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## Fertilization, cover crop and harvest date: Their effects on soil physicochemical traits in an olive orchard in Southwestern Buenos Aires

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#### **ABSTRACT**

There is a scarcity of information on the effects of fertilization combined with cover crops on the soil physicochemical properties in olive orchards. We assess the effects of fertilization, cover crop, and harvest time on various soil characteristics in an olive (Olea europaea) orchard. Studies were conducted during the growing seasons of 2021/2022 and 2022/2023 in southwestern Buenos Aires, Argentina. The general experimental design in 2021/2022 and 2022/2023 was split plots. Fertilization treatments included an organic manure applied to the soil, inorganic fertilization applied to the soil or leaves of olive trees, and an unfertilized control. Additionally, a mixture of Vicia benghalensis with Avena sativa was either planted or not (control) as a cover crop. The study demonstrated that soil pH increased on average from mid-spring to mid-autumn during both studied growing periods. Soil organic matter, available phosphorus (P), and extractable potassium (K) were greater at 0-20 cm than at 20-40 cm soil depth. In 2022/2023, the percentage of soil organic matter appeared to be higher in areas with a cover crop than in those without, from early summer to early winter. Soil concentrations of ammonium, nitrate, P, and K were either similar or most often higher never lower in soils with organic and inorganic fertilizations compared to the inorganic foliar fertilization. These three types of fertilization showed greater nutrient concentrations than the unfertilized control. Finally, soil available P concentrations appeared to be higher in areas without vegetation cover than in those with a cover crop at 0-20 cm soil depth.

**Contribution/Originality:** This study explores the combined effects of various fertilization and cover crop treatments at different sampling dates on the determination of various soil physiochemical properties. It offers new insights and enhances understanding of how these treatments affect the studied physicochemical soil traits in a semiarid region.

## 1. INTRODUCTION

The olive (Olea europaea L.) is a perennial tree species that occupies the greatest area in hectares worldwide (FAOSTAT, 2022). The fruit production of olives in Argentina concentrates in the northwestern region, specifically in the provinces of La Rioja, Mendoza, San Juan, and Catamarca (Bocchi, 2023). In this country, there are more than 150,000 hectares implanted with olives (Carciofi, Guevara Lynch, & Maspi, 2022). The southwestern region of the Province of Buenos Aires, Argentina, is composed of the semiarid, arid, and subhumid-dry Pampas, covering 6.5

million hectares divided into 12 districts. Its agricultural productivity is lower than that of the rest of the Pampa region due to the prevailing agroecological conditions (Cincunegui et al., 2019). Such a region is ecologically suitable for olive cultivation, which contributes to reducing the advancement of desertification in the region (Elias & Barbero, 2017). It also has competitive advantages since it has the port with the greatest depth in the country (Puerto Ingeniero White), adequate transportation systems, and the provision of associated services necessary for the commercialization and general development of the activity (Cincunegui et al., 2019).

A significant portion of olive grove producers in southwestern Buenos Aires rely solely on foliar fertilization with macronutrients, such as urea as a nitrogen source, phosphoric acid as a phosphorus source, and potassium nitrate as a source of both nitrogen and potassium, to enhance production. However, this method is primarily recommended for micronutrients like boron (B), iron (Fe), manganese (Mn), among others. Additionally, multiple foliar applications are necessary throughout the crop cycle due to several factors: (1) foliar fertilization depends on the nutrient requirements of the olive trees, with higher needs necessitating root absorption; (2) the low efficiency of leaf absorption caused by the pubescent undersides of leaves; and (3) the significant loss of fertilizers through dripping, evaporation, drifting, and other mechanisms. Many regional producers typically perform no more than two or three foliar applications annually, under the assumption that the crop is sufficiently nourished. Therefore, there is a need to identify more efficient fertilization strategies that not only incorporate inorganic nutrients but also enhance soil organic matter, thereby supporting sustainable olive production and improving nutrient use efficiency.

Usually, in olive orchards, the cover crop biomass is incorporated into the soil as a fertilizer (Aguilera-Huertas et al., 2023; Chehab et al., 2019). In addition, the biomass produced by the different cover crop species tested can be cut and used as feed for animal production before their incorporation into the soil, increasing the olive ecosystem's profitability and services, as well as the incomes of farmers. In this case, only residues are incorporated into the soil and serve as bio-fertilizers (Lee et al., 2023).

The nitrogen content in the surface soil layer can be influenced by the sampling date, the decomposition process, and the dry biomass incorporated into the soil (Ordóñez-Fernández, de Torres, Márquez-García, Moreno-García, & Carbonell-Bojollo, 2018). Conservation systems that reduce tillage and increase cropping intensity, diversity, and crop residue incorporation into the soil have the potential to improve soil health, as well as agro-ecosystem productivity and resilience. Further, soil health depends on complex biophysical and biochemical interactions in time and space. This emphasizes that soil is a living, dynamic system that provides multiple ecosystem services.

Roussos, Gasparatos, Kechrologou, Katsenos, and Bouchagier (2017) found that the application of the organic fertilizer 'Activit' combined with an inorganic fertilizer (NPK) significantly reduced the soil pH compared to the control (the inorganic fertilizer) in the olive cultivars 'Koroneiki' and 'Konservolia'. This result is in agreement with that reported by Busso, Rodríguez, and Suñer (2025) in the olive cultivar 'Arbequina' after applying the organic manure 'Bioorganutsa' in comparison with the unfertilized control. This reduction was probably due to the nitrification of ammonium and the production of organic acids (phenolic and carboxylic groups) during the decomposition of organic materials (García-Ruiz, Ochoa, Hinojosa, & Gómez-Muñoz, 2012).

Adekiya, Agbede, Aboyeji, Dunsin, and Ugbe (2019) and Adekiya et al. (2020) also reported that the lower pH of organic amended soil compared with the control could be due to the fact that during microbial decomposition of the incorporated manures, organic acids may be released, neutralizing the alkalinity of the manures, thereby lowering the pH of the soil below their initial value. However, Wang et al. (2019) showed that long-term manure application increases soil pH and reduces soil acidification. These opposite results may be attributable to the nature of the soil in the study of Wang et al. (2019), which was an acidic soil. Future research should pay more attention to the adjustments of soil pH by organic materials. This is because the previously mentioned studies observed that organic fertilizers may increase the pH of acidic soils and decrease it in alkaline soils.

