

RESEARCH ARTICLE

Do root interactions between wheat and non-mycorrhizal ancestral plants influence fungal activity and soil quality in an Andisol?

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ABSTRACT

Cover crops in sustainable agrosystems are becoming more and more applied. However, legacy effects on the growth of companion plants or following crops in rotation systems are still poorly understood, especially when Andean and ancestral crops are involved. In this work, two wheat (*Triticum aestivum* L.) cultivars differing in P efficiency (Kirón higher than Cheignon) were cropped together with two non-mycorrhizal plants, quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* spp.), to study the interactions and the effects produced in the wheat rhizosphere, plant growth and mycorrhizal parameters at harvest. The plants were grown in a three-compartment plexiglass rhizobox containing an Andisol. The central compartment separated by a nylon mesh was used for the cover crops, and wheat was sown on each side. A habitual pre-crop rapeseed (*Brassica napus* L.), also non-mycorrhizal, was used as control. At harvest, soil (pH, available P, acid phosphatase, microbial-P), plant (phytomass, organic acid exudation, P uptake efficiency, Zn and Mn in shoots), and mycorrhizal properties (root colonization, glomalin, spore number, and hyphal density) were measured in both wheat rhizospheres. Results show that wheat cultivars differed in soil and plant characteristics affected by non-host plants being the most efficient one, better. Surprisingly, mycorrhizal propagules were not depressed, and even fungal spores with amaranth (10543 spores 100 g⁻¹) increased 2.6-fold than control (3910 spores 100 g⁻¹) in the P-efficient cultivar. Data obtained suggest being cautious in selecting the best wheat cultivar when using these ancestral crops, especially for wheat natives' smallholders working in this type of soil.

Key words: Amaranth, cover crops, phosphorous, quinoa, rapeseed.

INTRODUCTION

The incorporation of cover crops into agricultural practices leads to a lot of benefits in production ecosystems, including C sequestration, improving soil structure, hydraulic soil properties, biologically active microbial communities biodiversity, and management of weeds, diseases, and insects, among others (Adetunji et al., 2020). Cover crops must be of fast growth, usually sown with or before a main crop, to obtain any direct or indirect benefits awarded in agrosystems to improve agricultural production

and sustainable management (Quintarelli et al., 2022). Cover crops can leave enough mycorrhizal propagules in the soil to be useful for the companion crop in intercropping or for the next crop in the rotation (Higo et al., 2019).

One of the most attractive approaches to enface the problem of scarcely bioavailable P in soils rich in total P could be the use of plants with long and active root lengths and hairs, together with their capacity to form symbiotic associations with mycorrhizal fungus (Lambers et al., 2018). All these attributes for a better P acquisition by the main crop could be performed using cover crop plants either as intercropping or in rotation. It is known that repeated soil applications of P fertilizers lead to the build-up of a large fraction of residual P, known as legacy P (Doydora et al., 2020), that is not immediately available for the plants to use. However, according to Menezes-Blackburn et al. (2018), residual P can be considered a potential P source for crop use, decreasing dependence on external agrochemical inputs. Therefore, the study of using new or ancient crops allows the main crop to be less dependent on chemical fertilizers, being crucial when these are scarce and international prices are continuously rising. Also, there is a consensus that the world's remaining phosphate reserves are declining.

As wheat is the most important cereal crop in Central-Southern Chile and is compatible to be cultivated with other plants (Aziz et al., 2015) it is crucial to study wheat-based agrosystems with other new non-mycorrhizal crops. Accordingly, many less-known crops with great nutritional potential can be used in small-scale farms, like quinoa (*Chenopodium quinoa* Willd.) for its exceptional adaptations to diverse stresses (García-Parra et al., 2020). Additionally, root exudation has been suggested to play a central role in some micro-nutrient acquisition mechanisms operating in some soils, particularly oxalate and citrate (Fujii et al., 2021). Amaranth (*Amaranthus* spp.) is another interesting crop that, despite their outstanding nutritional properties (Martinez-Villaluenga et al., 2020), have very few studies related to its use in intercropping or rotation designs even though their role as a P scavenger in poor P soils is known (Hallama et al., 2019). Amaranth is also a crop extremely adaptable to adverse growing conditions resisting heat and drought, which also has nonsignificant disease problem and is among the easiest plants to grow in agriculturally marginal lands (Rastogi and Shukla, 2013).

Consequently, taking into account that plant-to-plant interactions habitually occur between roots, the present study reports the effects produced on some soil characteristics, plant growth, and mycorrhizal propagules when roots of two wheat cultivars differing in P efficiency interact with non-mycorrhizal ancestral crops like quinoa and amaranth in an Andisol with high total and organic P. Rapeseed (*Brassica napus* L.) also a non-mycorrhizal crop was included in this study because of its common use by local farmers as pre-crop in wheat rotation.

MATERIALS AND METHODS

The experiment was carried out in a growth chamber using a system that consists of a Plexiglas rhizobox with three compartments. The central was used for the growth of quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* spp.), two ancestral crops used as cover crops. Both sides of the main compartment were separated from the adjacent chambers by a nylon mesh of 45 μm allowing exudates and arbuscular mycorrhizal fungi (AMF) hyphae to pass through but blocking the passage of plant roots. In the lateral compartments, seedlings of two wheat (*Triticum aestivum* L.) cultivars differing in P acquisition efficiency were grown ('Kiron' > P efficiency than 'Chevignon').

All seeds were surface-sterilized in a 1% sodium hypochlorite solution for 10 min, thoroughly rinsed with sterile distilled water, and germinated for 3 to 7 d on moistened filter paper or in peat soil according to each species, at 25 °C. Seedlings were sown into each compartment of a 1.8 L plexiglass rhizoboxes filled with Andisol Temuco Series (5.7 pH; 17% soil organic matter [SOM]; 23 mg kg⁻¹ Olsen P; 3256 mg kg⁻¹ total P; 1408 mg kg⁻¹ organic P; 12.87 cmol₊ kg⁻¹ effective cation exchange capacity) with five replicates. Plants were grown under controlled conditions in a growth chamber at 20 °C, watered with 20 mL modified Hewitt solution once every 14 d, and harvested 54 d after transplanting. A photosynthetic photon flux

density of $380 \mu\text{mol m}^{-2} \text{s}^{-1}$ was applied during 16:8 h. At harvest, soils and whole plants were carefully separated for further analysis.

