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Can Olive Pruning Forms Influence the Olive Rhizosphere? The Root Microbiota and the Rhizosphere Properties in the Alto Ricaurte (Colombia)

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Citation: García-Molano, J.F.; Cuervo-Bejarano, W.J.; Rodolfi, M.; Jaramillo-García, L.S.; Ganino, T. Can Olive Pruning Forms Influence the Olive Rhizosphere? The Root Microbiota and the Rhizosphere Properties in the Alto Ricaurte (Colombia). *Agronomy* **2022**, *12*, 1159. https://doi.org/10.3390/agronomy12051159

Academic Editors: Giacomo Ferretti and Barbara Faccini

Received: 15 April 2022
Accepted: 9 May 2022
Published: 11 May 2022

Abstract: (1) Background: Olive in Colombia is not a traditional crop, but in the Andean Region, ancient olive trees are widespread. The area is characterized by a climate condition with a high intensity of UV rays and meteoric events that negatively affect the olive grown. In this work, changes in the soil of olive trees subjected to different pruning will be established. (2) Methods: Olive trees of 2-years-old were cultivated in Boyacá (Colombia). Trees were pruned into a vase shape, globe shape, and natural shape. Physical, chemical, and biological soil analyses were carried out. (3) Results: In the olive tree, V and G pruning significantly increase the P content in the soil compared to NS, and these pruning forms reduce the OOC significantly in the rhizosphere soil by 87.5% and 78.3%, respectively. In all conditions, the roots established an association with Arbuscular Mycorhizal Fungi and stimulated the presence of other microorganisms, despite the trees being more vegetative than productive in this latitude. (4) Conclusions: The results of the study indicate that, in Colombian conditions, the pruning does not affect the rhizospheric soil conditions.

Keywords: *Olea europaea* L.; symbiosis; physical soil properties; chemical soil properties; nitrogen-fixing; enzymatic activity

1. Introduction

Olive cultivation in Colombia is not a traditional practice. Nevertheless, in the Andean region (over 2200 m above sea level), it is possible to find mature olive trees with more than 3 m in trunk circumference, and heights of 5 m high or more. In some orchards, the trees are not pruned, presenting a branched and dense canopy without fruit production [1]. Some olive cultivars from Portugal, Spain, and Italy have been propagated, but have not been identified biologically or morphologically; their phenological behavior is partially determined under the edaphic and climatic conditions of the high-Andean tropics, where the induction and floral differentiation occur at different times of the year [2]. Contrary to the Mediterranean climate, in the high-Andean tropic conditions, there are no seasons, the light period is much longer and constant (approximately 1614 h/year), there are high levels of UV-B radiation, and large temperature differences on the same day (with an average day

and night temperature of 26 and 7 °C, respectively), factors that probably cause the vertical growth of the olive tree branches while remaining it vegetative and, therefore, the floral induction season is uncertain [3]. Apparently, under these conditions, floral differentiation is mainly determined by rainfall [4].

The olive tree is a day-neutral plant and the flower bud induction and subsequent flower differentiation is a process influenced mainly by temperature [5], radiation [6,7], nutrition [8–10] and phytohormones concentrations, such as GA (gibberellins), ABA (abscisic acid), and certain cytokinins [11–13]. In seasonal climate conditions, olive trees show an alternate bearing, that is, the presence of "on" and "off" years, which means that the developing fruits during the summer have an inhibitory effect on the flowering of the next season [14–16]. In this way, the flowering intensity is inversely related to the previous year's yield.

Pruning is commonly done during winter by removing the branches that were productive in the previous season, and, therefore, promoting flowering in the branches that were induced at that time. This practice influences canopy characteristics, such as leaf area density, angular spatial distribution, and porosity [17–19] which will affect physiological processes, such as nutritional balance, hormonal regulation, the rate of photosynthesis and transpiration, and source–sink relationship [20,21], as well as the alternate bearing, homogenize fruitlet distribution, and improve the oil quality [22,23]. By changing the hormonal balance between ABA/GA, pruning can promote the association with arbuscular mycorrhizal fungi (AMF) [24,25] and thus survive under stress.

Associations with AMF could increase water availability and low mobility nutrients [26–28], promote growth [29] and improve resistance to diseases and pests [30–33]. Root colonization by AMF is affected by soil conditions, such as organic matter (OM), nitrogen, phosphorous, humidity, and pH [34,35]. Soil OM, due to its biochemical composition (proteins, carbohydrates, resins, fatty acids, among others), is a source of nutrients for the soil organisms that participate in element cycling and mineralization [36–38]. Organic soil amendments are common practice; however, it is recommended to take into account the origin of the material, the type of organisms present, and the content of organic molecules and elements to avoid nutritional imbalances in soil that could affect plants [39,40].

During the OM transformation, humic substances are produced; these molecules with high resistance to biochemical degradation and with a variable structure according to their origin, play an important role in the soil aggregate stability, the cation exchange capacity (CEC), the control of acidity, the nutrient recycling, and fertility [41,42]. In this way, the relationship between microorganisms and plants can affect soil characteristics.

