Geoderma 380 (2020) 114640

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Changes in carbon pools and enzyme activities in soil amended with pig slurry derived from different feeding diets and filtration process

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ARTICLE INFO

Handling Editor: Ingrid Kögel-Knabner

Keywords:
Pig Slurry
Feeding diet
Soil organic carbon
Carbon pools
Enzyme activities

ABSTRACT

Monitoring soil carbon content and pools and associated enzyme activities has become an important area of research in terms of the carbon cycle in agricultural lands in order to infer factors regulating soil carbon sequestration. Pig slurry (PS) is considered as a source of organic matter and nutrients for crop production. In recent years, because of the cost of conventional feed applications, manufacturers have preferred liquid feeding diets with decreases of 10–25% in production costs. Furthermore, physical and chemical filtration of PS by new techniques may prevent soil degradation and reduce the negative effects of PS, such as high salt, pathogen, and heavy metal contents. This research aimed to assess the effect of the application of raw pig slurry (R) and treated pig slurry (T) (derived from physical and chemical separation processes) from liquid (L) and solid (S) feeding diets on different soil organic carbon pools and enzyme activities in a sandy loam soil under a rainfed barley cropping system for two years. The solid diet consisted of maize/soya bean, tubers and roots, and supplementary vitamins and minerals. The liquid diet consisted of dairy products such as fresh whey, concentrated cheese or yogurt, skim milk powder, and beer by-products such as brewer's yeast. As a general pattern, the addition of the different types of PS (R or T) had no significant effect on most soil properties, except for microbial biomass C (Cmic), which increased with R. However, the type of diet did have a significant effect on most properties, both for R or T slurries, suggesting that this is more of a determinant factor in explaining changes in soil than the type of pig slurry. Solid diet favors the significant increase of soil organic carbon and arylesterase activity, suggesting higher stabilization of the organic compounds provided by the PS. However, the liquid diet contributed to increasing soluble C and Cmic, suggesting higher availability of nutrients and C sources. Hence, the use of treated pig slurry has no detrimental effect on organic carbon dynamics compared to raw pig slurry and can be suggested as an alternative to reduce the environmental impact, prevent soil pollution, and ensure sustainability.

1. Introduction

Pig slurry is a good source of plant nutrients (Antezana et al., 2016) and improves the chemical (Silva et al., 2016), physical (Agne and Klein, 2014) and biological characteristics of the soil if properly managed (Yanardağ et al., 2015, 2017). Land application of animal by-products is considered to be the most economical management practice that enables the nutrients in manure or slurry to be recycled (Assefa et al., 2006). Recent slurry production within Spain has been approximately 30 million tonnes year^{-1} (Moreno-García et al., 2017). As the scale and intensity of livestock production increase, environmentally safe and agronomically sound practices for the utilization of nutrients contained in the manure and slurry are needed (Chang and Janzen, 1996; Karlen et al., 1998). Animal by-products may contain salts, pathogenic organisms, and heavy metals. Thus, although pig slurry can be an ideal fertilizer, overdoses of slurry applications may cause salinization in semi-arid regions, increase toxic concentrations of metals in the soil, increase the risk of spreading pathogenic diseases in sludge and produce high ammonia gas emissions (Hjorth et al., 2011).

The integration of pig slurry as a fertilizer for crop production is the primary mechanism adopted for the disposal of this by-product. Nevertheless, the quantitative understanding of the relationship

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https://doi.org/10.1016/j.geoderma.2020.114640
Received 14 April 2020; Received in revised form 29 July 2020; Accepted 29 July 2020
Available online 12 August 2020
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between the animal feed composition and the agronomic and environmental consequences of the slurry produced from this feed is still very poor (Velthof et al., 2000). When liquid feed is used in stockbreeding, the production costs are reduced by 10–25% compared to conventional solid feed. Moreover, it provides a great advantage in terms of using and evaluating a wide range of food waste and agricultural sub-products (Rodríguez Estévez et al., 2007). Liquid nutrients used in pig nutrition are dairy products such as fresh whey, concentrated cheese or yogurt, bread and pastry, and beer by-products such as brewer's yeast. These products can be used directly in pig production without the need for expensive purification processes (Luis Criado et al., 2009).

Slurry management consists of various stages from animal nutrition to valorization such as its use as fertilizer (Sajeev et al., 2018). Research has always focused on the effects of manure and slurry on soil conditions and crop growth, without assessing the effect of feed on fertilizer composition and its value as fertilizer. Velthof et al. (2000) reported that animal feeding diets could easily change slurry properties. Furthermore, slurry characteristics are also changed by physical, chemical, and biological separation processes. In many cases, pig slurry is separated into solid and liquid fractions before being used directly in the field; this process is important for the ease of application and reduction of transportation costs. Adopting separation treatments, 70–80% of the total solids and 80–90% of volatile solids can be easily removed in pig slurry, with decreases of 70% in chemical oxygen demand (Fangueiro et al., 2012; Hjorth et al., 2011; Makara and Kowalski, 2015; Walker and Kelley, 2003). There are many techniques for fractions separation and pig slurry treatment, such as polyacrylamide flocculants (Walker and Kelley, 2003). In this study, we performed the physical and biological separation of pig slurries using biodegradable cationic polymers for flocculation developed by Martinez-Almela and Barrera (2005).

