Effect of land management on soil properties in ~~flood irrigated~~ citrus orchards in Eastern Spain

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Abstract

Agricultural land management greatly affects soil properties. Microbial soil communities are the most sensitive and rapid indicators of perturbations in land use and soil enzyme activities are sensitive biological indicators of the effects of soil management practices. Citrus orchards frequently have degraded soils and this paper evaluates how land management in citrus orchards can improve soil quality. A field experiment was performed in an orchard of orange trees (*Citrus Sinensis*) in the Alcoleja Experimental Station (Eastern Spain) with clay-loam agricultural soils to assess the long-term effects of herbicides with inorganic fertilizers (H), intensive ploughing and inorganic fertilizers (P) and organic farming (O) on the soil microbial properties, and to study the relationship between them. Nine soil samples were taken from each agricultural management plot. In all the samples the basal soil respiration, soil microbial biomass carbon, soluble carbon, water holding capacity, electrical conductivity, soil organic matter, total nitrogen, available phosphorus, available potassium, aggregate stability, cation exchange capacity, pH, texture, macronutrients (Na, Ca and Mg), micronutrients (Fe, Mn, Zn and Cu), calcium carbonate equivalent, calcium carbonate content of limestone, microbial indexes (BSR/C, Cmic/C and BSR/Cmic) and enzimatic activities (urease, dehydrogenase, ß-glucosidase and acid phosphatase) were determined. The results showed a substantial level of differentiation in the microbial properties, which were highly associated with soil organic matter content, kept by organic management. The management practices including herbicides and intensive ploughing had similar results on microbial soil properties. O management contributed to an increase in the soil biology quality, aggregate stability and organic matter content.

Keywords:

Agricultural management, Biochemical indicators, Biological activity, Mediterranean soils, Citrus.

1. Introduction

The land management in agricultural areas has an important influence ~~in~~ on microbial soil properties ~~—~~(García-Orenes et al., 2013).~~—~~ Different agricultural managements influence soil microorganism and soil microbial process trough changes in quantity and quality of organic residues in the soil, their spatial distribution, ~~and trough~~ changes in nutrient input and physical changes (Christensen, 1996). The management of soil can help to find good solutions to find the best agricultural practices.~~ to keep the soil quality.~~ Unsuitable land management can lead to a loss in soil fertility and a reduction in the abundance and diversity of soil microorganisms, for example intensive arable farming causes a progressive decline of soil organic matter (Caravaca et al., 2002) affecting to physical, chemical biochemical and microbiological properties. The excessive use of herbicides can modify drastically the function and structure of soil microbial communities and altering terrestrial ecosystem, with important changes in soil fertility and quality (Pampulha and Oliviera, 2006). However,

~~ecological practices and~~ some organic amendments can promote the activities of soil microbial communities and increase biodiversity (García-Orenes et al., 2010). ~~Mulches and catch/cover crops are often seen as one of the best strategies to improve the biological activity of soils (Martir-Torres et al., 2013; Maul et al., 2014).~~ The application of soil conservation management system keeping cover crops on soil surface and minimizing soil tillage, it becoming usually because of increasing interest of sustainable agriculture (Roldan et al., 2003). The application of different types of ~~mulching~~covers in the surface of soil can decreases the soil temperature changes, keeping it cool, keeping soil moisture during the dry term and promoting microbial activity and crop development ~~-~~(Souza and Andrade et al., 2003). ~~, and they are very efficient at reducing soil losses in agriculture land (Giménez Morera et al., 2010), on road embankments (Lee et al., 2013) and on fire affected rangelands (Fernández et al., 2012).~~

The Mediterranean ~~Belt~~ belt is suffering from an intense degradation of its soils due to the millennia old land use (Zornoza et al., 2007), the use and abuse of the soil system due to non-sustainable management, land abandonment in the 1950s and 60s, and now as a consequence of the intensification of agricultural management (Cerdà et al., 2010). This area is characterised by the depletion of SOM after millennia of ploughing and burning. In the 20th century, the arrival of herbicides, chemical fertilizers, fungicides and insecticides (biocides in general) drastically modified the function and structure of microbial communities, altering the terrestrial ecosystems, which has important implications for soil quality (Sofo et al., 2012; Imfeld & Vuilleumier, 2012). Soil biota also contributes substantially to the resistance and resilience of agroecosystems to abiotic disturbance and stress (Brussaard et al., 2007).

The microbial members of soil communities are the most sensitive and rapid indicators of perturbations and land use changes. In this sense, a quantitative description of microbial community structure and diversity has aroused great interest as a potential tool for soil quality evaluation (Zelles, 1999; Zornoza et al., 2009).

~~The land uses determine the fate of the soil properties and quality and they are time and spatially dynamic (Zhao et al., 2013). Soil erosion due to non-sustainable land management changes in land use and agriculture system evolution is the main reason for land degradation in many regions of the world, especially in semiarid areas (Cerdà et al., 2009a; Barbera et al., 2013; Jones et al., 2014). Land degradation processes are also linked to reduction in soil fertility and damage to the abundance and diversity of soil microorganisms (Caravaca et al., 2002) and plants (Raizada & Juyal, 2012). Soil erosion and soil biological activity decline are seen as the main cause of agricultural land degradation in Eastern Spain (García-Orenes et al., 2009; Cerdà & Doerr 2007).~~

Soil organic matter (SOM) improves a soil's chemical and physical properties, promoting biological activity and maintaining environmental quality (Brevik, 2009), and this is why organic fertilisers, such as manure~~, waste water and sewage sludge~~, promote the activities of soil microbial communities (Morugán-Coronado et al., 2011; Balota et al., 2013; Macci et al., 2013). Plants and microorganisms are key players within the soil ecosystem and are responsible for many important soil cycling processes, such as C mobilization and N mineralization (Zak et al., 2003). On the other hand, land use influences soil microbial processes and structure of microbial communities ~~by changing the quantity and quality of plant residues entering the soil and their spatial distribution, through changes in nutrients and inputs~~ (~~García-Orenes~~

~~et al., 20~~13~~09; ~~Blagodatskaya et al., 2011;~~ García-Orenes et al., 2012~~; García-Orenes et al., 2013).