The indigenous OM added to a soil increases their population and activities, as they found adequate quantities of resources, such as nutrients, water, and temperature. In these conditions, the OM mineralization rate is higher [43], enhancing soil–plant processes and relationships, for example, symbiosis, and root exudates released, which maintain microbial populations in the rhizosphere. In this context, pruning influences vegetative response and flower induction, but it is not known how it alters the rhizosphere soil. Moreover, in the tropics, the plant diversity, development, and behavior are different compared to plants in seasonal conditions [2,44,45]. For this reason, this research aimed to evaluate the changes in the physical, chemical, and biological properties of the rhizosphere soil of olive trees cv. 'Picual', pruned into three different shapes in high-Andean Colombian tropic conditions.

2. Materials and Methods

2.1. Experimental Area and Plant Material

This experiment is a part of research that began in 2012, with the aim to understand olive trees' behavior in high-Andean Colombian tropic edaphoclimatic conditions [2,44,45].

The research was carried out in the Huerto Olivanto orchard, located in Sutamarchán municipality, Boyacá, Colombia, in the Alto Ricaurte zone, summoned on the highland mountain chain of the Andes (5°37′14″ N, 73°37′17″ W, 2150 m above sea level). The soil has a limited pedological development with an ochric epipedon and a cambic endopedon. The

Ap, Bw, and C1 horizons present a high saturation of Ca^{2+}, Mg^{2+}, and low K^{+} concentration; the Ap horizon is fine textured with the presence of fine to medium gravels in the range of 15% to 35%, low OM content, and an average infiltration rate of 26 cm h^{-1}. The rooting space is characterized by a fine subangular block structure, being exceptionally fine in Ap, medium laminar in Bw, and unstructured in C1 (Table 1).

Table 1. Physical and chemical properties of the soil in the experimental area before orchard establishment. Data provided by Universidad Pedagógica y Tecnológica de Colombia (UPTC).

Variable	Depth of Horizon (cm)		
	Ap (0–12)	**Bw (12–27)**	**C1 (27–52)**
Sand (%)	22.88	16.88	6.88
Silt (%)	34.00	38.00	40.00
Clay (%)	43.12	45.12	53.12
Textural class	Ar	Ar	Ar
Pd (g cm^{-3})	2.65	2.65	2.65
Bd (g cm^{-3})	1.61	1.65	1.69
Tpor (%)	39.25	37.74	36.23
pH	4.76	4.80	4.93
EC (dS m^{-1})	1.19	0.98	0.80
Exchangeable acidity (Al^{3+}) ($cmol_c$ kg^{-1})	0.30	0.30	0.30
OOC (%)	0.67	0.53	0.53
OM (%)	1.15	0.92	0.92
TN (%)	0.06	0.05	0.05
P (mg kg^{-1})	5.46	6.86	3.52
Ca^{2+} ($cmol_c$ kg^{-1})	9.40	7.50	8.80
Mg^{2+} ($cmol_c$ kg^{-1})	3.36	3.04	3.02
K^{+} ($cmol_c$ kg^{-1})	0.15	0.08	0.10
Na^{+} ($cmol_c$ kg^{-1})	1.02	1.37	1.19
ECEC ($cmol_c$ kg^{-1})	13.93	11.99	13.11
Fe^{2+} (mg kg^{-1})	50.00	53.00	49.00
Mn^{2+} (mg kg^{-1})	0.94	0.00	0.00
Cu^{2+} (mg kg^{-1})	0.18	0.00	0.00
Zn^{2+} (mg kg^{-1})	2.04	1.76	1.76
Ca/Mg	2.80	2.47	2.91
Ca/K	62.67	93.75	88.00
Mg/K	22.40	38.00	30.20
Ca^{+}Mg/K	85.07	131.75	118.20

Pd: particle density, Bd: bulk density, Por: total porosity, EC: electrical conductivity, OOC: oxidizable organic carbon, OM: organic matter, TN: total nitrogen, ECEC: effective cation exchange capacity.

Between 2012 and 2016, the climate had low variability; on average, the diurnal, and nighttime temperatures ranged between 20–26 °C, and 7–9 °C, respectively; rainfall was 900 mm $year^{-1}$, and a photoperiod of 12.5 h day^{-1}.

In 2012, 1-year-old olive trees cv. 'Picual' were planted in 40 cm × 40 cm × 40 cm holes filled with a substrate consisting of a 70:30 mixture of soil and organic matter (manure and chaff), 1 kg of 15-15-15 fertilizer (NPK), and 1 kg of phosphate rock $Ca(PO_4)OH$. The plants received water only from rainfalls (Figure 1). In 2016, unpruned trees of about 3 m in height were pruned up to 2 m in height to induce bud sprouting.

