Domingo-Olivé, F., Bosch-Serra, A.D., Yagüe*, M.R., Poch, M.R., Boixadera. J. 2016. Long term application of dairy cattle manure and pig slurry to winter cereals improves soil quality. *Nutrient Cycling in Agroecosystems* 104: 39-51. *DOI: 10.1007/s10705-015-9757-7*

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Abbreviations: DCM, dairy cattle manure; DM: dry matter; MF, mineral nitrogen fertilizer; MWD, mean weight diameter; PS, pig slurry; SOC, soil organic carbon; SOM, soil organic matter; WSA, water stable aggregates.

Abstract

Organic fertilizers (manures and slurries) applied repeatedly over many cropping seasons favourably influence nutrient recycling, maintenance of soil organic matter (SOM), and improve soil quality parameters such as soil aggregation and porosity. These aspects are particularly relevant in Mediterranean environments characterized by low SOM. This study was set up in a subhumid Mediterranean area where two different trials, devoted to winter cereals, were fertilized with dairy cattle manure (DCM) or pig slurry (PS) for a period of 12 years. One objective of this research was to evaluate the impacts of these fertilization practices on aggregate stability and SOM fractions, when compared with a mineral N fertilizer and a control (no-N) treatment. Porosity and pore shape were also studied in PS plots. The use of DCM significantly increased water stable aggregates by up to 16.4%-18.0%. Slurry addition did not affect aggregation but it increased the area occupied by pores >65µm. Soil organic carbon (SOC) and light organic fraction (0.05-0.2mm) increased with DCM incorporation but in PS treatments the SOC increment was non-significant. Data from DCM and PS together showed a positive and significant linear relationship between SOC ($p<0.05$, $R^2=0.60$), SOC light fraction ($p<0.01$, $R^2=0.75$) and SOC light fraction at 0.05-0.2 mm size ($p<0.01$, $R^2=0.83$), with water-stable aggregate. The use of animal residues (DCM or PS), applied according to an N criterion, increased available phosphorus and potassium soil content while improving yields. The enrichment of soil nutrients with DCM and PS use requires further research in order to avoid potential environmental impacts.

Keywords: aggregate stability; organic carbon fractions; nutrient balance; slaking; soil porosity; pore size distribution; Mediterranean conditions; organic fertilizers

Introduction

Soil amendment with organic fertilizers of animal origin is a common habitual practice in order to improve soil fertility and productivity, particularly in agroecosystems with naturally low organic matter content which are very susceptible to soil degradation. The improvement of management practices to maintain or even to increase soil organic carbon (SOC) is of great interest as SOC losses are a hazard to soil quality and productivity (Jones et al., 2012). Pig (*Sus scrofa domesticus*) slurry (PS) is rich in N, and ammonium-N accounts for around 75% of it (Yagüe et al., 2012a). It also has a low organic carbon content (C:N ratio ranges between 4 to 8) in contrast to solid dairy cattle (*Bos taurus*) manure (DCM) (C:N ranges between 10 to 25). These organic materials are also quite different in terms of dry matter (DM) and N forms which may have different influences on microbial activity and chemical changes in soil (Ndayegame and Cotê, 1989; Velthof et al., 2000). When organic residues with a low C:N ratio are incorporated into soil, microorganisms have sufficient N for protein metabolism but not enough C as an energy source. Then, the microbial oxidation of native soil organic matter will occur (Trolldenier, 1975). Other studies suggest that a C:N ratio of manures greater than 15-19 results in net N immobilization (Van Kessel et al., 2000; Calderon et al., 2005), which affects its crop availability. Nevertheless, soil chemical alterations that occur due to manure incorporation are strongly influenced by soil texture, precipitation, quantity of manure applied and time between application and sampling (Choudhary et al., 1996).

The most common criterion used in trials on organic fertilizers is how well they substitute for N mineral fertilizers. Other aspects, such as the improvement of soil physical properties, are very frequently neglected. Long term effects of fertilization practices on aggregate stability and soil organic carbon have been studied. However, few articles have focused on aggregate stability according to the nature of the organic matter applied (Whalen and Chang, 2002; Yagüe et al., 2012b; Wang et al., 2014).

Soil aggregate stability is important for several ecosystem functions, such as water infiltration, reduction of erodibility and runoff, aeration for plant growth (Kemper and Rosenau, 1986), and physical protection of soil organic matter (SOM) (Tisdall and Oades, 1982). Disintegration of macroaggregates by “slaking” associated with the fast wetting process caused by penetration of water into soil dry aggregates, is the main destabilizing factor in rainfed soils (i.e. dry bare soil) in Mediterranean conditions. Dairy manure fertilization can improve soil aggregate stability against slaking, also it controls dissolution and dispersive actions (Nyamangara et al., 1999; Paré et al., 1999). As soil structure is the combination of different types of pores with solid particles (aggregates), characterization of the pore system is also interesting because many physical properties which are relevant in agronomic functions, are determined by the size distribution and shape of pores (Pagliai and Antisari, 1993).

In rainfed Mediterranean conditions, it is not well known how the long-term management of organic fertilizers affects soil quality parameters, particularly in terms of organic carbon fractions (heavy and light fraction), aggregate stability (mainly regarding the slaking disaggregation process) and porosity. Furthermore, only long-term experiments allow the required precision in the evaluation of changes in soil quality and their impacts on crop productivity (Peterson et al., 2012). A recent meta-analysis by Maillard and Angers (2014) on manure application and SOC stocks emphasized the need to further investigate the long-term impact of manure according its characteristics in relation to the animal species of origin.

Soil organic matter is considered the primary binding agent responsible for improving aggregate stability in microaggregates (<250 μm) and macroaggregates (>250 μm)

(Tisdall and Oades, 1982). The light fraction of SOM is sensitive to changes in management practice (Bremer et al., 1994) and it is considered to represent an early indicator for determining the long term impacts of management techniques on soil quality (Leifeld and Kögel-Kabner, 2005). Shukla et al. (2006) concluded that if only one soil attribute is used for monitoring soil quality changes every 3-5 years, SOC should be selected.

Organic fertilizer is usually applied to cover crop N needs. This criterion can enhance soil P build-up. Much of this phosphorus is bound in soil in less available forms, but some may be lost to the environment where it can contribute to the eutrophication of water bodies (Toth et al., 2006). This occurs because the N:P ratio in manure is narrower than the N:P ratio of nutrient demand by most crops.

Site-specific optimization of soil performance is included in the criteria for sustainable soil-use, a forefront of the agricultural policies in the European Union, framed by the thematic strategy for soil protection and ongoing activities. The EU trend is to widen research on factors such as land use, preservation of SOM and more efficient use of resources such as manure (COM 2012). The evaluation of soil quality and soil-use sustainability should support the synergies between local soil-use practices and regulatory conditions, land use and policy planning (Tóth et al., 2007). The appraisal of these soil-use fertilization practices must be done on a long-time scale.

Long-term experiments were initiated in a subhumid rainfed Mediterranean area of NE Spain in order to monitor the effects of organic (manure and slurry) and mineral fertilizers on crop productivity. We hypothesized that manure and even PS with low OM content may improve soil quality. The parameters chosen for study were aggregate stability, organic matter fractions (heavy and light) and soil fertility. In PS plots, due to the low OM content of slurries preliminary research on porosity was done through thin section methodology. The selected fertilization practices to be studied were associated with the highest yields and the accomplishment of the EU nitrate directives (European Union, 1991) in the area. At the start of the experiment, the maximum amount of N applied in organic fertilizers was 210 kg N ha^{-1} yr^{-1}, but later it was reduced to 170 kg N ha^{-1} yr^{-1} (Generalitat de Catalunya, 2009a). The chosen treatments were evaluated after 12 years of DCM or PS incorporation in each cropping season.