Soil organic matter (SOM) plays an important role in biogeochemical cycling processes in terrestrial ecosystems, and it is an essential source of available C and N for soil microorganisms and plants (Lal, 2020; Minasny et al., 2017; Plaza et al., 2018; Scaglia and Adani, 2009). In many cases, soil microbial biomass is closely linked to the primary productivity of an ecosystem and acts as a highly labile source of plant-available nutrients (Singh et al., 1989). The accumulation of SOM results from the activity of the soil biota: plants ensure the supply of organic compounds while soil fauna and microorganisms transform it. In the soil, most organic compounds are processed by heterotrophic microorganisms that use organic carbon as nutrient and energy sources (Fontaine et al., 2003). Soil microbial biomass and community structure are key drivers in mineralization and synthesizing processes. In this line, soil enzymes such as arylesterase, β-glucosidase, and β-galactosidase have a crucial role in C cycles (Karaca et al., 2010). The increased level of enzyme activities in the organic-amended soil may be a reflection of the increased protective sites within the soil, as a result of enhanced humus content (Pascual et al., 1999). The enzymes β-glucosidase and β-galactosidase play an important role in soils, as they are involved in catalyzing the hydrolysis and biodegradation of various glucosides and galactosides, releasing low molecular weight sugars that are important energy sources for microorganisms and plants (Bandick and Dick, 1999; Martinez and Tabatabai, 1997). Esterases (ester hydrolases) such as arylesterase are widely distributed in various organisms, including animals, plants, and microorganisms, and catalyze the hydrolysis and formation of ester bonds (Satoh and Hosokawa, 1998). Many of the esterases have wide substrate specificity, leading to the assumption that they have evolved to enable access to carbon sources or to be involved in catabolic pathways (Park et al., 2007). One of the tasks of the arylesterase is its involvement in the hydrolysis of toxic metabolites, organophosphates and recalcitrant aromatic compounds (Primo-Parmo et al., 1996).

Therefore, the objective of this study was to assess if repeated additions of raw and treated pig slurry derived from pigs fed with different diets significantly influenced soil organic carbon content and pools and carbon mineralization, and also to elucidate the relationships between organic carbon dynamics and some soil enzyme activities. In this study, we hypothesized that the use of pig slurry from pigs fed with a liquid nutrition diet could be an alternative to pig slurry obtained from pigs fed with a traditional (solid) nutrition diet with no significant differences in terms of soil carbon sequestration and dynamics in soil, because the liquid diet provides the essential nutrients required for pigs, with production of pig slurry with similar organic compounds. Additionally, treated pig slurries may decrease the toxicity of slurries by elimination, by physical separation, or by microbial degradation, and so, positive effects on microbial biomass and enzyme activities should be expected.

2. Material and Methods

2.1. Study site and experimental design

A two-year field experiment was performed with the application of raw pig slurry and treated pig slurry (with physical separation and chemical purification techniques) derived from two different pig diet managements (solid and liquid) in an agricultural soil cultivated with barley, to assess if the pigs' diet can have a significant effect on the soil carbon biogeochemical cycle and in the ability to enhance C sequestration in soil. The study was carried out for two crop cycles on a farm with pig production and agricultural land located in Cancarix, Albacete province, SE Spain (38°45′13″ N, 1°54′19″ W) (Fig. S1). The climate is semiarid Mediterranean, with a mean annual rainfall of 270 mm and a mean annual temperature of 17 °C. The soil is a Typic Xerorthents (Soil Survey Staff, 2014) with sandy loam texture, basic pH, high content of carbonates, and low organic matter content (Table 1). Barley (*Hordeum vulgare* L.), which is one of the most common crops in the region, had been grown in the field under rainfed conditions in the years prior to the experiment. The field experiment was set up in November 2009 in a complete randomized block with three replications, using plots of 5x5 m^2. Treatments were the application of: i) raw pig slurry from liquid feeding diet (RL), ii) raw pig slurry from solid feeding diet (RS), the liquid fraction of the treated pig slurry from liquid feeding diet (TL), the liquid fraction of the treated pig slurry from solid feeding diet (TL) and unamended control (CT). The application rate of pig slurry was 210 kg N ha^{-1} year^{-1}. Based on the N quantity of each pig slurry, the

Table 1
General characteristics of the studied soil (n = 48).

pH(H_2O)	EC dS m^{-1}	P_{Olsen} g kg^{-1}	Corg g kg^{-1}	TN g kg^{-1}	$CaCO_3$ %	Na cmol$^+$ kg^{-1}
7.80 ± 0.16	1.10 ± 0.17	0.69 ± 0.28	21.50 ± 3.96	2.10 ± 0.77	43.3 ± 3.9	1.60 ± 0.32
K cmol$^+$ kg^{-1}	**Mg** cmol$^+$ kg^{-1}	**Ca** cmol$^+$ kg^{-1}	**CEC** cmol$^+$ kg^{-1}	**Sand** %	**Silt** %	**Clay** %
3.10 ± 0.14	3.80 ± 0.67	19.70 ± 2.81	12.51 ± 1.47	50.24 ± 4.36	32.29 ± 4.87	16.98 ± 1.73

EC: Electrical Conductivity; Corg: soil organic carbon; TN: total nitrogen; CEC: Cation Exchange Capacity.

rates applied in this experiment were 5.82 L m^{-2} for RL, 5.07 L m^{-2} for RS, 9.05 L m^{-2} for TL, and 13.72 L m^{-2} for TS. Plots received no other fertilization besides pig slurry.

The solid diet consisted of sugar cane molasses, maize/soya bean, amino acids, tubers and roots, and supplementary vitamins and minerals. The liquid diet consisted of dairy products such as fresh whey, concentrated cheese or yogurt, bread, and pastry, skim milk powder, and beer by-products such as brewer's yeast.

