Long term changes in soil properties and enzyme activities after almond shell mulching in avocado organic production

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Abstract

The recycling of almond shell as mulch would allow avocado orchards to be managed organically, maintaining plant yield and improving soil conditions simultaneously. This study aims to analyze the long term effects of successive applications of almond shell as mulching for organically grown avocado. Effects on soil properties, enzyme activities and soil carbon storage as well as avocado yield and growth were studied in comparison to no-tillage conventionally managed (using mineral fertilizers and herbicides) avocado. In 2002, 2007 and 2012 almond shells were applied in a 7 cm thick layer ($26.0 \text{ kg dry matter m}^{-2}$) covering 4 x 8 m per tree. After a 10 year period a new soil organic horizon was established in the organic treatment, and the upper mineral soil layer (0-25 cm) enhanced its C content from 43 g kg^{-1} in F to 91 g kg^{-1} , and proportionally P and N contents. The high lignin content of almond shells, 362 g kg^{-1} , led to low organic matter degradation, 37% for the 10-year period. Nevertheless, this reduced rate of organic matter mineralization was sufficient to supply the needed nutrients and to maintain or even increase fruit yield in the organic treatment. Urease, β -glucosidase, dehydrogenase, protease and phosphomonoesterase showed high microbiological activity in the organic layer decreasing in mineral layers. Enzyme activities were significantly correlated to soil OC (organic C) content and site specific soil moisture. Reduced mineralization and high surface microbiological activity make almond shell an ideal mulch for avocado trees combining increased soil organic C content and agricultural benefits.

Keywords: soil organic matter; soil carbon sequestration; lignin; tropical fruit

Abbreviations: Organic matter: OM; Soil organic carbon: SOC

1. Introduction

Food quality and environmental concerns have increased the general interest for organic agriculture over the last decades. From an environmental standpoint, there is wide agreement that organic farming is linked to lower pesticide and energy consumption, better soil conservation and higher biodiversity than conventional farming (Kristiansen, 2006). Moreover, in the current climate change scenario, organic production systems are likely to be more resilient to the impacts of climate change on temperature and rainfall, due to the ability of the organic matter to counteract drought conditions (Metzger et al., 2006). Increasing periods of water shortages will probably be part of the new climate under Mediterranean conditions, driving to an agricultural change characterized by a reduction in the available land for cultivation and reduced yields. In addition, organic agriculture, especially when organic mulches are used, has a great potential as carbon sink and could become an interesting strategy to offset carbon emissions.

Appropriate mulching is a key tool in organic management of most woody perennial crops and it provides several environmental and agronomical advantages such as preventing weed growth (Verdú and Mas, 2007), controlling diurnal/seasonal fluctuations in soil temperature, protecting soil from erosion (Jafari et al., 2012) or increasing water use efficiency by reducing evaporation (Grundy and Bond, 2007). Unlike synthetic mulches, that need to be removed after a period of time, organic mulches decompose and they need to be re-applied, increasing soil organic matter and water holding capacity, releasing nutrients and enhancing soil aggregation (Haynes, 1980). Organic mulches also promote favorable soil biotic activities, such as soil

respiration and microbial biomass (Grundy and Bond, 2007), and improve overall soil productivity (Siczek and Frac, 2012).

From a sustainable perspective, residual organic materials from nearby crops or agro-industrial activities would be the best option as sources of mulch or soil amendments, increasing soil C storage and reducing the carbon footprint of the whole production. In recent years, environmental issues related to global climate change have demonstrated the need to assess soil organic matter dynamics and soil storage capacities more precisely (Vidal-Beaudet et al., 2012). One of the main factors involved in controlling the C budget is the microbial decomposition of organic mulches.

Nevertheless, the extent of CO₂ balance due to mulching will also depend on the kind of organic substances used, particularly its richness in lignin and phenolic compounds, because these compounds significantly influence the soil microbial enzyme activity (Theuerl and Buscot, 2010). Soil enzyme activities may respond to changes in soil management faster than other soil variables and, therefore, they might be useful as early indicators of biological changes. Enzyme activities have been studied to evaluate the response of soil functions to changing environmental conditions or management practices (e.g. tillage, type of crop, fertilization, etc.) (Nannipieri et al., 2012). Several studies have focused specifically on compost and crop residues, such as straw, used as mulch or soil amendment (Siczek and Frac, 2012; TerAvest et al., 2011). In spite of this and with the exception of plant litter in natural forests and wood chips, very few of these studies address lignocellulosic residues such as those present in almond shells.

Almond shells are the woody endocarps of the fruits produced by the almond tree (*Prunus amygdalus* Batsch), a woody perennial crop originated in Southwest Asia and currently cultivated worldwide in regions with a Mediterranean climate. The almond shells are obtained as a residue when the fruit is processed to obtain the edible

seeds (kernels). Its ligneous character makes this waste suitable to obtain activated C or biomass fuel (Fernández et al., 2012). Almond shells are normally incinerated or dumped without control due to logistic problems (seasonal production in small scale factories, transport costs, lack of energetic plants). Some alternative uses, near the productions sites of almond shells and, therefore, more advantageous to CO₂ savings, have been proposed and evaluated. Urrestarazu et al. (2005) indicated that almond shells seem to be an acceptable growing medium (100% pure) for soil-less cultures and they could be a viable and ecologically friendly alternative to widely used rockwool. Lao and Jiménez (2004) also obtained good results when using almond shells as a growing media component. Rigane et al. (2011) studied almond shell co-composting with other agricultural wastes and indicated that the resulting composts are compatible for use as constituents in growing media for horticultural soil-less cultures. Vargas-García et al. (2005) tested the capability to obtain inocula for composting from ligno-cellulolytic fungi growth on these shells. In a recent paper, Esfahlan et al. (2010) reviewed the possible uses of the almond by-products. Long lasting, availability, low cost and resistance against wind made this waste highly recommended as mulch. Nevertheless the information about the use of almond shells as mulch is still scarce and usually only plant response has been evaluated. Jafari et al. (2012) recommended the use of almond shells as a mulch in fig orchards and its possible use in orange trees for weed control has been also tested (Verdú and Mas, 2007).