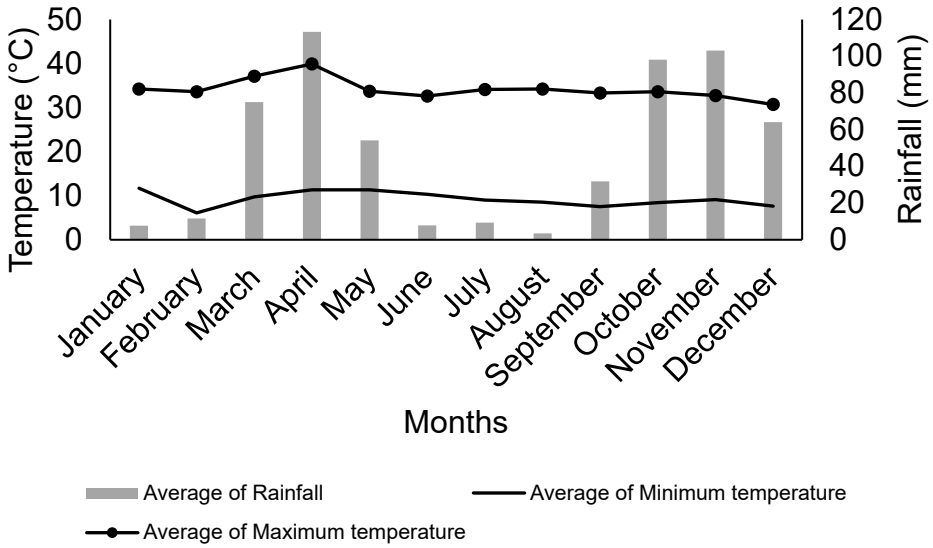

Figure 1. Monthly average temperature (line) and rainfall (bars) behavior in the experimental area for the 20-year period between January and December.

2.2. Experiment Design

A completely randomized design was used with three treatments corresponding to the tree pruning types into a vase shape (V), globe shape (G), and natural shape (NS) (Figure S1) with eight repetitions per treatment, where the experimental unit was a tree.

2.3. Soil Sampling

Rhizosphere samples were taken before the rainy season, about 105 days after pruning (dap). To classify the soil and determine changes in the rhizosphere, a trial pit was used. In the orchard, a soil sample of 500 g was taken from the tree drip line at a depth of 30 cm to determine its physical and chemical properties. For the quantification of the spores, at a depth of 20 cm, 200 g samples from the rhizosphere and 10 sub-samples of 2 g of young secondary and tertiary roots with absorbent hairs were taken. For the quantification of microorganisms at a depth between 10 and 20 cm, a 200 g sample was taken and refrigerated to be transported to the laboratory.

2.4. Physical and Chemical Soil Properties

The soil texture and structure were determined by using the Bouyoucos-hydrometer method and the wet sieving method [46,47], respectively. Particle density (Pd) was measured utilizing the pycnometer method [48], and the bulk density (Bd) by the core method [48] and was used to calculate the total porosity (Por). The coefficient of linear expansion (CLE) was determined following the IGAC methodology [48]. The consistency was calculated based on liquid (LL) and plastic limits (PL), and the color according to a Munsell table [49]. Soil pH was determined using the 1:1 soil to water ratio, and the exchangeable acidity (Al^{3+}) was extracted by KCl 1 N. The OM content was quantified by the Walkley–Black method [50], while available phosphorus was via the Bray II—Colorimetry method. The Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} contents were by ammonium acetate extraction and atomic absorption; electrical conductivity (EC) by saturation extract (conductivity meter), and the effective cation exchange capacity (ECEC) was calculated by summing cations.

2.5. Mycorrhizal Fungal Spore Counting, Nitrogen-Fixing, Cellulolytic, Phosphate Solubilizers Microorganisms, and Enzymatic Activity

For the spore's extraction, the method described by Sieverding [51] was used. Root processing was performed according to the staining method described by Posada and coworkers [52]. The percentage of total AMF colonization or inoculum (I) was calculated

as the quotient between the number of fields colonized by any AMF structure and the total number of observed fields, multiplied by 100.

Nitrogen-fixing microorganisms quantification was carried out following the Dobereiner and Day [53] protocol using a culture medium composed of KH_2PO_4, $MgSO_4$, $7H_2O$, NaCl, $CaCl_2$, $FeCl_3$, MoO_4Na $2H_2O$, malate, bromothymol blue, and agar–agar. Each sample was inoculated in triplicate and the counting was performed by using UV. For cellulolytic microorganisms counting, first, the soil samples were diluted with 0.1% peptone water up to 10^{-5}, inoculated in 1% carboxymethyl cellulose (CMC) agar culture medium, and incubated at 35 °C for 48 h, then, inoculated again in 1% CMC agar culture, and finally, using the Congo red tests, the colonies with larger hydrolytic haloes were selected [54]. The isolation of phosphate-solubilizing microorganisms was performed by incubating 500 mL from the sample with 250 mL of semolina and 50 mL of molasses. Then, the microorganisms were separated from the solid and liquid phases following the serial dilution method, using the culture medium suggested by Osorio and Habte [55], enriched with finely ground phosphate rock (from 90 to 500 µm). The next day, the colonies that produced a yellow halo were isolated.