~~Within agricultural lands, the Mediterranean Belt is characterised by the depletion of SOM after millennia of ploughing and burning. In the 20th century, the arrival of herbicides, chemical fertilizers, fungicides and insecticides (biocides in general) drastically modified the function and structure of microbial communities, altering the terrestrial ecosystems, which has important implications for soil quality (Sofo et al., 2012; Imfeld & Vuilleumier, 2012). Soil quality is the "foundation" of the sustainable development of terrestrial ecosystems as the soil can act as a source or as a sink of carbon or pesticides (Paz-Ferreiro & Fu, 2013; Vasconcellos et al., 2013). Soil biota also contributes substantially to the resistance and resilience of agroecosystems to abiotic disturbance and stress (Brussaard et al., 2007). The microbial members of soil communities are the most sensitive and rapid indicators of perturbations and land use changes. In this sense, a quantitative description of microbial community structure and diversity has aroused great interest as a potential tool for soil quality evaluation (Zelles, 1999; Zornoza et al., 2009).~~

Organic agriculture management promotes maintaining SOM levels for soil fertility, providing plant nutrients through the microbial decomposition of organic materials, and the control of pests, disease, and weeds with crops rotations, natural control agents and pets-resistant plant varieties (Lampkin~~g~~ et al., 2011). Also this soils have a significantly higher microbial biomass, larger fractions of mineralizable C and N, and greater microbial C (Clark et al 1998; Reganold et al; 2010). Also this management has a positive effect on soil structure by the improving of stability of aggregates (Garcí~~i~~a-Orenes et al., 2005)

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~~The traditional flood irrigated orchards and gardens are a very special component of Mediterranean landscapes. They are characterised by a recurrent controlled flood that allows vegetables and trees to grow during the typical Mediterranean drought. This is a man-made landscape, where the crops are not originally from the Mediterranean, the weeds are also foreigners (invasive or imported), and soils are the result of agricultural practices over millennia. The traditional flood irrigation garden systems changed their crop from vegetables to citrus during the 20th century. Most of them moved to chemical fertilization and herbicide use in the last five decades, although in the past ploughing was the most popular management. Since the 1990s, some farmers have moved to organic farming (3% of farmers). Meanwhile, the chemical farmers that plough the land (5%) are typically the oldest ones as they maintain the tradition of tillage, and 92% of farmers use herbicides. Herbicides arrived in the region in the 1980s and they were the most popular management after the 1990s, which is also connected to increased use of drip-irrigation (Cerdà et al., 2009a).~~

~~Although the traditional flood irrigated gardens are widespread in the Mediterranean type agroecosystems, and they are also found in other semiarid and arid ecosystems, there is not information about the impact of the above-mentioned changes in land management on soil quality and biological activity.~~ This is why we are applying an integrated approach by measuring the microbiological characteristics of the soil, the enzymatic activities and the soil physicochemical properties with different agricultural management including organic farming. This will allow an understanding of the impact of the land management on the soil system.

According to different studies, we hypothesise that organic farming treatment applied in field experiment, could mean a significant improvement on soil physical, chemical and biological properties.

The main goal of this study is to determine the impact of land management change (from ploughing to herbicides and to organic farming) on soil microbial and biochemical properties in citrus orchards ~~under flood-irrigation~~ in Eastern Spain.~~ and find the better managements agricultural system to keep the soil productivity and soil quality.~~

2. Materials and methods

2.1. Study site

This research was conducted at the Alcoleja Experimental Station located in L'Alcúdia de Crespins municipality, 60 Km from coastal land, in the Eastern Iberian Peninsula, southwest of the Valencia province (UTM: 709191X, 4316356Y; Zone 30), at 156 m.a.s.l. The climate is typically Mediterranean with a mean annual precipitation of 500 mm and a mean annual temperature of 16ºC. The soil is a Xerorthent (Soil Survey Staff, 2010). The texture of soil is clay-loam, consisting of 20% clay, 40% silt, and 40% sand.

2.2. Experimental design

The three plots studied located on one farm at the Alcoleja Experimental Station have been planted with citrus (*Citrus Sinensis*) the last 30 years. The planting pattern is 7x4 m this is the usual pattern for citrus in this agricultural area. All ~~The~~ the three orchards of this study have been flood-irrigated with fresh water from the Sants River, which is a spring of the Macizo del Caroig aquifer. The spring supplies the discharge for the irrigation and is 2 Km from the experimental station. No pollution, no sources of OM and no wastewater is mixed with the high quality water coming from

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Con formato: Fuente: +Cuerpo, 12 pto, Color de fuente: Automático, Inglés (Reino Unido) the spring. The three ~~orchards~~ treatments of this study are flooded every 20 days in summer (700 m^3 ha^{-1} per irrigation) from April to October and no irrigation takes place in winter~~ (700 m^3 ha^{-1} per irrigation) from April to October~~. The irrigation schedule is based on the farmer's experience. Three different agricultural managements were established in the experimental station 30 years ago: (H) Herbicides with inorganic fertilizers applied before irrigation (Glyphosate (*N*-(phosphonomethyl)glycine) 4 times per year; NPK 15%, 1 Mg ha^{-1} per year), (P) ploughing 5 days after the irrigation and inorganic fertilizers applied before the flooding (NPK 15%, 1 Mg ha^{-1} per year) during 30 years and (O) organic farming were established 8 years ago (chipped pruned branches and weeds, manure from sheep and goats, 20 Mg ha^{-1}: 0.07% N, 0.03% P_2O_5, and 0.09% K_2O ~~-~~applied once per year, in winter, after the harvest of the oranges, usually in January~~), the irrigation is every 20 days in summer and no irrigation takes place in winter.~~).

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2.3. Soil sampling

In July 2013, nine soil samples from individual trees were collected in a randomised design from every agricultural management: herbicide with inorganic fertilizers (H), ploughing with inorganic fertilizers (P) and O with manure, weeds and chipped pruned branches (O). The soil samples were taken from the 0-5 cm and were collected from three farms that are neighbours, and the distance between the individual sampling points was less than 10 meters. Field-moist soil samples were sieved at 2 mm and stored at environmental temperature to conduct the physicochemical analysis. Soil sample aliquots (a portion of a total amount of a field soil sample) were sieved between 0.25-4 mm to determine the percentage of stable

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Vegetation cover (Veg) was determined as the percentage of soil covered by plants (Cerdà et al., 2007). The plant recolonization was calculated at the 9 sampling points for each treatment by means of a 50 x 50 cm square frame, and vegetation cover was measured at 100 points (each 5 x 5 cm) by a pin.