Almond shell is an easily available residue in southern mainland Spain, the only region in continental Europe with a significant production of subtropical fruits such as avocado or mango. Avocado (*Persea americana* Miller, Lauraceae), in particular, is a crop with low nutrient requirements and weed pressure that shows relatively easy natural pest and disease control in Mediterranean climates and, consequently, it is

particularly suitable for organic production in areas such as continental Spain. One of the ways to enhance avocado organic production is through the use of organic mulching. The benefits of organic mulches in increasing root health, ameliorating stress, and increasing yield and fruit size in avocado are well known (Moore-Gordon et al., 1997). However, except for a recent paper (Bonilla et al., 2012) studying the effects of almond shells and other organic amendments on soil bacterial community composition, there is a lack of information about the long term effects of this organic residue on chemical and biochemical properties of soil under organic farming practices.

Supposedly, mulching with almond shell would allow avocado growth under organic farming practices maintaining plant yield and improving soil conditions. This study aims to analyze the long term effects of successive applications of almond shell as mulching for organically grown avocado. Effects on soil properties, enzyme activities and soil C storage as well as avocado yield and growth were studied in comparison to no-tillage conventionally managed (using mineral fertilizers and herbicides) avocado. The combination almond shell-avocado would give both sides high added value in an environmentally sound way.

2. Materials and Methods

2.1. Experimental site and design

The trial was carried out in a cv. Hass, grafted onto cv. Topa-Topa seedling rootstocks, avocado orchard located at the experimental Station ‘La Mayora’, on the coast of Malaga province (SE Spain). The trees, planted at 8 x 8 m, were 29-year-old at the starting of the trial (2002) when almond shell mulching was applied for the first

time. Previously the plot was cultivated in the usual way for avocado, under no-tillage receiving standard applications of N, K and B fertilizers, and herbicides through the microsprinkler irrigation system. Each tree had 2 microsprinklers that wet a 5 m wide strip along the tree row. Weed cover in the inter-row areas, maintained under rainfed conditions, was cut 2–3 times per year. Irrigation water was of good quality for avocado (electrical conductivity 0.5-0 to 0.6 dS m⁻¹, nitrate-N < 2 mg L⁻¹).

Before tree planting in 1973, the land in the experimental farm was terraced, forming benches 30-80 m wide and on the average about 50 cm deep, by adding topsoil from a nearby site. A heavy organic amendment (100 tonnes ha⁻¹ of manure) was also incorporated at that time. The resulting soil was derived from shale, having 41% of gravel. It presented a homogeneous sandy loam texture (sand 60.6%, silt 31.4%, clay 8.0%) and was well drained, without Ca and Mg carbonates.

Two no-tillage treatments, organic (O) and conventional with the application of inorganic fertilizers (F), were started in this orchard in May 2002. At that date no significant differences were found in soil matric potentials (measured by tensiometers) and leaf N content between the trees selected for both treatments. The O treatment consisted in a massive application of coarse almond shell as mulching. The almond shell was produced by Almendras Forclamar (Trapiche, Vélez-Málaga, Málaga, Spain) located near (10.7 km) the experimental orchard. The main physico-chemical characteristics of the applied almond shells are shown in Table 1. After this initial application, the almond shell application was repeated twice, in December 2007 and February 2012. Each time, the almond shell was applied as a 7 cm thick layer (30 kg m⁻², 26.0 kg dry matter m⁻²) covering a surface of 4 x 8 m per tree. No fertilizer was applied since the first application of almond shells in 2002, except Fe-EDDHA (3.1 kg

ha⁻¹ in 2002), ZnSO₄ (10.2 kg Zn ha⁻¹ in 2002 and 2012) and natural phosphate rock (47 kg P₂O₅ ha⁻¹ in 2010).

The F treatment continued receiving mineral fertilizers, according to leaf analysis, at the following yearly doses: 50-60 kg N ha⁻¹, using mainly NH₄NO₃, KNO₃ and Ca(NO₃)₂, every year, 11 kg P ha⁻¹ as phosphoric acid in 2010, 12.5-41.5 kg K ha⁻¹ as KNO₃ and 5 kg Fe-EDDHA ha⁻¹ (6% Fe, Ferrishell, Agrimor, Spain) in 2008-2012, 0.43 kg B ha⁻¹ (Solubor DF, Borax Europe Ltd., U.K.) in 2002-2004 and in 2010-2012, and ZnSO₄ (10.2 kg Zn ha⁻¹) in 2002 and 2012. Weed control in the irrigated area of the F treatment was done by applying a mixture of simazine (50% in commercial product) and oxifluorfen (25% in commercial product) through the irrigation system every 15-30 days to a maximum dose of 2 L ha⁻¹ of each product. Due to the shading of trees, no herbicides have been applied since July 2005.

The whole experiment was established in a randomized block design with 13 blocks each containing two elemental plots for O and F treatments. Average yield and avocado growth were calculated based on the 13 blocks (replications). Each elemental plot had two trees with each plot separated by a 0.7 m deep sheet of 0.2 mm thick polyethylene film in order to avoid contact between root systems. The length of the film was 5 m in accordance with the width of the irrigation system. Every year fruit harvesting was done at industry fruit maturity to obtain total yield. Tree trunk cross sectional area was also recorded yearly.