The phosphatase activity was measured according to the *p*-nitrophenyl phosphate method [56], and the cellulase activity was determined by the 3,5-dinitrosalicylic acid (DNS) method [57].

2.6. Data Analysis

Continuous response variables (MWD, Pd, Bd, CLE, pH, P, Ca^{2+}, Mg^{2+}, K^+, Na^+, Al^{3+}, EC, ECEC, Pho, and Cel) were analyzed by ANOVA or by the Kruskal–Wallis test if the homogeneity of variance and normality of residuals assumptions were violated. Count response variables (Spo, cel.µO, Nf, Ps) were modeled with negative binomial regressions, and, for the proportional response variables (Por, OM, I), a beta regression with the logit link function was conducted [58]. Means comparison of the count and proportion variables were analyzed with least-square means [59].

A principal component analysis (PCA) was used to reduce the dimensions of the data through the correlation between the physical, chemical, and microbiological variables measured in the rhizosphere soil samples in each treatment. Correlations were analyzed using Kendall's rank method for non-normal data [60] with Bonferroni-adjusted *p*-values, and a significance level of 0.05. The relationship between the cellulase activity (Cel) and percentage of inoculum (I), phosphate-solubilizing microorganisms (Ps) and nitrogen-fixing microorganisms (Nf), and the effective cation exchange capacity (ECEC) and calcium content (Ca^{2+}), were modeled by simple linear regressions. All statistical analyses were performed using the RStudio software [61] and FactoMineR [62], ggplot2 [63], corrplot [64], MASS [65], and betareg [58] packages. Figures showing inferential statistics were plotted according to Weissgerber et al. [66], who suggest that univariate scatterplots are the better choice to summarize small studies.

3. Results

3.1. Physical and Chemical Soil Properties

Mean values for the measured variables in each treatment are related in Table 2. The Kruskal–Wallis tests showed statistical evidence of the effect of tree pruning on soil P content (χ^2 = 10.29, $p < 0.01$, df = 2), and the Nemenyi multiple comparison tests [67] indicated that V and G pruning significantly increases the P content in the soil compared to NS (Figure 2A).

Table 2. Physical, chemical, microbiological and enzyme activity mean values for the pruning treatments: spherical vase shape (V), globe shape (G) and natural shape (NS).

Variable	Treatments		
	V	G	NS
Sand (%)	31.88	33.63	34.13
Silt (%)	37.25	37.25	34.75
Clay (%)	30.87	30.37	31.12
Pd (g cm^{-3})	2.41	2.55	2.37
Bd (g cm^{-3})	1.11	1.09	1.07
Por (%)	53.64	56.98	54.53
MWD (mm)	3.99	3.86	3.91
LL	39.26	40.73	40.07
PL	28.59	28.12	28.55
PI	10.67	12.61	11.52
CLE	0.06	0.06	0.06
pH	4.90	5.07	5.07
EC (dS m^{-1})	0.51	0.56	0.56
OOC (%)	0.39	0.45	1.05
P (mg kg^{-1})	56.31	52.06	33.14
Ca^{2+} ($cmol_c$ kg^{-1})	6.69	7.91	7.88
K^{+} ($cmol_c$ kg^{-1})	0.81	0.93	0.73
Mg^{2+} ($cmol_c$ kg^{-1})	3.15	3.34	3.25
Na^{+} ($cmol_c$ kg^{-1})	0.15	0.18	0.20
ECEC ($cmol_c$ kg^{-1})	12.60	13.58	13.44
I (%)	72.50	76.00	76.25
Spo (%)	3557.50	4551.25	4538.25
Al^{3+} ($cmol_c$ kg^{-1})	1.80	1.24	1.39
Nf (CFU)	1.71×10^5	4.76×10^5	1.52×10^5
Ps (CFU)	3.16×10^5	5.76×10^5	1.32×10^5
cel.µO	6.25	13.75	5.00
Pho (µM/min/g)	77.97	57.03	178.92
Cel (µM/min/g)	1.39	1.56	1.64

Por: Porosity percentage, MWD: mean weighted diameter, LL: liquid limit, PL: plastic limit, PI: plasticity index, CLE: coefficient of linear expansion, I: inoculum percentage, Spo: spores percentage, Nf: nitrogen-fixing, Ps: phosphate-solubilizing, cel.µO: cellulolytic microorganisms, Pho: phosphatase, Cel: Cellulase.

According to the beta regression, V and G tree pruning significantly reduces the OOC in the rhizosphere soil by 87.5% and 78.3%, respectively (Figure 2B). The negative binomial regression indicated that the Nf number is expected to have a 0.3 times lower rate in G concerning NS (Figure 2C). For Ps, the count is expected that V pruning has a rate 2.4 times higher compared with NS, and for G, it is expected to have a rate 0.44 times lower than NS (Figure 2D).