2.2. Pig slurry treatment

Farmer prefer screw press style separators mostly because of their lower cost. However, such separators make it possible to physically separate only the solid part of the pig slurry. We chose this seperator because it allows for more extensive and physical separation, as well as chemical separation, and can prevent PS's negative environmental effects. The SELCO Ecopurin® (SELCO MC, Advanced Engineering Services, Castellón, Spain) physical-chemical separator was used for the treating of the raw pig slurry, with the use of biodegradable cationic polymers for flocculation. The SELCO-Ecopurin® separation module (Fig. S2) consists of: 1) a polymer mixing section where the dry polymer is activated with water; 2) the ionic transfer reactor main module where polyacrylamide (PAM) is mixed with wastewater (5 kg m^{-3} dry PAM is activated in water and then mixed with the slurry for 20 to 30 min); 3) a rotating screen with 0.2 mm openings to separate the flocculated solids; 4) a filter press to further dewater the manure; and 5) a small dissolved air flotation unit to further separate residual solids in the liquid stream before exiting to the water treatment section. Solids skimmed from the dissolved air flotation (DAF) unit are returned to the mixing tank for subsequent separation (Martinez-Almela and Barrera, 2005), where 12 g of PAM per 1.5 kg of total suspended solids were used in the separation (Dinuccio et al., 2012). For the purification of the liquid phase after the decantation of flocculated solids, filters ($<$ 0.5–1 mm) were used to increase separation efficiency to $>$ 90%. These filters were filled with hollow spheres of plastic on which bacteria develop and are capable of removing the excess of ammonia (Martínez-Almeda et al., 2009).

The characteristics of the different pig slurries used in this experiment are shown in Table 2. The pH of the pig slurries was between 7.08 and 8.02, with high salinity, total nitrogen of 1.53–4.14 mg L^{-1} (where ~50–75% was ammoniacal nitrogen (AN), and P_2O_5 content of 36.3–1363 mg L^{-1}.

2.3. Soil sampling and analytical methods

There were three different soil samplings. The first sampling was carried out at the sowing of the barley in the first cycle (November 2009) and the two others were at the end of each of the two crop cycles (July 2010 and July 2011, respectively). Three composite samples were randomly collected in each plot at two different depths (0–20 cm and 20–40 cm). The soil samples collected were air-dried in the lab, passed through a 2-mm sieve, and stored at room temperature prior to laboratory analyses. The biochemical properties were also measured in air-dried samples, as the biochemical properties from Mediterranean semiarid soils are stable in air-dried samples for at least six months, and it is not necessary to measure them in field-moist soil samples (Zornoza et al., 2009b).

Soil pH and electrical conductivity (EC) were measured in deionized water (1:1 and 1:5 w/v, respectively). Soil organic carbon (Corg) was determined by potassium dichromate oxidation, whilst total nitrogen (TN) was determined by the Kjeldahl method (Duchaufour, 1970). The volumetric method (Bernard calcimeter) was used to determine the equivalent calcium carbonate (Hulsemann, 1966). Plant available P was measured according to Olsen and Dean (1965). Cation exchange capacity (CEC) was determined according to Chapman (1965). An atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer) was used to determine the selected element (Na, K, Ca, and Mg) concentrations. Recalcitrant carbon (Crec) content was determined by hydrolysis with H_2SO_4 (Rovira et al., 2009; Rovira and Vallejo, 2000). Microbial biomass carbon (Cmic) was determined using the fumigation-extraction procedure after extraction with 0.5 M K_2SO_4 (Vance et al., 1987b). The non-fumigated fraction was considered as soluble carbon (Csol). The arylesterase activity was quantified following the method of Zornoza et al. (2009a). β-glucosidase and β-galactosidase activities were measured according to Eivazi and Tabatabai (1988).

2.4. Pig slurry analysis

Characterization of the pig slurry was carried out according to the Standard Methods for the examination of water and wastewater (APHA, 2005). Raw PS was diluted (1/10) in a 100 ml flask, manually shaken for 1 min, then filtered with the filter paper (Albet paper No 242.). This extract was kept refrigerated at 4 °C for conservation. For further analyses of PS, the following analytical techniques suggested by APHA (2005) were used. pH was measured by pH meters (LPG 21 Crisol), electrical conductivity (EC) (LPG Conductivity Crisol 31), total nitrogen, and NH_4^+-N were measured by Duchaufour (1970) method. Trace elements (Cu, Zn, Fe, and Mn) were measured by Atomic Absorption Spectrometer (AAS) (AAnalyst-800 AAS, Perkin Elmer Precisely). Phosphorus forms (P_2O_5 and PO_4^{3-}) were measured as molybdenum blue after acid hydrolysis and oxidation at 100–120 °C. This test used Nanocolor 0–55 and was measured with the photometer PF-11, both from Macherey-Nagel.

Table 2

Selected chemical properties of the pig slurries studied (n = 48).

Samples	pH	EC	Corg	Total N	C:N	NH_4^+-N
		dS m^{-1}	g L^{-1}	g L^{-1}		g L^{-1}
RL	7.23 ± 0.35	22.39 ± 6.06	33.40 ± 14.16	3.61 ± 1.53	9.25 ± 3.93	2.78 ± 1.17
RS	7.08 ± 0.35	23.76 ± 7.31	33.29 ± 13.75	4.14 ± 1.71	8.05 ± 3.33	3.00 ± 1.21
TL	7.64 ± 0.12	20.28 ± 0.17	26.29 ± 1.48	2.32 ± 0.13	11.34 ± 0.64	2.01 ± 0.20
TS	8.02 ± 0.07	15.40 ± 1.50	20.60 ± 4.72	1.53 ± 0.35	13.47 ± 3.09	1.27 ± 0.13
	P_2O_5 mg L^{-1}	PO_4^- mg L^{-1}	Mn mg L^{-1}	Zn mg L^{-1}	Fe mg L^{-1}	Cu mg L^{-1}
RL	831.6 ± 155.9	1163 ± 549	1.34 ± 1.31	2.62 ± 1.51	3.40 ± 1.48	0.73 ± 0.62
RS	165.3 ± 12.1	107.7 ± 9.5	3.95 ± 1.70	5.84 ± 2.16	5.06 ± 1.70	1.85 ± 0.96
TL	1363.2 ± 255.7	1733 ± 262	0.97 ± 0.27	2.81 ± 1.13	2.13 ± 0.69	0.64 ± 0.36
TS	36.3 ± 21.3	27.5 ± 6.3	0.44 ± 0.34	0.33 ± 0.17	0.00 ± 0.00	0.19 ± 0.21

RL: Raw pig slurry from the liquid feeding diet; RS: Raw pig slurry from the solid feeding diet; TL: Treated pig slurry from the liquid feeding diet; TS: Treated pig slurry from the solid feeding diet; EC: electrical conductivity.