2.2. Soil sampling

Soil samples were collected in April 2012 from three randomly selected blocks out of the 13 replications (the 13 replications were used for yield determination and the

3 soil samples for soil properties). In the F treatment, samples were taken at two depths, 0-25 cm and 25-50 cm (named as F25 and F50 respectively). Though in microbiological studies sampling thickness uses to be thinner, soil OC content results obtained in a preliminary sampling campaign (data not shown) advocated for the thicker soil samples used in this study. In the O treatment samples were taken at three depths: the upper degraded organic horizon below the undegraded 2012 almond shell mulch, approximately 8 cm depth (O-8), and the following 0-25 and 25-50 cm mineral layers which will be named O25 and O50. The thickness of the whole organic layer (undegraded plus degraded) was determined at each sampling point. Each individual sample was a composite sample from 4 points around a tree, located 1 m from the trunk base and 0.5 m from the microsprinkler. After careful mixing, each composite sample was divided in two halves. One half was air dried and grinded to pass through a 2 mm sieve and destined to chemical analysis where the gravel was separated and weighted. The second half was stored at 4°C until biochemical analyses were done. In this case, samples were sieved in moist condition.

2.3. Almond shell and soil analysis

Almond shell samples were analyzed following standard procedures for soil improvers and growing media of the European Committee for Standardization (CEN). Electrical conductivity and pH were determined in a 1:5 aqueous extract (CEN, 1999a; CEN, 1999b). Organic matter was determined by dry combustion at 450°C (CEN, 1999c) and N was determined by distillation after Kjeldahl digestion. Mineral nutrients and trace elements were determined after aqua regia digestion in a microwave oven by ICP-OES. Laboratory compacted bulk density was determined following EN 13040

(CEN, 1999d) and the result was referred on a dry matter basis. Laboratory compacted bulk density was determined using a 1 L cylinder under specific pressure conditions and it was used to take samples for other analysis. Compost samples from the WEPAL programs (Houba et al., 1996) were also analyzed for quality control of analytical procedures. The results obtained for these samples agreed $\pm 5\%$ with the certified results. The method used for measuring lignin content (ASTM, 2013) utilizes a strong sulfuric acid solution (72 % wt) in a primary hydrolysis, followed by dilution with water and a secondary high-temperature (100–125 °C) hydrolysis. This procedure hydrolyzes the polymeric carbohydrates into soluble monosaccharides, leaving behind a lignin-rich residue that is vacuum-filtered and measured gravimetrically.

Particle-size distribution and bulk density (BD) in soil samples were determined by the hydrometer method (Gee and Or, 2002) and the core method (Grossman and Reinsch, 2002) respectively. The determination of BD was carried out in non-disturbed soil samples (4 replications in each soil layer). In the case of the organic soil layer (O-8) the metal cylinder was inserted in the deeper section, where the material was highly decomposed, after separating the non-decomposed almond shell on the surface. Soil chemical properties were determined following standard procedures (Sparks et al., 1996): pH in water, electrical conductivity in 1:5 water extract, available-P by the Olsen method, available-K after ammonium acetate extraction and Kjeldahl-N was determined by titration after Kjeldahl digestion. Ammonium-N and nitrate-N were extracted from fresh soil samples using 1:10 soil to 2M KCl and water ratio, respectively. After extraction, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were spectrophotometrically determined in an automated analyzer system (AutoAnalyzer III, Bran+Luebbe, Norderstedt, Germany). Organic-C (OC) was determined following the classical Walkley and Black method (Walkley and

Black, 1934). OC data were converted to soil organic matter (OM) using the conventional conversion

$$OM = OC \times 1.724 \quad \text{Eq. 1}$$

In the upper organic layer of O treatment (samples O-8), OC was determined using a TOC analyser (Simadzu TOC-VCSH) following the standard procedure for biowastes (CEN, 2002). These organic samples were also analyzed for total contents of nutrients following the standard procedures for soil improvers and growing media indicated for almond shell samples. The OC, expressed in g kg^{-1} soil, is transformed to total amount of organic C in each soil layer, in kg m^{-2} , by using the equation 2, to have a C balance in soil.

$$OC (\text{kg m}^{-2}) = OC (\text{g kg}^{-1}) \times \text{Soil depth} \times BD \times (1000 - \text{gravel}) / 10^6 \quad \text{Eq. 2}$$

Where Soil depth is expressed in m, BD in g L^{-1} , and gravel of soil was 410 g kg^{-1} .

The activity of five enzymes relating to C, N and P cycles, one oxido-reductase (dehydrogenase) and four hydrolases, were determined. For the enzyme activities the following methods were used: Acid phosphomonoesterase and β -glucosidase activities were determined following the methods reported by (Tabatabai, 1994) after soil incubation at 37°C for 1 h with MUB (modified universal buffer, pH 6.5) and p-nitrophenyl phosphate (0.025 mol L^{-1}), for phosphatase activity assay and MUB (pH 6) and p-nitrophenyl- β -D-glucoside (0.025 mol L^{-1}), for β -glucosidase activity; the activity of p-nitrophenol (PNP), the product of the reactions, was determined spectrophotometrically at 400 nm; dehydrogenase activity was determined by the reduction of 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) to

iodo-nitrotetrazoliumformazan (INTF) following Trevors (1984) with slight modifications (incubation during 20 h at 22°C), and INTF produced was measured spectrophotometrically at 490 nm; urease activity was determined as reported by Kandeler et al. (1999), modifying the Kandeler and Gerber (1988) method, based on the colorimetric determination of $\text{NH}_4\text{-N}$ released from soil-urea incubation during 2 h at 37°C; protease activity was determined by the Ladd and Butler (1972) method as described by Alef and Nannipieri (1995), based on the determination of amino acids released after incubation of soil with caseinate for 2 h at 50°C using Folin reagent being the absorbance determined at 700 nm. To standardize each enzyme activity, an enzyme activity index, i , was calculated by dividing the average value in each layer by the corresponding value in the F25 soil layer. The index for the layer was calculated as the sum of the five indices.