According to PCA (Figure 3), three main components or dimensions were extracted, Dim 1, Dim 2, and Dim 3, which explain approximately 20.54%, 15.19%, and 11.88% of the total variance in the data, respectively (Figure 3, Table 3). Dim 1 was significantly correlated with ECEC, EC, cel.µO count, and the concentration of Na^{+} and Ca^{2+} ions; while the correlation with the microorganisms Nf and Ps was negative. Dim 2 was significantly influenced by pH, Cel activity, I, CLE, Spo, and negatively by Na^{+}, Al^{3+}, and Pho. For Dim 3, the positive correlation was with P, CLE, Bd, and MWD, and negative with Ca^{2+}. This means that Dim 1 is mostly explained by bases and its effect on EC and ECEC, Dim 2, is influenced by pH and biological activity in terms of Cel, I, and Pho. Dim 3 is dominated by P and physical properties of the soil.

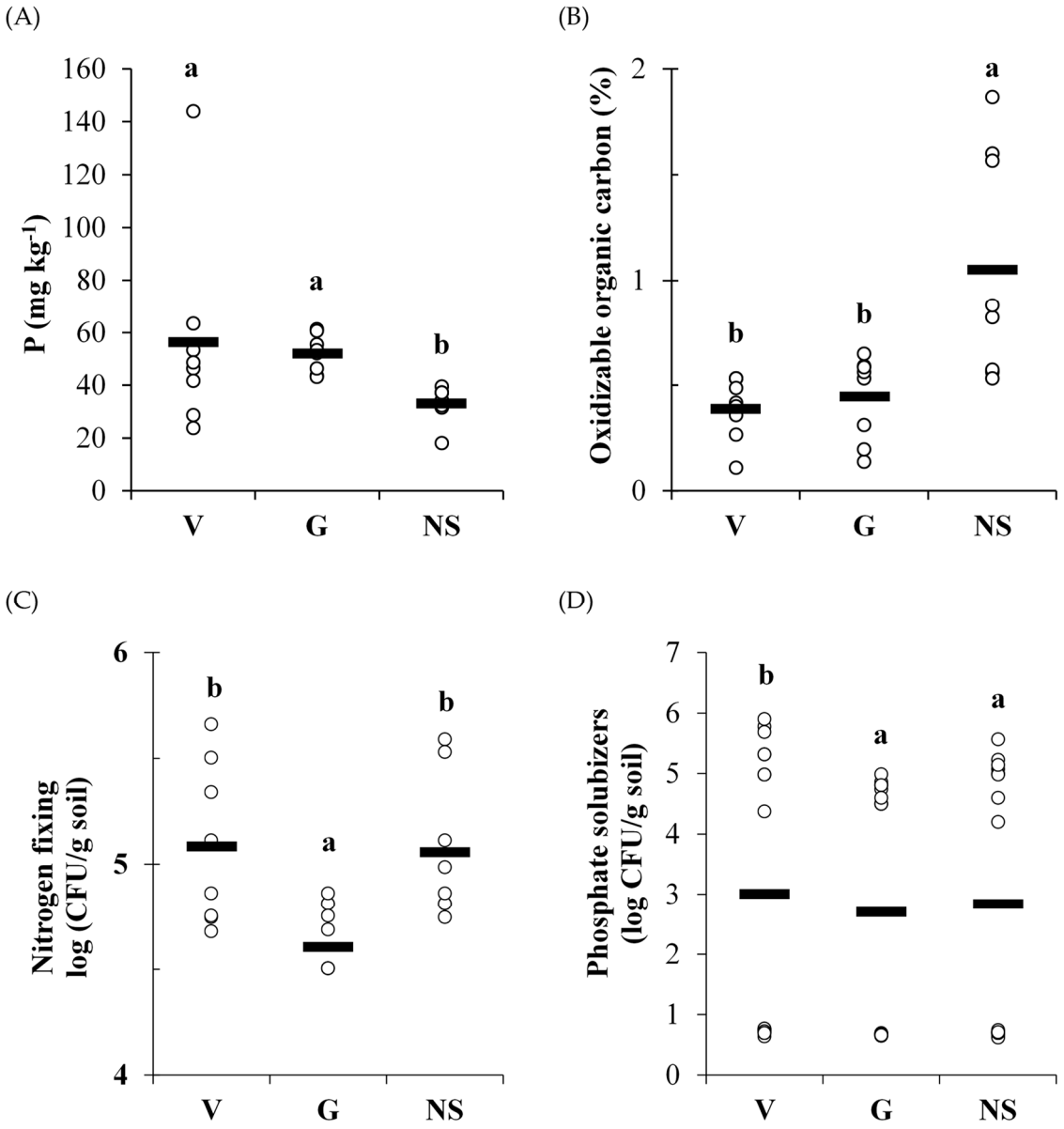

Figure 2. Effects of the olive pruning shape, vase (V), globe (G), and natural shape (NS), in olive trees rhizosphere soil on (**A**) P contents. Nemenyi test following Kruskal–Wallis H test was used to compare P content in rhizospheric soil between pruning treatments. (**B**) Oxidizable organic carbon. After beta regression, pairwise comparisons using Tukey's adjustment were used for comparing pruning treatments. (**C**) Nitrogen-fixing microorganisms count and (**D**) Phosphate solubilizers microorganism count were fitted to a negative binomial model and the differences between pruning treatments were tested with pairwise comparisons using Tukey's adjustment. Different letters denote significant ($p < 0.05$) differences between the means. Figures are plotted according to Weissgerber et al. (2015) [66].