In a two-year study conducted by Elhaddad, González, Abdelhamid, Garcia-Ruiz, and Chehab (2024), the incorporation of various cover crops into the soil was examined to assess their effects on soil characteristics. Cover

crops were incorporated from 3 to 5 months after sowing different grass and legume species in the field. The cover crops included (1) spontaneous vegetation, (2) oat (Avena sativa), (3) wheat (Triticum aestivum), (4) fenugreek (Trigonella foenum-graecum, a legume), (5) vetch (Vicia sativa, a legume), and (6) a mixture of vetch-oat (50/50%). During the first cropping season, cover crops were cut for animal feed, and only residues were incorporated into the soil. In the second year, all cover crop biomass was incorporated into the soil. The authors found that the increase in soil organic matter in treatments with cover crops was primarily due to the incorporation and decomposition of cover crop residues, along with the cumulative effects during the second cropping season and manure application. Their study showed that soil organic matter in all cover crop treatments and spontaneous vegetation exceeded 2.5%, attributable not only to the dry biomass of cover crops but also to manure application (10 tonnes per hectare). The desirable organic matter content in soil generally ranges between 2 and 3%, depending on soil texture, and can reach up to 6% in most agricultural soils (Stegarescu, Reintam, & Tõnutare, 2021). In summary, the results of Elhaddad et al. (2024) suggest that grass cover crops can positively influence soil organic matter, potentially enhancing soil nutrient status and functional quality in olive farms. Sanchez et al. (2007) also reported increases in soil organic matter in the upper soil layer (0-15 cm depth) after six years of experiments on areas seeded with various cover crops. These increases in the topsoil were 31, 27.9, 23, and 18.6 g kg<sup>-1</sup> in areas covered with Trifolium fragiferum L., alfalfa/fescue, Vicia sativa, and control treatments, respectively. Aranda, Macci, Peruzzi, and Masciandaro (2015) reported that after 17 years of organic management with olive-mill pomace co-compost application, olive grove soils exhibited higher quality than those managed conventionally without co-compost. They observed significant increases in key soil chemical parameters in soils treated with organic amendments. Therefore, applying olive-oil extraction by-products to soils could provide important mid- to long-term agro-environmental benefits, supporting the sustainability of such management practices.

Roussos et al. (2017) found that the application of the organic fertilizers 'Activit' and 'Agrobiosol', nor different types of fertilization, respectively, did not cause significant differences in the levels of soil organic matter in comparison with the control. The non-remarkable changes in soil organic matter registered by these authors were attributed to the short trial period and to high summer temperatures, which accelerated the organic matter decomposition at the studied sites. In two subsequent studied years, Adekiya et al. (2020) also did not find significant differences in the percentage of soil organic matter between the control and the inorganic soil fertilization treatment.

Shelton, Jacobsen, and McCulley (2018) determined that on average, NH4-N tended to be greater in both the inorganic (i.e., urea) and organic (a blended, pelletized, commercial animal byproduct fertilizer) fertilization treatments than in the unfertilized control of corn conservation agroecosystems in Kentucky, USA; nonetheless, they informed that mean comparisons failed to identify specific months during which soil NH4-N significantly differed, finding only that urea N was marginally greater than the unfertilized treatment at the end of autumn.

The dry biomass and the soil organic matter generated by the cover crop play an important role in soil characteristics and nutrient dynamics (Soriano, Cabezas, & Gómez, 2023). The availability of soil macronutrients in the topsoil layer (30 cm depth) is the result of soil organic matter decomposition from the cover crop residues incorporated into the soil (Elhaddad et al., 2024). In their study, during the 2021 and 2022 cropping seasons, the percentages of soil total N, and available P and K were greater in areas where different cover crops were incorporated into the soil than in the control (which was tilled periodically three times per year without intercropping by chisel ploughing soil at 20 cm depth). The percentage increase in nutrient concentrations among the cover crops and the control was cover-crop dependent (Elhaddad et al., 2024). Whether N is mineralized or immobilized greatly depends on the C/N ratio of the organic residues being decomposed (Rodrigues et al., 2013).

High soil available P values were reported in the soils of the plots where wheat rather than the legume species was incorporated into the soil (Elhaddad et al., 2024). Cover crops and spontaneous vegetation significantly increased the soil available K levels compared to those of a control and were correlated with the dry biomass incorporated into the soil (Elhaddad et al., 2024). These authors indicated that the decreased soil K levels during the second season

(2022) can be explained by the low amount of the dry matter decomposition process and the K fraction exported by olive trees. Indeed, some studies have revealed that olive trees export high levels of potassium during their vegetative and fruiting life cycle (Ferreira, Arrobas, Moutinho-Pereira, Correia, & Rodrigues, 2018).

Legume cover crops in general improve soil quality, providing more favorable conditions for the growth, development, and yield of main crops, and playing a significant role in reducing weed infestation (Elsalahy, Döring, Bellingrath-Kimura, & Arends, 2019; Kocira et al., 2020). For uniform coverage of the soil surface, it is critical that the species used as cover crops produce a substantial amount of biomass. The exposure of the soil promotes weed infestation and increases erosion susceptibility, while high C/N ratios can extract nitrogen from the system, reducing its availability to plants (Rizzardi & Silva, 2006).

Herencia (2018) examined the effects of cover crop management on soil chemical parameters across 3 years in samples collected in spring and autumn, at three different soil depths (0–5, 5–10, and 10–20 cm) in an organic olive orchard in SE Spain. The organic management regimes were: an annual cover crop of vetch (Vicia sativa L.) (i) leaving the debris on the soil surface or (ii) mixing the debris into the soil, and (iii) mixing the annual weed cover into the soil. For pH, they found the results were more influenced by seasonal variability than by treatments. In general, the highest values resulted from the use of leguminous cover crops, leaving the crop debris on the soil surface, and the differences were more notable in the uppermost layers of the soil.

Cover crops improve the content of soil organic matter and the availability of nutrients (Da Silva et al., 2021; Duan, Liu, Yang, Tang, & Shi, 2020; Sanchez et al., 2007). Stein, Hartung, Perkons, Möller, and Zikeli (2023) found that cover crops, including natural vegetation, under sustainable management systems had higher biomass production and carbon contents, indicating enhanced carbon storage and a potential increase in soil organic matter. In Tunisia, the use of legumes as a cover crop in olive orchards has been reported to increase the soil organic matter up to 1.5%, three times higher than a control (Chehab et al., 2019). More recently, Reyes-Martín, Fernández-Ondoño, Ortiz-Bernad, and Abreu (2023) tested intercropping olive trees with vetch and a barley-vetch mixture, which resulted in a higher soil organic matter content. Moreover, grass cover crops, when used between olive trees, can act as a sponge to conserve water, prevent flash floods, and increase soil organic matter content, contributing to soil health and potentially mitigating climate change (Feng, Sekaran, Wang, & Kumar, 2021).

Chehab et al. (2019) reported that the use of legumes as cover crops increased the soil N by about 25% as compared to a control plot. Vetch improved the soil nitrate content by over 35% for barley at a 0–20 cm soil depth throughout the studied period (Soriano et al., 2023).

Hindersmann et al. (2023) reported that Paspalum notatum Flüggé (Poaceae), when used as a cover crop in olive orchards, competed with olive trees for mineral forms of nitrogen available in the soil, resulting in decreased growth and nitrogen content in organs of the tested olive cultivars. This effect could be explained, at least in part, by the density of plants in a community, which determines the intensity of competition and the efficiency in exploiting available resources (Li et al., 2020). As plant density increases, competition among plants for soil water and nutrients in the belowground root systems is expected to intensify (Zhai, Xie, Ming, Li, & Ma, 2018).

Long-term cover crops have been attributed to improving soil P bioavailability and increasing the content of total phosphorus, microbial phosphorus, organic phosphorus, and certain forms of inorganic phosphorus in surface soil (Peregrina, Pérez-Álvarez, & García-Escudero, 2014). Cover crops have been shown to significantly impact soil P dynamics. Koudahe, Allen, and Djaman (2022) determined that crop residue additions from cover crops may enhance soil organic C and N accretion, as well as increase the availability of P and K in some soil types under certain climatic conditions.