2.4. Soil physicochemical, microbiological and biochemical analyses

Soil pH and electrical conductivity (EC) were measured with a 1:5 (w/v) aqueous solution. The basal soil respiration (BSR) was measured using a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA). Soil organic matter (SOM) was determined by Walkley & Black (1934), available Na, K, Mg and Ca were extracted with 1N ammonium acetate (Knudsen et al., 1982) and Fe, Cu, Zn and Mn were extracted with DTPA (Lindsay & Norvell, 1978) and measured by atomic absorption and emission spectrophotometry. The reason for the inclusion of Na in the study is because the research is focused mainly on potential soil degradation and Na is related to sodicity processes, which lead to the loss of soil structure due to clay dispersion. Cation exchange capacity (CEC) was measured by the method described by Roig et al. (1980). Microbial biomass carbon (Cmic) was extracted using the chloroform fumigation and extraction procedure (Vance et al., 1987). Soluble carbon (Csol) from the soil solution was extracted through potassium dichromate digestion, following the Jenkinson & Powlsow method (1976). Carbon mineralization coefficient (BSR/C), Cmic/C ratio and metabolic quotient (BSR/Cmic), indexes able to evaluate microbial activity under different land management, were determined. Aggregate stability (AS) was measured using the method of Roldán et al. (1994); this method examines the proportion of

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aggregates that remain stable after a soil sample (sieved between 0.25-4 mm) is subjected to an artificial rainfall of known energy (270 J m^{-2}). Total nitrogen (Nk) was determined by the Kjeldahl method (Bremmer & Mulvaney, 1982). Available phosphorus (AP) was determined by the Burriel-Hernando method (Díez, 1982). Water holding capacity (WHC) was assayed by the method of Forster (1995). Calcium carbonate equivalent (~~CCE~~Carb) and calcium carbonate content of limestone ($CaCO_3$) were measured by Porta et al., 1986. Urease activity (EC 3.5.1.5) was assayed according to the method of Tabatabai (1994), using urea as the substrate. Dehydrogenase activity (DHA) was determined according to García et al. (1997). Phosphatase (PHP) (EC 3.1.3.1) and β-glucosidase (β-Glc) (EC 3.2.1.21) activities were determined using p-nitrophenyl phosphate disodium (PNPP, 0.115 M) and p-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrates, respectively. The assay is based on the release and detection of p-nitrophenol (PNP) according to Tabatabai (1994~~).~~).

2.~~6~~5. Statistical analyses

The fitting of the data to a normal distribution for all soil properties was checked with the Kolmogorov-Smirnov test at $p<0.05$. To compare the effect of agricultural management, an ANOVA test was done. We tested significance between treatments for each soil to observe changes over treatments. The separation of means was carried out according to the average post-hoc Tukey test $p<0.05$, assuming equal variance. Pearson's correlation coefficients (R) were calculated to quantify the linear relationship between parameters. All soil properties from all samples were subjected to principal components analysis (PCA) to elucidate major variation patterns in terms of the three treatments and to establish relationships between variables and

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treatments of the study. To perform correlation-based PCA ($p \leq 0.05$), the data was normalized in order to have the same (=1) variance for the samples along all species axes~~.~~ The correlation criterion >0.4 in different components. ~~.~~Sampling adequacy of individual and set variables by KMO (>0.5) and Bartlett's test of sphericity (<0.05) were done, ~~all~~WHC was removed from PCA analysis because this variable ~~variables~~ obtained communality value~~s~~ ~~>~~<0.5. The selection of main components was regulated by the latent root criterion (eigenvalues > 1.0) and the classification of soil properties by component was performed by varimax rotation. Alexakis (2011) concluded that principal component analysis proves a successful tool for the interpretation of results. All statistical analysis was performed with the SPSS program (Statistical Program for the Social Sciences 18.0).

3. Results

3.1. Physicochemical parameters

Table 1 shows the vegetation cover, texture, pH, EC, ~~CCE~~Carb, WHC, $CaCO_3$, CEC, macronutrients (Na, Ca and Mg) and micronutrients (Fe, Mn, Zn and Cu). The maximum vegetation cover was observed in the O plot (42.2%) with less cover in the other agricultural managements (H: 1.2 % and P: 1.1%), the main weeds species observed at organic farming management were Brachypodium retusum (pers.) Beauv. and Cistus albidus L. (Table 1). The ploughed soil (P) had the highest clay content, and the highest values of WHC and $CaCO_3$ in comparison to the herbicide (H) and organic farming (O) land managements, $CaCO_3$ was statistically higher for both, P and O treatments (Table 1). The pH level in the plots ranged from 7.9 in O plot to 8.3 in P plot. In the O plot, soils showed a statistically significantly higher content of salts (EC) and a slight decrease in pH was observed compared with the other agricultural

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managements studied. Although the pH and EC were significantly different between treatments, if we observe the pH and EC classifications (Soil Survey Staff, 2010) pH was moderately alkaline (between 7.9 and 8.4) and EC was not saline (< 2 dS/m) (Table 1). The lowest value of CEC was found in H plot and the highest value of CEC was found in O plot, CEC was statistically low for both, H and P treatments- (Table 1).

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The H plot did not show ~~a great improvement~~differences in the fertility parameters (Table 1 and Figure 1). The percentage of sand in this plot (H) was higher than the other agricultural managements (Table 1).

Figure 1 shows the content of the main nutrients parameters studied (N, P and K) in the three different agricultural management soils. This information shows that the soil under O management had a higher content of these important elements ~~important~~ for soil fertility. The H plot was the one that had the lowest values in SOM, N, P and K content. Furthermore, O plot showed the highest content in SOM reaching almost 8% against the low content of the other agricultural managements studied, which reached 2%, SOM was statistically low for both, H and P treatments (figure 1). Agricultural management with O increased the N content five times compared to the other management plots. Available P and available K obtained their maximum in the O plot, for available P, H and P treatments showed the lowest values while for available K only P treatment showed the lowest value. ~~and this agricultural management had statistical differences with the other farming practices (H and P), P plot had the lowest values for these two soil parameters.~~

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Figure 1 also shows the percent aggregate stability at each plot. The O management obtained the highest level of AS which seems to be related with the organic matter content.

3.2. Microbial biomass carbon, basal respiration, microbial indexes and enzymatic characterization of the soil.

The microbial biomass carbon is considered the most active and living part of OM and is composed of microorganisms that participate in different processes in the soil. Soils under O management had ~~higher~~ highest levels of Cmic, Csol and BSR. The statistical analysis for Cmic shows the lowest value in P treatment, ~~and~~ Csol and BSR only H treatment showed the lowest value ~~than P and H plots~~ (~~Figure 2A and B~~Table 2). To evaluate microbial activity under different land management we analysed carbon mineralization quotient (BSR/C), Cmic/C ratio and metabolic quotient (BSR/Cmic), very low values w~~as~~ere obtained for BSR/C and BSR/Cmic. However there are ~~with~~ statistical differences between treatments, for Cmic/C soils under O treatment showed the lowest values (Table 2).

