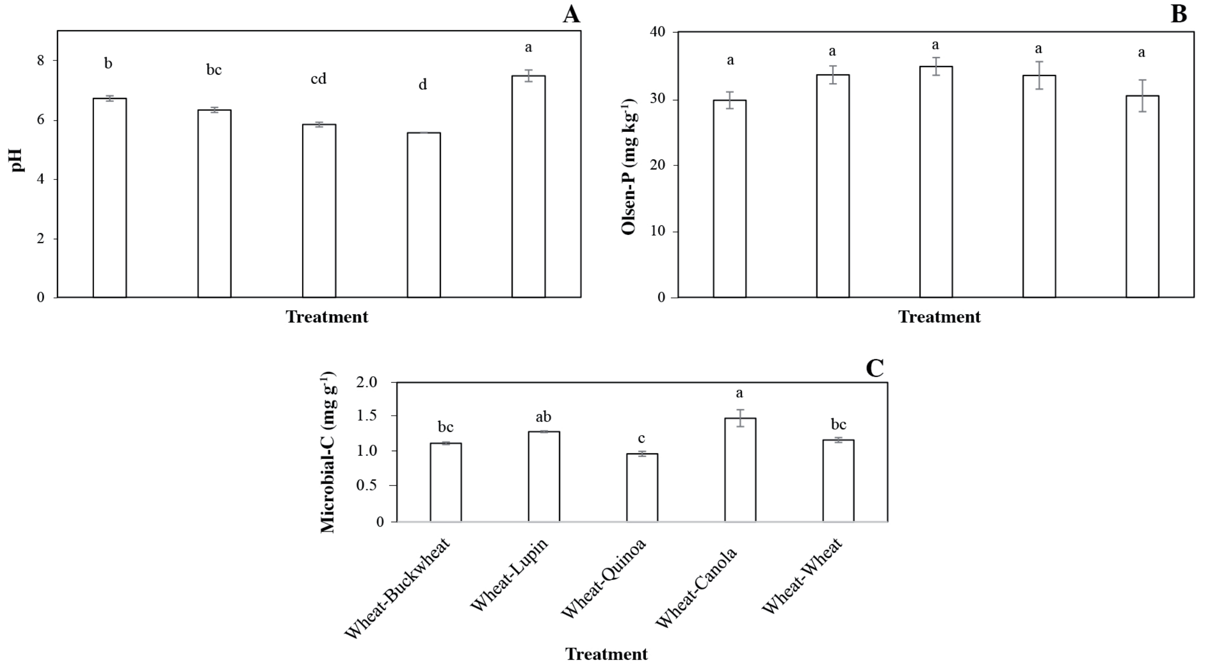
Soil parameters

All companion plants used in intercropping with wheat produced a lower rhizosphere pH compared with wheat alone following the order monocropping > buckwheat > lupin > quinoa > canola, suggesting a tendency to show more or better adaptation mechanisms for a higher plant P acquisition (Figure 2A) which is not correlated with P Olsen measured at harvesting (Figure 2B). However, a small increment in available P was observed in the shared rhizospheres of wheat, quinoa, lupin, and canola. Microbial population prospers around the rhizosphere, developing many beneficial activities for the plant, resulting in a higher rate of mineral cycling at such level. Microbial activity can be determined through a lot of direct or indirect activities, one of them being C-microbial biomass. The highest C-biomass was observed with canola and the lowest with quinoa, both significantly different for wheat in monocropping (Figure 2C). How much P could be transitorily immobilized in microbial protoplasm in canola wheat combination is a very interesting finding to be deeper studied for future intercropping management programs.