#### 1.1. Objective

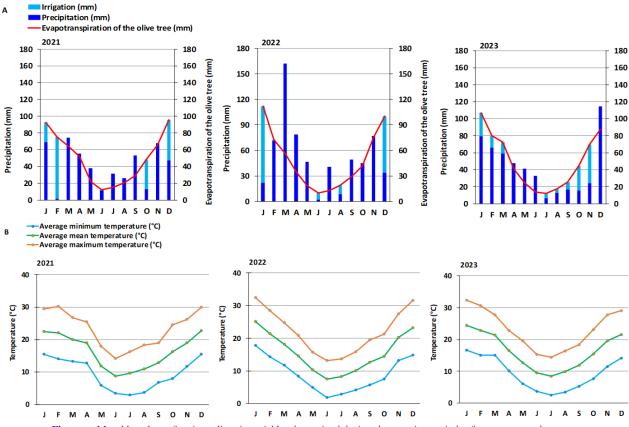
To compare the effects of organic soil, inorganic soil, or foliar fertilization treatments and areas with or without a cover crop during two growing seasons on various soil physicochemical traits in an olive orchard of southwestern

Buenos Aires, Argentina. The results obtained during the first growing cycle (2020/2021) were already published by Busso et al. (2025). Thereafter, this paper reports the results obtained during the second (2021/2022) and third (2022/2023) growing seasons.

#### 2. MATERIALS AND METHODS

#### 2.1. Study Site

The study was conducted in the District of Bahía Blanca, Province of Buenos Aires (38°34′S, 61°59′W) in an olive (Olea europaea L.) orchard under irrigation belonging to the private agropecuarian establishment of "Nobles Caciques" during the growing cycles of 2021/2022 and 2022/2023. Figure 1 shows the values of various climatic variables recorded during the two studied growing seasons by a meteorological station located at the Regional Center for Basic and Applied Research in Bahía Blanca (i.e., CRIBABB). This institution, which is part of CONICET, is 10 km from the study site. Monthly evapotranspiration of the olive crop was calculated following Alegre et al. (2002). Soils are classified as Petrocalcic Kastanozems according to the World Reference Base system (IUSS Working Group WRB, 2015).



 $\textbf{Figure 1.} \ Monthly \ values \ of \ various \ climatic \ variables \ determined \ during \ the \ growing \ periods \ of \ 2021, \ 2022 \ and \ 2023 \ and \$ 

#### 2.2. Land History, and Orchard Establishment and Management

The enterprise "Nobles Caciques" purchased the land in 2000. The land was a natural rangeland at that time, which was replaced by an olive orchard cultivar "Arbequina". Transplanting of rooted cuttings was carried out in 2015, when they were one year old. The trees were approximately five years old at the start of the study in 2020. Spacing among plants was 1.5 m, and spacing among tree rows was 4 m. Therefore, the orchard had a super-high-density (SHD) of 1667 plants ha<sup>-1</sup>. The orchard has been in production since 2021. Fungicides containing Azoxystrobin and Tebuconazole as active ingredients are preventively applied at the end of winter and early spring. Insecticides are not applied, as pests that could affect the olive have not been identified to date.

#### 2.3. Experimental Design

The general experimental design in 2021/2022 and 2022/2023 was a split-plot design. Based on the experimental design utilized by Busso, Rodríguez, and Suñer (2024) in 2020/2021, soil sampling continued in 2021/2022 and 2022/2023, with the samples pooled to reduce costs for chemical analyses. During 2021/2022 and 2022/2023 (Figure 2), soil sampling was conducted across the four seasons of the year: November (spring), January (summer), April (autumn), and June (winter). Since the interactions between Cover and Treatment were either absent or relatively low in the previous period (i.e., 2020/2021: Busso et al. (2024)) for all studied physicochemical properties, including pH, organic matter, ammonium, nitrate, phosphorus, and potassium, soil sampling was divided into two parts during the subsequent growing seasons (i.e., 2021/2022 and 2022/2023). One part studied the effects of the cover crop (Part 1: Figure 2 A), and the other part analyzed the treatment effects (Part 2: Figure 2 B). Part 1: Soil cover and depth were major factors, and months (month x period) were secondary factors. The experimental unit on each date was the six large plots of the original experimental design (Figure 2 A). Since there were 8 trees per subplot at each soil sampling date on each replicate block (8 trees/subplot/sampling date x 4 sampling months/type of cover crop x 2 types of cover crop x 3 replicate blocks = 192 trees), a total of 192 trees were utilized in the studies conducted in 2021/2022 and 2022/2023. Part 2: Treatments and soil depth were major experimental factors, while months (month x period) served as a secondary factor. The experimental units at each date consisted of a mixture of the corresponding subplots, obtained from the two contiguous large plots in the original experimental design (Figure 2 A). During the 2022/2023 growing season, the same 192 trees were utilized, with 4 trees per subplot per sampling date, across 4 sampling dates, 4 treatments, and 3 replicate blocks, totaling 192 trees (Figure 2 B).

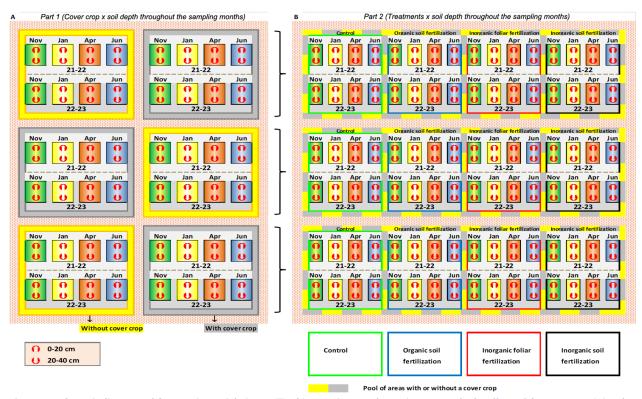


Figure 2. Schematic diagrams of the experimental designs utilized in 2021/2022 and 2022/2023 to study the effects of the cover crop (A) and fertilization treatments (B).

## 2.4. Soil Sampling and Fertilization

A soil sampling was conducted at 0-20 and 20-40 cm soil depths using a soil auger in 2021/2022 and 2022/2023. Soil sampling occurred in November (spring), December (summer), April (autumn), and June (winter) during each of the two studied growing seasons. It must be recognized that soil responses obtained in 2021/2022 might be partly

due to residual effects of the fertilization conducted in 2020/2021 (Busso et al., 2024). In turn, soil responses obtained in 2022/2023 might be due, in part, to the residual effects of the fertilizations conducted in 2020/2021 and 2021/2022. Sampling was made to determine some physicochemical properties of the soil (i.e., pH, organic matter, ammonium, nitrate, available P, extractable K). The major part of the root system is located at this depth, near the wet bulb generated by the drip irrigation system (Medina, 2013). Soil sampling was conducted underneath the canopy of irrigated olive trees. The unit of measurement was one pool of eight samples taken at each of the three replicate points (Busso et al., 2024).