Fresh shoots and roots were weighed, oven-dried at 60°C for 48 h, ground and ashed at 550°C , acid-digested in an HCl solution, and analyzed for P by spectrophotometry using the molybdenum blue method. Mobilized P was expressed as mg plant^{-1} using P concentration in shoots and its dry weight. Phosphorous uptake efficiency (PUE) was calculated as the amount of accumulated P in shoots and roots per phytomass. Zinc and Mn were analyzed using atomic absorption spectroscopy in the same acid solution. Soil organic matter was determined by the universal ignition method of Walkley-Black. Total and organic P was determined by applying the methodology of Carter and Gregorich (2006). Available P was determined by the Olsen method with NaHCO_3 pH 8.5 solution as extractant. Microbial biomass P (MBP) was determined according to Brookes et al. (1982) using alcohol-free- CHCl_3 vapors where fresh, moist soils were subjected to sterilization in a vacuum desiccator at room temperature for 24 h, repeated evacuations of gaseous chloroform were performed and extracted with 0.5 M NaHCO_3 . Microbial protoplasm P was calculated using the difference between P extracted from fumigated and unfumigated soils applying a conversion factor (k_p) of 0.4 and corrected by P adsorption.

Acid phosphatase (APase) activity in soil was determined using a modified universal buffer (pH 6.5), with 25 mM *p*-nitrophenyl phosphate (pNPP) as substrate at 35°C for 1 h (Seguel et al., 2017). Carboxylates from rhizosphere soils (0.5 g) were extracted with CaCl_2 (0.2 mM), vacuum filtered through a $0.22 \mu\text{m}$ pore membrane, and quantified by high-performance liquid chromatography (HPLC) in a Shimadzu Prominence LC-20A (Shimadzu Corporation, Kyoto, Japan) with the aid of commercial standard.

Mycorrhizal root colonization was determined by the gridline intersect method and staining with 0.05% (w/v) trypan blue (Phillips and Hayman, 1970). Indigenous AMF spores were isolated from soils using the wet sieving and decanting method in a sucrose suspension (Seguel et al., 2017). Hyphal length was determined by adapting the filtration-gridline method described by Rubio et al. (2003). Total glomalin as glomalin-related soil protein (GRSP) was extracted according to Wright and Upadhyaya (1996) using citrate buffer pH 8.0 and autoclaved twice for 1 h at 121°C ; the supernatant was filtered and the extracted protein was analyzed using the Bradford assay with bovine serum albumin as standard.

The effects of companion crops on wheat plants were tested through ANOVA. Before data analysis, the Shapiro-Wilk and Levene's tests were used to check the normality and homoscedasticity of the data, respectively. Significant differences between means were analyzed using the Tukey HSD test ($p \leq 0.05$) performed with JAMOVI Windows v 2.3.16. A principal component analysis (PCA) was performed using R Studio Windows v 2012.12.0 to visualize and describe data patterns for each wheat cultivar.

RESULTS

Here we applied such a rhizobox microcosm system to study plant root effects of non-mycorrhizal plants such as quinoa and amaranth on the rhizosphere of wheat, a common mycorrhizal crop for imitating the interaction between cover crops and a cereal as a cash crop in an agrosystem. In addition, rapeseed was included as a control because their roots contain glucosinolates (GSLs) that are potentially hydrolyzed in the soil to release isothiocyanates (ITCs) which are thioglycosides toxic to some soil fungi, including the inhibition of mycorrhizal fungal spore germination (Sotelo et al., 2015).

The release of acidic compounds in plant roots is habitually produced by multiple processes carried out in the rhizosphere. Acidification produced by plant roots can decrease rhizosphere soil pH in relation to the bulk soil, resulting in the dissolution of sparingly soluble soil P or making more P bioavailable in the soil solution (Campos et al., 2017), among other activities developed there. Changes in rhizosphere soil pH are mainly associated with soil, plant species, and genotypes driving the size and activity of the microbial population. Both wheat cultivars' rhizosphere pH roughly maintained the same levels relative to bulk soil (5.7). However, when 'Chevignon' wheat was associated with rapeseed, the pH level decreased compared with 'Kiron' wheat (Figure 1A). We identified a few effects of soil acidification related to other variables (Figure 4).

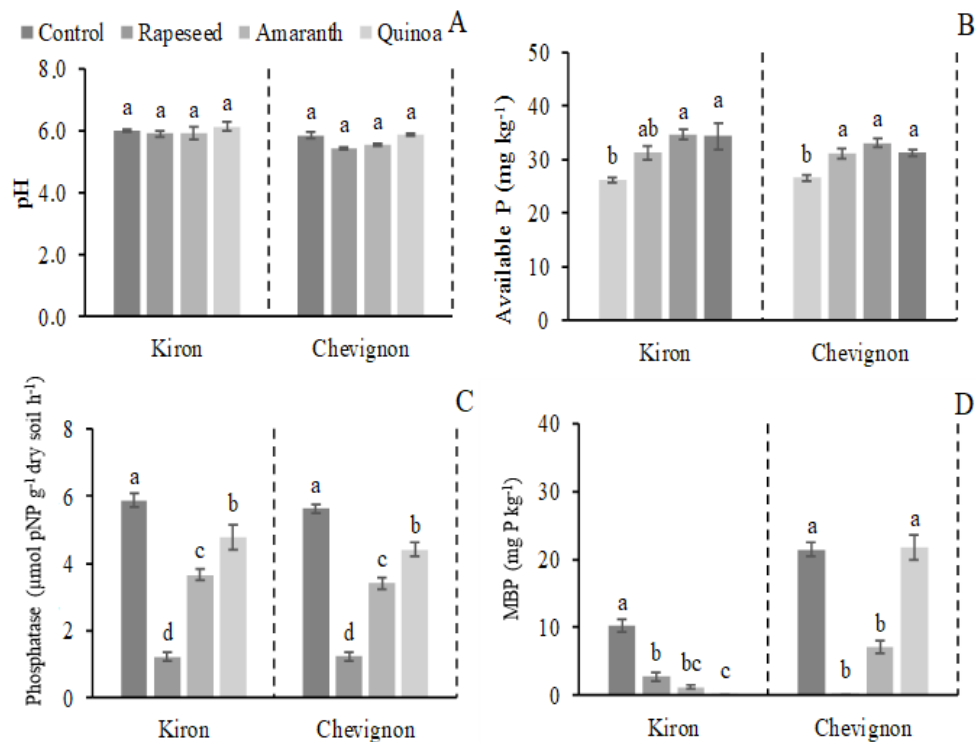


Figure 1. Rhizobox bioassay edaphic determinations in ‘Kiron’ and ‘Chevignon’ wheats: pH (A), available P (B), phosphatase activity (C), and microbial biomass P (MBP) (D). Different letters indicate significant differences between pre-crops according to Tukey’s test ($p \leq 0.05$). Bars representing the average of three biological replicates. pNP: Paranitrophenol.