Mycorrhizal parameters

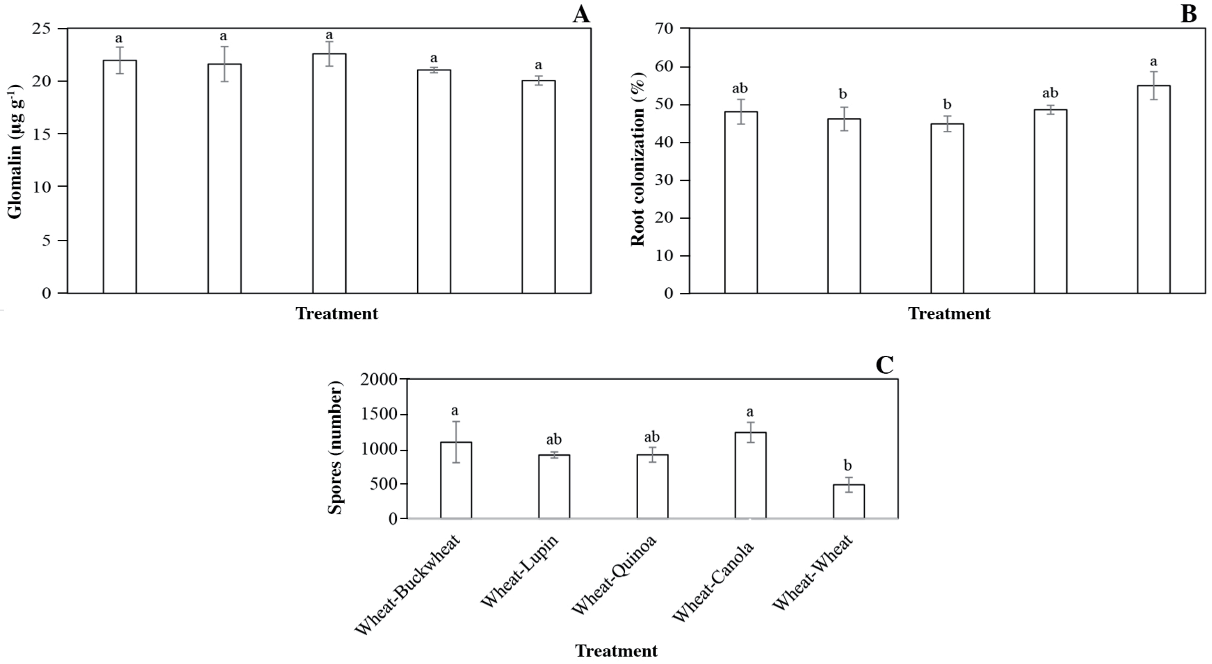
Arbuscular mycorrhizal fungi (AMF) constitute a group of microorganisms that form beneficial symbiotic relationships with almost plant roots in most ecosystems. In this study, all companion plants used do not form symbiotic relationships, but how they affected mycorrhizal activity in the shared rhizosphere is observed in Figure 3. Glomalin, a glycoprotein associated with the presence of AM, showed a slight increase in intercropped wheat plants compared with monocropping. Surprisingly, spore numbers were significantly increased but did not correlate with root colonization.

Figure 2. Soil parameters of wheat plants grown either in monoculture or intercropped with buckwheat, lupin, quinoa and canola plants in a low-P volcanic soil at 44 DAS. Graphics represent soil pH (A), Olsen-P (B), and microbial C-biomass (C).



Data represent mean ± SE, n = 3. Means for each response followed by the same letter do not differ significantly by Tukey's HSD test ($p \leq 0.05$). DAS: Days after sowing.

Figure 3. Main mycorrhizal parameters of wheat plants grown either in monoculture or intercropped with buckwheat, lupin, quinoa and canola plants in a low-P volcanic soil at 44 DAS. Graphics represent glomalin related soil protein (A), root mycorrhizal colonization (B), and spores' number (C).



Data represent mean ± SE, n = 3. Means for each response followed by the same letter do not differ significantly by Tukey's HSD test ($p \leq 0.05$).
DAS: Days after sowing.

DISCUSSION

Intercropping is a common agricultural practice, particularly in countries with high subsistence agriculture and low mechanization levels. In Latin America, small-holder farmers grow 70% to 90% of beans with maize, potatoes, and other crops, while maize is intercropped on about 60% of the area by this crop. All the above companion plants are mycorrhizal, differing from this study where the plant root combination could significatively change the strategies of both partners for acquiring nutrients, especially P. It appears that physiological traits developed by crops to maximize resource acquisition seem to be the same in mono and intercropping systems. However, the challenge of intercropping is how to select the best traits combination of different plants to improve overall performance or, on the contrary, to promote one over the other. Beneficial studies of intercropping have been almost focused on aboveground plantplant interactions parameters but much lesser studies have been reported on belowground interactions (Brooker et al., 2015) and how of such interactions could affect the development of the following crop management in an agricultural system.