2.4. Statistical analyses

Mean comparisons were performed by using T-test. Equality of variances was checked using Levene's test and a follow up T-test was then performed. Relationships among soil chemical properties and enzyme activities were studied by using linear Pearson correlation coefficients. Taking into account each individually analysed soil sample (each of the replications is a separate case), all soil enzyme activities showed highly significant linear correlation to soil moisture content, soil organic carbon and other nutrients (data not shown). Subsequently, an attempt was made to model each enzyme activity (dependent variable) by using multiple linear regression examining soil moisture and soil physicochemical properties as independent variables. The high degree of collinearity among independent variables (OC was the controlling factor) made the

statistical analysis unreliable. To avoid collinearity problems, the regression line between soil moisture content and OC was calculated, and unstandardized residuals were used instead of raw moisture content data.

All statistical analyses were carried out using SPSS 19.0 analytical software (SPSS, 2010).

3. Results and Discussion

3.1. Degraded-mulch description

In the O treatment, the different additions of almond shells resulted in the development of a superficial organic layer. This organic layer can be divided into three organic horizons that correspond to the soil organic horizons described as standard in forest soils. The average (3 plots x 4 replicates) depth of this organic layer was 14.8 ± 2.9 cm. The upper part of the organic layer (7 cm) was the un-decomposed most recent almond shell added in 2012. This part can be considered similar to a natural OL soil horizon, composed of fresh litter material coming from the accumulation of leaves, twigs and mainly woody materials, with most of the original plant material being easily discernible (Zanella et al., 2011). A black layer could be observed underneath the fresh material (O-8). The average depth of this black layer was 7.8 cm. The characteristics of the O-8 layer are shown in Table 1. The upper part of this black layer was constituted by some partially degraded pieces of almond shells, likely originating from the 2007 shell addition, mixed with humic material. This could be considered an OF horizon (Zanella et al., 2011). Under the partially degraded material, with not clear transition to it, the black layer consisted of spongy finely textured organic material likely originating from

the 2002 shell addition. This could be described as an OH horizon, characterized by an accumulation of transformed material and well-decomposed litter due to the action of soil microorganisms (Zanella et al., 2011). Average bulk density of the O-8 layer was 216 g L^{-1} significantly lower than that of the added mulch (371 g L^{-1} , Table 1). Bulk densities in the range $100\text{-}200 \text{ g L}^{-1}$ were usual in natural organic peat soils (Parry and Charman, 2013).

3.2. Chemical fertility of soil and plant yield

Main soil chemical properties in the F and O treatments (mineral soil below organic layer) are shown in Table 2. In the upper 0-25 cm layer, the almond shell treatment (O25) significantly reduced soil pH by 0.5 units to a neutral value, and increased Kjeldahl-N and available-P (Olsen-P) by 65% and 48% respectively. The increase in available soil P was observed in spite of the reduced P supply by the almond shells (equivalent to 7 kg P ha^{-1}), which suggests that the organic residue is able to mobilize unavailable soil P, probably due to pH reduction. This increase in available soil P is of high interest because this plant nutrient is considered a non-renewable resource, creating concerns about future P availability in the EU and worldwide (European-Commission, 2013). The differences between treatments in these properties seem to be due, directly or indirectly, to the added organic matter as it is also indicated by the highly significant Pearson correlation coefficients obtained for the complete soil dataset (Table 2). The high Pearson correlation coefficients reveal the importance of the added organic matter in the build-up of soil fertility. High Pearson's correlation coefficient for the pair OC-available K also indicated that the small increase observed (20 mg kg^{-1}) was probably due to almond shell mulch although the treatment means

were not statistically different. Lack of significant differences between the treatments in the deeper 25-50 cm layer shows that, during the time period of the trial, transportation of soluble nutrients to subsoil (25-50 cm) layer has not taken place.

In the O-8 organic layer, the inorganic-N content ($\text{NH}_4\text{-N}$ plus $\text{NO}_3\text{-N}$, Table 1) amounted to 17 mg kg^{-1} that indicated a net N mineralization. At least during the first stages of degradation similar woody amendments usually produced N immobilization because of their wide C/N ratio and the inorganic N requirement due to C mineralization and microorganism metabolism (TerAvest et al., 2011). This process mainly affects the soil surface layer and deeper layers may have different net N mineralization rates (Grundy and Bond, 2007). In the present case, the C/N ratio in the O-8 degraded layer (below the most recent un-decomposed mulch) diminished enough (162 to 20.6, Table 1) to allow for net N release in mineral forms. Forest ecosystem studies have found the same behavior in forest litter layers (Persson and Wirén, 1995). Valenzuela-Solano et al. (2005), using eucalyptus yardwaste as mulch for avocado, found that mulched intact soil cores released 53 kg ha^{-1} more N annually than control treatments, although the net mineralization of the applied mulch started approximately 8.5 months following application. The same authors found a C/N ratio of 19.5 in the more degraded organic layer, which was in contact with the topsoil mineral layer. In a study on litter decomposition under field conditions Manzoni et al. (2008) indicated that decomposers of low-N residues are able to begin mineralization even when the litter N concentration is still relatively low, and without necessarily having to immobilize much inorganic N. These researchers hypothesize the existence of a common mechanism of carbon utilization across diverse ecosystems and trophic levels, where C ‘waste’ occurs under restricted nutrient availability. In the present study, organic farming practices and heavy organic mulch in the O treatment may have favored conditions that were close to those

of natural forest soils (for example high biodiversity) enabling the action of the ‘common mechanism’ as opposed to poorer soil quality in the F agricultural soil. In the 0-25 cm layer of both treatments $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were present at low concentrations, being negligible in the subsoil (25-50 cm) layer. Soil N depletion in O25 could result from inorganic N absorption carried out by the dense, shallow root system of avocado trees located below the O-8 mulch layer.