Soil available P at harvest results from plant absorption and the plant’s capacity to dissolve some legacy P of the soil used. In this experiment, different plants that differ in P needs and absorption efficiency were used. Soil available P at harvest shows that both wheat cultivars left higher P levels compared with the control. However, no effects of companion plants were observed on both wheat cultivars (Figure 1B). No apparent differences in phosphatase activity between both wheats influenced by the cover crops were observed. The most significant detrimental effect observed in the enzymatic levels was when wheat was companioned with rapeseed. Still, both wheat cultivars demonstrated significantly lower enzymatic activity when associated with all three cover crops than wheat alone (Figure 1C). In general, ‘Kiron’, the more P-efficient wheat, presented lesser microbial P than ‘Chevignon’ (Figure 1D). Also, in both wheats, the effect of the cover crops decreased significantly in comparison with the non-associated wheat, except for ‘Chevignon’ associated with quinoa.

Cover crops might affect companion plants through mechanisms involving better nutrient absorption, mainly determined by changes in their physiological root traits. Therefore, in both wheat cultivars, the highest shoot yield was obtained with quinoa as a cover crop being higher in ‘Kiron’; although in ‘Chevignon’ such increment was near 50%, in relation to control (Figure 2A). Additionally, as expected, the observed shoot:root ratio (not shown) was higher in ‘Kiron’, alone or with cover crops as a companion plant. The same pattern was observed with mobilized P, where rapeseed caused a significant decrease, while the highest increase was obtained with quinoa (Figure 2B). Cover crops can increase the P uptake efficiency (PUE) by different mechanisms analyzed above. In this context, PUE determined in this study was more efficient with cover crops than alone in ‘Kiron’, especially with rapeseed (Figure 2C).

Organic acid exudation is indicated as the cause of local rhizosphere acidification by plants as a reaction to P-starvation and a possible mechanism of its root phosphate-solubilizing capacity. In this experiment, succinic, malic, citric, and oxalic organic acids exudations were measured. However, the levels of the first two acids were below detection limits; thus, only the latest were presented (Figures 2D, 2E). In general, the exudation of organic acids differs between wheat cultivars and treatments. Oxalic acid was observed in all wheat treatments, while citric acid was barely detected in rapeseed and amaranth when grown with 'Kiron' and 'Chevignon', respectively. Nonetheless, the oxalic acid exudation in 'Kiron' was affected by companion plants, being the exudation higher in rapeseed compared with the control. Oxalic acid levels were about tenfold higher than citric ones, despite these last acid amounts being higher for both cultivars with quinoa as the cover crop; while the highest levels of oxalic acid were associated with rapeseed. The quality and quantity of organic acid released by plant roots appear to be related to nutrient plant acquisition, mainly P, Zn, or Mn. The Zn content decreased when 'Chevignon' was accompanied by cover crops, while in 'Kiron' it only decreased with rapeseed (Figure 2F). On the other hand, 'Kiron' alone and when associated with cover crops showed the highest amount of Mn accumulation compared with 'Chevignon' (Figure 2G).

The influence of cover crops on the rhizosphere microbial community greatly depends on the species used, mainly those producing high quality and quantity of released photosynthates. It is also important if these crops are or are not mycotrophic to maintain or increase inoculum potential to benefit cash crop root mycorrhization. The beneficial or detrimental effect can be visualized through: a) AMF colonization percentage; b) mycorrhizal hyphal length; c) spore number; and d) glomalin-related soil protein (GRSP) content. All these effects produced by the activity of non-mycorrhizal crops on the mycorrhizal status of two wheat cultivars can be visualized in Figure 3. In general, wheat mycorrhizal status was not seriously affected by the interaction of non-mycorrhizal plants, even by rapeseed, a known reducer of mycorrhizal propagules. Arbuscular mycorrhizal fungi colonization in wheat roots decreased with rapeseed and amaranth crops in 'Kiron' (Figure 3A); while, the mycelium density increased with amaranth and quinoa in 'Chevignon' (Figure 3B). Surprisingly, amaranth produced antagonistic effects increasing close to 2.5-fold the mycorrhizal spore number in 'Kiron' wheat rhizosphere, but decreased by about a third in 'Chevignon' (Figure 3C). In general, 'Kiron' presented higher levels of glomalin than 'Chevignon', but decrease in the number of spores AMF was observed (Figure 3D).

We found interesting correlations between soil, plant, and mycorrhizal characteristics. The 'Kiron' wheat presented significant correlations between the phosphatase and AMF colonization ($r = 0.833^{***}$), citric acid ($r = 0.831^{***}$), Zn ($r = 0.761^{**}$), and oxalic acid ($r = -0.778^{**}$). The MBP correlated with PUE ($r = 0.843^{***}$) and available P ($r = -0.823^{**}$). Meanwhile, shoot biomass correlated with citric acid ($r = 0.812^{**}$), mobilized P ($r = 0.809^{**}$) and Mn ($r = -0.816^{**}$). On the other hand, 'Chevignon' wheat showed more significant correlations, especially with mycorrhizal activity, demonstrating interactions between MBP ($r = 0.931^{***}$), and phosphatase ($r = 0.814^{**}$). Similarly, correlations were observed between available P and PUE ($r = -0.891^{***}$), Zn ($r = -0.736^{**}$), Mn ($r = -0.736^{**}$) and AMF colonization ($r = -0.736^{**}$); MBP with phosphatase ($r = 0.927^{***}$). The plant parameters correlations observed were between the shoot biomass with the number of spores ($r = -0.864^{***}$) and hyphal density ($r = -0.717^{**}$); PUE with hyphal density ($r = -0.734^{**}$); citric acid with spore number ($r = -0.751^{**}$). Nutritional variables correlations were observed, such as Zn with Mn ($r = 1^{***}$) and hyphal density ($r = 0.717^{**}$), and finally, Mn showed a correlation with hyphal density ($r = -0.747^{**}$).