Among the four non-mycorrhizal crops used as companion plants in this experiment the most studied has been lupin and canola, but the bulk of such studies has been on wheat-based pre-crops (Angus et al., 2015). However, much lesser information is found related to the direct interactions of both partners in intercropping, and its capacity to mobilize poorly available nutrients in the soil, particularly P and micronutrients, which highlights the potential of this crops to improve P-availability for P-inefficient plant species when sharing rhizosphere functions in intercropping (Lambers et al., 2018).

Our results showed that although wheat plant height and shoot biomass was not significantly affected by companion plants, some root morphological traits such as total root length, total root area, and root branching showed around 50% decrease, indicating that below-ground competition was more severely compared with above-ground and consequently the effect on plant nutrition could be necessarily affected. However, the results obtained here did not show such behavior. Therefore, shoot-related P parameters were almost the same as monocropping, and even P concentration was significantly higher with canola intercropped. It is recognized that plants develop two strategies for increasing P mobilization at the rhizosphere level, be through H^+ root excretion and chelating anions as malate and citrate and/or by the symbiotic association with mycorrhizal fungi (Hallama et al., 2019). Due to the inability of these associated crops to form this symbiosis, it appears that the only way for increasing P concentration at the rhizosphere level would be the mentioned root exudation strategy (Lambers et al., 2018). Accordingly, the obtained Olsen-P levels did not show difference between mono and intercropping, but clearly, all the shared rhizosphere pH were more acidic compared with wheat rhizosphere.

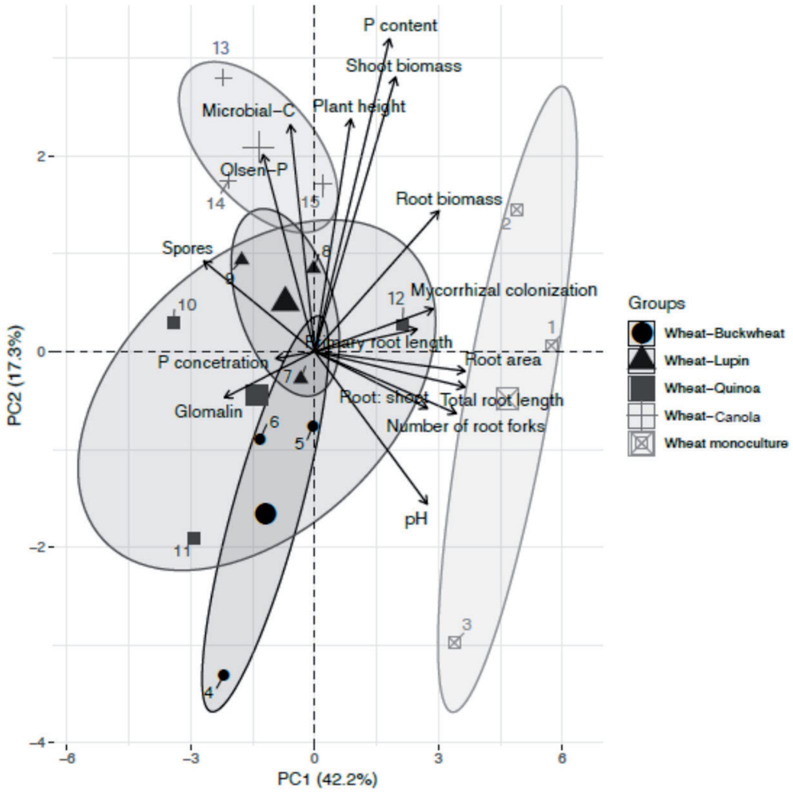
It has been reported that buckwheat can solubilize P in low-P soils through tartrate root exudation affecting rhizosphere P dynamics (Teboh and Franzen, 2011) but not altering soil P-availability (Possinger et al., 2013), which agrees with our results where we observed a discrete but significant pH decrease. Lupin, instead, exhibits typically strong root morphological and physiological responses to P deprivation through cluster root activity (Cheng et al., 2011) and increased proton, citrate, and acid phosphatase exudation (Tang et al., 2013). Therefore, it was expected to find an acute rhizosphere pH decrease on lupin-wheat association but quinoa and canola even showed higher acidity in our experimental conditions, probably due to that lupin genotype used presented scarce proteoid roots. This performance is not surprising, since they agree with the results recently reported in a field experiment of wheat-white lupin intercropping carried out by Schoebitz et al. (2020) in an Andisol of Southern Chile with similar characteristics. They found that rhizosphere pH not differed significantly between mono- and intercropping even at the three plant densities studied. Nonetheless, in opposite to our results, they reported that Olsen-P levels were significantly lower in wheat intercropped compared to wheat monocrop.