2.5. Statistical analysis

The fit of the data to a normal distribution for all properties measured was checked with the Kolmogorov-Smirnov test. Data were submitted to two-way repeated measures ANOVA, with time (start, cycle 1, and cycle 2) as within-subject factor, and pig slurry type (raw and treated) and diet type (liquid and solid) as between-subject factors. Relationships among properties were studied using Pearson correlations. Soil chemical properties related to carbon content and pools and enzyme activities were subjected to principal components analysis (PCA) to elucidate major variation patterns in terms of C pools. The PCA reduced all the variables by using linear combinations and explained most of the variance that the initial results had. Statistical analysis was performed with the software SPSS for Windows (Version 26.0).

3. Results

3.1. Soil organic carbon content and pools

The highest value in Corg was recorded in RS after the last crop cycle in surface soil, while the lowest value was observed in RL after the first crop cycle in subsurface soil (Fig. 1). There was no significant effect of pig slurry type (raw or treated) on Corg, while diet type significantly affected Corg on the surface soil. The pig slurry type × diet type interaction was not significant. Thus, pig slurry derived from the solid diet, raw or treated, significantly increased Corg compared to slurry coming from the liquid diet on the surface. This effect was not observed in the subsurface layer.

Csol showed the highest values during the initial sampling, whilst the lowest value was for TS at the end of the second cycle in the subsurface soil (Fig. 1). The sampling time was the factor with the highest significant effect on Csol, mostly in surface soil; this was also observed in Cmic. As for soil Corg, the pig slurry type did not significantly affect Csol at either of the soil depths. However, diet type was significant in surface soil, with significantly higher values under the liquid diet in the last crop cycle, regardless of the pig slurry type. Csol was positively correlated with Corg, TN, P, EC, Mg, Ca, β-glucosidase, arylesterase, and Crec. Conversely, Csol was negatively correlated with pH and C:N ratio.

The highest Crec content was observed in TL at the surface at the end of the first cycle, while the lowest value was observed at the subsurface in the initial sampling (Fig. 1). Sampling time significantly affected Crec at both depths, with higher values in the last two samplings. This trend was the opposite to that of the previous properties, indicating that Crec increased in summer time under Mediterranean conditions in rainfed cropping systems. The type of pig slurry and diet did not significantly affect Crec at either depth. We found positive correlations between Crec and Corg, TN, P, CEC, Mg, Ca, β-glucosidase, β-galactosidase and arylesterase. There were negative correlations