Enzymatic activities (dehydrogenase, urease, phosphatase and β-glucosidase) were measured in the three types of agricultural management (Figure ~~2C~~2A, ~~D~~B, ~~E~~ C and ~~F~~D). Significant differences were found between the organic management and the other management systems for all enzymatic activities studied.~~, with phosphatase highest in the soil under O when compared to the H and P plots.~~ The ~~dehydrogenase~~ DHA enzyme activity (Figure ~~2C~~2B) in the soil reflects the total oxidative activity of the microbes, and its concentration was high in O plot indicating that the activity of the microbial community increases with agriculturally sustainable management.

We have not recovered data about the productivity of harvest in the different agricultural managements because the objective of this study was evaluate effects on soil properties not in crop productivity.~~However, we observed and stimated that there had not been important differences of yield between treatments in the last five years.~~

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3.3. Bivariate correlation coefficients.

Table ~~2~~ 3 shows the correlation coefficients between the most important physicochemical and biochemical properties studied in all agricultural treatments of this research. ~~Vegetation cover~~Veg had strong correlation coefficients with BSR, SOM, AS, Csol, Nk, AP, K and CEC. On the other hand, Cmic/C and pH were negatively correlated with Veg. pH was negatively correlated with the soil fertility parameters (SOM, Nk, AP, K, CEC), microbiological activity (BSR, Cmic and Csol ~~enzimes~~enzymes), AS and vegetation cover. Referring to fertility properties, SOM shows a strong correlation with Csol, AS, Nk, AP, K, CEC, phosphatase and ~~vegetation cover~~veg. Nk was strongly correlated with Csol, AP, AS, K, SOM, CEC, ~~phosphatase~~ PHP and veg~~etation cover~~. AP showed strong correlations with K, AS, Nk, SOM, Csol, PHP, CEC and ~~vegetation cover~~veg, and available K showed strong correlation coefficients with AS, Nk, SOM, AP, CEC and ~~vegetation cover~~veg, K was negatively correlated with Cmic/C. CEC had strong positive correlation coefficients with Csol, ~~vegetation cover~~veg, AS and fertility properties (Nk, SOM, AP and K). ~~Aggregate stability~~AS had strong correlation coefficients with ~~vegetation cover~~veg, Csol, PHP and soil fertility parameters (SOM, Nk, AP, K, CEC). $CaCO_3$, BSR, ~~and~~ Cmic, β-Glc, Urease, DHA and BSR/C did not show strong correlations with the other parameters studied, although it should be noted that a relationship was observed between BSR and SOM, Csol, CEC, BSR/Cmic and ~~vegetation cover~~veg. ~~No strong correlation coefficients were found between enzymatic activities and any soil properties studied. We only founded positive correlation between phosphatase and AS, Nk, SOM, AP, K and CEC.~~

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3.4. PCA.

With the PCA performed on the soil physical, chemical, and biochemical properties ~~69~~60% of the total variation could be explained by the first ~~three~~ two principal components. Figure 3 shows the soil property clusters analyzed through principal components analysis, performed with the different agricultural managements studied. The first principal component (PC) explained ~~40~~42% of the variation and it separates the O soil samples from those of the other agricultural managements (P and H). The second component explained ~~20~~18% of the variance and separates the soil samples from H and P management while~~, the third component explained 9% of the variation~~. The first component was determined by N, SOM, Csol, AS, Veg, CEC, Ca, Cmic/C, AP, pH, K, PHP, BSR, Cu, EC, Carb, Mn, Na, $CaCO_3$, Mg, Cmic and silt (correlation >0.4) ~~vegetation, CEC, SOM, Nk, AS, BSR, acid phosphatase, available P, Ca and K~~. The second component was correlated with Sand, extractable Fe, $CaCO_3$, Mg, Clay, relation C/N, silt, EC, Carb, Zn and ~~-~~pH~~extractable Fe. The third component was associated with urease~~ (~~table~~ Table ~~3~~4). In figure 3 it is shown that the analysis has clearly clustered the soils by type of agricultural management received, and the majority of fertility and microbiological activity properties analyzed are closely associated with soils under O management.

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4. Discussion

The soil of field study is an Xerorthent, (a) Ent: young soil with little or no profile development, (b) orthents: entisols lacking in pedogenetic development and (c) xerorthents: these soils have a xeric soil moisture regimen (cool and moist in winter and warm and dry in summer) (Illustrated guide to soil taxonomy, version 1.0, 2014, USDA).

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A combination of high throughput approaches along with physical, chemical, and microbiological methods were used to investigate three different agricultural practices in citrus orchards~~ under flood irrigation~~. This research approach provides insight into how key fertility parameters and microbiological activity respond to the different agricultural managements, how the O management (weeds, manure, and no chemical fertilization) improved most soil properties, and how inorganic fertilisation drastically changed soil microbial activity. These hypotheses were supported by the results obtained. First, SOM is responsible for microbiological processes and organic compound turnover. Indeed, in agricultural soils that are intensively managed, microbial activity tends to change quicker in response to organic management than community composition (Burger & Jackson, 2003). Secondly, distinct enzyme activities indicated that O increased the association of enzyme activity with fertility properties (Piotrowska & Wilczewski, 2012; Bowles et al., 2014). Enzymatic activities may be considered as biochemical indicators of soil quality, mainly based on OM content and related biological and biochemical parameters (García-Ruiz et al., 2008).