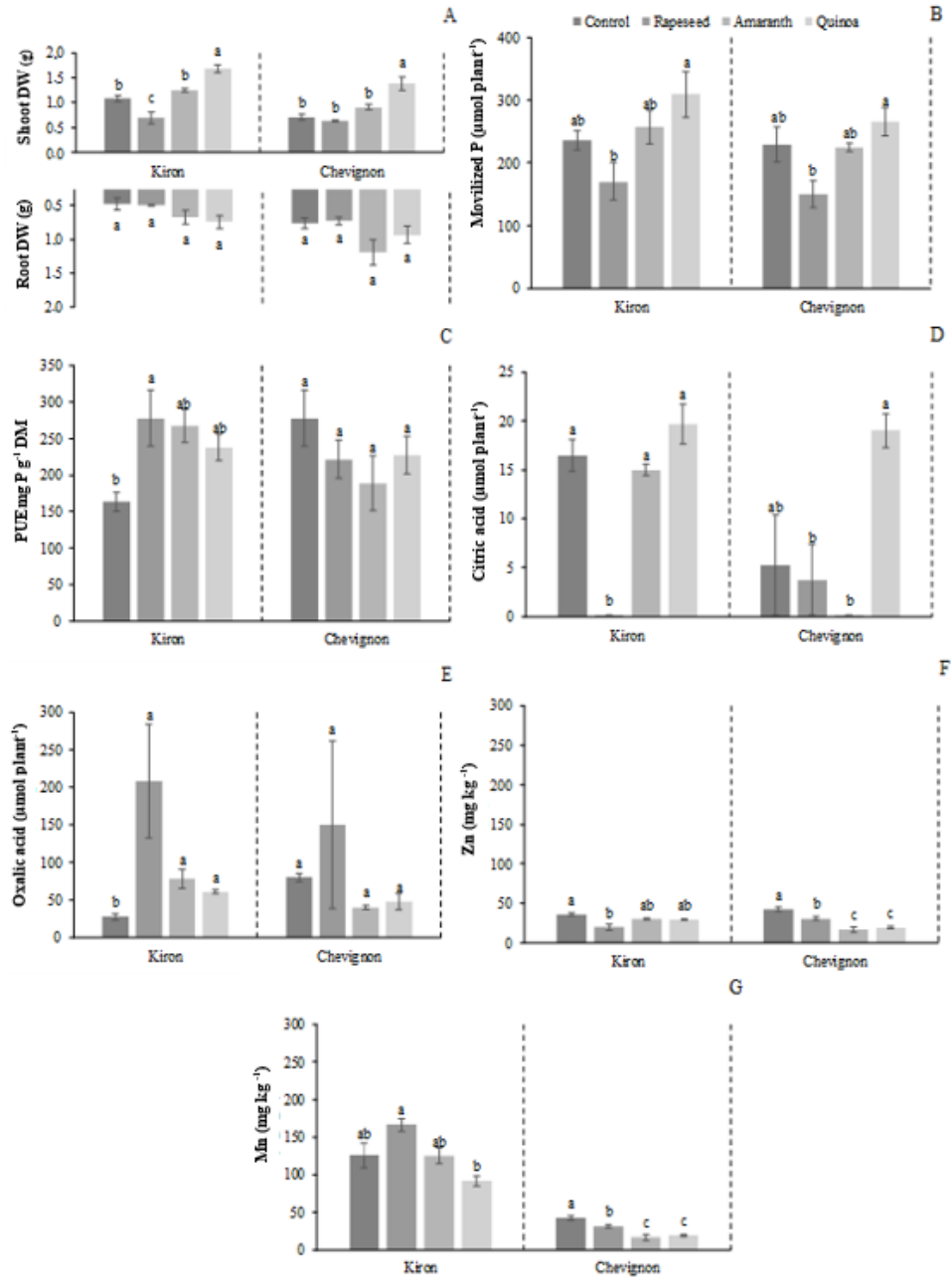


Figure 2. Rhizobox bioassay plant determinations in ‘Kiron’ and ‘Chevignon’ wheats: Shoot and root dry weight (A), mobilized P (B), P uptake efficiency (PUE) (C), citric acid (D), oxalic acid (E), shoot Zn (F), and Mn content (G). Different letters indicate significant differences between pre-crops according to Tukey’s test ($p \leq 0.05$). Bars representing the average of three biological replicates.