Materials and methods

Soil and climate description

The experiments were established in 2001 (La Tallada d'Empordà, Girona, NE Spain). The altitude of the site is 17 m a.s.l. and coordinates are 42° 03' 15" N, 03° 03' 46" E. The soil is very deep (>1.2 m), well drained, non-saline, calcareous and without pebbles. Soil bulk density was 1565 kg m^{-3} for the first 0.30 m and 1700 kg m^{-3} from 0.30 to 0.90 m depth. Water holding capacity was 176 mm (0-0.90 m). In the upper layer (0-0.30 m) soil texture is loamy and the SOM content is about 17 g kg^{-1} (10 g SOC kg^{-1}). It decreases with depth to 7 g kg^{-1} (Table 1). The soil is classified as an Oxyaquic Xerofluvent (Soil Survey Staff, 1999). The field has a gentle slope, so that the aquic character (saturation of the surface in most years) was more relevant in the bottom part of the field.

The area has a dry Mediterranean climate according to Papadakis' classification (MAPA, 1989). The annual average temperature is 15.8ºC and summer temperatures are high (on average 23.0ºC). Average annual precipitation is 602 mm. Potential evapotranspiration is also high, based on Thornthwaite's equation (~827 mm yr^{-1}). Most rain falls in autumn with important storm events in September-October-November, which can cause runoff if the soil is bare (Fig. S1).

Description of the experiment

The experimental field was cropped with a rotation of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) during the experimental period 2001/02 to 2012/13. The standard rotation was wheat-barley-barley. The field was annually sown in November-December and harvested in late June-early July. During each cropping season, conventional tillage management (main tillage with mouldboard plough or disc-harrow between 0.20-0.25 cm depth) were employed. The straw was removed from fields according to farmers' practice. The stubble was incorporated during summer time.

Two experimental trials were established in the same field. The experiment with cattle manure (DCM) was located in the upper part of the field and the experiment with pig slurry (PS) was located at the bottom. Both locations were representative of soil conditions in the area. In both trials DCM and PS were applied before sowing. The trials included treatments with mineral N fertilization (MF) at sidedressing. Treatments in the DCM trial consisted of a control (named $0\text{-}0_{DCM}$; no-N addition), mineral N rate of 40 kg N ha^{-1} applied at sidedressing (named $0\text{-}MF_{DCM}$), DCM treatments at presowing (DCM-0) only or combined with a mineral N sidedressing (40 kg N ha^{-1}; $DCM\text{-}MF_{DCM}$). In the PS trial treatments consisted of a control (named $0\text{-}0_{PS}$; no-N addition), a mineral N rate of 50 kg N ha^{-1} applied at sidedressing (named $0\text{-}MF_{PS}$) and PS treatments at presowing (PS-0) only or combined with mineral N applications as sidedressing (50 kg N ha^{-1}; $PS\text{-}MF_{PS}$). The average values of main chemical parameters of DCM and PS are described in Table S1.

In each plot, rates of animal residues were adjusted by weighing the manure and the slurry applied. The average annually applied rate was 22.5±8.0 t ha^{-1} (±SD) in the DCM trial, which equalled a total average of N applied of 189±101 kg N ha^{-1}. In the PS trial, the average slurry rate was 47.3±13.7 t ha^{-1}, which equalled a total average of N applied of 187±108 kg N ha^{-1}. The average values of total N applied were between the limits of

170 to 210 kg N ha^{-1} yr^{-1}. At the start of the experiment the area was included in a non-vulnerable zone. Thus, 210 kg N ha^{-1} yr^{-1} was the advised upper threshold from N of organic origin. Later on, the area was included in a “nitrate vulnerable zone” and 170 kg N ha^{-1} yr^{-1} was the new upper threshold when using livestock residues (Generalitat de Catalunya, 2009b). In plots where PS was applied, the amount of 37.5 kg K ha^{-1} yr^{-1}, as potassium sulphate (50% K_2O) was added because of the low K content of PS (Table S1).

At sowing, the controls (0-0_{DCM} and 0-0_{PS}) and the mineral N fertilizer treatments (0-MF_{DCM} and 0-MF_{PS}) received phosphorus as calcium superphosphate (18% P_2O_5). The amount of P applied was equivalent to 34.9 kg P ha^{-1} yr^{-1} in the DCM and PS experiments. They also received potassium as potassium sulphate (50% K_2O) at a rate equivalent to 120.8 kg K ha^{-1} yr^{-1}.

Plot size was 48 m^2 (6 m wide and 8 m long) in DCM trial and 30 m^2 (3 m wide and 10 m long) in the PS trial. The treatments in each trial were arranged according to a randomized block design with three replicates.

Sampling and analysis of manures and soil properties

Characteristics of the manures and the slurries applied

Every cropping season, in the field, just before fertilizer application, a composite sample of PS and DCM from each trial was taken. The samples were analysed in the laboratory. The analytical methods used were gravimetric dry matter content at 105ºC, organic matter by ignition at 550ºC, organic nitrogen by the Kjeldahl method, ammonium nitrogen by distillation and titration according to methods 4500-NH_3 B-C ALPHA (2012). Total phosphorus and potassium were analysed by acid digestion (wet) and further determined using inductively coupled plasma atomic emission spectroscopy (USEPA, 1992).

Soil porosity and pore-size distribution and shape in PS trial

In the PS trial, in order to study the effect of small amounts of OM additions on soil porosity (> 25μm) and pore shape, undisturbed soil was sampled on March 25th 2012 (~3 months after the last presowing fertilization). Treatments 0-MF_{PS}, PS-0 and PS-MF_{PS} were sampled.

For each treatment, three undisturbed samples (0-10 cm depth) were obtained, one from each block. They were dried at room temperature and impregnated with polyester resin with a fluorescent dye (Uviex©). One vertical thin section (5 cm wide x 13 cm long) was made from each block. From each thin section, three fields 42.0 x 31.5 mm were selected for obtaining images, in three light conditions: parallel polarisers (PPL), crossed polarisers (XPL) and incident UV light. The latter was processed with ImageJ (Rasband, 2008) to obtain digital binary images from which the total porosity, associated with pores with an apparent diameter (AD) > 25 μm (the minimum threshold allowed by the procedure) was statistically analysed. Each image set was used to perform a pore-size distribution analysis based on an "opening" algorithm of mathematical morphology using the Quantim4 library (Vogel, 2008). The area occupied by pores was divided into four ranges according to the pores' AD: 25-65 μm; 65-100 μm, 100-200 μm, 200-400 μm, > 400 μm. Images were analysed and four shape descriptors, defined in Ferreira and Rasband (2012) were determined: Circularity (Circ.), Aspect Ratio (AR), Roundness (Round) and Solidity (S).

Soil aggregate stability, organic matter fractionation and other chemical analysis

The preliminary results (25th March 2012 sampling) showed differences in soil porosity associated with PS addition. This fact justified a new sampling. Thus, soil was sampled after harvest (July 2013), and DCM extended treatments were included for aggregate stability and SOC fractionation.

Soil was sampled on July 23th of 2013 after cereal harvest (~9 months after last presowing fertilization with organics). Samples were taken from 0-10 cm depth for each treatment and each replication in the three field blocks. Selected treatments were: 0-0_{DCM}, 0-MF_{DCM}; DCM-0, and DCM-MF_{DCM} from the DCM trial and 0-0_{PS}, 0-MF_{PS}, PS-0, PS-MF_{PS} from the PS trial (Table 2).

In these samples, SOC fractionation and main chemical parameters (EC, pH, N, P, and K) were analyzed. In addition to this, two aggregate stability tests, named mean weight diameter (MWD) and water-stable aggregates (WSA), were applied. The first allowed aggregate-size distribution evaluation after a fast wetting. The MWD was obtained following Le Bissonnais (1990) and the further modification established by Amézqueta et al. (1996). It was expressed in microns (µm) as the sum of four multiplications. Each multiplication was obtained as a product of the relative mass percentage of four size aggregate classes (<250 µm, ≥250 µm to 500 µm; ≥ 500 µm to 1000 µm; ≥1000 µm to 2000 µm) and the associated mean diameter of aggregates in each class (125 µm, 375 µm, 750 µm and 1500 µm).