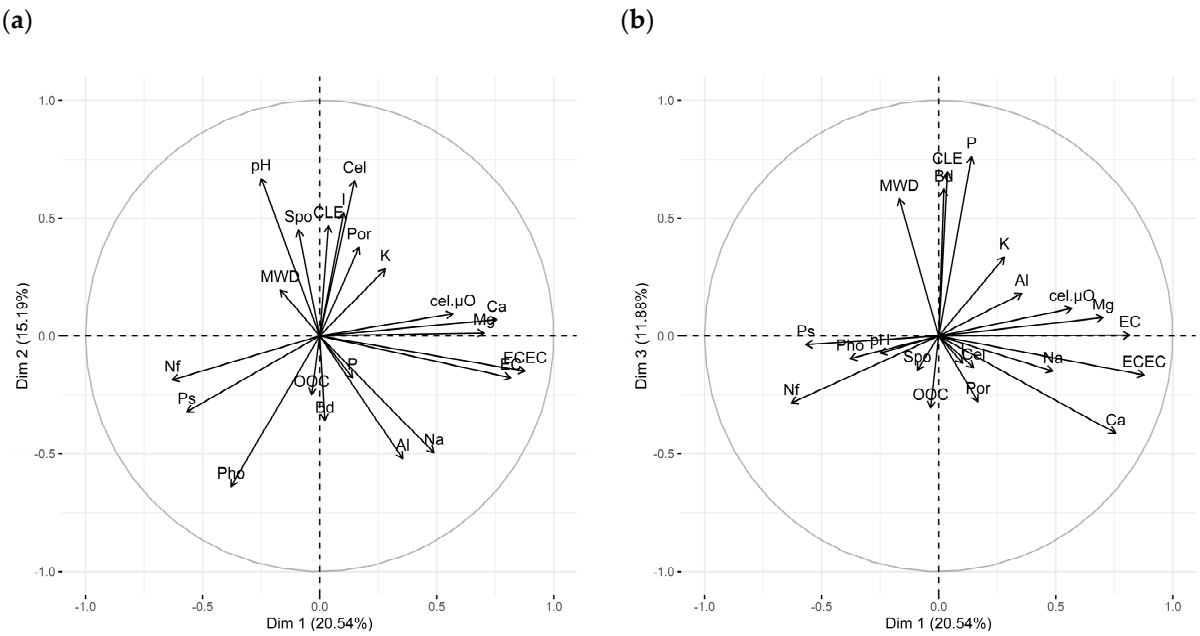

Figure 3. Principal components analysis variable factor map for physical, chemical, and biological variables measured in the rhizospheric soil of olive trees 105 days after pruning. The three first components or dimensions were extracted (Dim 1, Dim 2, and Dim 3). (**a**) Dim 1 vs. Dim 2; (**b**) Dim 1 vs. Dim 3.

Table 3. Explained variance by principal components or dimensions Dim 1, Dim 2 and Dim 3, and correlation values of the measured variables with respect to each Dim.

Variable	Correlation
Dim 1 (20.54%)	
ECEC	0.88 ***
EC	0.82 ***
Ca^{2+}	0.76 ***
Mg^{2+}	0.70 ***
cel.µO	0.57 ***
Na^{+}	0.50 *
Ps	−0.56 ***
Nf	−0.63 ***
Dim 2 (15.19%)	
pH	0.67 ***
Cel	0.66 ***
I	0.52 **
CLE	0.47 *
Spo	0.45 *
Na^{+}	−0.50 **
Al^{3+}	−0.52 **
Pho	−0.64 ***
Dim 3 (11.88%)	
P	0.76 ***
CLE	0.70 ***
Bd	0.62 ***
MWD	0.58 ***
Ca^{2+}	−0.41*

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$.

The Kendall correlation coefficients (r_τ) (Figure 4) showed moderate positive and significant correlations ($p < 0.05$) between EC and Mg^{2+} (0.34), Mg^{2+} and ECEC (0.43), Ca^{2+} and ECEC (0.55), P and CLE (0.45), I and Cel (0.37), Al^{3+} and ECEC (0.34), Spo and Ca^{2+} (0.29), Spo and pH (0.45), MWD and CLE (0.44), Por and Cel (0.33), Pho and Nf (0.34), and Ps and Nf (0.5). On the other hand, high and significant negative moderate correlations were found between pH and Al^{3+} (−0.76), Pho and Cel (−0.32), Nf and P (−0.3), Nf and cel.µO (−0.34), Nf and EC (−0.41).