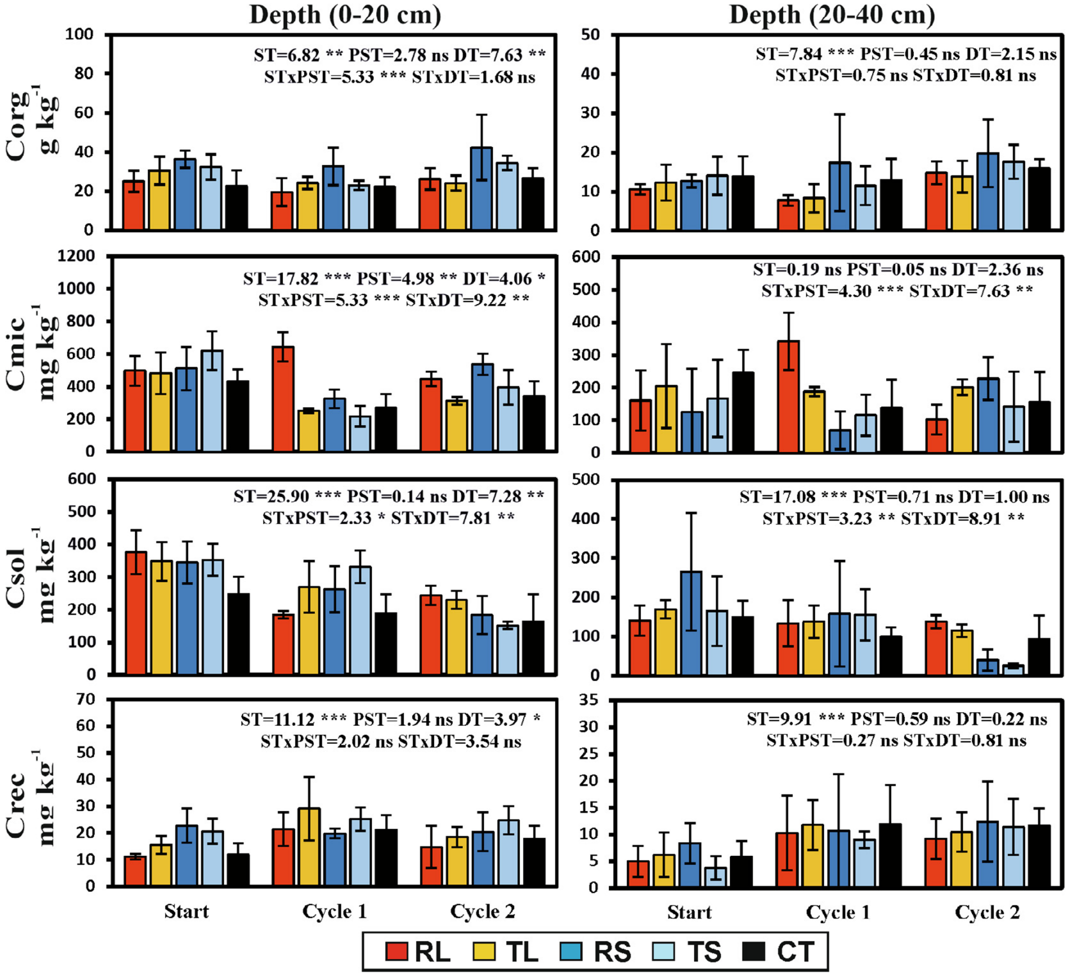

Fig. 1. Effect of pig slurry application on total soil organic carbon (Corg), microbial biomass carbon (Cmic), soluble carbon (Csol) and recalcitrant carbon (Crec) in the surface and subsurface soil layers (n = 3). RL = Raw Pig Slurry Liquid Feeding Diet, RS = Raw Pig Slurry Solid Feeding Diet, TL = Treated Pig Slurry Liquid Feeding Diet, TS = Treated Pig Slurry Solid Feeding Diet, CT = Control, F values and significance of the two-way repeated ANOVA measures are shown in each graph. ST = Sampling Time, PST = Pig Slurry Type, DT = Diet Type. Significant at *P < 0.05, **P < 0.01 and P < 0.001; ns = not significant (P > 0.05).

Table 3
Correlation matrix among the different soil properties. Sample size = 48.

	TN	P	pH	EC	CaCO$_3$	C:N	CEC	K	Mg	Ca	β-Glu	β-Gal	Aryl	Csol	Cmic	Crec
Corg	0.94**	0.75**	−0.72**	0.15	−0.02	−0.22	0.37**	0.18	0.71**	0.68**	0.68**	0.43**	0.52**	0.54**	0.61**	0.70**
TN	*	0.82**	−0.74**	0.27*	−0.06	−0.47**	0.30*	0.10	0.83**	0.69**	0.65**	0.36**	0.59**	0.65**	0.64**	0.75**
P		*	−0.54	0.52**	−0.25*	−0.30**	0.10	0.16	0.80**	0.59**	0.30*	0.23	0.47**	0.64**	0.59**	0.44**
pH			*	−0.19	−0.06	0.27*	−0.30*	0.01	−0.58**	−0.55**	−0.53**	−0.24*	−0.39**	−0.51**	−0.46**	−0.63**
EC				*	−0.24*	−0.32**	−0.12	0.33**	0.56**	0.31**	−0.22	−0.19	0.16	0.52**	0.15	0.03
CaCO$_3$					*	0.13	0.06	0.00	−0.22	−0.14	0.13	−0.03	−0.15	−0.14	−0.27*	−0.04
C:N						*	0.08	0.16	−0.49**	−0.33**	−0.31**	−0.05	−0.42**	−0.46**	−0.30*	−0.38**
CEC							*	0.23*	0.17	0.22	0.28*	0.14	0.05	−0.18	0.13	0.39**
K								*	0.26*	0.31**	0.15	0.01	−0.05	0.00	0.04	−0.04
Mg									*	0.76**	0.40**	0.21	0.58**	0.75**	0.59**	0.60**
Ca										*	0.56**	0.25*	0.54**	0.61**	0.52**	0.51**
β-Glu										*	0.50**	0.46**	0.31**	0.57**	0.57**	
β-Gal											*	0.37**	0.12	0.45**	0.39**	
Aryl													*	0.56**	0.61**	0.60**
Csol														*	0.45**	0.47**
Cmic														*	0.46**	
Crec																*

Corg: soil organic carbon; TN: total nitrogen; EC: electrical conductivity; CEC: cation exchange capacity; Cmic: microbial biomass carbon; Csol: Soluble carbon; Crec: Recalcitrant carbon; β-Glu: β-Glucosidase enzyme activity; β-Gal: β-Galactosidase enzyme activity; Aryl: Arylesterase enzyme activity; Sub: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

between Crec and pH and C:N ratio (Table 3).

3.2. Microbial biomass carbon and enzyme activities

The highest value in Cmic was recorded for RL in surface soil at the beginning of the experiment, while the lowest value was observed for RS in subsurface soil at the end of the first cycle (Fig. 1). The type of pig slurry significantly affected Cmic in surface soil, with higher values after application of raw pig slurry. Diet type was also significant in surface soil, as was the pig slurry type × diet type interaction, showing that the liquid diet contributed to higher Cmic when raw slurry was applied, mostly during the first crop cycle. No significant effect of individual factors was observed in subsurface soil. Cmic was significantly and positively correlated with Corg, TN, P, Mg, Ca, β-galactosidase, β-glucosidase, arylesterase and Crec. Furthermore, Cmic was negatively correlated with pH, CaCO$_3$ content and C:N ratio (Table 3).

Enzyme activities are shown in Fig. 2. Sampling time significantly affected β-glucosidase and β-galactosidase activities at both depths, with higher values in the last sampling. The type of pig slurry and diet did not significantly affect these two enzymes at any depth, and interactions were not significant either. Sampling time showed no significant effect on arylesterase activity in the surface soil layer, indicating that arylesterase activity remained stable along time. However, for subsurface soil, the sampling time significantly affected arylesterase, with a higher value after the first crop cycle after the application of pig slurry derived from the liquid diet. The pig slurry type showed no significant effect on arylesterase. Nonetheless, diet type showed a significant effect at the surface, with higher activity under the solid diet.