In the second aggregate stability test, the WSA methodology followed Kemper and Rosenau (1986) with the exception of the initial gentle pre-wetting of aggregates which was avoided, as some authors recommend (Pulido-Moncada et al., 2013). In our case, it was avoided in order to focus on the slaking disaggregation effect which predominates under Mediterranean rainfed conditions. Four laboratory replicates were used for each sample and WSA was expressed as a mass percentage, discounting the mass associated with sand.

For each fertilization treatment, five soil density (light and heavy) and physical (<0.05 mm, ≥0.05-0.2 mm and ≥0.2-2 mm) fraction OM sizes were obtained according to the procedure NF X 31-516 established by AFNOR (2007). The SOC from the light fraction was analysed following the total volatile solids (TVS) methodology. The oxidizable SOC from the heavy fraction was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate (Yeomans and Bremner, 1988).

The other analysed chemical parameters were pH (potentiometry; 1:2.5 soil: distilled water), electrical conductivity at 25ºC (1:5 soil:distilled water), available P (Olsen method) and available K (ammonium acetate 1N, pH=7), following MAPA (1994).

Data analysis

All statistical analyses were performed using the SAS V8 (SAS Institute, 1999-2001) statistical software. When differences, according to the analyses of variance (ANOVA), were considered significant ($p<0.05$), Duncan's Multiple Range Test (DMRT) was computed for comparing all possible pairs of means at the 0.05 probability level. Total porosity and pore shape data were normalized using square root transformation. Soil carbon fractions and aggregate stability results were an exception, for which a threshold of $p<0.10$ was adopted in ANOVA analyses. For regressions, fit was considered acceptable if the coefficient of determination (R^2) was 0.75 or higher.

Results and discussion

After 12 years with similar fertilization schedules, plant yields justified the use of manures (Table 2), but the sidedressing with MF did not add a significant yield increase. The residual effects during the crop season, causes the savings in fertilizer sidedressing (Schröder et al., 2005). The DCM trial attained better agronomic conditions than the PS trial, as is reflected by the high yields (>5.5 t ha^{-1}) achieved in the DCM control without applied N (Table 2).

Total porosity, size classes and shape parameters

After 11 years of annual addition of PS, the soil samples obtained in March 2012 (~2-3 months after last application) did not show differences between MF and PS treatments in porosity associated with pores of apparent diameter higher than 0.25 µm (Table 3). However, in the 65 and 400 µm range, porosity was significantly higher in PS treatments than in the MF one (Table 3). The opposite was detected for pores larger than 400 µm. Pagliai and Antisari, (1993) found similar results with a higher PS addition (100-300 m^3 ha^{-1} yr^{-1}). In their study, slurry resulted in increased porosity in the range of 50-500 µm compared with the control. The detected porosity changes in our experiment are relevant because pores in the range from 65 to 400 µm are transmission pores associate to aggregate packing. They are important for water flow during drainage, and moreover, they are the pores needed by roots to grow into (Greenland, 1977). The higher percentage of pores bigger than 400 µm in the MF treatment indicates the presence of small planar voids or fissures that separate larger aggregates.

As the addition the OM improves aggregate stability, as well as soil porosity (Pagliai and Antisari, 1993; Pagliai et al., 2004), our findings on porosity suggest a potential effect of PS on aggregate stability despite the low OM addition. Thus, it was justified to go deeper into the potential influence of PS on physical properties such as aggregate stability. Shape parameters were not affected by fertilization treatments (Table S2).

Soil organic carbon fractions, soil aggregate stability and their relationships

After 12 years of annual DCM application, our results show a net increase of SOC by DCM addition (average increment value of 42%) which equalled an increase of 4.5 g C kg^{-1} soil, when compared with the control and MF treatments (Table 4). The SOC light fraction at the 0.05-0.2 mm size was that most affected (p=0.055; Table 4). In PS treatments, the SOC tend to increase although only the PS-0 treatment was significantly different from the mineral (0-MF_{PS}) treatment (Table 4). These results can be explained by the low OM addition in PS compared with DCM (Table S1 and Table 2) and the fact that straw was removed in all treatments. Thus, in our PS experiment, the effect is due to direct C input by the slurry itself. We consider the indirect C input through increased net primary production (including roots and crop residues) stressed by different authors (Whalen and Chang, 2002; Maillard and Angers, 2014) to be less important.

The light fraction size of 0.2-0.05 mm represented between 36 to 42% SOC in the DCM trial and between 23 to 29% of the SOC in PS trial. This indicates that this fraction is an early indicator of SOC changes in soil (Leifeld and Kögel-Kabner, 2005). This higher significance on SOC changes after DCM application is consistent with the idea that its organic matter is more stable than that from PS (Velthof et al., 2000). Also, due to the low C:N ratio of PS, the mineralization of its OM is faster. This makes it rather difficult to observe changes in the light fraction OM pool nine months after PS application. Time of sampling is a factor in detecting changes in soil chemical composition, as stated by (Choudhary et al., 1996), mainly because residues with low C:N ratio only have a temporary effect (Yagüe et al., 2012b).

The resistance of aggregates against the slaking effect, assessed by means of WSA, was significantly improved in DCM treatments when comparing with that of mineral fertilization or the control (Table 5). These results are in accordance with Paré et al. (1999), who found that the application of DCM for a three year period resulted in the production of cementing agents. These agents stabilized aggregates against slaking forces independently of the tillage system (conventional tillage or no-tillage). In the pig slurry trial, differences were found when stability was evaluated by means of MWD. The MWDs tended to increase as the amount of applied N increased, independent of its origin (Table 5).

The MWD was a better indicator of stability in PS trials than WSA because PS enhances the presence of aggregates in the intervals between 250 and 500 µm and from

500 to 1000 μm (Fig. 1). Also because the addition of PS had a "transient effect" of cementing agents (Yagüe et al. 2012b) which could be insufficient to maintain stability at the moment (9 months after incorporation) when a strong disruption over dry aggregates was applied (WSA procedure).

Different and positive linear relationships between WAS and SOC, SOC light fraction and SOC light fraction from 0.05 to 0.2 mm size were found (Figs. 2a, 2b, and 2c). In fact, total SOC is important for soil aggregation although it includes more specific active fractions which are those most directly involved in aggregation (Huang et al., 2010). The light fraction of SOC has an important role in the formation and stability of soil structure, especially in the stabilization of soil macroaggregates (Kay, 1998; Yagüe et al., 2012b).

Changes in main chemical parameters

Dairy cattle manure, applied annually, increased soil salinity (with respect to the control) and P and K soil content (with respect to the control and MF), but there were no significant differences between treatments which included DCM (Table 6).

The phosphorus increase in DCM trials was 33.3-44.0 mg P kg soil^{-1} (equivalent to an annual accumulation of 2.8-3.7 mg P kg soil^{-1}). The potassium increase was 130.0 and 187.9 mg K kg soil^{-1} (equivalent to an annual accumulation of 10.8-15.7 mg K kg soil^{-1}). In the DCM plots, the maximum increment in average yields with respect to the control (5.5 t ha^{-1}) was 2.1 t ha^{-1}. Thus, nutrient supply from soil was important and it can justify, in DCM plots, the increments in P and K soil content. This fact should alert us to the dangers of giving too much weight solely to N criteria in fertilization practices, and it implies that a more accurate fertilization management system must be found in order to avoid problems associated with an excess of macronutrients (P, K) in the near future. The introduction of leguminous crops or high P and K demanding crops could be an interesting means to reduce N and P excesses.

Pig slurry applied just at sowing also increased P and K soil content. However, when combined with mineral fertilizer it only increased K soil content (Table 6) with respect to the control and the MF treatment. In PS plots, the P content increased from 14.9 to 30.2 mg P kg soil^{-1} (equivalent to an annual accumulation of 1.2-2.5 mg P kg soil^{-1}). The potassium K increase was from 77.9 to 152.0 mg K kg soil^{-1} (equivalent to an annual accumulation of 6.5-12.7 mg K kg soil^{-1}).