Total fruit yield and trunk cross sectional area are shown in Table 3. Yield data were grouped every two years to avoid the effect of alternate bearing, common in avocado trees. In general terms, yield in both treatments were not significantly different except for the period 2006-2007. During 2006-2007 the total yield in O treatment was greater than in F treatment but both treatments had the lowest yields among the considered bienniums. Reduced yields for the 2006-2007 biennium could be due to frost fall in January 2005. It could be that the mulch effect was more beneficial to the avocado trees during this adverse circumstance. Values of trunk cross sectional area were similar for both treatments during all the considered periods.

Organic mulching is highly beneficial to avocado trees in most situations, because it simulates a forest litter layer. The origin of avocado in neotropical rainforests suggests that recycling of litter has played an important role in its evolution, and there are many reasons to refer to the tree as a ‘litter feeder’, in which surface ‘feeder’ roots proliferate and absorb released nutrients (Moore-Gordon et al., 1997). Reinforced mulching created by almond shell, in addition to natural litter, mainly from dropped leaves, provides roots with more natural edaphic growing conditions. The low degradability of the almond shell mulch can create and maintain an ideal surface environment for the avocado root system providing an adequate N mineralization rate and nutrient supply to sustain avocado yields at least in the order of $8.5 \text{ tonnes year}^{-1}$

hectare⁻¹. Better hydrophysical properties (aeration, porosity) in the low bulk density organic layer probably have also contributed to this adequate root environment.

3.3. Soil Carbon balance

Soil organic carbon balances for both treatments are shown in Table 4. Excluding from the calculation the 2012 almond shell addition, and focusing on the 2002 and 2007 additions, thickness of almond shell mulch was reduced to almost half its initial value (14 cm to 7.8 cm) that corresponded to 67.5 % weight reduction in the O-8 layer. Since part of OM migrated to the O25 layer (Table 4), weight reduction in O-8 cannot be fully attributed to organic matter degradation.

The 2002 and 2007 almond shell additions incorporated 23.4 kg m⁻² OC of which 5.6 kg m⁻² OC remained in the O-8 layer, 9.0 kg m⁻² OC migrated to O25 topsoil mineral layer, and only 0.2 kg m⁻² OC increased C in O50 subsoil in relation to F50 (Table 4). As a result, mulching caused 14.7 kg m⁻² OC more increment in the O soil than in the F soil, which corresponded to 36.9% degradation of the almond shell added-OC during the 5+5 years period. Mulch degradation rate can vary widely depending on product and site characteristics but reported data for similar lignocellulosic materials indicate shorter degradation periods or higher degradation rates than in the present case. Grundy and Bond (2007) indicated that chipped bark or wood mulch decompose over a period of around 2 years, and Vidal-Beaudet et al. (2012) reported 48% C degradation in green waste compost mixture (lignin content=28.4%) used as soil amendment after 5 years.

Total soil OC was 2.4 times greater in the O soil than in the F soil. This result is in accordance with the findings of Valenzuela-Solano et al. (2005) who, in a study

about the nitrogen mineralization from eucalyptus yardwaste mulch in avocado, found that soil OC content was three times higher in the mulched than in the un-mulched soil after 3 years. The greater OC increment was observed in the O25 layer, where the OC content was more than twice the content in the F25 layer (91.3 g kg^{-1} and 43.4 respectively, Table 2). Sequestered OC in the O25 cm layer was also almost twice the OC in the O-8 decomposed organic layer (9.0 kg m^{-2} and 5.6 kg m^{-2} , Table 4). The increased organic matter in the O25 layer maintained a C/N ratio (11.7) similar to that of the F25 (11.5) corresponding to steady soil humus; A C/N ratio of around 11 is common for most of the world topsoils (Batjes, 2014). This finding suggests that the new organic matter in the O25 layer was not merely incorporated due to physical mixing or hauling (for instance through earthworm cavities) from the surface O-8 organic layer, since this layer presented a higher C/N ratio (20.6, Table 1). Values below C/N 20 are traditionally considered suitable for a good compost (Paradelo et al., 2013). Mulch could have increased dissolved organic C in the upper mineral soil horizon after decomposition of plant materials and leaching (Huang et al., 2008). In the O25 mineral layer, C/N ratio indicated that OC could be considered more resistant than that in the upper O-8 organic layer.

In the O treatment soil OC build-up could be a result of several effects acting during degradation:

1) The recalcitrant nature of almond shell due to its high lignin content. Lignin is highly refractory and resistant to chemical and biological degradation due to its complex cross-linked structure (Martínez et al., 1995; Theuerl and Buscot, 2010). In the present experiment lignin has been concentrated as almond shells degraded, and its content increased from 362 g kg^{-1} of added almond shell to 589 g kg^{-1} in the O-8 organic soil horizon (Table 1). Lignin content of almond shells has been reported to range from 283

g kg⁻¹ (Martínez et al., 1995) to 384 g kg⁻¹ (Quesada et al., 2002). In litter, lignin often makes up between 150 and 400 g kg⁻¹ and, in some extreme cases, litter can have lignin contents as high as 500 g kg⁻¹ (Berg and McClaugherty, 2008). Lignin content in the O-8 layer surpassed the above cited upper limit for litter which was in accordance with the low C degradation rate observed in this trial.