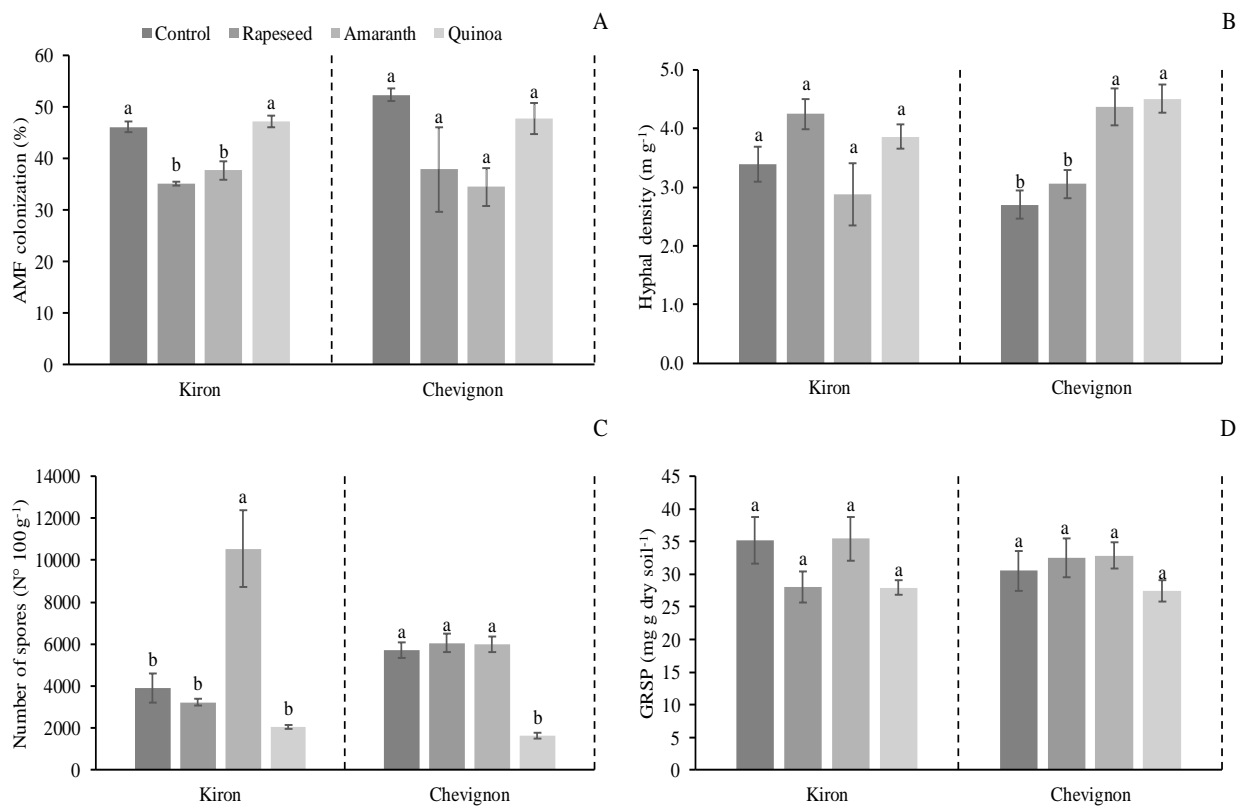


Figure 3. Rhizobox bioassay mycorrhizal parameters in ‘Kiron’ and ‘Chevignon’ wheats: Arbuscular mycorrhizal fungi (AMF) colonization percentage (A), AMF mycelium (B), spore number (C), and glomalin-related soil protein (GRSP) content (D). Different letters indicate significant differences between species according to Tukey’s test ($p \leq 0.05$). Bars representing the average of 3 biological replicates.

Principal component analysis (PCA) was performed for each wheat cultivar, and explained 62% and 69% of the total experimental variance in the first two components (PC1 and PC2) for ‘Kiron’ and ‘Chevignon’, respectively. Interestingly, ‘Kiron’ data from intercropping with quinoa and amaranth showed a similar pattern and were highly related to shoot and root biomass, as well as with changes in pH, mobilized P, and available P. ‘Kiron’ control samples formed a homogenous and distanced group compared to the other crops. On the other hand, PCA analysis of ‘Chevignon’ samples showed no clearly grouping between the different companion crops, suggesting that ‘Chevignon’ responses to intercropping was specific and highly dependent on different crops used here (Figure 4).

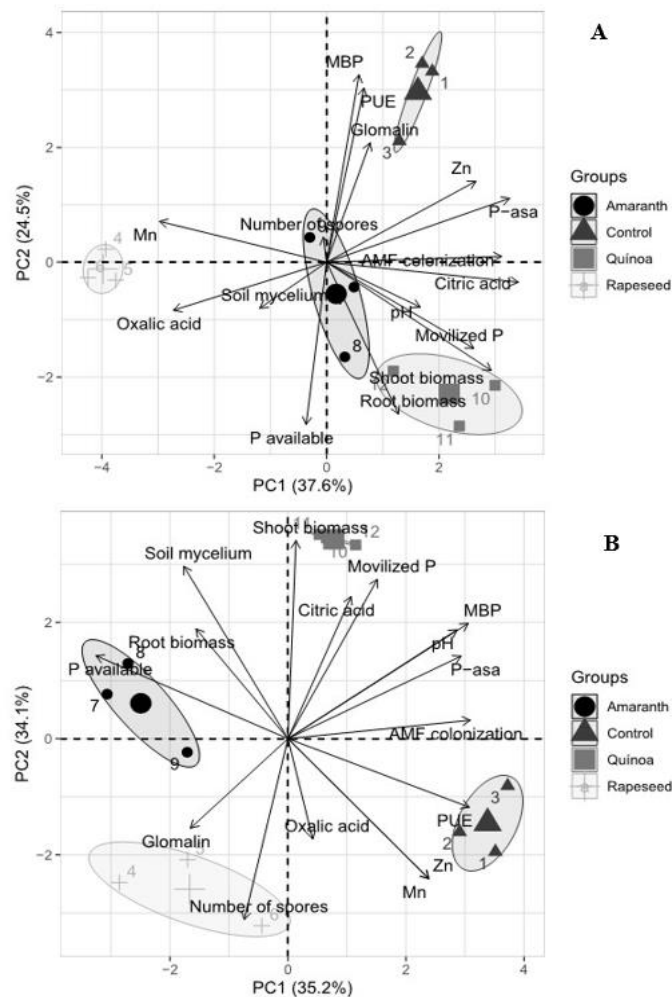


Figure 4. Principal component analysis for soil, plant and mycorrhizal variables in ‘Kiron’ (A) and ‘Chevignon’ (B) wheats. AMF: Arbuscular mycorrhizal fungi; MBP: microbial biomass P; PUE: P uptake efficiency.

DISCUSSION

It is recognized that cover crops, either in intercropping or in rotation, provide several ecosystemic impacts from the soil health, plant growth, and water quality point of view, all very well documented (Lamichhane et al., 2023). Choosing the best cover crops from the most widely used will depend on the nature of the problem to be solved. Here, our focus has been centered on selecting new ancestral species to be used as cover crops in wheat production in high P-fixing soils to minimize the use of P fertilizers, especially for smallholders. Root strategies to acquire and utilize P have been intensively described by Lambers et al. (2022), coinciding that the main processes and traits involved are morphological adaptations, phosphatase and H^+ exudation, including organic chelating acid anions, and mycorrhizal formation. Such intense activity is carried out in the rhizosphere, which is the critical zone where tripartite interactions among plants, soils, and microorganisms take place (Gianfreda, 2015).

The effects of the three cover crops on soil characteristics of the rhizosphere from both wheat cultivars showed that acidity levels and P availability were not significantly different. Phosphatase and MBP in the wheat’s rhizosphere were significantly affected by the effect of the companion plants, especially with

rapeseed (Figure 1). The effect of rapeseed as a pre-crop has been well documented as the reduction of microbial biomass. However, there is no clear information on the direct influence of rapeseed on acid phosphatase activity. Still, it seems logical that the decrease in microbial biomass affects rhizosphere enzymatic activity (Hansen et al., 2019). Quinoa produced a small but significant reduction in acid phosphatase activity, coinciding with Campos et al. (2022), that in this species root growth and morphology are more important than biochemical activities associated.

Additionally, there is scarce information about soil pH influenced by amaranth root exudation and its relationship with P acquisition compared with other studies reporting its role in allelopathy or as useful plant in bioremediation (Haseeb et al., 2022). The small changes in soil pH would influence the available P levels, which were almost the same for both cover crops (Figure 1B) due to high phosphatase activity (Figure 1C) and mobilized P (Figure 2B). In another parallel experiment (not shown), we found that phosphatase exuded by amaranth was close to eight-fold higher than those of quinoa, which would indirectly demonstrate that phosphatase activity observed in Figure 1C would mainly be derived from microbial origin according to Janes-Basset et al. (2022).