In the laboratory, soil samples taken during each of the studied growing cycles were air-dried and then screened through a 2 mm mesh. The following edaphic properties related to fertility were analyzed: pH (measured in a soil: water ratio of 1:2.5), organic matter, ammonium and nitrate levels, extractable phosphorus (P), and exchangeable potassium (K). The methods used to determine each of these variables were reported by Busso et al. (2024). Table 1 presents the mean values of these edaphic properties in the interrow areas among the olive trees, which were neither fertilized nor seeded with a cover crop during each of the studied growing seasons.

Table 1. Mean edaphic properties taken in the interrow areas among the olive trees which were neither fertilized nor seeded with a cover crop during each of the studied growing seasons. Soil sampling was conducted at two depths (0-20 and 20-40 cm) on four sampling dates (spring, summer, autumn, and winter) in 2021/2022 and 2022/2023. Values for these variables in 2020/2021 were published by Busso et al. (2024). There were three replicates on each sampling date and soil depth. Each value represents the mean of n= 48 for 2021/2022 and 2022/2023.

Period	pН	Organic matter (%)	Ammonium(‰)	Nitrate (‰)	P (ppm)	K (ppm)
2021-2022	6.95	2.95	4.96	3.42	11.76	573.38
2022-2023	6.89	2.82	5.16	4.14	13.02	535.15

The details for making the fertilization treatments (i.e., control, organic and inorganic soil fertilization, inorganic foliar fertilization), the seeding or non-seeding (control) of a cover crop, and the timing for applying these treatments in 2021/2022 and 2022/2023 were reported by Busso et al. (2024) and Busso et al. (2025). Details on the irrigation system were also provided by Busso et al. (2024).

#### 2.5. Statistical Analysis

A four-way ANOVA table (2 green cover types x 4 fertilization treatments x 2 soil depths x 2 dates) was conducted for each factor in 2020/2021 (Busso et al., 2024). At this period, the fertilization treatment × cover crop interactions were not significant or they represented a negligible effect. As a result, these sources of variation (i.e., fertilization treatments and cover crops) were considered separate in the ANOVA tables for the two subsequent studied periods. Because of this, three-way ANOVA tables were conducted during 2021/2022 and 2022/2023 [2 cover crop conditions (with, without) × 2 soil depths × 4 sampling dates (i.e., Part 1) or 4 fertilization treatments × 2 soil depths × 4 sampling dates (i.e., Part 2)]. See the Appendix 1 for more details. When F tests were significant, means were compared using Fisher's LSD test at a significance level of 5%. The program used to conduct the statistical analyses was Infostat (Di Rienzo et al., 2015).

#### 3. RESULTS

## 3.1. Cover Crops X Soil Depths Throughout the Sampling Dates 3.1.1. Soil pH

pH was significantly greater (p<0.05) on the areas without a cover crop than on those with it (Figure 3 A). In addition, there were differences, on average, among the sampling months (Figure 3 B). According to the decomposition of this factor, the differences (p<0.05) were due to the months (Figure 3 B). There was an increase (p<0.05) in pH with the advancement of the growing seasons in 2021/2022 and 2022/2023 (Figure 3 B).

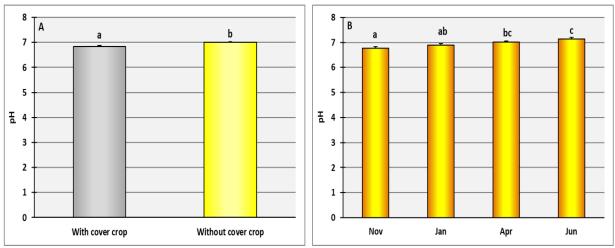


Figure 3. (A) Soil pH in the areas either with or without a cover crop was the average of n=48. (B) Soil pH among months was the average of n=24. Different lowercase letters above the histograms indicate significant differences (p<0.05). Vertical lines on the histograms represent 1 S.E. of the means.

#### 3.1.2. Soil Organic Matter

No evidences of interaction (p>0.05) in the ANOVA table were found for soil depth.

However, organic matter was greater (p<0.05) at 0-20 than at 20-40 cm soil depth (Figure 4 A).

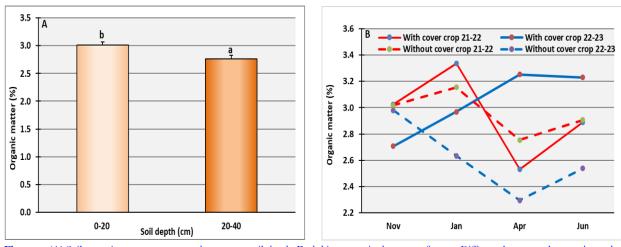


Figure 4. (A) Soil organic matter at 0-20 and 20-40 cm soil depth. Each histogram is the mean of n=48. Different lowercase letters above the histograms indicate significant differences (p<0.05). Vertical lines above the histograms represent 1 S.E. of the means. (B) Variation among months on the areas either with or without a cover crop in 2021/2022 and 2022/2023. Each symbol is the mean of n=6. The S.E. of each mean was 0.16.

There was a significant (p<0.05) interaction date x cover crop. The behavior over time was different, either with or without a cover crop (Figure 4 B). If months are decomposed, the behavior was also not the same in both periods (Figure 4 B). Organic matter values ranged between 2.3% (without a cover crop, April 2022/2023) and 3.3% (with a cover crop, January 2021/2022) (Figure 4 B).

#### 3.1.3. Ammonium

There was neither evidence of interactions nor differences for any of the studied major factors in the ANOVA table. Areas with or without a cover crop had a similar percentage of ammonium (Figure 5).

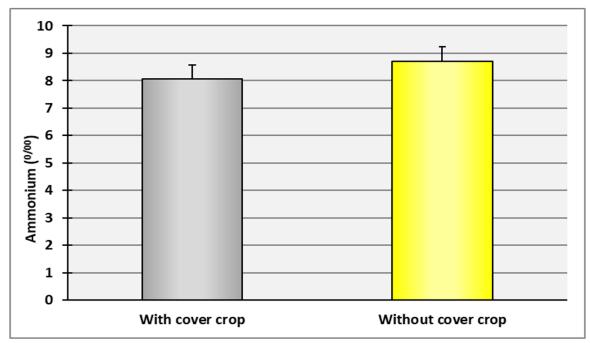


Figure 5. Ammonium concentration (‰) in the areas either with or without a cover crop. Each histogram is the average of n=48 + 1 S.E.

#### 3.1.4. Nitrate

There were differences, on average, among sampling months. According to the decomposition of this factor, the behavior of months was not the same in both periods. Because of this, the interpretations will be made separately for each period. During 2021/2022, soil nitrate concentration (‰) was similar (p>0.05) in November and January (Figure 6). These concentrations were greater (p<0.05) than those in April and June (Figure 6). In these two later months, nitrate concentrations were similar (p>0.05) (Figure 6). Nitrate concentrations were similar (p>0.05) among months in the period 2022/2023 (Figure 6).