In our study ~~all the enzymes studied~~PHP ~~are ~~is closely related with N, SOM and biological parameters such as ~~Cmic ~~Csol~~and BSR~~, as indicated by their correlation coefficients shown in Table ~~2~~3. These results are consistent with other studies that showed high correlations between different enzymatic activities and the microbial response (Acosta-Martinez et al., 2008; Bonanomi et al., 2011). The high level of enzymatic activities observed in the ~~organic ~~O management plot indicates that this soil is biologically and biochemically more active than the other agricultural managements. This activity in O treatment could be related with the higher level of SOM ~~and~~that also contains high levels of Csol~~ in O treatment~~, the Csol fraction is an important pool with

Con formato: Fuente: +Cuerpo, 12 pto, Color de fuente: Automático, Inglés (Reino Unido), Diseño: Claro respect to SOM turnover in agricultural soils, it acts as readily-available substrate for soil microorganisms responsible of the enzymatic activities –(García-Orenes et al., 2010). ~~, it is also able to process labile organic components and to protect more stable organic fractions (Emran et al., 2012; Gispert et al., 2013) as shown by the high SOM of this treatment.~~ The ~~phosphatase~~ PHP activity that has been reported in the literature usually shows a negative correlation with the high presence of ~~available phosphorus~~AP (Allison et al., 2007). In contrast, in this work we found high ~~phosphatase~~ PHP activity in O plot even with a high presence of ~~available~~ AP. This behaviour could be due to the great correlation between the enzyme ~~and~~ with ~~the~~ Csol and SOM (Table ~~2~~3), suggesting that ~~soil~~ SOM and Csol ~~has~~ have been more important than P availability in regulating investment in ~~phosphatase~~ PHP in this soil (Bowles et al., 2014). The decrease of the enzymatic activities in H soil could be explained by the low levels of SOM, nutrients and also microbiological activity found. In P soil, this decrease is worsened by tillage management and could be modified by the potential of soil enzyme-mediated substrate catalysis (Kalender et al., 1996).

Furthermore, it was clearly observed in our survey that O improved the ~~microbial biomass carbon~~Cmic and ~~basal soil respiration~~BSR; the quantity and quality of SOM and nitrogen inputs are the overriding controls on soil microbial biomass carbon (Fierer at al., 2009, Kallenbach & Grandy, 2011). Thus, different agricultural amendments (e.g. manure, leguminous cover crops, and composted materials) can stimulate Cmic differently through increases in OM (Marriot & Wander, 2006; Smukler et al., 2008). In contrast, García-Orenes et al. (2010) already detected low basal soil respiration quotients in agricultural soils with null inputs of OM under rainfed conditions in the rangelands and agriculture lands of the mountainous areas of Eastern

Spain. The success of manure additions in the citrus orchards in recovering SOM, vegetation cover, and microbiological activity, must also be due to the fact that there was high soil water content as a consequence of the irrigation (Sorensen et al., 2013). The contribution of the irrigation to improving soil microbial biomass has been mainly studied on soil treated with wastewater (Chaerun et al., 2011; Prado et al., 2011, Morugán-Coronado et al., 2011) and all of these studies confirm improvements as water and OM are key factors for healthy soil development (Morugán-Coronado et al., 2013). Soil moisture tends to have a positive influence on soil quality due to its contribution to the improvement of soil microbial activity when there are improvements in the SOM (de Oliveira et al., 2014; Oo et al., 2014).

SOM additions from organic farming help stabilize soil from runoff losses and protect the soil surface from erosion increasing infiltration and ~~water holding capacity~~WHC (Apezteguía et al., 2009). ~~There is also an increase in biological activity as insects such as ants are more common (Cerdà et al., 2009b).~~ Moreover, the increase in SOM can lead to greater soil aggregation, which increases pore space and further promotes infiltration (Williams & Petticrew, 2009; Larsen et al., 2014). Additionally, SOM is the major aggregate agent and provides structure in temperate soils (Mataix-Solera et al., 2011; Gispert et al., 2013). The importance of soil aggregation and OM is true in both agricultural and rangeland soils (Brevik & Fenton, 2012). The low SOM observed in the herbicide plot and the corresponding decrease in microbial activity affects the soil's physicochemical properties, as well as the productivity of agricultural ecosystems (De Grood et al., 2005). The aggregation of soil (AS) and SOM observed in plots H and P were lower than AS in O plot, ploughing and the herbicide application facilitates decomposition of SOM due to the disruption of aggregate-embedded organic matter (Tisdall & Oades, 1982). Intensive tillage can be a major cause of erosion due to disruption of the soil surface and removal of protective cover that would be effective in reducing runoff and soil loss (Kok et al., 2009). Organic farming systems and sustainable agricultural management systems use organic inputs, leaving residue on the fields that cover at least 30% of the soil surface, and this conservation practice has been shown to reduce soil erosion (Brevick, 2009) and increase SOM in agricultural soils (Brevik, 2009).

In comparison with conventional tillage, organic farming has potential benefits in encouraging soil structure formation (Pulleman et al., 2003), and enhancing soil microbiology (Mäder et al., 2002; Oehl et al., 2004) such as has been shown in the Alcoleja research site. Organic farming avoids the inputs of inorganic fertilizers and their consequences (Tu et al., 2006), offers organic C inputs to soil, and has been used to successfully reduce soil erosion (Bilalis et al., 2003; Jordan, 2004). It is also effective in preserving soil moisture and buffering severe changes in soil temperature (Brevick, 2009), which can be important in Mediterranean soils to improve biological activity.

CONCLUSIONS

The main conclusion of the research carried out at the L'Alcoleja experimental station is that on traditional ~~flood-irrigated~~ citrus orchards, the organic farming strategy (manure, no tillage, high vegetation cover) contributed to better soil conditions, including high biological activity, increased enzymatic activities, more organic matter and more stable aggregates. Meanwhile, ~~the chemical (~~herbicide treated~~)~~ and ploughing with inorganic fertilization managements resulted in ~~low vegetation cover,~~ low aggregate stability and low microbial biomass.

The implication of this work potentially entails environmental positive applications in soil agricultural management (soil protection, residue crop reuse and stop soil degradation). Hence, the present research represents a further step towards a more sustainable and crop production in Mediterranean agricultural soil.

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Figure captions

Figure 1. Mean values (± standard deviation) of (A) total nitrogen, (B) soil organic matter (SOM), (C) C/N relation, (D) available potassium, (E) available phosphorus, (F) aggregate stability (AS) in soil. Different letters indicate significant differences (P<0.05) between soil management – from black (organic farming-O), light gray (herbicide-H) to dark gray (ploughing-P) – for each treatment according to one-way ANOVA.

Figure 2. Mean values (± standard deviation) of enzymatic activities (A) β-Glc, (B) DHA, (C) Urease and (D) PHP ~~Cmic, BSR, Dehydrogenase, β-Glucosidase, Urease and Acid phosphatase~~ in soil. Different letters indicate significant differences (P<0.05) between soil management – from black (organic farming-O), light gray (herbicide-H) to dark gray (ploughing-P) – for each treatment according to one-way ANOVA.