In PS trials, the addition of P by PS (100 kg P ha^{-1} yr^{-1}) was higher than that applied in DCM (53 kg P ha^{-1} yr^{-1}). However, the final average figures of P soil content (Table 6) did not reflect this difference. These results could be explained by an enhancement of P absorption by plants. Furthermore, when PS was complemented with MF, P soil content tended to decrease in PS trials, probably because the ammonium N fraction of MF favoured, even more plant P absorption.

Phosphorus availability is a constraint in soils with a pH between 8.1 and 8.3, as it is easily fixed in calcium compounds. Besides, it is well known that the addition of ammonium-N in a fertilization formula enhances P absorption (Brewster et al., 1991) because it produces H^+ in the soil solution-rhizosphere. These ions may temporarily bind the negative charged lime, organic matter and clay in soil (buffering ability). If the H^+ ions are not neutralized or bound to soil particles, they create an acid environment close to roots (Hinsinger, 2001). The pH decreases and P uptake by the crop is enhanced. Pig slurry addition, with an important ammonium-N content (Yagüe et al., 2012a), could positively affect wheat uptake of P and, consequently, it can slow down P accumulation in soil. Furthermore, organic materials with high P and, low C/P ratios release more P (Gadgon and Simard, 1999), which can facilitate its availability. With respect to K addition, this was lower in slurry additions (83 kg K ha^{-1} yr^{-1}) than in manure ones (205 kg K ha^{-1} yr^{-1}).

After 12 years, in control ($0\text{-}0_{DCM}$; $0\text{-}0_{PS}$) and mineral fertilizer ($0\text{-}MF_{DCM}$; $0\text{-}MF_{PS}$) treatments, the addition of 34.5 kg P ha^{-1} yr^{-1} and 120.8 kg K ha^{-1} yr^{-1} did not affect the amounts of P and K in soil. This means that the P and K soil equilibrium was maintained. By contrast, the addition of DCM and PS increased the availability of these nutrients (Table 6).

Conclusions

Long-term (12-yr) application of DCM (average rate of 23 t ha^{-1} yr^{-1}) gave a significant increase in soil organic carbon (4.5 g C kg^{-1}), mainly in the light fraction. Aggregates which were water stable against slaking disrupting forces, increased with the addition of DCM (up to 16.4-18.3%) when compared with control or mineral fertilizer plots (14.7-15.0%). The effect of PS with respect to the previous parameters was not significant, probably because the effects of PS are more transient than those of DCM. The MDW test was in sufficiently accurate to allow detection of differences between fertilization treatments.

The light fraction of organic matter (0.05-0.2 mm size) was positively and linearly related with WSA (R^2=0.83; $p<0.01$). This fact indicates that changes in water stability of aggregates and organic matter fractions (i.e. light fraction particularly 0.05 to 0.2 mm) may serve as indicators of soil quality related to agricultural fertilization practices.

Porosity in the 65-400 μm size range was increased with the use of pig slurry, thus PS application will probably increase water flow.

The build-up of phosphorus and potassium in soil, when PS and DCM are applied following the N demand criteria, clearly deserves further attention and should be considered in fertilization management strategies. Recommendations need to include widening crop rotations (e.g. by the introduction of leguminous crops) or other fertilization managements within a rotation (e.g. biennial application of manures which can alternate with fertilization using N only, applied as mineral N at sidedressing, if required). Further research is needed to improve nutrient management in a rotation concept from the agronomic and environmental aspects when using animal residues.

Acknowledgements

The authors thank IRTA Mas Badia staff, all along the period of the experiment for field assistance and Montse Antúnez and Belén Martínez for laboratory assistance. The initial field maintenance was done through a project from Interministerial Science and Technology Research commission (CICYT) of Spain (AGL2001-2214-C06). The subsequent field maintenance and measurements development were supported through the Plans for the improvement of crop fertilization, led by the Department of Agriculture, Livestock, Fisheries, Food and Natural Environment from Generalitat de Catalonia and through different projects from the National Institute for Agricultural and Food Scientific Research and Technology of Spain (INIA): RTA04-114-C3; RTA2010-00126-C02-02 and RTA2013-57-C5-05.

References

ALPHA (2012) Nitrogen (ammonia): 4500-NH_3 B, preliminary distillation step and 4500-NH_3 C, titrimetric method. In: Rice, E.W., Bridgewater, L (Eds.). Standard methods for the examination of water and wastewaste. Second edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C. pp 401-411.

AFNOR (2007) NF X 31-516. Qualité du sol. Fractionnement granulodensimétrique des matières organiques particulaires du sol dans l´eau. Association Française de Normalisation, La Plaine Saint-Denis, France.

Amézqueta E, Singer MJ, Le Bissonnais Y (1996) Testing a new procedure for measuring water stable aggregation. Soil Sci. Soc. Am. J. 60:888-894.

Bremer E, Jensen HH, Johnston AM (1994) Sensitivity of total light fraction and mineralizable organic matter to management practice in a Lethbridge soil. Can. J. Soil Sci.74:131-138.

Brewster J.L., Rowse H.R., Bosch, A.D. 1991. The effects of sub-seed placement of liquid N and P fertilizer on the growth and development of bulb onions over a range of plant densities using primed and non-primed seed. J. Hortic. Sci. 66, 551-557.

Calderon FJ, Mccarty GW, Reeves III J (2005) Analysis of manure and soil nitrogen mineralization during incubation. Biol. Fertil. Soils 41: 328-336. DOI 10.1007/s00374-005-0843-x.

Choudhary M, Bailey LD, Grant CA (1996) Review of the use of swine manure in crop production: effects on yield and composition and on soil and water quality. Waste Manag. Res. 14: 581-595.

COM 2012. Report from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions, The implementation of the soil thematic strategy and ongoing activities. Available: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52012DC0046&from=EN (accessed 5 October 2015).

European Union, 1991. Council Directive 91/676/EEC, of 12 December 1991, concerning the protection of waters against pollution caused by nitrates from agricultural sources. Off. J. Eur. Communities L375:1991, pp. 1-8.

Ferreira T, Rasband W (2012) ImageJ User guide. Availability: http://imagej.nih.gov/ij/docs/guide/user-guide.pdf_(accessed 5 October 2015).

Gadgon, B., Simard, RR (1999). Nitrogen and phosphorus release from on-farm and industrial compost. Can. J. Soil Sci. 79:481-489.

Generalitat de Catalunya (2009a). Decret 136/2009, d'1 de setembre, d'aprovació del programa d'actuació aplicable a les zones vulnerables en relació amb la contaminació de nitrats que procedeixen de fonts agràries i de gestió de les dejeccions ramaderes. Diari Oficial de la Generalitat de Catalunya 5457, 65858-65901.

Generalitat de Catalunya (2009b). ACORD GOV/128/2009, de 28 de juliol, de revisió i designació de noves zones vulnerables en relació amb la contaminació per nitrats procedents de fonts agràries. DOGC 5435, 61692-61695.

Greenland DJ (1977). Soil damage by intensive arable cultivation: temporary or permanent?. Philosophical Transactions Royal Society of London, 281: 193-20. DOI: 10.1098/rstb.1977.0133.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237: 173-195.

Huang S, Peng XX, Huang QR, Zhang WJ (2010) Soil aggregation and organic carbon fractions affected by long term fertilization in a red soil in subtropical China. Geoderma 154: 364-369. DOI: 10.1016/j.geoderma.2009.11.009.

Jones A, Panagos P, Barcelo S, Bouraoui F, Bosco C, Dewitte O, Gardi C, Erhard M, Hervás J, Hiederer R, Jeffery S, Lükewille A, Marmo L, Montanarella L, Olazábal C, Petersen JE, Penizek V, Strassburger T, Tóth, M, Van Den Eeckhaut, Van Liedekerke M, Verheijen F, Viestova E, Yigini Y (2012) JCR reference reports. The state of soil in Europe. A contribution of the JCR to the European Environment Agency's Environment state and outlook report_SOER 2010. Availability: http://ec.europa.eu/dgs/jrc/downloads/jrc_reference_report_2012_02_soil.pdf (accessed 5 October 2015).