2) The rate-retarding effect normally ascribed to high concentration of N that has a suppressing effect on the formation of ligninase and, thus, on lignin mass loss (Berg and McClaugherty, 2008). In the present case inorganic-N accumulation has been observed in the O-8 organic layer (Table 1).

3) The greater stability of finer particles constituent of O-8 degraded layer than that of greater particles in the added mulch. Several authors (Doublet et al., 2010; Vidal-Beaudet et al., 2012) observed slower C mineralization in finer than coarser fractions of soils and that organic matter becomes increasingly stable and more resistant to degradation after fragmentation, attributing the difference to greater accessibility of coarse fractions of organic matter to soil fauna.

4) The protecting effect of soil mineral components in the O25 layer. In soils, organic matter may be protected inside aggregates (Tisdall and Oades, 1982) and also the association with soil minerals enhances protection and stabilization (Marschner et al., 2008).

The low degradability of almond shells makes their use as mulch very advisable, since a large OC proportion is sequestered in the soil and mulch replacement will be needed only every several years reducing economic costs associated with fertilization.

3.4. Soil enzyme activities

Acid phosphomonoesterase, β -glucosidase, dehydrogenase, urease and protease activities in the different soil layers of both treatments are shown in Table 5. In the O-8 organic layer the enzyme activities were far greater than those in the underneath O25 layer and in control F25 layer. Enzyme activity index was 5 in F25 (reference value), 7.0 in O25 and 43 in O-8. In subsoil layers, activity indices were lower than in the topsoil layers and similar in both treatments (0.7 and 1.1 in F50 and O50, respectively). Almond shell mulch clearly stimulated soil enzyme activities in the O-8 degraded layer in spite of the high lignin content and the low degradation of almond shells. Current C/N ratio (20.6) in the O-8 layer, corresponding to an easily accessible substrate for soil microorganisms, could explain the increased biological activity. Urease and β -glucosidase indices were highest in the O-8 organic layer (21 and 9, respectively). Urease activity in soils is tightly bound to soil organic matter and soil minerals (Alef and Nannipieri, 1995). β -glucosidase activity has been suggested to be a good indicator of soil management and microbial functioning and its significant correlation with the soil organic matter is known (Alef and Nannipieri, 1995). The lignocellulosic character of almond shells (Martínez et al., 1995) justifies the predominance of this enzyme activity.

In a previous study, Bonilla et al. (2012) studied bacterial community composition and diversity in avocado soils observing that the addition of almond shells caused an increase in heterotrophic bacteria population levels which could be related to the higher organic C content detected and enzyme activities in the soil. These results are in agreement with the findings of the present study.

Except phosphatase and urease activities, enzyme activities in the O25 layer were similar to those in the F25 layer. In spite that the OC content in O25 was more than twice that observed in F25 (table 2), the response of several enzyme activities and

of the activity index was limited, which confirms the high and similar OM stability in both layers that was derived from their similar C/N ratio. This fact also indicated the resilience of the mineral layers of the original soil to change their biochemical status and help to explain the C accumulation in the topsoil mineral layer. In case of the mulch-increased enzyme activities, phosphatase and urease, soil water content could have affected them. Mean soil water content was 154 g kg^{-1} in F25 un-mulched soil layer and increased to 231 g kg^{-1} in O25 mulched soil. Siczek and Frąć (2012) found that mulch application enhanced soil enzyme activities compared to un-mulched soil and they partially attributed the enhancement to increased water availability by mulching through evaporation reduction.

Soil enzyme activities showed highly significant linear correlation to soil moisture content, soil organic carbon and other nutrients (data not shown). Soil moisture content also was highly correlated to OC (Pearson's correlation coefficient $r=0.989$), Unstandardized residuals from moisture could be considered as 'site-specific moisture content' and those residuals were no longer linked to OC and perhaps they are due to heterogeneity in the irrigation system efficiency or to soil micro-site characteristics. Soil enzyme activities were linearly related to OC and residual moisture content and the results of the statistical analyses are shown in Table 6 and Figure 1. The β -coefficients for OC and their high significance corroborate that enzyme activities depended mainly on OC. To a lesser extent, as indicated by lower β -coefficients, site-specific moisture content affected mainly phosphatase and urease activities, the two mulch-increased enzyme activities in the O25 layer. The relationship among OC and site-specific moisture on both activities are shown in Figure 1 depicting the partial regression plots. F and adjusted R^2 values in Table 6 also demonstrate the high impact of the considered independent variables on the enzyme activities. These results were

consistent with previous findings of Bonilla et al. (2012) which indicated that organic treatments impacted soil bacterial community composition and that this effect was more evident in the organic superficial layer of the soil, whereas in deeper soil layers the amendment did not affect bacterial communities. These authors concluded that the main factor affecting bacterial profiles in superficial soils was the treatment with almond shells and the behavior depends on the nature of the amendment, whereas in deep soil, the amendment did not seem to affect bacterial communities and the behavior depends upon soil type and particular conditions for decomposition. Present results indicate that among these particular conditions, micro-site soil moisture was particularly relevant with regard to phosphomonoesterase and urease activities.