Soil microbes, including bacteria, fungi, and microfauna, play important roles in nutrient biochemical cycling because of their involvement in processes of immobilization/mineralization, especially in the case of P. Concretely, P concentrations in soil microbial biomass are highly variable depending on the soil type, vegetation, and agricultural practices. These concentrations range between 3% and 6% being higher in grasslands compared to woodland soils. Soil microbial-P constitutes 1.6% or 15.5 mg P kg⁻¹ of the total pool in intensive agricultural soils presenting close correlations with C microbial biomass (Luo et al., 2022). Some reports have suggested that soil MBP can serve as an index reflecting soil P fertility, while other authors claimed its use as an indicator of P availability in soils like volcanic Andisols replacing other traditional extracting methods (Sugito and Shimano, 2013). In this study, MBP ranged from 1.2 to 21.5 mg P kg⁻¹ soil, where the highest value was observed in 'Chevignon', possibly due to a greater C exuded by its roots (Figure 1D). As we expected, rapeseed-wheat interaction produced the lowest values of MBP as a consequence of the known fungicide role of *Brassicaceae*. Furthermore, a relationship was observed with soil available P (-0.8233**), but no correlation was evidenced with phosphatase activity. The high available P observed in the wheat rhizosphere soil suggests that plants can mobilize P from less labile soil P forms.

The best results related to wheat plant growth were obtained when accompanied with quinoa compared with amaranth and rapeseed as a cover crop, especially in 'Kiron' (Figures 2A, 2B). This is better visualized considering the shoot:root ratio of plants, since one of the main symptoms of P deficiency in plants is the enhancement of such ratio. Our results suggest that quinoa and canola give a major P absorption efficiency to 'Kiron' and 'Chevignon', respectively.

One of the most important roles played by roots to acquire P from unavailable P sources is the exudation of short-chain organic acids solubilizing, desorbing, or competing for active sites, increasing P availability in the rhizosphere. In general, oxalate, citrate, and malate are produced by almost all plants in variable quantities depending on the environmental conditions, being the most effective organic acid anions in mobilizing P in soils, in descending order: Tricarboxylate citrate and dicarboxylates, oxalate, and malate (Adeleke et al., 2017). In this study, malate and succinate were also found in the rhizosphere of all treatments assayed, but our focus will only be on citrate and oxalate due to their incidence in P cycling and the higher concentrations found here (Figures 2D, 2E). Oxalate was the predominant carboxylate detected within the wheat rhizosphere in interaction with the three cover crops, mainly *B. napus* observing two- and four-fold higher levels of exudation in 'Chevignon' and 'Kiron', respectively. The carboxylate levels observed in rapeseed are in accordance with other studies under similar experimental conditions, roughly the same as those observed in this study with 'Chevignon'.

There is very little information related to the organic acid exudation of amaranth and its incidence in P nutrition. The scarce reports found are connected with the amelioration of Al soil phytotoxicity (Fan et al., 2016). Citric acid concentration was lower in 'Chevignon' than 'Kiron', was absent in rapeseed grown with 'Kiron' and amaranth grown with 'Chevignon' (Figure 2D). The organic acid exudation pattern is involved with plant mineral nutrition, especially Cu, Zn, and Mn, due to chelate formations and quick root absorption.

Shoot Mn concentrations were higher in ‘Chevignon’ alone than when interacting with cover crops compared to ‘Kiron’, which is known for its higher P efficiency. The present study revealed an interesting correlation between Mn concentrations in shoots and oxalate levels.

In a cropping system, adverse effects on non-mycorrhizal species may persist and affect the next crop, which may be associated with allelochemical adverse effects (Sotelo et al., 2015). This could be the potential role played by quinoa and amaranth and their allelopathic properties on mycorrhizal propagules left in the soil. However, the mechanisms involved in rapeseed are different considering that their roots exude glucosinolates, further hydrolyzed to isothiocyanates with antifungal activity, affecting mycorrhizal fungal behavior (Miklavčič et al., 2021). In this study, we found that root colonization in wheat cultivars alone were 45% and 52% in ‘Kiron’ and ‘Chevignon’, respectively, being the effect of companion plants different. In ‘Kiron’ with rapeseed and amaranth decreased, but not in ‘Chevignon’ (Figure 3A). In this sense, some authors reported that AMF colonization of wheat following a Brassica crop is greatly reduced compared with wheat after mycorrhizal pre-crops (Ryan, 2012; Bakhshandeh et al., 2017). Notwithstanding, it did not affect wheat nutrition, growth, or yield. The same performance has been reported by Castillo et al. (2016) with the application of non- and mycorrhizal pre-crops in a wheat rotation in an Andisol in the second year. Wheat root colonization was higher after lupin and rapeseed compared with oats which is a highly mycotrophic crop.

Nonetheless, to maintain the mycotrophic soil status in a rotation system including non-mycorrhizal crops, holding enough viable spores and mycelium to produce good colonization in the next crop is essential. Surprisingly, spore number and hyphal length were not depressed in both wheat cultivars by rapeseed presence (Figures 3B, 3C). In this study, viable spores were maintained in a similar number under the influence of rapeseed and quinoa which could be due to the antifungal compound’s concentration. In the case of rapeseed, these compounds may have been below the necessary concentration to affect spore germination, or that harvest time was too short for an adequate diffusion.

The high increase of AMF spore with amaranth in the ‘Kiron’ rhizosphere contrasts sharply with that obtained in ‘Chevignon’ (Figure 3B), suggesting that the choice of the wheat cultivar used is very important when using this type of cover crops. Concerning the latter, Schreiner and Koide (1993), using 12 plant species from both mycotrophic and non-mycotrophic hosts to examine their ability to produce antifungal compounds, reported that amaranth had nonsignificant effect on the germination of mycorrhizal fungal spores.

Although cover crops are known to affect root colonization of the subsequent crop and soil mycorrhizal propagules in organic agroecosystems, little is known about their effect on AMF biodiversity. In this respect, it is interesting to mention the study by Poomipan (2014), working with maize and cabbage (*Brassica oleracea*) and analyzing 12 AMF species, observed that despite the negative effect of cabbage on fungal spores’ number in comparison with maize, such effect was lesser on some strains like *Glomus* sp.