Kay BD (1998) Structure and organic carbon: a review. In: Lal, R., J.M. Kimble, R.F. Follet, B.A. Stewart. Editors. Soil processes and the carbon cycle, Boca Raton: CRC Press 169-197 pp.

Kemper WD, Rosenau RC (1986) Aggregate stability and size distribution. In: A. Klute, editor, Methods of Soil Analysis. Part I. Physical and Mineralogical Methods. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI. p. 425-442.

Le Bissonnais Y (1990) Experimental study and modelling of soil surface crusting processes. Catena Suppl. 17: 13-28.

Leifeld J, Kögel-Knabner I (2005) Soil organic matter fractions as early indicators for carbon stock changes under different land-use? Geoderma 124:143-155. DOI: 10.1016/j.geoderma.2004.04.009.

MAPA (1994). Métodos químicos de suelos. In: Ministerio de Agricultura, Pesca y Alimentación, editor. Métodos oficiales de Análisis. Tomo III. MAPA, Madrid, Spain. p. 281-283.

MAPA (1989). Caracterización climática de la provincial de Girona. Ministerio de Agricultura, Pesca y Alimentación. Secretaria Técnica provincial. Centro de publicaciones, Madrid, Spain, p.256.

Maillard E, Angers DA (2014) Animal manure application and soil organic carbon stocks: a meta-analysis. Global Change Biology 20: 666-679. DOI: 10.1111/gcb.12438.

Ndayegamiye A, Côte D (1989) Effect of long-term pig slurry and solid cattle manure application on soil chemical and biological properties. Can. J. Soil. Sci. 69:39-47.

Nyamangara J, Piha MI, Kirchmann H (1999) Interactions of aerobically decomposed cattle manure and nitrogen fertilizer applied to soil. Nutr. Cycl. Agroecosys. 54, 183–188.

Paré T, Dinel H, Moulin AP, Townley-Smith L (1999) Organic matter quality and structural stability of a Black Chernozemic soil under different manure and tillage practices. Geoderma 91, 311–326. DOI: 10.1016/S0016-7061(99)00011-7.

Pagliai M, Vignozzi N, Pellegrini S (2004) Soil structure and the effect of management practices. Soil Till. Res. 79:131-143. DOI: 10.1016/j.still.2004.07.002.

Pagliai M, Antisari LV (1993) Influence of waste organic matter on soil micro- and macrostructure. Biores. Techn. 43:205-213. DOI: 10.1016/0960-8524(93)90032-7.

Peterson GA, Lyon DJ, Fenster CR (2012) Valuing long-term field experiments. Quantifiying the scientific contribution of long-term tillage experiment, Soil Sci. Soc Am. J. 76. 757-765. DOI:10.2136/sssaj2011.0413.

Pulido-Moncada M, Gabriels D, Cornelis W, Lobo D (2013) Comparing aggregate stability tests for soil physical quality indicators. Land Degradation and Development. DOI: 10.1002/ldr.2225 (In press).

Rasband, W. 2008. ImageJ 1.40. National Institute of Health, USA. Availability: http://imagej.nih.gov/ij/ (accessed 5 October 2015)

SAS Institute Inc.1999-2001 SAS/TAT. Software V 8.2. Cary, NC.

Schröder, J.J., A.G. Jansen, and G.L. Hilhorst. 2005. Long-term nitrogen supply from cattle slurry. Soil Use Manage. 21:196-204.

Shukla MK, Lal R, Ebinger M (2006) Determining soil quality indicators by factor analysis. Soil Till. Res. 87: 194-204. DOI: 10.1016/j.still.2005.03.011.

Soil Survey Staff (1999) Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys. 2nd ed. Agriculture Handbook nº 436. United States Department of Agriculture. Natural Resources Conservation Service, U.S. Government Printing Office, Washington DC.

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163. DOI: 10.1111/j.1365-2389.1982.tb01755.x.

Toth, JD, Dou Z, Ferguson, JD, Galligan DT,. Ramberg CF Jr (2006) Nitrogen- vs. phosphorus-based dairy manure applications to field crops: nitrate and phosphorus leaching and soil phosphorus accumulation. J. Environ. Qual. 35: 2302-2312. DOI: 10.2134/jeq2005.0479.

Tóth, G, Stolbovoy, V., Montanerella, L. 2007. Soil quality and sustainable evaluation. An integrated approach to support soil-related policies of the European Union. European Commission EUR 22721 EN. Available at: http://eusoils.jrc.ec.europa.eu/esdb_archive/eusoils_docs/other/EUR22721.pdf (accessed 5 October 2015)

Trolldenier, G (1975). Influence de la fumure minérale sur l'équilibre biologique dans la rhizosphêre. Bulletin de la Société Botanique de France, 122(2) : 157-167. DOI : 10.1080/00378941.1975.10839362

USEPA (1992) United States Environmental Protection Agency. Method 6010A. Inductively coupled plasma-atomic emission spectroscopy. Kovar, J.K. Chapter 6. Methods of determination of P, K, Ca, Mg and others trace elements. In: Peters. J (Ed). Recommended methods of manure analysis. Publ. A3769. Univ. of Wisconsin Extension, Madison, WI.

Van Kessel JS, Reeves JB, Meisinger JJ (2000) Nitrogen and carbon mineralization of potential manure components. J. Environ. Qual. 29:1669–1677. DOI: 10.2134/jeq2000.00472425002900050039x.

Velthof GL, Bannink A, Oenema O, Van Der Meer HG, Spoelstra SF (2000) Relationships between animal nutrition and manure quality - A literature review on C, N, P and S compounds. Alterra-rapport 063, Alterra, Green World Research, Wageningen, 44 pp. Available at: http://edepot.wur.nl/28901 (accessed 5 October September 2015).

Vogel HJ. (2008), Quantim4 C/++ Library for scientific image processing, UFZ-Helmhotz Center for Environment Research, Helmholtz, Germany.

Wang X, Cammeraat ELH, Cerli C, Kalbitz K (2014). Soil aggregation and the stabilization of organic carbon as affected by erosion and deposition. Soil Biol. Biochem. 72: 55-65. DOI: 10.1016/j.soilbio.2014.01.018.

Whalen JK, Chang C (2002) Macroaggregate characteristics in cultivated soils after 25 annual manure applications. Soil Sci. Soc. Am. J. 66:1637-47.

Yagüe MR, Bosch-Serra AD, Boixadera J (2012a) Measurement and estimation of the fertilizer value of pig slurry by physicochemical models: usefulness and contraints. Biosyst. Eng. 111:206-216. DOI: 10.1016/j.biosystemseng.2011.11.013.

Yagüe MR, Bosch-Serra AD, Antunez M, Boixadera J (2012b) Pig slurry and mineral fertilization treatments' effects on soil quality: macroaggregate stability and organic matter fractions. Sci. Total Environ. 438:218-224. DOI: 10.1016/j.scitotenv.2012.08.063.

Yeomans JC, Bremner JM (1988) A rapid and precise method for routine determination of organic carbon in soil. Comm. Soil Sci. Plant Anal. 19:1467-1476. DOI:10.1080/00103628809368027.

Figures legend:

Figure 1. Mass of aggregates for each of the four size classes remaining after the implosion caused by the penetration of water into soil aggregates (slaking) in dairy cattle manure (DCM) and pig slurry (PS). Treatments include minerals (a) MF_{DCM} and MF_{PS}: mineral N fertilizer, applied at a rate of 40 or 50 kg N ha^{-1} yr^{-1}, respectively, as calcium ammonium nitrate (27%) at sidedressing; and organics (b) DCM and PS: dairy cattle manure and pig slurry applied just before sowing at an average rate of 22.5 or 47.3 t ha^{-1}, respectively. Mean values in each size class and for each trial followed by a different capital letter are significantly different at the $\alpha=0.05$ probability level based on the Duncan Multiple Range Test. NS: no significant ($p>0.05$). Bars represent the standard error of three replicates.

Figure 2. Relationship between (a) soil organic carbon (SOC), (b) SOC light fraction (0.05-2 mm); (c) SOC light fraction (0.05-0.2 mm) and water stable aggregatest (WSA; % w/w). Data from the dairy cattle manure and the pig slurry plots were included. Bars represent the standard error of four replicates (* $p<0.05$; ** $p<0.01$).