There were positive correlations between β-glucosidase and Corg ($r^2 = 0.68P < 0.01$), TN, P, CEC Mg, Ca, β-galactosidase and arylesterase. The β-glucosidase was negatively correlated with pH and the C:N ratio (Table 3). The β-galactosidase was positively correlated with Corg, TN and Ca, while negatively correlated with pH. Arylesterase was positively correlated with Corg, TN, P, Mg, and Ca, and negatively correlated with pH and the C:N ratio.

The PCA performed with soil physicochemical and biological properties in the surface layer (Fig. 3a) showed that 81.77% of the total variation could be explained by the first two PCs. PC1, which explained 49.77% of the variation, was strongly associated with Csol, Cmic, and arylesterase with positive values and Crec with negative values. PC2, which explained 32% of the variation, was strongly related to Corg and β-galactosidase. Very similarly, the PCA performed with the subsurface layer (Fig. 3b) also showed that 79.62% of the total variation could be explained by the first two PCs. PC1, which explained 52.97% of the variation, was strongly associated with Crec, arylesterase, and β-galactosidase with positive values and Csol and Cmic with negative values. PC2, which explained 26.68% of the variation, was strongly related to Corg and β-glucosidase. At both soil depths, neither of the two PCs clustered samples by treatments, highlighting that soil organic carbon, organic carbon pools, and enzyme activities were not highly affected by treatments, with similar behavior being found among the different plots.

4. Discussion

The impact of pig slurry on SOC content is a long-term process that cannot be assessed in a two-year experiment, but other indicators such as labile organic C pools, Cmic and enzyme activities respond to changes quickly and could give evidence about the short-term effect of pig slurry addition (Aon et al., 2001; van Bruggen and Semenov, 2000). The organic matter content in pig slurry is usually low (between 2 and 5%), with low C:N ratios (between 8 and 13%) as observed in this study (Table 2) and confirmed in other studies (Martinez-Almela and Barrera, 2005; Santos et al., 2018). This characteristic makes pig slurry highly degradable by soil microorganisms, and the effect on soil organic carbon content and pools could not be appreciated short-term (Plaza et al., 2004).. It is important to highlight that the type of pig slurry (raw or treated) had no significant effect on organic carbon content, and pools none on enzyme activities. Thus, once applied to the soil, the organic matter provided by the pig slurry is rapidly mineralized, likely due to the low C:N ratio with no effect being observed at harvest time. This fact has been previously reported in areas with pig slurry applications, contributing to decreased soil C:N ratios, indicating greater N availability (Rauber et al., 2012). The majority of C loss that occurred during the first week following the application of pig slurry was due to the metabolism of sugars and amino acids, which are readily available to soil microorganisms owing to the high N content (Rochette and Angers, 2000). It is true that during the pig slurry phase separation process, organic matter content decreases, but the rate of application was based on the N content of the pig slurry. Therefore, the application rate for treated pig slurry was higher than that for raw pig slurry to reach the same amount of N (see Section 2.1), although the final effect on the soil was the same. These results suggest that the quality of organic matter in both types of pig slurry (raw and treated) is similar, and its dynamics in the soil (degradation, mineralization, humification) may also be similar, leading to the lack of differences in Corg and