Figure 3. PCA factor scores from each agricultural management and loadings from soil properties in all management practices: Organic farming (O) (●), Ploughing (P) (●) and Herbicide (H) (▼)
1: pH; 2: EC (Electrical conductivity); 3: BSR (Basal soil respiration); 4: Cmic (microbial biomass Carbon); 5: AS~~:~~ (aggregate stability); 6: N~~:~~ (total nitrogen); 7: SOM~~:~~ (soil organic matter); 8: CN; 9: AP~~:~~ (Available phosphorus); 10: Na; 11: K; 12: Ca; 13: Mg; 14: Fe; 15: Cu; 16: Zn; 17: Mn; 18: CEC~~:~~ (Cation exchange capacity); 19: Carb~~:~~ (calcium carbonate equivalent); 20: $CaCO_3$~~:~~ (Calcium carbonate content of limestone); 21: β-Glc (β-Glucosidase); 22: Urease; 23: PHP (acid phosphatise); 24: Clay; 25: Silt; 26: ~~s~~Sand; 27: DHA (Dehydrogenase); 28: Veg (Vegetation cover); 29: Csol (Soluble carbon); 30: Cmic/C.

Table 1. Main soil characteristics and vegetation cover of different treatments (Herbicides with inorganic fertilizers (H), intensive ploughing and inorganic fertilizers (P) and organic farming (O)). Values are mean ± standard deviation~~ (n= 27)~~.

Table 2. Main biochemical soil properties of different treatments (Herbicides with inorganic fertilizers (H), intensive ploughing and inorganic fertilizers (P) and organic farming (O)). Values are mean ± standard deviation.

Con formato: Inglés (Reino Unido)

Con formato: Justificado, Espacio Después: 0 pto, Interlineado: sencillo

Table ~~2~~3. Correlation coefficients (R-values) for relationships between physical, chemical and biochemical properties for all the managements.

Table ~~3~~4. Matrix of PCA obtained with all soil samples (n=27)

Con formato: Inglés (Reino Unido)

Con formato: Justificado, Espacio Después: 0 pto, Interlineado: sencillo

Con formato: Fuente: +Cuerpo

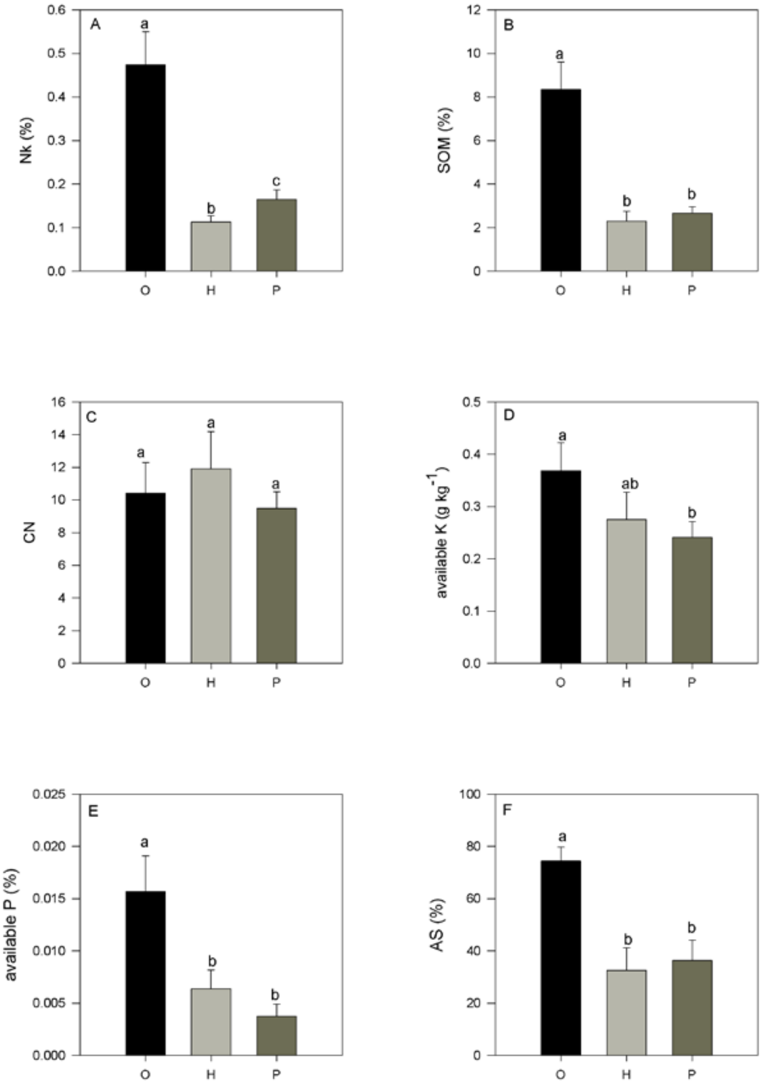
A
Nk (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
a
b
c
B
SOM (%)
0
2
4
6
8
10
12
a
b
b
C
CN
0
2
4
6
8
10
12
14
16
a
a
a
D
available K (g kg^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
a
ab
b
E
available P (%)
0.000
0.005
0.010
0.015
0.020
0.025
a
b
b
F
AS (%)
0
20
40
60
80
100
a
b
b
O
H
P