Tables legend:

Table 1. Physical and chemical characteristics of the soil in the field trial.

Table 2. Averages† of total N, organic N and organic matter (OM) applied annually in dairy cattle manure (DCM) and pig slurry (PS) trials, where mineral N (MF) as a fertilization treatment was included at sowing or as sidedressing (SideD). Grain yields (13% humidity) of 2012-2013 sampling season are also presented.

Table 3. Average values† (n=6) of total porosity (>25µm) and different porosity fractions, for each fertilization treatment, in pig slurry trial.

Table 4. Average values† (n=3) of soil carbon in different physical sizes and density fractions and total oxidizable organic carbon by dichromate oxidation. Values were obtained from dairy cattle manure (DCM) and pig slurry (PS) trials maintained for a period of 12 years.

Table 5. Average values† (n=4) of the mean weight diameter after a fast wetting (MWD) and the mass percentage of water-stable aggregates (WSA) for both trials.

Table 6. Average† of main soil chemical parameters measured after 12-yr of similar fertilization practices.

Supplemental material:

Figure S1. Monthly precipitation (P), and mean air temperature (T) during the crop season samplings (2012-2013) and for the historical period (1993-2014)

Table S1: Main physicochemical average values (±standard deviation)† of dairy cattle manure (DCM) and slurry from fattening pigs (PS) in the period from 2001 to 2013.

Table S2. Average values (n=6), for different apparent pore diameter intervals, of porosity shape parameters: Circularity (Circ.), Aspect Ratio (AR), Roundness (Round) and Solidity (S). Samples were obtained the 25th March 2012 for each fertilization treatment of the pig slurry trial.

Table 1. Physical and chemical characteristics of the soil, in the field trial.

	Depth (m)		
Parameter	**0-0.3**	**0.3-0.6**	**0.6-0.9**
Particle size distribution (g kg^{-1})†			
Sand (2000 <Ø< 50 µm)	458	412	538
Silt (50 <Ø< 2 µm)	413	465	351
Clay (Ø < 2 µm)	129	123	111
pH (water; 1:2.5‡)	8.4	8.4	8.2
Electrical conductivity (1:5‡; dS m^{-1}, 25ºC)	0.13	0.18	0.22
Organic matter (g kg^{-1})	17	14	7
Total N (g kg^{-1}; Kjeldahl)	0.10	0.08	0.05
Phosphorus (mg P kg^{-1}; Olsen)	32	14	7
Potassium (mg K kg^{-1}; NH_4OAc, 1N, pH=7)	306	180	89
Calcium carbonate equivalent (%)	12.9	12.3	11

Composite samples (0-0.30m, 0.30-0.60m and 0.60-0.90m) were obtained at the start of the fertilization experiment (October 2001).

† Ø: particle apparent diameter.

‡ Relation of soil: distilled water.

Table 2. Averages[†] of total N, organic N and organic matter (OM) applied annually in dairy cattle manure (DCM) and pig slurry (PS) trials, where mineral N (MF) as a fertilization treatment was included at sowing or as sidedressing (SideD). Grain yields (13% humidity) of 2012-2013 sampling season are also presented.

Trial	**Treatments**[‡]	**Fertilizer treatment**		**Total N applied**	**MF**	**DCM or PS**			**Grain yield. 2013 harvest**
		Sowing	**SideD**			**Org-N**	**NH_4^+N**	**OM**	
				---- kg N ha^{-1} ---		**------------- kg ha^{-1} ---------------**			**(kg ha^{-1})$^{\#}$**
DCM	**0-0$_{DCM}$**	0	0	0	0	0	0	0	5531B
	0-MF$_{DCM}$	0	MF	40	40	0	0	0	5443B
	DCM-0	DCM	0	189	0	163 (±92)	26(±13)	3897(±1875)	7269A
	DCM-MF$_{DCM}$	DCM	MF	229	40	163(±92)	26(±13)	3897(±1875)	7603A
	Significance								*
PS	**0-0$_{PS}$**	0	0	0	0	0	0	0	2382B
	0-MF$_{PS}$	0	MF	50	50	0	0	0	3447B
	PS-0	PS	0	187	0	71(±46)	116(±66)	1913(±1694)	6262A
	PS-MF$_{PS}$	PS	MF	237	50	71(±46)	116(±66)	1913(±1694)	6457A
	Significance								***

Significant: ***$p<0.001$.

[†] Numbers in brackets are the standard deviation.

[‡] MF$_{DCM}$ and MF$_{PS}$: mineral N fertilizer, applied at a rate of 40 or 50 kg N ha^{-1} yr^{-1}, respectively, as calcium ammonium nitrate (27%) at sidedressing; DCM and PS: dairy cattle manure and pig slurry applied just before sowing at an average rate of 22.5 or 47.3 t ha^{-1}, respectively.

[#] For yields, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p=0.05).

Table 3. Average values[†] (n=6) of total porosity (>25µm) and different porosity fractions, for each fertilization treatment, in pig slurry trial.

Treatment	**Total Porosity**	**Size porosity (%)[#]**				
Sowing-sidedressing[‡]	**> 25 µm**	**25-65µm**	**65-100µm**	**100-200µm**	**200-400µm**	**>400µm**
0-MF$_{PS}$	31.9	1.40 (0.12)	1.95 (0.14)B	4.33 (0.21)B	7.01 (0.27)B	17.20 (0.41)A
PS-0	31.7	2.35 (0.15)	3.33 (0.18)A	7.51 (0.27)A	9.60 (0.31)A	8.91 (0.28)B
PS-MF$_{PS}$	30.9	2.01 (0.14)	2.77 (0.17)A	6.78 (0.26)A	9.32 (0.30)A	10.07 (0.41)B
Significance	NS	NS	**	***	**	**

Soil sampling was done the 25th March 2012.

NS: non significant, p>0.05.

[†] Numbers between parenthesis indicate the transformed values [x^(1/2)] of porosity.

[‡] MF$_{PS}$: mineral nitrogen fertilizer, applied at a rate of 50 kg N ha^{-1} yr^{-1} as calcium ammonium nitrate (27%) at sidedressing; PS: pig slurry applied just before sowing at an average rate of 47.3 t ha^{-1}.

[#] Within columns, means followed by the different letter are significantly different according to Duncan Multiple Range Test at the α=0.05 level of significance.

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Table 4. Average values[†] (n=3) of soil carbon in different physical sizes and density fractions and total oxidizable organic carbon by dichromate oxidation. Values were obtained from dairy cattle manure (DCM) and pig slurry (PS) trials maintained for a period of 12 years.

		Fractions (mm)					**Total C**
		0.2-2		**0.05-0.2**		**<0.05**	
Trials	**Treatment[‡]**	**Heavy**	**Light**	**Heavy**	**Light**		
		----------------------------- g C kg soil^{-1} ------------------------					
DCM	**0-0$_{\text{DCM}}$**	0.03	0.86	0.57	3.77B	5.34BC	10.58B
	0-MF$_{\text{DCM}}$	0.07	1.06	0.48	4.66AB	4.77C	11.05B
	DCM-0	0.06	2.02	0.79	6.14A	6.05AB	15.06A
	DCM-MF$_{\text{DCM}}$	0.05	2.37	0.74	5.69A	6.83A	15.69A
	Significance	NS	NS	NS	*	**	***
PS	**0-0$_{\text{PS}}$**	0.08	1.12	0.33	2.82	6.24	10.59B
	0-MF$_{\text{PS}}$	0.17	1.33	0.36	2.29	5.55	9.72B
	PS-0	0.22	2.21	0.45	3.66	6.09	12.63A
	PS-MF$_{\text{PS}}$	0.08	1.39	0.47	3.16	5.83	10.93AB
	Significance	NS	NS	NS	NS	NS	*

Sampling was done at cereal harvest on the 23rd July of 2013.

NS: not significant ($p>0.05$); Significant: *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

[†] Within columns, means having a common letter are not significantly different according to DMRT at the $\alpha=0.05$ level of significance.