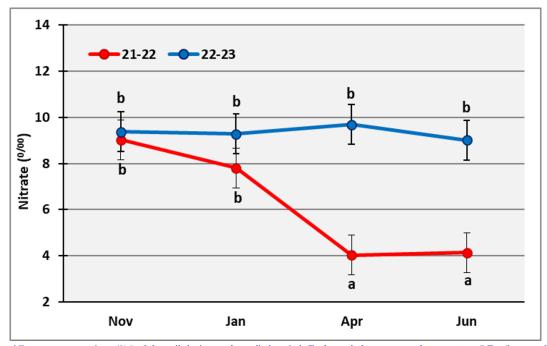


Figure 6. Nitrate concentrations (‰) of the soil during each studied period. Each symbol represents the mean  $\pm$  1 S.E. of n= 12. Different lowercase letters within each studied period (2021/2022 or 2022/2023) indicate significant differences (p<0.05).

#### 3.1.5. Phosphorus

Although there was interaction among the three factors, the results on average between soil depths are not invalidated. Soil phosphorus was greater at 0-20 cm than at 20-40 cm soil depth (Figure 7 A).

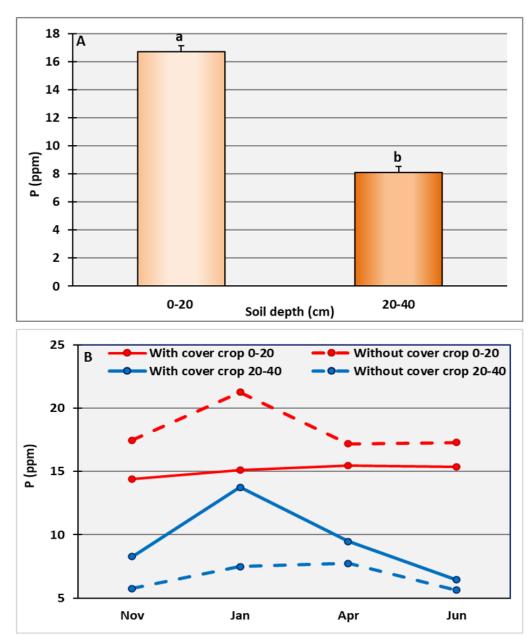


Figure 7. (A) Phosphorus concentration (ppm) at 0-20 and 20-40 cm soil depth. Each histogram is the mean of n=48. Vertical lines indicate 1 S.E. of the means. Different lowercase letters above the histograms indicate significant differences (p<0.05). (B) Variation in soil phosphorus concentration (ppm) among months on the areas either with or without a cover crop in 2021/2022 and 2022/2023 at 0-20 and 20-40 cm soil depth. Each symbol is the mean of n=6. The standard error of the means was 1.30.

The interaction date x cover crop was significant (p<0.05). The behavior with the cover crop was different from that without the cover crop throughout the sampling months (Figure 7 B). If sampling months are decomposed, the behavior was also not the same in both studied periods (Figure 7 B). The P concentration at 0-20 and 20-40 cm soil depths in areas with or without plant cover across different months ranged from 5.64 ppm (without a cover crop, 20-40 cm soil depth, June) to 21.26 ppm (without plant cover, 0-20 cm soil depth, January) (Figure 7 B).

#### 3.1.6. Potassium

Despite the interaction date x soil depth was significant (p<0.05), this does not invalidate the results on average between the two studied soil depths. The concentration of K was greater (p<0.05) at 0-20 than at 20-40 cm depth (Table 2).

**Table 2.** Concentration of K (ppm) at 0-20 and 20-40 cm soil depth. Each mean is the average of n=48. Different letters indicate significant differences (p<0.05) between the studied soil depths.

Treatment	n	К (ррт)	LSD 5%
0-20	48	16,6950	b
20-40	48	8,0783	a

The behavior of depths with respect to the soil K concentrations was different throughout the months (Figure 8). If dates are decomposed, the behavior was also not the same in both periods (Figure 8). Soil K concentration at 0-20 and 20-40 cm depth in 2021/2022 and 2022/2023 ranged between 480.04 ppm (0-20 cm depth, 2022/2023, November) and 687.33 ppm (0-20 cm depth, 2021/2022, November) (Figure 8).

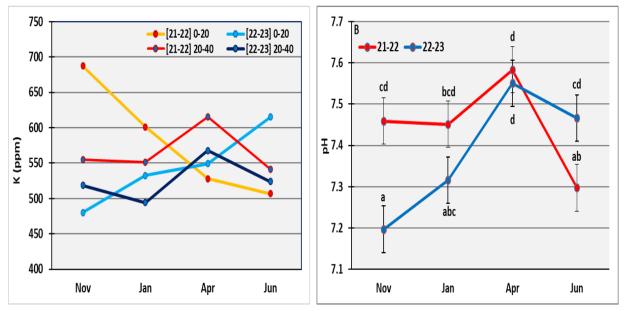


Figure 8. Variation on the different months in the K concentration (ppm) at 0-20 and 20-40 cm soil depth during the periods 2021/2022 and 2022/2023. Each symbol is the mean of n=6. The standard error of each mean was 21.46.

# 3.2. Treatments X Soil Depths Throughout the Sampling Dates 3.2.1. pH

No evidences (p>0.05) of interactions were found in the ANOVA table. However, the control and inorganic foliar fertilization treatments had a greater (p<0.05) pH than the organic and inorganic fertilization treatments for the soil (Figure 9 A). There were significant differences (p<0.05), on average, among sampling months (Figure 9 B). According to the decomposition of this factor, these differences were due to the months and their interaction with the sampling periods. The behavior of the months was not the same in the two studied periods (Figure 9 B). The greatest (p<0.05) pH was obtained in April 2022, and the lowest (p<0.05) in November 2022 (Figure 9 B).

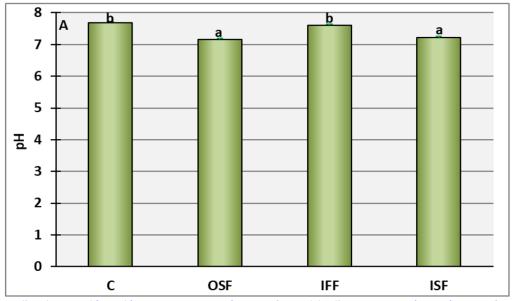


Figure 9. (A) Soil pH in areas with or without a cover crop was the mean of n=48. (B) Soil pH among months was the mean of n=24. Different lowercase letters above the histograms and the symbols indicate significant differences (p<0.05). Vertical lines on the histograms and symbols represent 1 S.E. of the means.

#### 3.2.2. Soil Organic Matter

The percentage of organic matter was greater (p<0.05) at 0-20 than at 20-40 cm soil depth (Figure 10 A). There were differences, on average, among sampling months (Figure 10 B). According to the decomposition of this factor, such differences were due to months and their interaction with the periods [values of organic matter ranged between 2.4 % (April 2022) and 3.2 % (January 2022) (Figure 10 B)]. This interaction was not important enough to invalidate the results on average for the months. The percentage of soil organic matter, on average (n=48), was greater (p<0.05) in November (3.0 %) and January (3.1 %) than in April (2.5 %) and June (2.9 %). Even more, the percentage of organic matter was greater (p<0.05) in June (2.9 %) than in April (2.5 %) (Figure 10 B).