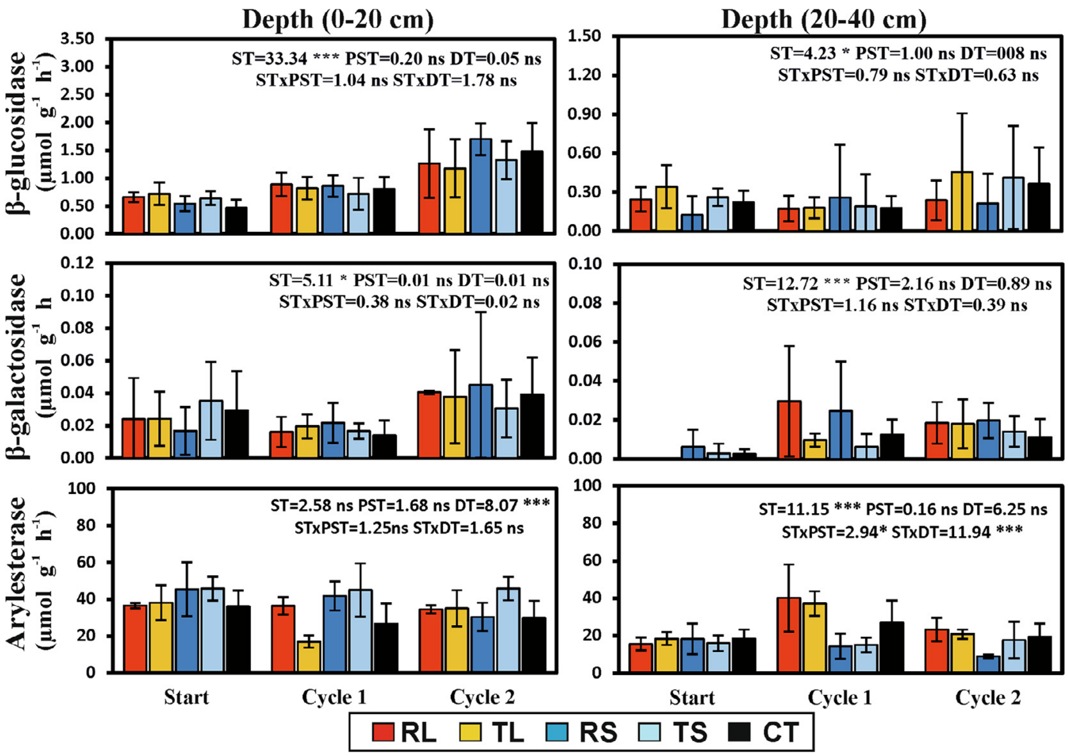

Fig. 2. Effect of pig slurry application on arylesterase, β-galactosidase and β-glucosidase enzyme activities in the surface and subsurface soils (n = 3). RL = Raw Pig Slurry Liquid Feeding Diet, RS = Raw Pig Slurry Solid Feeding Diet, TL = Treated Pig Slurry Liquid Feeding Diet, TS = Treated Pig Slurry Solid Feeding Diet, CT = Control, F values and significance of the two-way repeated ANOVA measures are shown in each graph. ST = Sampling Time, PST = Pig Slurry Type, and DT = Diet Type. Significant at *P < 0.05, **P < 0.01 and P < 0.001; ns = not significant (P > 0.05).

organic carbon pools. This fact makes sense because the treatment the pig slurry was submitted to was only a phase separation after flocculation, which does not provide any chemical modification in soil organic matter. Contrarily to our results, Bol et al. (2003) reported that soil enzyme activity increased with the application of raw pig slurry under grassland (> 40 years) in a clayey soil in Southwest England. Increases in enzyme activities were suggested to be due to the improvement in soil conditions (such as reaching a near to optimal pH value) and due to the application of microorganisms by the slurry amendment in the case of the activity (Gianfreda and Ruggiero, 2006).

The only C pool significantly affected by the type of pig slurry was Cmic, with higher content with the addition of raw pig slurry. With the application of pig slurry, the presence of microbial habitats in the soil and access to microbial food sources become easier, as a result of which the microbial population can grow decomposing soil organic particles (de Mora et al., 2005; Zornoza et al., 2013b). Raw pig slurry may provide higher availability of nutrients to favor the growth of microbial communities, as confirmed by the lower C:N ratio compared to the treated pig slurry. This is also supported by the positive correlations between Cmic and soil nutrients (Table 3). The negative relationship

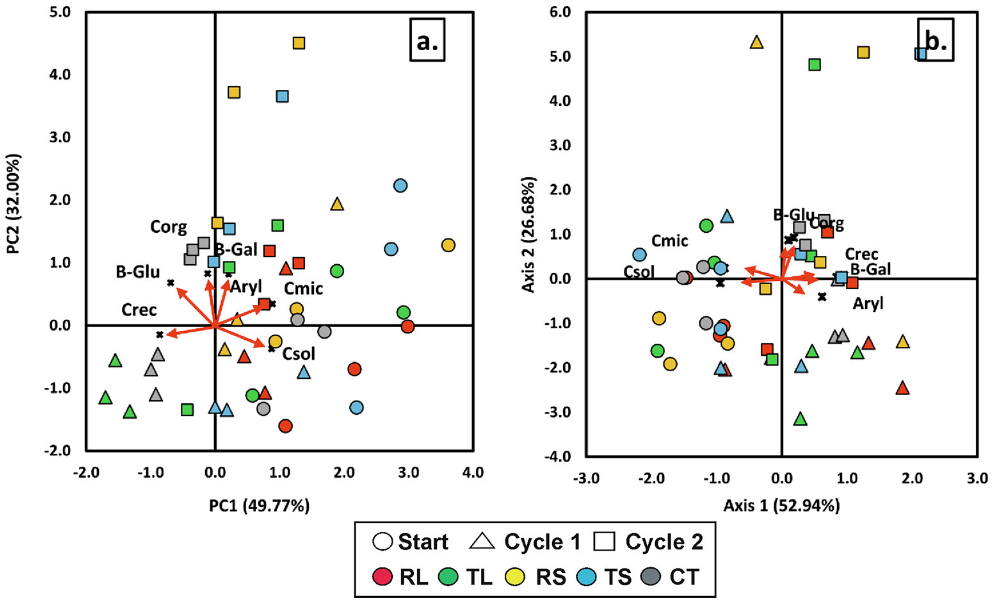

Fig. 3. Biplot of principal component analysis (PCA) performed with C pools and enzyme activities in surface soil layer (a) and subsurface layer (b). Cmic: microbial biomass C, Corg: total organic C; Crec: recalcitrant C; Csol: soluble C; B-Glu: β-glucosidase activity; B-Gal: β-galactosidase activity; Aryl: arylesterase activity. RL = Raw Pig Slurry Liquid Feeding Diet, RS = Raw Pig Slurry Solid Feeding Diet, TL = Treated Pig Slurry Liquid Feeding Diet, TS = Treated Pig Slurry Solid Feeding Diet, CT = Control.

between Cmic and pH observed in this study has also been observed in previous studies (Pietri and Brookes, 2008; Vance et al., 1987a). The main reason for this could be the limited activity of the microbial population in alkaline soil, and that the activity increased with decreasing pH. Microbial metabolism and enzyme activities are highly dependent on soil pH (Eivazi and Tabatabai, 1990; Xiao-Chang and Qin, 2006).

Cmic was nevertheless more affected by sampling time than by the type of pig slurry (Fig. 3). Cmic content was higher at the beginning of the experiment than at the end of both crop cycles. This was likely related to the different sampling season since the first sampling was performed in November, while the last two samplings were carried out in July. July is summertime in the study area, with high temperatures and solar radiation and practically null precipitation. Under rainfed systems, this time of year is associated with the lowest microbial biomass due to climatic constraints under the Mediterranean climate (Plaza-Bonilla et al., 2015). Similarly, Csol followed the same pattern, suggesting that Csol increases with microbial biomass growth, owing to an active soil organic matter degradation (Zornoza et al., 2007). Crec showed higher values in November, the opposite trend to Csol and Cmic. This fact may indicate that Crec increases in summertime under Mediterranean conditions in rainfed cropping systems, likely due to lower microbial activity. Pig slurry applications had a positive effect on the accumulation of Cmic in the surface layer but without significant changes in the subsurface. Accordingly, Dambreville et al. (2006) reported that applications of pig slurry for nine years tended to increase Cmic content, but this positive effect was limited only to the surface layer. Cosandey et al. (2003) also reported that Cmic content decreased with increasing soil depth in a terric Histosol.