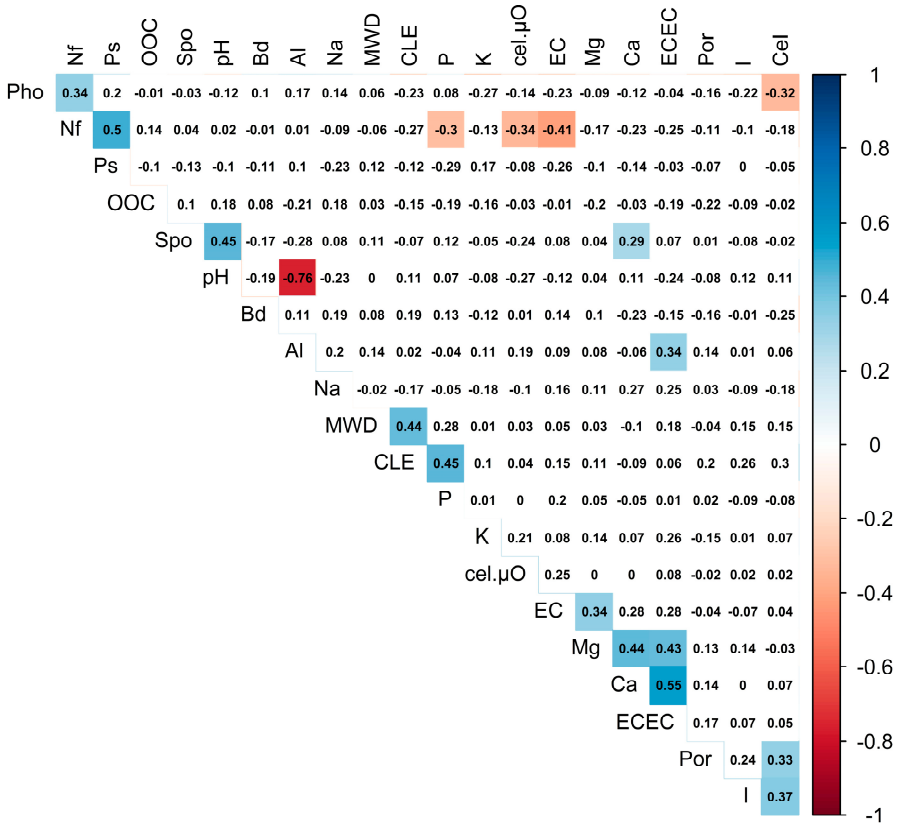

Figure 4. Correlogram reporting the relationship between physical, chemical, and biological variables measured in the rhizospheric soil of olive trees. Colored cells indicate a significant correlation at a confidence level of 95%. Blue cells indicate a positive correlation and red cells indicate a negative correlation. The value inside each cell corresponds to Kendall (r_τ) correlation coefficient with Bonferroni-adjusted *p*-values.

3.2. Mycorrhizal Fungal Spore Counting, Nitrogen-Fixing, Cellulolytic, Phosphate Solubilizers Microorganisms, and Enzymatic Activity

Simple linear regressions (Table 4) established that the percentage of inoculum (I) can significantly predict Cel (t = 2.59, $p = 0.017$); moreover, it explained 23% of the variability of Cel (adjusted R^2). On the other hand, the Nf contributed (58%) significantly ($p < 0.001$) to the variability of Ps, meaning that a change of one unit of Nf will increase Ps by 1.22 units. The Ca^{2+} concentrations in the rhizosphere soil solution have a significant effect ($p < 0.0001$) on the ECEC. In this way, an increase of one unit of Ca^{2+} is expected to increase ECEC by 1.17 units.

Table 4. Results of the simple linear regressions.

Variables		Equation	R^2	Significance
Dependent	**Independent**			
Cellulase activity (Cel)	Inoculum percentage (I)	$y = 0.41 + 1.49x$	0.23	0.017
Phosphate-solubilizing (Ps)	Nitrogen-fixing (Nf)	$y = 18,070.2 + 1.22x$	0.58	<0.0001
Effective cation Exchange capacity (ECEC)	Calcium (Ca^{2+})	$y = 4.45 + 1.17x$	0.74	<0.0001

R^2: coefficient of multiple correlation; Significance: two-tail observed significance levels (p) for t-statistic.

4. Discussion

Olive trees can grow in poor soil [68]. To produce 10 kg of olive fruits, trees require between 1.5 to 2.0 g of N, 0.1 to 0.3 g of P, 0.8 g of K^+, 1.0 g of Ca^{2+}, and 0.1 g of Mg^{2+} [69–71]. In this experiment, the rainfall was less than 300 mm per semester. In addition, some soil conditions could have restricted the orchard's growth and development [72–74].

In the literature, there are no studies that have investigated the correlation between the tree pruning shape (V, G, and NS) and the properties of the soil. The results obtained in this research do not show significant differences between the plants with different pruning shapes.