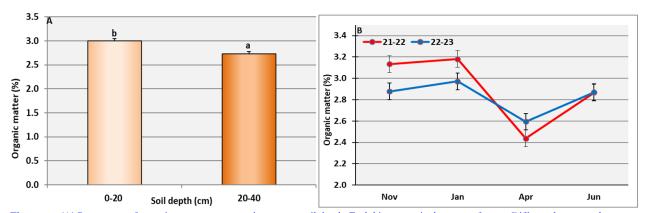
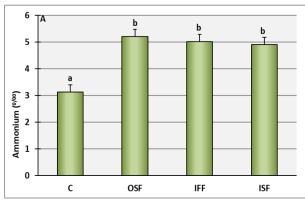


Figure 10. (A) Percentage of organic matter at 0-20 and 20-40 cm soil depth. Each histogram is the mean of n=48. Different lowercase letters above the histograms indicate significant differences (p<0.05). (B)Percentage of soil organic matter at different months in both studied periods. Each symbol is the mean of n=24. The S.E. of the mean was 0.08.

#### 3.2.3. Ammonium

There was a treatment x date interaction (p<0.05) in the ANOVA Table. However, this does not invalidate the results among the treatments on average (Figure 11 A).



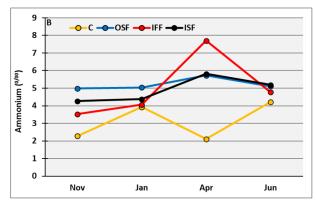


Figure 11. Ammonium concentration (‰) (A) on the different fertilization treatments (each histogram is the mean + 1 S.E. of n=48) and (B) on the different months in the studied fertilization treatments. Each symbol is the mean of n=12. The S.E. of the symbols was 0.53. Different letters above the histograms indicate significant differences (p<0.05) among the fertilization treatments.

The lowest (p<0.05) ammonium concentration was obtained in the control treatment (Figure 11 A). Ammonium concentrations were similar (p>0.05) in the soil organic and inorganic, and in the leaf inorganic fertilization treatments (Figure 11 A). At different months and across various fertilization treatments, the ammonium concentration ranged from 2.1 (control, April) to 7.7 % (inorganic leaf fertilization, April) (Figure 11 B).

#### 3.2.4. Nitrate

Although there was an interaction between treatments at x months (p<0.05), this did not appear to invalidate the results among treatments on average (Figure 12A).

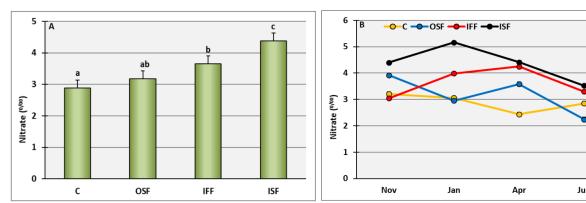


Figure 12. Nitrate concentration (%) among the different treatments (A), and months in such treatments (B). Histograms are the average + 1 S.E. of n=48. Symbols are the mean  $\pm$  1 S.E. of n=12. Different lowercase letters above the histograms indicate significant differences (p<0.05) among the different treatments.

Nitrate concentration was similar (p>0.05) in the control and the organic soil fertilization (Figure 12A). However, nitrate concentration in the control was lower (p<0.05) than that obtained in the inorganic leaf and soil fertilization treatments. The greatest (p<0.05) nitrate concentration was shown in the inorganic soil fertilization treatment (Figure 12A). Nitrate concentrations in the different months and treatments ranged between 2.2 % (organic soil fertilization, June) and 5.2 % (inorganic soil fertilization, January) (Figure 12 B).

## 3.2.5. Soil Phosphorus

Even though there was an interaction (p<0.05) among the three factors, this does not invalidate the results on average among the treatments. The lower (p<0.05) soil P concentration was obtained in the control and in the inorganic leaf fertilization treatment (Figure 13 A). Phosphorus concentration in these treatments was lower (p<0.05) than that in the organic and inorganic soil fertilization treatments (Figure 13 A). The greatest (p<0.05) soil P concentration was obtained in the inorganic soil fertilization treatment (Figure 13 A). Despite the greater mean values

obtained in the organic and inorganic soil fertilization treatments at 0-20 and 20-40 cm soil depths, the magnitude of differences was lower at 20-40 cm soil depth (Figure 13 B). Values of phosphorus concentration in the different treatments at both studied soil depths ranged between 43.16 ppm (control, 0-20 cm soil depth) and 73.49 ppm (inorganic soil fertilization, 0-20 cm soil depth) (Figure 13 B).

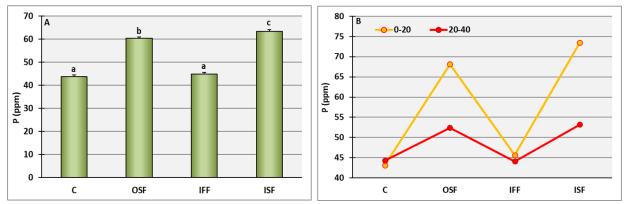
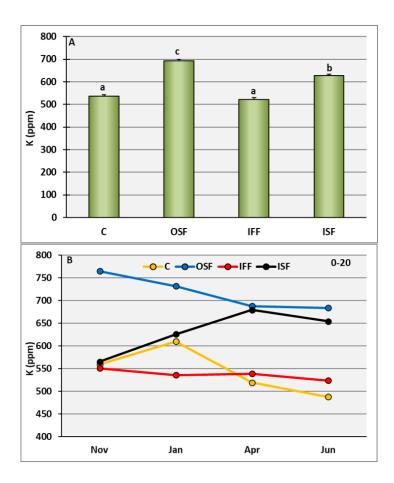


Figure 13. (A) Phosphorus concentration (ppm) in the different treatments in 2021/2022 and 2022/2023. Each histogram is the mean of n=48. Vertical lines on the histograms are  $\pm$  1 standard error of the mean. Different lowercase letters on the histograms indicate significant differences (p<0.05). (B) Phosphorus concentration (ppm) among the treatments at 0-20 and 20-40 cm soil depth. Each symbol is the mean of n= 24.

#### 3.2.6. Soil Potassium

Despite an interaction occurring among the three factors, this did not invalidate the results among treatments on average (Figure 14).



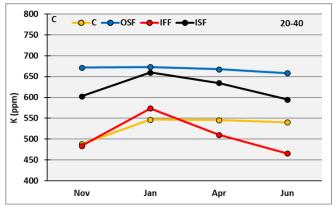


Figure 14. K concentration (ppm) (A) among the different treatments. Each histogram is the mean of n=48. Vertical bars above the histograms are + 1 standard error of the mean. Different lowercase letters above the histograms represent significant differences (p<0.05) among the treatments. Variation in the K concentration among months in the different treatments at 0-20 (B) and 20-40 cm soil depth (C). Each symbol is the mean of n=6. The standard error of each mean was 19.01.

The lowest (p<0.05), but similar (p>0.05), K concentration (ppm) was shown in the control and inorganic soil fertilization (Figure 14 A). The highest K concentration was observed in the organic soil fertilization treatment (Figure 14 A). The K concentration was greater in the organic than in the inorganic fertilization treatment (Figure 14 A).