Glucosidases play a key role in degrading organic compounds such as crop residues or animal manure in soils, and β-glucosidase activity is strongly related to soil organic matter cycling (Gascó et al., 2016). Moreover, β-glucosidase has been used to monitor quick changes in soil organic matter caused by changes in soil management (Bandick and Dick, 1999). The increase in β-glucosidase enzyme activity may have a positive relationship with the increasing amount of organic matter in the soil. Gianfreda and Ruggiero (2006) reported that enzyme activity in the soil slightly increases with the application of RPS. The enzymes absorbed in the degradation of carbohydrates (b-galactosidase and b-glucosidase) showed higher activities with PS application because 50% of the organic carbon present in the slurry was soluble (Zornoza et al., 2013a). However, we could not observe a remarkable increase in enzyme activity due to the late sampling in our study results. This may indicate that the effect of PS applications is effective in the short term, or enzyme activities are limited due to the low organic matter content of our studied soil. Arylesterase enzyme hydrolyzes highly recalcitrant aromatic compounds of soil organic matter (Primo-Parmo et al., 1996). Furthermore, increases in this enzyme activity are a clear indicator that organic matter applied by solid pig slurry is more recalcitrant and prone to be accumulated in the soil. Zornoza et al. (2013a) reported that increments in arylesterase activity after the application of the pig slurry were not as intense as in the other enzymes, since pig slurry provides highly available organic compounds, rather than recalcitrant ones. However, no effect was observed in Crec, suggesting that two crop cycles may not be long enough to assess changes in Crec under rainfed cropping systems in a semiarid Mediterranean climate. Crec concentration in our study was similar to that reported in the surface layer of other agricultural soils (Currie et al., 2002; Masiello and Druffel, 2003).

The most important outcome of this experiment is the fact that pig diet (solid or liquid) had an important effect on some soil properties, with this factor being more significant than if pig slurry was applied raw or treated. In fact, this is the first attempt, as far as we are concerned, in which slurry derived from liquid diet is used as organic fertilizer. The liquid diet led to a pig slurry that significantly increased Csol and Cmic, for both raw and treated pig slurry. Contrarily, the solid diet led to significant increases in Corg and arylesterase activity. When the organic C content of pig slurries was examined, it was observed that the solid diet contained a greater amount of stable C compared to the liquid food diet, it therefore had a positive effect on Corg and Crec. In addition, the liquid diet originated a slurry with higher quantity of other nutrients such as P, Zn and Fe (Table 2). Hence, the organic sources of the liquid food diet, such as the fresh dairy products and brewery by-products given in the liquid food diet, originated a slurry with more degradable compounds, associated to higher concentration of essential nutrients, which were directly used by the microbial population by applying high doses to the soil. As a consequence the use of a slurry derived from a liquid diet had a positive effect on Csol and Cmic. This is related to the activation of the biochemical cycles in the soil and higher mineralization rates, which can finally contribute to increase the availability of nutrients for the crop (Plaza et al., 2004). Contrarily, a solid diet seems to provide more recalcitrant organic compounds that slow down soil organic carbon mineralization, thus favoring C sequestration. Thus, diet type is an important factor determining the characteristics of the slurry (Hansen et al., 2014), more determinant that posterior treatments to purify the slurry. In this sense, Sánchez-Martín et al. (2017), for example, observed that the inclusion of fibrous compounds in the pig diet can lead to a reduction of urea-N excretion, with lower N compounds in the slurry. So, the manipulation of diet provides an available strategy to change organic and mineral compounds within the slurry (Dijkstra et al., 2013), which may be used to tailor slurries to be used as potential fertilizers in agriculture. This experiment highlights the high influence of diet on the excreta produced by the animals. Thus, not only strategies must be assessed to treat the slurry to reduce the possible environmental effects once applied in agriculture as fertilizers, but also strategies to manipulate the diet in a way it is healthy for the animal, fulfilling the required standards for animal production, but also generates a slurry with the organic and mineral compounds needed to enhance soil quality and fertility and foster vegetal production. The liquid diet used in this study favors the growth of microorganisms and the increase of soluble organic compounds, which can be associated to increases in soil fertility by the mineralization of the easily degradable compounds.

5. Conclusions

Generally speaking, the addition of different types of pig slurry had no significant effect on soil organic carbon content and pools and enzyme activities involved in the C cycle under rainfed barley monoculture in a sandy loam Mediterranean soil. Although the type of pig slurry (raw or treated) had no significant effect on the studied properties except for microbial biomass carbon, the type of diet significantly affected many of the properties. The solid diet led to increases in soil organic carbon and arylesterase activity, indicating higher recalcitrance of the organic compounds provided. Contrarily, the liquid diet led to increases in soil soluble organic carbon and microbial biomass carbon, suggesting more available nutrients. Thus, the use of treated pig slurry has no detrimental effect on organic carbon dynamics compared to raw pig slurry and can be suggested as an alternative to decrease the environmental impact, prevent soil pollution, and ensure sustainability.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Raúl Zornoza acknowledges the financial support from the Spanish Ministry of Science, Innovation, and Universities through the “Ramón y Cajal” Program [RYC-2015-18758].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114640.

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