In agricultural systems, cropping patterns strongly affect glomalin concentrations which are closely related to the mycorrhizal dependence of the crops for their growth. Whereas there are a lot of field crops strongly dependent on symbiosis (corn, potato, legumes) that obtain benefits from this association, there are others, like cereals, which, even though have benefits, are not dependent on it. A third group, including Polygonaceae, Amaranthaceae, and Brassicaceae, that do not form symbiosis are not benefited either. In our study, we expected to find higher glomalin and mycorrhizal propagules in wheat rhizospheres compared with rapeseed and amaranth. However, soil glomalin concentrations in all treatments ranged from 2.8% to 3.5% (Figure 3d), suggesting that companion plants do not affect this soil quality indicator corroborating our early findings in an intercropping study (Castillo et al., 2022). Glomalin contributes to soil formation and stability of stable water aggregates, and its levels are closely related to hyphal length and spore density (Li et al., 2022). These relationships are also observed in Figure 3, especially with ‘Kiron’.

Results observed for glomalin agree with those reported by Woignier et al. (2014) in volcanic Andisols from Martinique, who found a range of 2.0%-3.6%, accounting for about 8% of total soil organic C. Even so, these levels are much higher compared with those determined in Chilean Ultisols, with lower organic matter content compared with Andisols.

CONCLUSIONS

Our study indicates that it is possible to crop wheat using amaranth and quinoa without major alteration of soil characteristics and maintaining soil mycorrhizal activity. Our results lead us to think that to improve the sustainability of agroecosystems used in wheat production by local small agriculture farmers, the use of their own plant genotypes and the employment of their knowledge of ancestral plant management are required. In this context, to minimize income costs, especially in phosphate fertilization and biocide reductions, it is urgent to dig deeper into further studies, including field trials using the better wheat cultivar together with the best ecotype of these ancestral crops. At the same time, in awareness of the new market and worldwide nutritional tendencies, such as Andean crops could be the most cost-effective species for native smallholders and more attractive to be cropped.

Author contributions

Conceptualization & draft writing: F.B. Writing-review; C.C. Validation: J.S. Software: S.D., P.C. Methodology: P.A., A.R. Funding acquisition: R.C. Supervision: E.S. All co-authors reviewed the final version and approved the manuscript before submission.

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References

- Adeleke, R., Nwangburuka, C., Oboirien, B. 2017. Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany* 108:393-406. doi:10.1016/j.sajb.2016.09.002.
- Adetunji, A.T., Ncube, B., Mulidzic, R., Lewud, F.B. 2020. Management impact and benefit of cover crops on soil quality: A review. *Soil and Tillage Research* 204:104717. doi:10.1016/j.still.2020.104717.
- Aziz, M., Mahmood, A., Asif, M., Ali, A. 2015. Wheat based intercropping –A review. *The Journal of Animal and Plant Science* 25(4):896-907.
- Bakhshandeh, S., Corneo P.E., Mariotte P., Kertesz M.A., Dijkstra, F.A. 2017. Effect of crop rotation on mycorrhizal colonization and with yield under different fertilizer treatment. *Agriculture, Ecosystem and Environment* 247:130-136.
- Brookes, P.C., Powlson, D.S., Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* 14:319-329. doi:10.1016/0038-0717(82)90001-3.
- Campos, M., Martins, A.R.H., Rosolem, C.A. 2017. Cover crops and soil phosphorus availability. *Communications in Soil Science and Plant Analysis* 48(10):1240-1246. doi:10.1080/00103624.2017.1341918.
- Campos, P.M.S., Meier, S., Morales, A., Borie, F., Cornejo, P., Ruiz, A., et al. 2022. Root traits distinguish phosphorus acquisition of two wheat cultivars growing in phosphorus-deficient acid soil. *Rhizosphere* 22:100549. doi:10.1016/j.rhisph.2022.100549.
- Carter, M., Gregorich, E.G. 2006. *Soil sampling and methods of analysis*. Lewis Publication, Boca Raton, Florida, USA.
- Castillo, C.G., Borie, F., Oehl, F., Sieverding, E. 2016. Arbuscular mycorrhizal fungi biodiversity: prospecting in Southern-Central zone of Chile. A review. *Journal of Soil Science and Plant Nutrition* 16:400-422. doi:10.4067/S0718-95162016005000036.
- Castillo, C.G., Solano, J., Collinao, M., Catalán, R., Campos, P., Aguilera, P., et al. 2022. Intercropping wheat with ancestral non-mycorrhizal crops in a volcanic soil at early growth stage. *Chilean Journal of Agricultural Research* 82:663-672. doi:10.4067/S0718-58392022000400663.
- Doydora, S., Gatiboni, L., Grieger, K., Hesterberg, D., Jones, J.L., McLamore, E.S., et al. 2020. Accessing legacy phosphorus in soils. *Soil Systems* 4(4):74. doi:10.3390/soilsystems4040074.
- Fan, W., Xu, J.M., Lou, H.Q., Xiao, Ch., Chen, W.W., Yang, J.L. 2016. Physiological and molecular analysis of aluminium induced organic acid anion secretion from grain amaranthus (*Amaranthus hypocondriacus* L.) roots. *International Journal of Molecular Science* 17(5):608. doi:10.3390/ijms17050608.
- Fujii, K., Hayakawa, C., Ningsih, S. 2021. Effects of tree species on root exudation and mineralization of organic acids in a tropical forest. *Research Square*. doi:10.21203/rs.3.rs-544741/v1.
- García-Parra, M., Zurita-Silva, A., Stechauner-Rohringer, R., Roa-Acosta, D., Jacobsen, S.E. 2020. Quinoa (*Chenopodium quinoa* Willd.) and its relationship with agroclimatic characteristics: A Colombian perspective. *Chilean Journal of Agricultural Research* 80:290-302. doi:10.4067/S0718-58392020000200290.