The behavior of treatments was not the same throughout the months at both soil depths (Figures 14 B and C). The potassium (K) concentration ranged from 487.65 ppm (control, June) to 764.92 ppm (organic soil fertilization, November) at a soil depth of 0-20 cm across different treatments and months (Figure 14 B). At 20-40 cm soil depth in the different treatments and months, the K concentration ranged between 465.67 ppm (inorganic leaf fertilization, June) and 673.10 ppm (organic soil fertilization, January) (Figure 14 C).

### 4. DISCUSSION

The cover crop is a conservation practice that can improve soil conditions among the rows in an olive orchard (Koudahe et al., 2022). This can be achieved by improving the soil fertility and health (Da Silva et al., 2021; Duan et al., 2020).

#### 4.1. pH

In 2021/2022 and 2022/2023, soil pH was greater in areas without a cover crop than in those with it (Figure 3 A). However, the study showed that soil pH increased as the rates of application of crop residues (Vicia villosa Roth and Avena sativa L.) to the soil also increased. Wang et al. (2019) reported that long-term manure application increased soil pH and reduced soil acidification. These results may be attributable to the nature of the soil in the study of Wang et al. (2019), which was an acidic soil, while in the present study, it was characterized as soil with a pH of approximately 7 (Table 1). Use of cover crops in olive orchards showed the beneficial impacts that vegetation among tree rows can provide through improving the soil properties. For example, Keesstra et al. (2016) reported that elimination of spontaneous vegetation in olive orchards is an undesirable management practice. These authors showed that use of spontaneous vegetation as a cover crop is a good alternative to improve soil properties. On the other hand, Hindersmann et al. (2023) reported that Paspalum notatum Flüggé (Poaceae) when used as a cover crop in olive orchards, competed with olive trees for mineral forms of N available in the soil, resulting in decreased growth and N content in organs of the tested olive cultivars. This effect could be explained, at least in part, by the density of plants in a community, which determines the intensity of competition and the efficiency in exploiting available resources (Li et al., 2020). As plant density increases, competition among plants for soil water and nutrients in the belowground root systems is expected to intensify (Zhai et al., 2018).

Roussos et al. (2017) reported that the application of the organic fertilizer 'Activit' significantly reduced the soil pH in comparison with the control. This is similar to our results obtained in 2021/2022 and 2022/2023 Part 2 (Figure 9 A), and those reported by Busso et al. (2024). This reduction was likely due to the ammonium nitrification and the production of organic acids (phenolic and carboxylic groups) during the decomposition of the organic materials (García-Ruiz et al., 2012). Adekiya et al. (2020) also reported a lower pH in soil fertilized with an organic amendment compared to the control; they suggested that during the microbial decomposition of the incorporated manures, organic acids could be released, which neutralized the alkalinity of the manures and lowered the soil pH. Adekiya et al. (2019) observed a similar trend in their work on organic amendments to the soil. The lower pH in the inorganic fertilization treatment (Figure 9 A) could be due to the leaching of bases from the soil surface. Adekiya et al. (2020) reported a reduced pH in the inorganic fertilization treatment compared to the control, similar to our results (Figure 9 A) and those of Busso et al. (2024).

Soil pH increased from mid-spring to early winter in 2021/2022 and 2022/2023 Part 1 (Figure 3 B) and 2022/2023 Part 2 (Figure 9 B). In southwestern Buenos Aires, rainfall is lower in winter than in spring (Figure 1). At least part of the pH increments and their regional variation could be explained by lower sulfur deposition from the atmosphere through precipitation (Kirk, Bellamy, & Lark, 2010).

#### 4.2. Soil Organic Matter

In agreement with the results of Busso et al. (2024) at the same study site as ours in 2020/2021, we did not find significant differences among fertilization treatments in the percentage of soil organic matter in 2021/2022 and 2022/2023. Similarly, Roussos et al. (2017) determined that the application of the organic fertilizers 'Activit' and 'Agriobiosol' did not cause significant differences in the soil organic matter levels in comparison with the control, which could be partially attributed to the short-term duration of the investigation. This was also indicated by the work of García-Ruiz et al. (2012), who also reported that the high summer temperatures at their study site would accelerate the organic matter decomposition. Gosling and Shepherd (2005) also did not find significant differences in the soil organic matter with different types of fertilization. Additionally, and in agreement with our results, Adekiya et al. (2020) did not find significant differences in the percentage of soil organic matter between the control and the inorganic fertilization treatments in their two studied years.

The soil surface layer (0–20 cm) had a greater percentage of soil organic matter than at 20–40 cm soil depth in 2021/2022 and 2022/2023 Part 1 (Figure 4), and in 2021/2022 and 2022/2023 Part 2 (Figure 10). Similar results were reported by Busso et al. (2024) in 2020/2021 in the previous study year at the same study site. The variation in the soil organic matter percentage throughout months was not consistent in the areas either with or without a cover crop in the periods 2021/2022 and 2022/2023, Part 1 (Figure 4 B). However, Sanchez et al. (2007) determined increments in soil organic matter after 6 years on areas seeded with various cover crops. These increments in the surface soil layer were 31.0, 27.9, 23.0, and 18.6 g kg<sup>-1</sup> on the areas covered with Trifolium fragiferum L., alfalfa/festuca, Vicia sativa L., and the control, respectively. In that region, the control was natural vegetation of grasses and legumes with the soil tilled twice at the end of winter, which is the conventional management system used by the farmers in the region.

In November and January 2021/2022 and 2022/2023, mean monthly temperatures were greater than those in April and June (Figure 1). The greater temperatures in mid-spring and early summer than in mid-autumn and early winter could help to explain the greater organic matter percentages during the warmer than the cooler seasons (Figure 10). Kirschbaum (1995) suggested that a 1°C increase in temperature could ultimately lead to a loss of only 3% of soil organic C for a soil at 30°C, whereas the same temperature increase would lead to a loss of over 10% of soil organic C in regions of the world with an annual mean temperature of 5°C.

#### 4.3. Ammonium

In 2021/2022 and 2022/2023 Part 1, significant differences were not found in ammonium concentrations when organic and inorganic fertilizers were applied on the areas either with or without a cover crop between the olive tree rows in any of the sampling dates and studied soil depths. This result is similar to that reported by Busso et al. (2024) in 2020/2021 at the same study site. Climatic conditions at the study site might have played a role in the mineralization of soil organic matter (Brunetto et al., 2018) and the decomposition of plant residues (Leon et al., 2015) since temperature and rainfall distribution varied during such experimental periods (Figure 1).

The lower ammonium concentration in 2021/2022 and 2022/2023 Part 2 was obtained in the control. The results obtained in these years (Figure 11 A) are similar to those reported by Shelton et al. (2018). These authors determined that, on average, NH4-N tended to be greater in the inorganic (urea) and organic (a commercial animal by-product) fertilization treatments than in the unfertilized control in agroecosystems of maize conservation in Kentucky, USA. However, they reported that the average comparisons were unable to identify specific months during which the NH4-N concentration differed significantly. This is similar to our results in 2021/2022 and 2022/2023 (Figure 11 B). In their study, N as urea was only marginally greater than the unfertilized control by the end of autumn.