Quinoa has a high tolerance to several abiotic stresses (i.e., salinity, water deficit, and cold), being the mechanisms not yet totally elucidated (Jakobsen, 2003). According to Singh et al. (2021), different *Chenopodium* species have a specialized herringbone root system. No reports are found related to P nutrition or other important abiotic stress. The significant pH decrease observed in Figure 2A needs to be deepened to better know the exudation type. The rhizosphere acidification here produced by quinoa roots agrees well with the highest acidification reported by Solano et al. (2021) in a field experiment carried out in the same soil and be at 40 d growth as well at 90 d harvesting.

In contrast with the above ancestral plants analyzed, there is a little more information related to wheat and rapeseed intercropping, which are commonly used for their complementary use of resources, providing an excellent mixture for weed and disease control (Kirkegaard et al., 2008). However, the bulk of that studies have been mainly focused on agronomic management (Ebrahimi et al., 2016) and not on root interactions. An exception is a report of Wang et al. (2007), who conducted a study in acidic and alkaline soils to answer if P mobilized by three *Brassicas* genotypes was available to wheat, exhibiting that none of the *Brassica* genotypes improved wheat growth and P uptake agreeing well with our results (Figures 1J and 2A), showing that P availability in the wheat rhizosphere was similar in intercropping compared to monocropping.

It is known that several Brassicaceae contain glucosinolates and isothiocyanates, which have negative effects on soil-borne fungal pathogens but also affect AMF activity by inhibiting spore germination and/or hyphal growth (Vierheilig et al., 2000). Plants develop many strategies for enfacing soil P deprivation where AMF plays a vital role in improving P ability to acquire inorganic P from the rhizosphere (Aslam et al., 2020) in acidic soils. According to soil glomalin levels, root colonization percentage, and spore number, it appears that non-mycorrhizal plants did not affect mycorrhizal symbiosis activity allowing wheat to grow normally in intercropping even with canola contrarily to Wang et al. (2007) where despite plant growth was not reduced wheat mycorrhization was severely affected. Microbial biomass C was mainly unaffected by companion plants with the exception of canola, possibly due to higher excretion of carbonaceous compounds, which together structure of microbial population needs to be studied. Principal component analysis (PCA) of response variables shows that intercropping has general effects, some of them not showing dependence such as root growth and mycorrhizal colonization but also has specific effects on the microbiota and P dynamics according to the combination with companion plants (Figure 4).

Figure 4. Principal component analysis (PCA) of response variables (arrows). Different treatments are wheat-buckwheat (circles), wheat-lupin (triangles), wheat-quinoa (squares), wheat-canola (crosses), wheat monoculture (cross box); and individual samples are indicated in numbers.



Percentage values in parentheses indicate the variation explained by each principal component (PC).

CONCLUSIONS

Although this study shows some effects on interactions occurring in the rhizosphere of wheat ancestral plants intercropped, we are aware that other morphological traits like glucosinolates, phosphatase, H^+, organic anions exudations, among others, must be complementarily evaluated together the best proportion of wheat/companion plant to have a better picture in search of new alternatives for offering to smallholders working in volcanic soils.

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