4. Conclusions

Almond shell application as mulch to organically cultivated avocado trees originated a new soil organic horizon. The almond shells degraded slowly due to their high lignin content although maintaining a high biological activity in the mulch layer. Furthermore, the yield of the organically managed avocado trees was similar to or higher than that under conventional management, indicating that the mineralization rate of organic matter was enough to supply the necessary plant nutrients. Most of almond shell-added OC was transferred and accumulated in stabilized OM forms in the mineral topsoil layer. Both characteristics, reduced mineralization and high surface biological activity, makes almond shell mulch ideal for avocado trees, resulting in both environmental (increasing soil organic carbon) and agricultural benefits (lower costs, higher yielding capacity) reverting the soil OM depletion common in many agricultural soils. Moreover, it is expected that similar results could be obtained with other similar

residues in Mediterranean regions such as shells derived from peach or apricot juice production. In the medium term (10 years) mulching was shown as a way of storing C in soil although further studies should be conducted to prove whether or not mulching further helps creating the conditions which favor low OM mineralization extending C storage for the long term (100 years). The relationships among the micro-environmental conditions associated to mulching, the organic input characteristics and the related soil biological quality would be key parameters to reach the goal.

Acknowledgements

The authors want to thank to Jose M. Farré which initiated the present avocado trial and F. Cabrera by his critical comments. This work was partially supported by the Operative Program FEDER of the Junta de Andalucía (PAIDI-AGR108, PAIDI-AGR156 and P08-AGR-3694). Thanks to two anonymous reviewers for their valuable contributions.

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Figure captions

Fig. 1. Partial regression plots of the linear regression of phosphatase and urease activities on soil organic carbon and on the residual (uncorrelated to soil carbon) soil moisture.

Table 1 Characterization of almond shells and soil organic layer (degraded almond shell).

	Unit	ALMOND	SHELLS	SOIL	O-8 ^a
		Value	± sd ^b	Value	± sd ^b
Dry matter	g kg ⁻¹	86.9	± 3.7	33.5	± 6.5
BD ^c	g L ⁻¹	371	± 17	216	± 61
PARTICLE SIZE					
>25 mm	g kg ⁻¹	0.0	± 0.0	--	
25-10 mm	g kg ⁻¹	482.3	± 35.3	--	
10-5 mm	g kg ⁻¹	279.6	± 19.0	--	
5-2 mm	g kg ⁻¹	179.5	± 23.8	--	
<2 mm	g kg ⁻¹	58.6	± 0.8	--	
pH		7.4	± 0.1	7.0	± 0.1
E.C. 1:5 vol	mS m ⁻¹	23.8	± 0.4	22.1	± 8.1
OM ^d	g kg ⁻¹	975.2	± 11.3	656.6	± 55.3
OC ^e	g kg ⁻¹	450.5	± 8.5	332.0	± 34.6
Lignin	g kg ⁻¹	362	± 9	589	± 6
Kjeldahl-N	g kg ⁻¹	3.00	± 1.03	16.3	± 1.3
C/N		162	± 50	20.6	± 3.6
Lignin/N		130	± 41	364	± 31
NH ₄ -N	mg kg ⁻¹	--		7.29	± 0.22
NO ₃ -N	mg kg ⁻¹	--		9.67	± 7.59
P	g kg ⁻¹	0.262	± 0.122	1.85	± 0.49
K	g kg ⁻¹	3.95	± 1.15	7.01	± 0.75
Ca	g kg ⁻¹	2.31	± 1.36	22.4	± 1.6
Mg	g kg ⁻¹	0.37	± 0.21	5.55	± 0.81
Na	g kg ⁻¹	0.06	± 0.00	0.37	± 0.05
Fe	mg kg ⁻¹	144	± 11	12236	± 2732
Cu	mg kg ⁻¹	4.1	± 1.1	34.0	± 6.7
Mn	mg kg ⁻¹	7.1	± 0.2	300	± 78
Zn	mg kg ⁻¹	12.1	± 4.1	89.2	± 24.8
Cd	mg kg ⁻¹	<0.1	--	0.32	± 0.07
Cr	mg kg ⁻¹	9.5	± 1.3	18.5	± 4.0
Ni	mg kg ⁻¹	4.4	± 0.3	14.1	± 3.1
Pb	mg kg ⁻¹	0.7	± 0.2	10.0	± 2.9

^a Soil degraded organic layer; ^bStandard deviation, n=3; ^cCompacted bulk density on a dry matter basis for almond shells and core bulk density for soil organic layer; ^dOM: Organic Matter; ^eOC = Total Organic Carbon; -- not determined.

Table 2 Differences in soil chemical properties between the organic (O) and the conventional (F) treatments.

	Unit	Sig ^a	Mean±	sd ^b	Mean±	sd	Pearsons' coeff ^c	Sig
			F25		O25			
pH		**	7.80 ± 0.12		7.29 ± 0.09		-0.814	**
OC ^d	g kg ⁻¹	*	43.4 ± 6.5		91.3 ± 20.6		--	
Kj-N ^e	g kg ⁻¹	**	3.38 ± 0.55		5.56 ± 0.57		0.983	**
C/N ratio		*	11.5 ± 0.06		11.7 ± 0.06		--	
Olsen-P	mg kg ⁻¹	*	12.8 ± 2.5		19.0 ± 3.0		0.913	**
avail-K	mg kg ⁻¹	ns	146 ± 22		166 ± 14		0.779	**
NH ₄ -N	mg kg ⁻¹	ns	1.54 ± 0.35		1.95 ± 0.24		0.980	**
NO ₃ -N	mg kg ⁻¹	ns	2.49 ± 2.22		0.0			
			F50		O50			
pH		ns	8.17 ± 0.32		8.10 ± 0.35			
OC ^c	g kg ⁻¹	ns	11.1 ± 2.6		12.1 ± 2.1			
Kj-N	g kg ⁻¹	ns	1.07 ± 0.19		1.23 ± 0.04			
C/N ratio		ns	10.6 ± 0.3		10.8 ± 0.2			
Olsen-P	mg kg ⁻¹	ns	8.1 ± 1.7		6.4 ± 3.9			
avail-K	mg kg ⁻¹	ns	84.7 ± 17.8		96.0 ± 29.5			
NH ₄ -N	mg kg ⁻¹	ns	1.23 ± 0.13		1.30 ± 0.22			
NO ₃ -N	mg kg ⁻¹	ns	0.0		0.0			

