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ABSTRACT

In the European Union, the maintenance of soil quality is a key point in agricultural policy. The effect of additions of dairy cattle (*Bos taurus*) manure (DCM) during a period of 11 years were evaluated in a soil under irrigated maize (*Zea mays* L.) monoculture. DCM was applied at sowing, at wet-weight rates of 30 or 60 Mg $ha^{-1}yr^{-1}$ (30DCM or 60DCM). These were compared with a mineral-N treatment (300 kg N ha^{-1}, MNF), applied at 6-8 emerged leaves and with a control (no N, no manure). Treatments were distributed in a randomized block design. Factors analysed were stability against wetting stress disaggregation, porosity, soil organic carbon (SOC) fractions and earthworm abundance, studied eight months after the last manure application. The application rate of 30DCM increased aggregate stability and the light SOC fraction, but not the pore volume, nor the earthworm abundance, compared with MNF. The DCM rates did not result in unbalanced agronomic advantages versus MNF, as high yields (17-18 Mg ha^{-1} yr^{-1}) were obtained. In Mediterranean environments, the use of DCM should be encouraged mainly because of its contribution to the light SOC fraction which protects dry macro-aggregates from implosion (slaking) during the wetting process. Thus, in intensive agricultural systems, it protects soil from physical degradation.

INTRODUCTION

The European Union and its Member States are addressing soil quality issues, and setting targets for sustainable land use and soil quality (EU, 2013). Worldwide, vegetation (Cerdà, 1996), revegetation (Yu & Jia, 2014) or revegetation for agricultural uses (Barua & Haque, 2013), specific soil tillage management practices (Nieto et al., 2012), the application of different carbonaceous materials (Cely et al., 2014; Wick et al., 2014) or organic amendments are widely used to improve the physical attributes of degraded agricultural soils (Diacono & Monterruno, 2010; Quilty & Cattle, 2011; Castro et al., 2012). The use of manures to maintain or increase soil organic carbon (SOC) is of great interest, as SOC losses are a real threat (Jones et al., 2012; Batjes, 2014; Srinivasarao, 2014). In Europe, around 45% of mineral soils have very low or low (0-20 g kg^{-1}) SOC content (Rusco et al., 2001), but in southern Europe the percentage of topsoil horizons with less than 20 g $kg^{-1}$of SOC can be up to 74% (Zdruli et al., 2004). The coarse textured soils are the most affected (Muñoz-Rojas et al., 2012) although soil management practices can drive SOC changes (De Moraes Sá et al., 2015; Parras-Alcántara et al., 2014). Soil organic carbon is an attribute of soil quality because it is one of the principal aggregating agents in soil (Tisdall & Oades, 1982). Furthermore, different authors (Jaiarree et al., 2014) pointed out that organic materials should be applied as a priority to soils with low fertility and low SOC in order to enhance soil C sequestration. Also, they favor nutrient recycling in agricultural systems where such residues are produced (i.e. promoting sustainable field management).

Physical fractionation of SOC, using density separation, assumes that biologically significant pools, differing in structure and function, can be obtained. In a long-lasting cultivated soil where stubble is not burned, it separates newly incorporated, partially decomposed debris named the “light fraction” (which includes free and occluded organic C within aggregates) from a more decomposed organic matter (heavy fraction), with a lower C:N ratio, which includes organic matter adsorbed onto mineral surfaces or sequestered within soil aggregates.

The presence of earthworms is another attribute of soil quality because of their contribution in the conversion of plant residues into soil organic matter. Earthworms help to increase porosity and decrease bulk density, leading to greater soil water infiltration (Lee & Foster, 1991).