For the mycorrhizal fungal spore counting, the observable high percentages of I (Table 2) could be due to the low rainfall (Figure 1). The correlation between I and Cel, highlighted in Table 3 and Figure 4, can be explained by the fungi's Cel, which behaved to degrade and penetrate the root cell walls and exchange nutrients with carbon compounds [75–77]; moreover, these nutrients, are useful to maintain and improve the symbiotic relationship [78]. Since the low populations of cel.µO are related to the scarcity of OM, the correlation between Cel and I is an indicator of the trees' water requirements.

For the physical and chemical soil properties evaluations, the low P availability could be caused by the experimental orchard characteristics, which consisted of an average day temperature of 28 °C and a very strongly acidic pH. Moreover, the observed low P availability, the high activity of Nf, and the release of N from the soil in the form of NH^4 can explain the negative correlation between P and Nf. The low levels of N found in the rhizosphere soil of the different pruning forms of trees (Figure 1) could be due to temperatures, which represent the main factor that affects the enzymatic activity in soils [79].

Low N and OM contents and a very strongly acidic soil pH affected P, K^+, Ca^{2+}, and Mg^{2+} availability [9,80–86]. The low demand for P for olive trees [84], and the high clay sorption capacity [85], could explain the high soil P levels. Furthermore, Ca^{2+} content and its relationship with ECEC could be due to Ca^{2+}, which is the one with the highest incidence in the exchange complex, even if it is part of the soil parent material (Table 1) and all cations are in excess. The Ca^{2+}, Mg^{2+}, and K^+ concentrations are higher in the pruned trees, but despite the ECEC having medium values (5–10 cmolc/kg), the correlation with Ca^{2+} and Mg^{2+} is significant (Figure 4).

The relationship between P and MWD, CLE, and Bd, observable in the correlogram (Figure 4), might be due to the acidic pH. At acid pH, indeed, P is present with the most assimilable P chemical species, that is HPO_4^{2-}. This phosphate form binds to clay surfaces, replacing OH- groups. The strongly acidic pH in the experiment suggests that HPO_4^{2-}binds to clay or OM, thus increasing cation adsorption as Ca^{2+} and Mg^{2+} [86,87]. In this condition, P is on microsites that are part of the aggregates that increased in size, presumably, as an effect of the glomalin [88–90]. As a result, increasing MWD, expanding the clays (CLE), and, as a consequence, decreasing Bd, contribute to the adaptation of olive trees in this type of soil.

These stressing conditions could have promoted the presence of beneficial microorganisms in the soil because nitrogen-fixing and phosphate solubilizes microorganisms, and AMF are biological indicators of the low nutrient availability [91–95]. Therefore, the root

microbiota and the rhizosphere found in the Alto Ricaurte olive trees could increase the availability of P for plants [96].

Due to a strongly acidic pH, the concentrations of Al^{3+} and Fe^{3+} immobilize P, thus pH and I showed a positive correlation (Figure 4). P immobilization induces the plant to establish a symbiosis with fungi to acquire P. Furthermore, symbiosis can induce significant changes in soil pH that enhance P availability [76,97–99]. Some soil microorganisms require elements like P to construct membranes and synthesize ATP, and proteins useful in the N_2 fixation process. This last process requires additional Fe and Mo for synthesizing leghemoglobin and nitrogenase [100–103]. Therefore, P mobilization could be improved by microbial activity, and microbial activity could be promoted by phosphatase activity [86,104,105] that, in turn, is affected by the total N in the soil. Moreover, the relationship between Nf, Ps, and Pho activity [106] (Figure 3 and Table 3) in this experiment, could be due to the transport carried out by AMF to the plants of N, as NH^{4+} or NO^{3-} [107].

5. Conclusions

The soil where the olive trees were planted showed physical, chemical, and microbiological changes due to root activity. The roots established an association with AMF (Arbuscular Mycorhizal Fungi) and stimulated the presence of other nitrogen-fixing (Nf), phosphate-solubilizing (Ps), and cellulolytic microorganisms (cel.μO) microbial populations which is evident by the enzymatic activity of phosphatase (Pho) and cellulase (Cel). However, pruning forms do not seem to significantly affect the root activity.

The olive trees' growth in the experiment are affected by the nutritional imbalances, the low rainfall, and the strongly acidic pH existing in the experimental area.

Nevertheless, the ability of olive trees to adapt to adverse climate and soil conditions has allowed its presence in the Zaquencipa valley, located in Alto Ricaurte, Boyacá-Colombia, thus confirming the location as an alternative for site olive trees revegetalization, and, possibly, olive trees cultivation with productive purposes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051159/s1, Figure S1: Tree pruning types [108].

Author Contributions: J.F.G.-M.: conceptualization, research, methodology, software, writing—original draft, writing—review and editing; W.J.C.-B.: formal analysis, research, methodology, software; M.R.: research, methodology, software, writing—review and editing; L.S.J.-G.: research, methodology, formal analysis, software; T.G.: conceptualization, methodology, writing—review and editing, project management. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We gratefully thank the company Frutalia de Colombia™, and the owner of the Huerto Olivanto orchard for their support to carry out this research.

Conflicts of Interest: The authors declare no conflict of interest.

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