^a **p<0.01, *p<0.05, ns: non significant; ^bsd:standard deviation, n=3; ^cCorrelation of OC with the rest of parameters using all replicates data; ^dOC: Soil organic Carbon; ^eKj-N: Kjeldahl-nitrogen

Table 3 Avocado total yield (kg tree⁻¹ year⁻¹) and trunk cross sectional area (cm²) for the organic (O) and the conventional (F) treatments

	Yield (kg tree ⁻¹ year ⁻¹)			Trunk area (cm ²)		
	O	F	Sig	O	F	Sig
2004-2005	76.6	61.0	ns	1221	1245	ns
2006-2007	33.2	22.6	**	1314	1313	ns
2008-2009	98.0	89.9	ns	1316	1327	ns
2010-2011	106.5	104.3	ns	1419	1345	ns
2004-2011 ^a	628.6	555.6	ns			

^a Cumulative yield in kg tree⁻¹

Table 4 Soil organic carbon balance sheet corresponding to the organic (O) and the conventional (F) treatments in the different soil layers

	Depth	BD	Treatment O	Treatment F	O – F
	cm	g L ⁻¹	OC content kg m ⁻²	OC content kg m ⁻²	OC content kg m ⁻²
MULCH					
2007 added AS ^a	7	370	11.7	0	
2002 added AS	7	370	11.7	0	
2002+2007 added			23.4	0	23.4
SOIL					
O-8	7.8	216	5.6	--	5.6
Mineral Topsoil	0-25	1270	17.1	8.1	9.0
Mineral subsoil	25-50	1530	2.7	2.5	0.2
TOTAL in soil			25.4	10.6	14.7

^a AS, almond shell. 2012 AS mulch not included

Table 5 Soil enzyme activities (in $\mu\text{mol g}^{-1} \text{hour}^{-1}$), standardized activity index (i) and water content (g kg^{-1}) in the different soil layers corresponding to the organic (O) and the conventional (F) treatments

	Sig ^{a,b}	Mean $\pm$	sd ^c	i ^d	Mean $\pm$	sd	i
					O-8		43
Water content	**	--			665 $\pm$ 65		
Phosphatase	**	--			225 $\pm$ 45		4.4
Glucosidase	**	--			10.3 $\pm$ 0.8		8.9
Dehydrogenase	*	--			0.070 $\pm$ 0.027		3.2
Urease	**	--			76.8 $\pm$ 15.6		20.8
Protease	**	--			3.92 $\pm$ 0.73		5.6
					F25		5.0
Water content	*	154 $\pm$ 23			231 $\pm$ 32		
Phosphatase	*	51.1 $\pm$ 3.2	1		71.8 $\pm$ 10.1		1.4
Glucosidase	ns	1.16 $\pm$ 0.38	1		1.67 $\pm$ 0.55		1.4
Dehydrogenase	ns	0.022 $\pm$ 0.003	1		0.025 $\pm$ 0.002		1.1
Urease	*	3.7 $\pm$ 1.3	1		6.5 $\pm$ 0.0		1.8
Protease	ns	0.70 $\pm$ 0.26	1		0.89 $\pm$ 0.26		1.3
					O25		7.0
					F50		0.7
Water content	ns	81.0 $\pm$ 9.2			80.1 $\pm$ 9.8		
Phosphatase	ns	14.5 $\pm$ 0.5	0.3		16.0 $\pm$ 6.3		0.3
Glucosidase	ns	0.082 $\pm$ 0.015	0.1		0.143 $\pm$ 0.049		0.1
Dehydrogenase	*	0.0056 $\pm$ 0.0009	0.3		0.0082 $\pm$ 0.0014		0.4
Urease	ns	0.50 $\pm$ 0.34	0.1		1.11 $\pm$ 0.73		0.3
Protease	ns	0.00 $\pm$ 0.00	0.0		0.00 $\pm$ 0.00		0.0
					O50		1.1

^a **p<0.01, *p<0.05, ns: non significant; ^bSignificance symbols compared pairs O-8 and O25, F25 and O25, F50 and O50 using independent samples T-test. ^csd:standard deviation, n=3; ^dstandardized activity index

Table 6 Linear regression of each soil enzyme activity (dependent variable) against soil organic carbon and residual soil moisture content (independent variables)

	β -coefficients ^a		Coefficients significance ^b				Adjusted R ²	F
	SOC ^c	Residual moisture ^d	SOC		Residual moisture			
Phosphatase	0.983	0.145	0.000	**	0.001	**	0.985	491
Glucosidase	0.973	0.044	0.000	**	0.515	ns	0.940	111
Dehydrogenase	0.920	0.192	0.000	**	0.076	ns	0.864	45
Urease	0.957	0.209	0.000	**	0.004	**	0.952	141
Protease	0.981	0.092	0.000	**	0.091	ns	0.965	193

^a Standardized regression coefficients, ^b **p<0.01, ns: non significant; ^c SOC: soil organic carbon;

^d Residual moisture content after soil moisture was calculated from a linear regression using organic carbon as independent variable