Figure 1

Figure 2

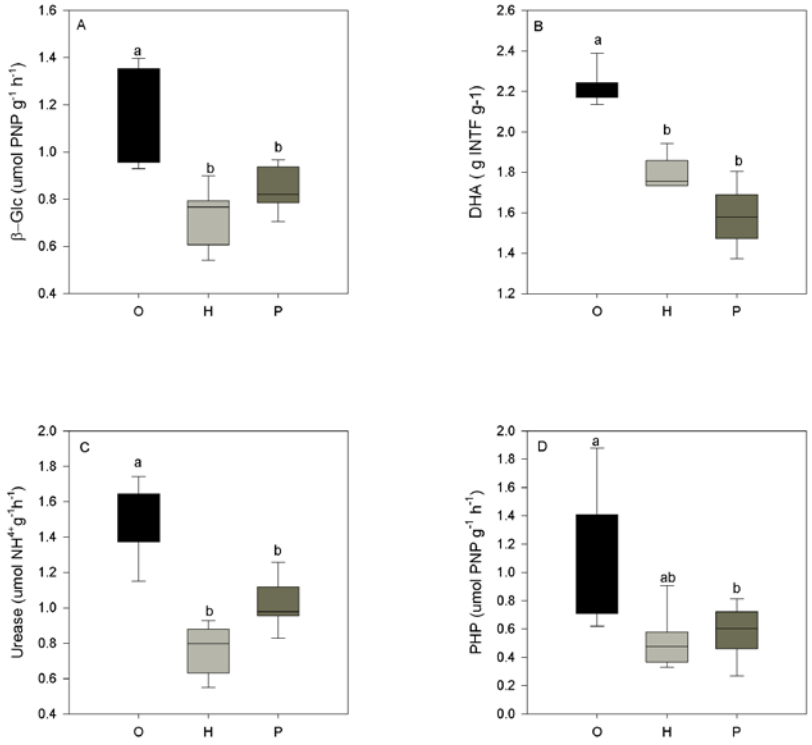

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Figure 3

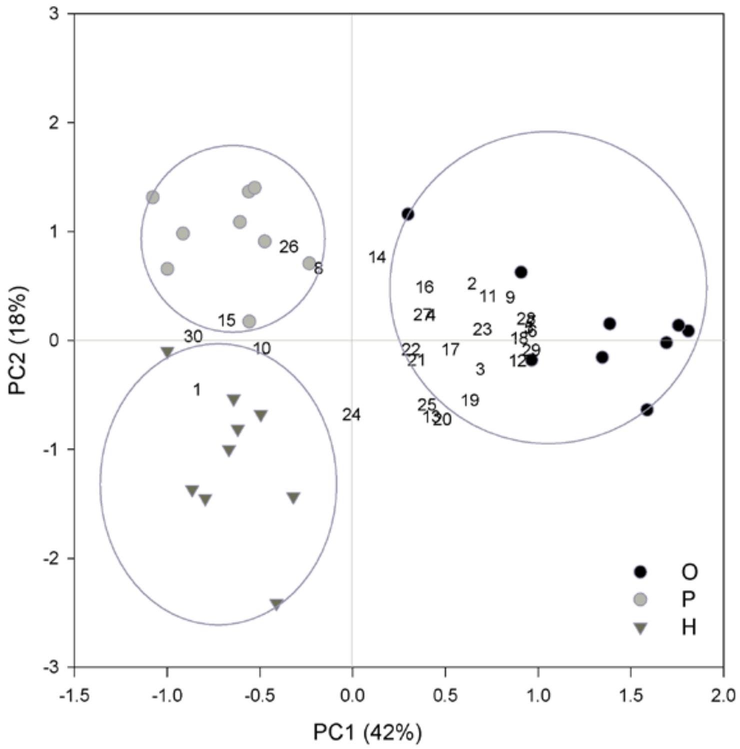

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Table 1

Table 1. Main soil characteristics and vegetation cover of different treatments (Herbicides with inorganic fertilizers (H), intensive ploughing and inorganic fertilizers (P) and organic farming (O)). Values are mean ± standard deviation.

	O*	H	P
~~Vegetation cover~~Veg (%)	42.2 ± 8.9^{a}	1.3 ± 0.6^{b}	1.1 ± 0.6^{b}
Texture (% clay, silt, sand)**	19^{ab}, 40^{a}, 39^{a}	17^{a}, 34^{b}, 48^{b}	23^{b}, 43^{a}, 34^{c}
pH (extract 1:5,w/v)	7.9 ± 0.15^{a}	8.2 ± 0.15^{b}	8.3 ± 0.07^{b}
EC (1:5, µS cm^{-1})	244 ± 28^{a}	201 ± 44.4^{b}	175 ± 14.2^{b}
Carb (%)	48 ± 5.8^{a}	33 ± 4.4^{b}	46 ± 4.3^{a}
WHC (%)	50 ± 9.5^{ab}	44 ± 2.7^{a}	52 ± 2.3^{b}
$CaCO_3$ (%o)	147 ± 16.4^{a}	104 ± 18.7^{b}	157.8 ± 13.8^{a}
CEC (cmol kg^{-1})	11.8 ± 2.8^{a}	6.7 ± 0.8^{b}	8.10 ± 2.0^{b}
Macronutrients (g kg^{-1}) Na, Ca, Mg	0.99^{a}, 3.27^{a}, 0.52^{a}	1.11^{a}, 2.70^{b}, 0.41^{b}	1.10^{a}, 2.90^{c}, 0.55^{a}
Micronutrients (g kg^{-1}) Fe, Mn, Zn, Cu	0.04^{a}, 0.01^{a}, bdl, bdl	0.04^{a}, 0.01^{b}, bdl, bdl	0.02^{b}, 0.01^{ab}, bdl, bdl

~~n=27~~

~~*0-5 cm depth.~~

~~**Sand: 2-0.02 mm, Silt: 0.02-0.002 mm, Clay: <0.002 mm.~~

~~bdl: below detection limit of lowest concentration used in absorption chromatography analysis (. Zn: 0.01 g kg^{-1}; Cu: 0.01 g kg^{-1})~~

n=27

*0-5 cm depth.

**Sand: 2-0.02 mm, Silt: 0.02-0.002 mm, Clay: <0.002 mm.

bdl: below detection limit of lowest concentration used in absorption chromatography analysis (Zn: 0.01 g kg^{-1}; Cu: 0.01 g kg^{-1})

EC: Electrical conductivity; WHC: Water holding capacity; Carb: calcium carbonate equivalent ; $CaCO_3$: Calcium Carbonate Content of Limestone; CEC: Cation exchange capacity, Veg: Vegetation cover.

(%o) parts per mil' symbol

A one-way ANOVA ($P<0.05$) were used to compare differences between managements.

Different letters indicate significant differences ($P<0.05$) between soil management for each treatment according to one-way ANOVA.

Table 2

Table 2. Main biochemical soil properties of different treatments (Herbicides with inorganic fertilizers (H), intensive ploughing and inorganic fertilizers (P) and organic farming (O)). Values are mean ± standard deviation. ~~(n= 27). Different letters indicate significant differences (P<0.05) between soil management for each treatment according to one-way ANOVA.~~

Con formato: Inglés (Reino Unido)

	O*	H	P
Cmic	2293.4 ± 366.2 ^{a}	2087.3 ± 362.3 ^{ab}	1923.1 ± 148.7 ^{b}
Csol	263.8 ± 47.7 ^{a}	61.2 ± 6.7 ^{b}	128.0 ± 10.2 ^{c}
BSR	1.1 ± 0.3 ^{a}	0.5 ± 0.2 ^{b}	0.8 ± 0.3 ^{a}
BSR/C	$2.2\cdot10^{-2} \pm 4.5\cdot10^{-3}$ ^{a}	$3.7\cdot10^{-2} \pm 2.1\cdot10^{-2}$ ^{ab}	$5.4\cdot10^{-2} \pm 2.2\cdot10^{-2}$ ^{b}
Cmic/C	47.9 ± 8.0 ^{a}	163.0 ± 49.4 ^{b}	125.3 ± 15.9 ^{c}
BSR/Cmic	$4.8\cdot10^{-4} \pm 1.3\cdot10^{-4}$ ^{a}	$2.2\cdot10^{-4} \pm 6.8\cdot10^{-5}$ ^{b}	$4.3\cdot10^{-4} \pm 1.6\cdot10^{-4}$ ^{a}

n=27

*0-5 cm depth.