- Gianfreda, L. 2015. Enzymes of importance to rhizosphere processes. *Journal of Soil Science and Plant Nutrition* 15(2):283-306. doi:10.4067/S0718-95162015005000022.
- Hallama, M., Pekrun, C., Lambers, H., Kandeller, E. 2019. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant and Soil* 434:7-45. doi:10.1007/s11104-018-3810-7.
- Hansen, J.C., Schillinger, W.F., Sullivan, T.S., Paulitz, T.C. 2019. Soil microbial biomass and fungi reduced with canola introduced into long-term monoculture wheat rotations. *Frontiers in Microbiology* 10:1488. doi:10.3389/fmicb.2019.01488.
- Haseeb, M., Iqbal, S., Hafeez, M.B., Saddiq, M.S., Zahra, N., Raza, A., et al. 2022. Phytoremediation of nickel by quinoa: Morphological and physiological response. *PLOS ONE* 17(1):e0262309. doi:10.1371/journal.pone.0262309.
- Higo, M., Tatewaki, Y., Gunji, K., Kaseda, A., Isobe, K. 2019. Cover cropping can be a stronger determinant than host crop identity for arbuscular mycorrhizal fungal communities colonizing maize and soybean. *Peer Journal* 7:e6403. doi:10.7717/peerj.6403.
- Janes-Bassett, V., Blackwell, M.S.A., Blair, G., Davies, J., Haygarth, P.M., Mezeli, M.M., et al. 2022. A meta-analysis of phosphatase activity in agricultural settings in response to phosphorus deficiency. *Soil Biology and Biochemistry* 165:108537. doi:10.1016/j.soilbio.2021.108537.
- Lambers, H., Albornoz, F., Kotula, L., Laliberté, E., Ranathunge, K., Teste, F., et al. 2018. How belowground interactions contribute to the coexistence of mycorrhizal and non-mycorrhizal species in severely phosphorus-impooverished hyperdiverse ecosystem. *Plant and Soil* 324:11-33. doi:10.1007/s11104-017-3427-2.
- Lambers, H., Costa, P.B., Cawthray, G.R., Denton, M.D., Finnegan, P.M., Hayes, P.E., et al. 2022. Strategies to acquire and use phosphorus in phosphorus-impooverished and fire-prone environments. *Plant and Soil* 476:133-160. doi:10.1007/s11104-022-05464-8.
- Lamichhane, J.R., Alleto, L., Cong, W., Dayoub, E. 2023. Relay cropping for sustainable intensification of agriculture across temperate regions: Crop management challenges and future research priorities. *Field Crops Research* 291:108795. doi:10.1016/j.fcr.2022.108795.
- Li, Y., Xu, J., Hu, J., Zhang, T., Wu, X., Yang, Y. 2022. Arbuscular mycorrhizal fungi and glomalin play a crucial role in soil aggregate stability in Pb-contaminated soil. *International Journal of Environmental Research and Public Health* 19(9):5029. doi:10.3390/ijerph19095029.
- Luo, H., Yu, J., Li, R., Gu, J.D., Luo, L., Zhang, Y., et al. 2022. Microbial biomass C:N:P as a better indicator than soil and ecoenzymatic C:N:P for microbial nutrient limitation and C dynamics in Zoige Plateau peatland soils. *International Biodeterioration and Biodegradation* 175:105492. doi:10.1016/j.ibiod.2022.105492.
- Martínez-Villaluenga, C., Peñas, E., Hernández-Ledesma, B. 2020. Pseudocereal grains: Nutritional value, health benefits and current applications for the development of gluten-free foods. *Food and Chemical Toxicology* 137(4):40-57. doi:10.1016/j.fct.2020.111178.
- Menezes-Blackburn, D., Giles, C., Darch, T., George, T.S., Blackwell, M., Stutter, M., et al. 2018. Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: A review. *Plant and Soil* 427:5-16. doi:10.1007/s11104-017-3362-2.
- Miklavčič, A., Tamayo, A., Steenkjær, A.C., Hansen, N.M.L., Peeters, K., Schwarzkopf, M. 2021. Glucosinolates and isothiocyanates in processed rapeseed determined by HPLC-DAD-qTOF. *Plants* 10(11):2548. doi:10.3390/plants10112548.
- Phillips, J.M., Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161. doi:10.1016/S0007-1536(70)80110-3.
- Poomipan, P. 2014. Effect of non-host plant on the community of arbuscular mycorrhizal fungi. *Thai Journal of Science and Technology* 3(1):57-65. doi:10.14456/tjst.2014.1.
- Quintarelli, V., Radicetti, E., Allevato, E., Stazi, S.R., Haider, G., Abideen, Z., et al. 2022. Cover crops for sustainable cropping systems. A review. *Agriculture* 12(12):2076. doi:10.3390/agriculture12122076.
- Rastogi, A., Shukla, S. 2013. Amaranth: A new millennium crop of nutraceutical values. *Critical Reviews in Food Science and Nutrition* 53(2):109-125. doi:10.1080/10408398.2010.517876.
- Rubio, R., Borie, F., Schalchli, C., Castillo, C., Azcón, R. 2003. Occurrence and effect of arbuscular mycorrhizal propagules in wheat as affected by source and amount of phosphorus fertilizer and fungal inoculation. *Applied Soil Ecology* 23:245-255. doi:10.1016/S0929-1393(03)00045-3.
- Ryan, M.H. 2012. The effects of Brassica crops on the level of mycorrhizal inoculum in soil. *Proceedings of the Australian Agronomy Conference, 10th "Science and Technology: Delivering Results for Agriculture?"*, Hobart, Tasmania, Australia. January 2001 Available at <http://www.regional.org.au/au/asa/2001/2/a/ryan.htm#TopOfPage>.

- Schreiner, R.P., Koide, R.T. 1993. Stimulation of vesicular-arbuscular mycorrhizal fungi by mycotrophic and nonmycotrophic plant root systems. *Applied and Environmental Microbiology* 59:2750-2752. doi:10.1128/aem.59.8.2750-2752.1993.
- Seguel, A., Cornejo, P., Ramos, A., von Baer, E., Cumming, J., Borie, F. 2017. Phosphorus acquisition by three wheat cultivars contrasting in aluminium tolerance growing in an aluminium-rich volcanic soil. *Crop Pasture Science* 68 (4):305-316. doi:10.1071/CP16224.
- Sotelo, T., Lema, M., Soengas, P., Cartea, M.E., Velasco, P. 2015. *In vitro* activity of glucosinolates and their degradation products against brassica-pathogenic bacteria and fungi. *Applied Environmental Microbiology* 81(1):432-440. doi:10.1128/AEM.03142-14.
- Sugito, T., Shimano, T. 2013. Estimating P availability in andosols using soil microbial biomass as an indicator. *Japan Agricultural Research Quarterly* 47:347-351. doi:10.6090/jarq.47.347.
- Woignier, T., Etcheverria, P., Borie, F., Quiquampoix, H., Staunton, S. 2014. Role of allophanes in the accumulation of glomalin-related soil protein in tropical soils (Martinique, French West Indies). *European Journal of Soil Science* 65:531-538. doi:10.1111/ejss.12151.
- Wright, S.F., Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science* 161(9):575-586. doi:10.1097/00010694-199609000-00003.