[‡] MF$_{\text{DCM}}$ and MF$_{\text{PS}}$: mineral N fertilizer, applied at a rate of 40 or 50 kg N ha^{-1} yr^{-1}, respectively, as calcium ammonium nitrate (27%) at sidedressing; DCM and PS: dairy cattle manure and pig slurry applied just before sowing at an average rate of 22.5 or 47.3 t ha^{-1}, respectively.

Table 5. Average values[†] (n=4) of the mean weight diameter after a fast wetting (MWD) and the mass percentage of water-stable aggregates (WSA) for both trials.

Trial	Treatment	MWD (µm)	WSA (%)
Dairy Cattle Manure (DCM)	**0-0$_{\mathrm{DCM}}$**	288	14.70C
	0-MF$_{\mathrm{DCM}}$	321	15.00C
	DCM-0	307	18.31A
	DCM-MF$_{\mathrm{DCM}}$	303	16.37B
	Significance	NS	***
Pig Slurry (PS)	**0-0$_{\mathrm{PS}}$**	326B	11.93
	0-MF$_{\mathrm{PS}}$	341AB	13.53
	PS-0	346AB	13.66
	PS-MF$_{\mathrm{PS}}$	363A	12.43
	Significance	*	NS

Sampling was done at cereal harvest on the 23rd July of 2013.

NS: not significant ($p>0.05$); Significant: *$p<0.05$, ***$p<0.001$.

[†] Within columns, means having a common letter are not significantly different according to DMRT ($\alpha=0.05$).

[‡] MF$_{\mathrm{DCM}}$ and MF$_{\mathrm{PS}}$: mineral N fertilizer, applied at a rate of 40 or 50 kg N ha^{-1} yr^{-1}, respectively, as calcium ammonium nitrate (27%) at sidedressing; DCM and PS: dairy cattle manure and pig slurry applied just before sowing at an average rate of 22.5 or 47.3 t ha^{-1}, respectively.

Table 6. Average[†] of main soil chemical parameters measured after 12-yr of similar fertilization practices.

Trial	**Treatment**	**pH$_{1:2.5}$**	**EC$_{1:5, 25°C}$ (dS/m)**	**N (%)**	**P (Olsen)**	**ΔP**	**K (NH$_4$OAc, 1N, pH=7)**	**ΔK**
					------------------ mg kg soil^{-1}			**---------------------**
DCM	**0-0$_{DCM}$**	8.3	0.11B	0.15	37.7B	-	328.7B	-
	0-MF$_{DCM}$	8.2	0.11B	0.15	32.3B	-	310.8B	-
	DCM-0	8.2	0.13AB	0.22	71.0A	+33.3	458.9A	+130.0
	DCM-MF$_{DCM}$	8.2	0.14A	0.23	81.7A	+44.0	516.6A	+187.9
	Significance	NS	*	NS	***	-	***	-
PS	**0-0$_{PS}$**	8.2	0.11	0.14	35.8 B	-	291.8B	-
	0-MF$_{PS}$	8.2	0.12	0.19	35.0 B	-	259.4B	-
	PS-0	8.1	0.12	0.19	66.0 A	+30.2	443.2A	+152.0
	PS-MF$_{PS}$	8.1	0.12	0.18	50.7AB	+14.9	369.7A	+77.9
	Significance	NS	NS	NS	*	-	***	-

Soil samples came from trials where dairy cattle manure (DCM) or pig slurry (PS) were applied.

NS: not significant ($p>0.05$); Significant: *$p<0.05$, ***$p<0.001$.

Δ: increment in the P or K soil content (plot value under organic fertilization – plot control value).

[†] Within columns, means having a common letter are not significantly different according to DMRT ($\alpha=0.05$).

[‡] MF$_{DCM}$ and MF$_{PS}$: mineral N fertilizer, applied at a rate of 40 or 50 kg N ha^{-1} yr^{-1}, respectively, as calcium ammonium nitrate (27%) at sidedressing; DCM and PS: dairy cattle manure and pig slurry applied just before sowing at an average rate of 22.5 or 47.3 t ha^{-1}, respectively.

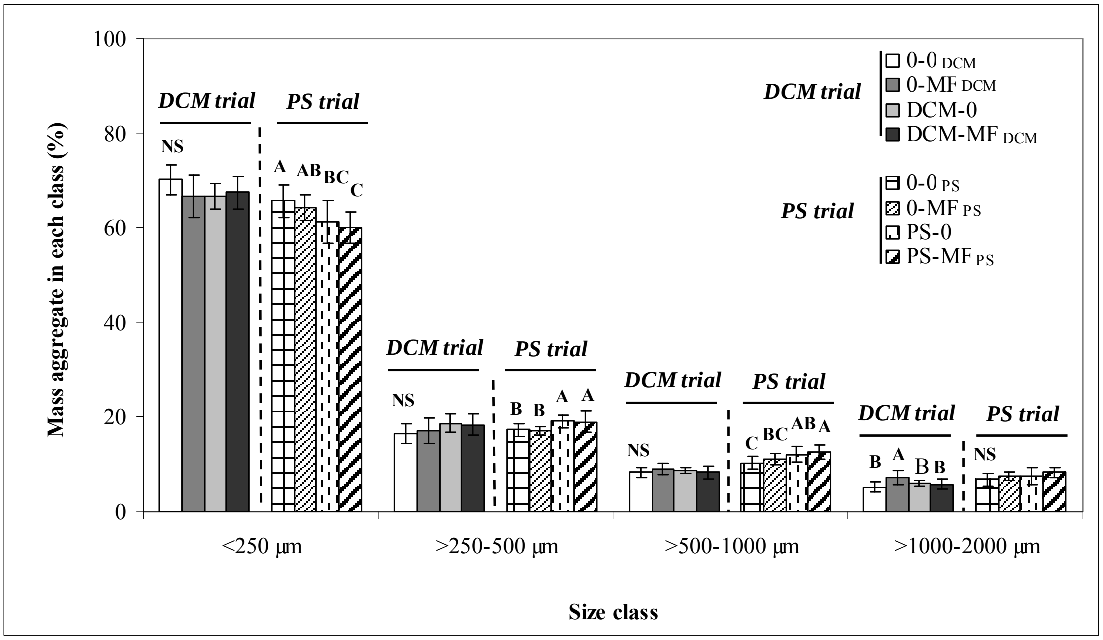

Figure 1. Mass of aggregates for each of the four size classes remaining after the implosion caused by the penetration of water into soil aggregates (slaking) in dairy cattle manure (DCM) and pig slurry (PS). Treatments include minerals (a) MF_{DCM} and MF_{PS}: mineral N fertilizer, applied at a rate of 40 or 50 kg N ha^{-1} yr^{-1}, respectively, as calcium ammonium nitrate (27%) at sidedressing; and organics (b) DCM and PS: dairy cattle manure and pig slurry applied just before sowing at an average rate of 22.5 or 47.3 t ha^{-1}, respectively. Mean values in each size class and for each trial followed by a different capital letter are significantly different at the α=0.05 probability level based on the Duncan Multiple Range Test. NS: no significant (p>0.05). Bars represent the standard error of three replicates.