Cmic: Microbial biomass carbon~~,~~ ; Csol: Soluble carbon~~,~~ ; BSR: Basal soil respiration~~; BSR/C,~~ ; ~~Cmic/C and BSR/Cmic~~.

A one-way ANOVA (P<0.05) were used to compare differences between managements.

Different letters indicate significant differences (P<0.05) between soil management for each treatment according to one-way ANOVA.

Table ~~2~~3

Table ~~2~~3. Correlation coefficients (R-values) for relationships between physical, chemical and biochemical properties for all the managements.

	pH	EC	BSR	Cmic	Csol	CaCO$_3$	AS	Nk	SOM	AP	K	CEC	BSR/Cmic	Cmic/C	BSR/C	β-Glc	Urease	PHP	DHA	Veg
pH																				
EC	-.891***																			
BSR	-.418*	.311^{ns}																		
Cmic	-.392*	.414*	.238^{ns}																	
Csol	-.740***	.515**	.748***	.359 ns																
CaCO$_3$	-.293^{ns}	.088^{ns}	.535*	.253^{ns}	.489*															
AS	-.839***	.619**	.569*	.363^{ns}	.908***	.536*														
Nk	-.833***	.587**	.607**	.403*	.928***	.585**	.949***													
SOM	-.878***	.665***	.642***	.432*	.938***	.502*	.950***	.967***												
AP	-.865***	.686***	.466*	.595**	.795***	.354^{ns}	.827***	.875***	.900***											
K	-.838***	.655***	.298^{ns}	.148^{ns}	.670***	.191^{ns}	.779***	.767***	.773***	.793***										
CEC	-.725***	.527**	.631***	.407**	.902***	.502*	.845***	.858***	.887***	.838***	.686***									
BSR/Cmic	-.242^{ns}	.133^{ns}	.930***	-.103^{ns}	.451*	.432^{ns}	.409*	.445*	.458*	.235^{ns}	.223^{ns}	-.454*								
Cmic/C	.758***	-.535*	-.524*	.022^{ns}	.826***	-.498*	-.846***	-.580***	-.847***	-.644***	-.723***	-.766***	-.491*							
BSR/C	.656***	-555*	.238^{ns}	-.166^{ns}	.368 ns	-.035^{ns}	-.561*	-.531*	-.543*	-.544*	-.624**	-.459*	.373^{ns}	.569*						
β-Glc	-.259^{ns}	.274^{ns}	.246^{ns}	-.247^{ns}	.298ns	.184^{ns}	.312^{ns}	.260^{ns}	.244^{ns}	.084^{ns}	.278^{ns}	.351^{ns}	.278^{ns}	-.507*	-.253^{ns}					
Urease	-.298^{ns}	.169^{ns}	.258^{ns}	.046^{ns}	.291 ns	.333^{ns}	.281^{ns}	.307^{ns}	.252^{ns}	.191^{ns}	.407*	.261^{ns}	.229^{ns}	-.302^{ns}	-.157^{ns}	.246^{ns}				
PHP	-.602**	.332^{ns}	.271^{ns}	.242^{ns}	.667***	.525*	.680***	.775***	.699***	.712***	.562**	.629***	.171^{ns}	-.618**	-.515*	-.009^{ns}	.151^{ns}			
DHA	-.468*	.359^{ns}	.013^{ns}	.133^{ns}	.234 ns	.158^{ns}	.483*	.436*	.373^{ns}	.310^{ns}	.364^{ns}	.184^{ns}	-.067^{ns}	-.459*	-.486*	.408*	.307^{ns}	.288^{ns}		
Veg	-.851***	.668***	.682***	.443*	.917***	.441^{ns}	.930***	.940***	.971***	.846***	.750***	.840***	-.493*	-.786***	-.486*	.259^{ns}	.320^{ns}	.600**	.388*	

SOM: soil organic matter; Nk: total Nitrogen; AS: aggregate stability; C: Organic Carbon; Cmic: microbial biomass carbon; Csol: Soluble Carbon; BSR: basal soil respiration; AP: available phosphorus; K: available Potassium; EC: Electrical conductivity; CaCO$_3$: Calcium Carbonate Content of Limestone; CEC: Cation exchange capacity; DHA: Dehydrogenase activity; Veg: Vegetation cover; PHP: Phosphatase activity; β-Glc: β-Glucosidase.

Significant correlation at: p<0.05*; p<0.01 ** and p<0.0001***; ns: not significant correlation at p>0.05.

Tabla con formato

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Table ~~3~~4

Table ~~3~~4. Matrix of PCA obtained with all soil samples (n=27)

Variance explained	PC1 (42%)	PC2 (18%)
Nk	0.97	0.085
SOM	0.965	0.171
Csol	0.963	-0.091
AS	0.951	0.116
Veg	0.937	0.199
CEC	0.898	0.019
Ca	0.89	-0.186
Cmic/C	-0.858	0.037
AP	0.85	0.394
pH	-0.838	-0.453
K	0.73	0.405
PHP	0.701	0.101
BSR	0.69	-0.266
Cu	-0.68	0.184
EC	0.645	0.522
Carb	0.631	-0.551
Mn	0.527	-0.085
Na	-0.491	-0.072
$CaCO_3$	0.485	-0.725
Mg	0.424	-0.704
Cmic	0.423	0.232
Silt	0.402	-0.592
Zn	0.388	0.49
DHA	0.379	0.235
B-Glc	0.347	-0.179
Sand	-0.342	0.861
Urease	0.317	-0.086
Fe	0.132	0.762

Variance explained (60%)

Nk: total Nitrogen; SOM: soil organic matter; Csol: Soluble carbon; AS: aggregate stability; Veg: Vegetation cover; CEC: Cation exchange capacity; AP: available phosphorus; K: available Potassium; PHP: Phosphatase activity; BSR: basal soil respiration; EC: Electrical conductivity; Carb: calcium carbonate equivalent; $CaCO_3$: Calcium Carbonate Content of Limestone; Cmic: microbial biomass carbon; DHA: Dehydrogenase activity; β-Glc: β-Glucosidase.~~.~~

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