#### 4.4. Nitrate

In disagreement with our results in 2021/2022 and 2022/2023 (Figure 12 A), Shelton et al. (2018) found that during the warm season, soil nitrate concentrations were greater in the organic fertilization treatment (a by-product of commercial animal fertilizer) than in the unfertilized control on areas where Vicia villosa was used as a cover crop in agroecosystems of maize conservation in Kentucky, USA. However, in agreement with our results (Figure 12 A), these authors showed that soil nitrate concentrations were greater in the inorganic fertilization treatment (i.e., urea) than in the unfertilized control in areas where Triticum aestivum was used as a cover crop. Angle, Gross, Hill, and McIntosh (1993) also found that soil nitrate concentration in one of the studied years was more than three times greater in the soil inorganic fertilization than in the control on a Zea mays L. culture.

Similar to our results in 2021/2022 (Figure 6), other authors (Sanchez et al., 2007) reported that nitrate concentrations remained below 7.5 mg kg<sup>-1</sup> during the winter months in all treatments, and then increased in spring and summer. A significant increase in soil nitrate, up to 100 ppm, was observed at the end of spring in areas seeded with Vicia villosa due to the rapid biomass decomposition after the maturation of this species. These authors concluded that even with the use of permanent cover crops, it is necessary to add organic fertilizers to sustain tree yield and vigor.

## 4.5. Phosphorus

In agreement with the results of this study (Figure 7 B), Beniaich et al. (2023) showed similar (p>0.05) concentrations of available P at 0-5 cm soil depth when the olive plantation was interspersed with spontaneous vegetation than when it was made on uncovered soil, and only uncovered soil in their first studied year. Also, Roussos et al. (2017) determined similar soil P concentrations at different sampling months during the whole year, similar to that obtained in 2021/2022 and 2022/2023 (Figure 7 B).

This study demonstrated that the application of organic fertilizer and inorganic soil fertilization treatments resulted in a significantly higher P concentration than the control and inorganic foliar fertilization treatment in the 2021/2022 and 2022/2023 periods, on average for the soil sampling dates (November, January, April, June), cover crop, and soil depths (Figure 13 A). A greater availability of nutrients in the soil could be determined because of the organic amendment and the inorganic soil fertilizer increased the soil P concentrations in comparison to the other treatments (Figure 13 A). Roussos et al. (2017) and Adekiya et al. (2020) also determined a greater soil P concentration after application of various organic fertilizers than in the control. The application of organic material can increase P availability through an effective reduction of P adsorption to the soil and an increase in P solubility by the organic acids present in the organic amendment (Sanyal & DeDatta, 1991).

Phosphorus concentrations were greater at 0-20 than 20-40 cm in 2021/2022 and 2022/2023 Part 1 (Figure 7 A).

#### 4.6. Potassium

Similarly, for P, soil exchangeable K concentration was greater in the soil organic and inorganic fertilization treatments than in the control and the inorganic foliar fertilization treatment (Figure 14 A). Roussos et al. (2017) reported a greater soil K concentration after applying the commercial organic fertilizer 'Activit' in comparison to the unfertilized control in early and late winter, and mid-spring. This is similar to the results obtained by Adekiya et al. (2020) when they compared various organic fertilizers with the control in relation to the soil K concentrations; their results were similar to ours. The organic fertilizer significantly increased soil K concentration compared to other treatments on average across all sampling dates, soil depths, and cover crop types (i.e., with or without) (Figure 14 A). Adekiya et al. (2020) reported that the significant increase in soil K concentration in the soil organic treatment compared to the inorganic fertilization treatment was due to leaching of K in the inorganic fertilization treatment.

An efficient tool for monitoring soil management systems can be the evaluation of the impact of cover crops and the type of aggregate on the soil (for example, organic material, foliar fertilization, or inorganic soil fertilization) on chemical soil properties. It is expected that additional studies will improve our understanding of olive orchard ecosystems. Also, the evaluation of soil indicators, the biological impact of cover crops, the effects of cover crops on multiple ecosystem services, and the integration of socio-economic evaluations must also be determined in future studies (Castellano-Hinojosa & Strauss, 2020; Gomez & Soriano, 2020; Rodríguez Sousa, Barandica, Sanz-Cañada, & Rescia, 2019; Shackelford, Kelsey, & Dicks, 2019).

#### 5. CONCLUSIONS

This study demonstrated that soil pH increased on average from mid-spring to mid-autumn during 2021/2022 and 2022/2023 (Figure 3 B) and in 2022/2023 (Figure 9 B). The soil organic matter (Figure 4 A and 10 A), available P (Figure 7 A), and extractable K (Table 2) were greater at 0-20 cm than at 20-40 cm soil depth. In 2022/2023, the percentage of soil organic matter appeared to be greater in areas with a cover crop than in areas without a cover crop from the early summer to the early winter period (Figure 4 B). This suggests the advantage of establishing a cover crop between the rows of the olive plants as a result of the increase in organic matter. Additionally, soil concentrations of ammonium, nitrate, P, and K were either similar or most often greater, but never lower, at the organic and inorganic soil fertilizations than at the inorganic foliar fertilization (Figure 11 A, 12 A, 13 A, and 14 A). This indicates that farmers should take advantage of organic and/or inorganic soil fertilizations rather than continuing to use inorganic foliar fertilizations in their olive orchards. Also, these three types of fertilization showed greater soil ammonium, nitrate, phosphorus, and potassium concentrations than the unfertilized control (Figure 11 A, 12 A, 13 A, and 14 A). Higher potassium concentrations in the fertilization treatments indicated above appeared to be greater from midautumn to early-winter at 0-20 cm soil depth, and from mid-spring to early-winter at 20-40 cm soil depth (Figure 14 B and C). The soil ammonium (Figure 11 B) and nitrate concentrations (Figure 12 B) also appeared to be greater in the organic and/or inorganic soil fertilizations than in the unfertilized control from mid-spring to early-winter. Finally, soil available P concentrations appeared to be greater in the vegetation uncovered areas than in those with a cover crop at 0-20 cm soil depth (Figure 7 B). This is because the roots of the olive trees and those of the cover crop might compete for soil resources (e.g., water and nutrients), which could result in lower soil nutrient concentrations in areas close to the tree stems.

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**Competing Interests:** The authors declare that they have no competing interests.

**Authors' Contributions:** All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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#### Appendix 1. Statistical analysis.

In the general tables of variance (THREE-WAY ANOVAs) = (1) 2 cover crop types (with, without) x 4 sampling months x 2 soil depths, and (2) 4 fertilization treatments x 4 sampling months x 2 soil depths, tests for each factor (plant cover or treatment, sampling date, and soil depth) and their interactions should be tested with their corresponding error mean squares (MS). However, because all error mean squares resulted very similar in the general table of variance of each studied factor, it was tested whether those error mean squares were statistically equal using the Bartlett test. Since the error mean squares for all factors were statistically equal (p>0.05), a weighted mean square error was used for all factors with the total degrees of freedom (=62 when studying the effects of the soil cover crop, and =126 when studying the effects of treatments). These weighted mean square errors, along with their degrees of freedom, were used to perform the new THREE-WAY ANOVA Tables 1 and 2 for each factor.

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