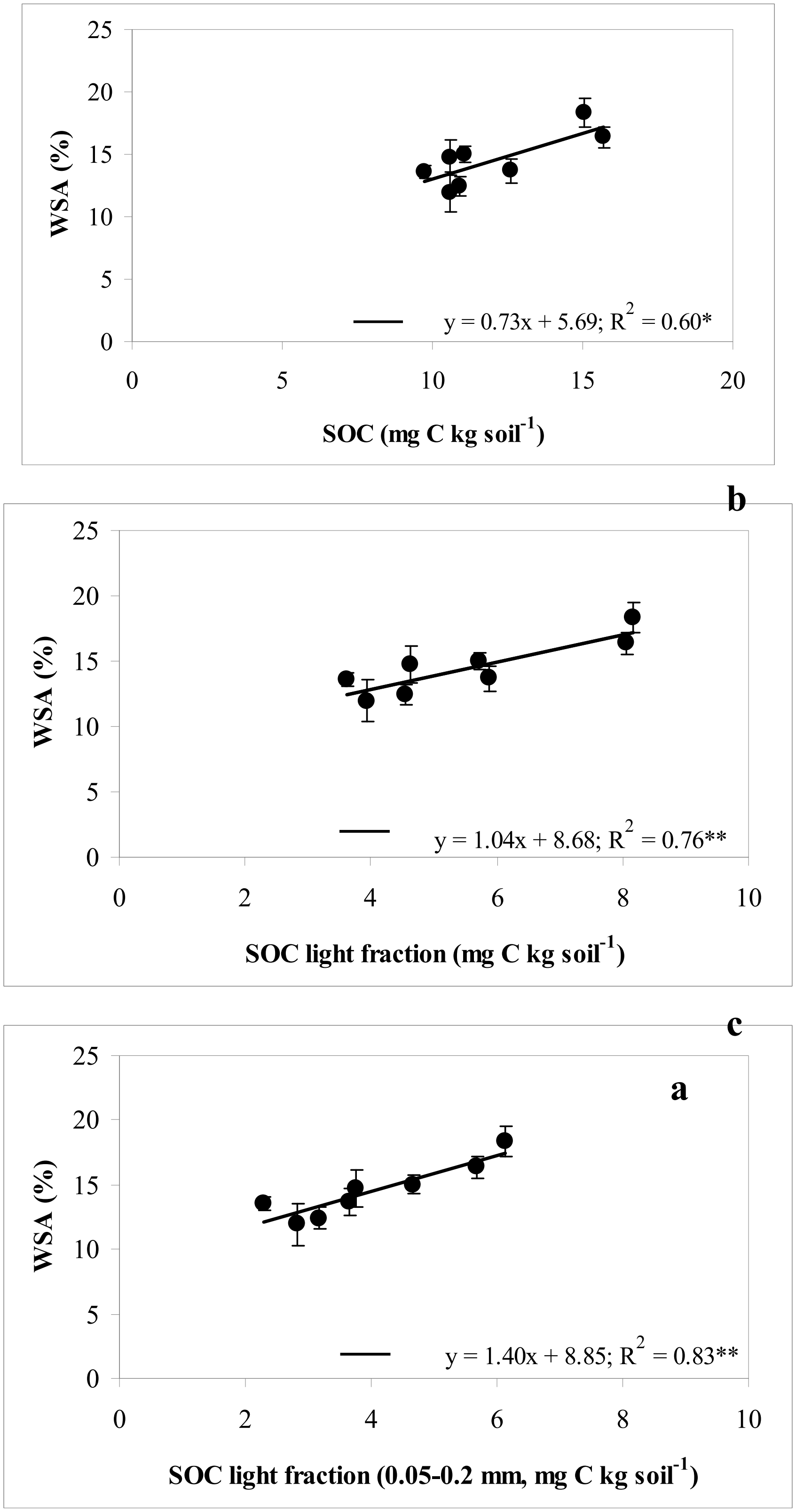

Figure 2. Relationship between (a) soil organic carbon (SOC), (b) SOC light fraction (0.05-2 mm); (c) SOC light fraction (0.05-0.2 mm) and water stable aggregatest (WSA; % w/w). Data from the dairy cattle manure and the pig slurry plots were included. Bars represent the standard error of four replicates (* $p<0.05$; ** $p<0.01$).

Table S1: Main physicochemical average values (±standard deviation)† of dairy cattle manure (DCM) and slurry from fattening pigs (PS) in the period from 2001 to 2013.

Parameter	**DCM**	**PS**
Dry matter (%)	29.6 ±8.6	6.2 ±2.9
Organic matter (% dm)	59.3 ± 9.8	59.6 ± 9.4
Kjeldahl- N (% dm)	2.4 ± 0.4	2.4 ± 0.3
Ammonium-N (% dm)	0.4 ± 0.1	6.0 ± 6.5
Total N (% dm)	2.8 ± 0.5	8.4 ± 6.7
Phosphorus (P; % dm)	0.8 ± 0.2	2.8 ± 0.8
Potassium (K, % dm)	3.2 ± 1.0	4.9 ± 5.5
Ratio C:N	12.2 ± 0.9	6.1 ± 3.9

† % dm: expressed on a dry matter basis.

Table S2. Average values (n=6), for different apparent pore diameter intervals, of porosity shape parameters: Circularity (Circ.), Aspect Ratio (AR), Roundness (Round) and Solidity (S). Samples were obtained the 25th March 2012 for each fertilization treatment of the pig slurry trial.

25-65 µm	**Circ.**	**AR**	**Round**	**S**
0-MF$_{PS}$	0.89	1.77	0.63	0.87
PS-0	0.91	1.70	0.66	0.86
PS-MF$_{PS}$	0.89	1.79	0.62	0.85
Significance	NS	NS	NS	NS
65-100 µm	**Circ.**	**AR**	**Round**	**S**
0-MF$_{PS}$	0.72	1.97	0.57	0.79
PS-0	0.75	1.90	0.58	0.80
PS-MF$_{PS}$	0.71	1.95	0.57	0.78
Significance	NS	NS	NS	NS
100-200 µm	**Circ.**	**AR**	**Round**	**S**
0-MF$_{PS}$	0.61	2.07	0.55	0.78
PS-0	0.62	2.06	0.54	0.77
PS-MF$_{PS}$	0.58	2.05	0.55	0.76
Significance	NS	NS	NS	NS
200-400 µm	**Circ.**	**AR**	**Round**	**S**
0-MF$_{PS}$	0.48	2.25	0.52	0.73
PS-0	0.48	2.16	0.54	0.73
PS-MF$_{PS}$	0.44	2.24	0.51	0.70
Significance	NS	NS	NS	NS
> 400 µm	**Circ.**	**AR**	**Round**	**S**
0-MF$_{PS}$	0.28	2.33	0.50	0.62
PS-0	0.28	2.21	0.52	0.61
PS-MF$_{PS}$	0.26	2.15	0.53	0.60
Significance	NS	NS	NS	NS

NS: non significant, p>0.05.

† MF$_{PS}$: mineral nitrogen fertilizer, applied at a rate of 50 kg N ha^{-1} yr^{-1} as calcium ammonium nitrate (27%) at sidedressing; PS: pig slurry applied just before sowing at an average rate of 47.3 t ha^{-1}.

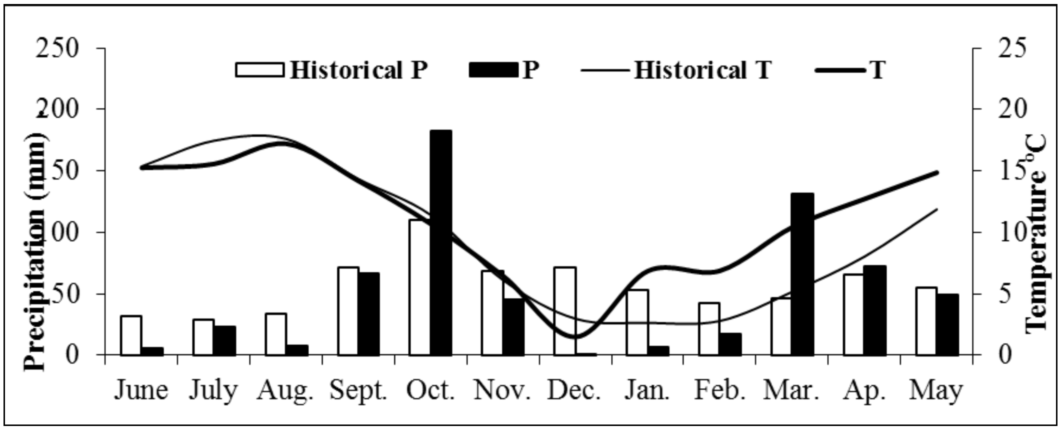

Figure S1. Monthly precipitation (P), and mean air temperature (T) during the crop season samplings (2012-2013) and for the historical period (1993-2014)