Medium and long-term studies on manure fertilization relating to aggregate stability and SOC have been reported (Tripathi et al., 2014). But under Mediterranean climates in particular, there is a gap in knowledge about these potential relationships because management studies have mainly focused on the effects of tillage (Plaza-Bonilla et al., 2013) where “no tillage” or “minimum tillage” increased the proportion of macroaggregates together with gains in SOC content, or on the effect of slurries (Yagüe et al., 2012) which have a positive impact on aggregate stability and soil microbial biomass. The gap is relevant, because highly productive Mediterranean climates exist on several continents (Bosch-Serra, 2010). Climatic conditions can also induce different responses (Whitbread, 2003) although aridity, which leads to soil structure degradation processes becoming more active (Cerdà, 2000), is one of the most relevant.

The experimental area is representative of northeastern Spain where soils are characterised by low SOC content and climate by erratic rainfall (Beguería et al., 2011). Manures are readily available because dairy cattle are raised locally (Idescat, 2009). Recently, as the area has been classified as a nitrate vulnerable area (Generalitat de Catalunya, 2014), management policies focus on water quality preservation. However, soil disaggregation exists and crust formation in the fields is evidence of soil quality problems, which lead to difficulties in crop emergence and poor water infiltration, and enhance laminar flux, which can transport disaggregated materials.

The breakdown of soil aggregates by implosion (slaking), caused by the penetration of water into soil dry aggregates, is increased when the soil surface dries between rain or irrigation events. Slaking is the main destabilizing mechanism for aggregate disintegration in the Ebro basin (Amézqueta et al., 2003). At field level, disaggregation by wetting stress increases the risk of soil erosion (Mataix-Solera et al., 2011).

Macropores and mesopores (100-1000 µm) increase as manure rates increase (Miller et al., 2002) but microporosity also depends upon the organic material applied (Pagliai & Antisari, 1993). Pore size distribution and total porosity affects and is affected by almost everything that occurs in soil: movement of water and air, the transport and reaction of chemicals, and the presence of roots and other biota (Nimmo, 2004). Our hypothesis is that the regular use of manures in a Mediterranean agricultural system with low SOM content, helps in avoiding slaking. Furthermore, it could affect other physical (i.e. porosity and pore size and shape) and biological (i.e. presence of earthworms) properties related to aggregation.

The aim of this study was to assess the effect of fertilization management (mineral or dairy cattle manure) on soil quality parameters under a maize (*Zea mays* L.) crop that was maintained as a monoculture during 11 growing seasons, under irrigation. Soil quality studies focused on SOC fractions and the stability of aggregates to wetting stress (slaking), soil porosity and earthworm abundance. The defined objectives were: (i) to assess changes in soil organic carbon physical fractions; (ii) to evaluate soil macroaggregate stability under a slaking breakdown mechanism; (iii) to study potential relationships between macroaggregate stability to wetting stress and the different soil organic carbon fractions; (iv) to evaluate other related impacts of SOC such as porosity distribution and pore size and shape; (v) to quantify changes in earthworm abundance and their potential relationship with macroaggregate stability or porosity.

MATERIALS AND METHODS

Soil and climate description

The experiment was established in 2002 in Tallada d'Empordà, Girona, NE Spain (altitude 18 m a.s.l., lat. 42° 03' 02" N, long. 03° 03' 37" E.) The soil studied has a loam texture in the upper layer (0-0.30 m) and a soil organic carbon content of 7.6 g SOC kg^{-1}. It is well drained and no salinity is present (Table I). The soil is classified as Oxiaquic Xerofluvent (Soil Survey Staff, 2014).

The area has a dry Mediterranean climate according to Papadakis classification (MAPA, 1989). The annual average temperature is 15.6ºC and summer temperatures are high (on average 23ºC). 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 storm events in September-October-November which can enhance runoff processes if the soil is bare. But these events may also occur in spring-time, during the maize sowing period, when crust formation can strongly affect crop emergence and establishment. The dry period includes July and August.

Description of the experiment

The experimental field was cropped with maize (*Zea mays* L.) as an irrigated monoculture, until November 2012. Every year seeding was done in March-April and harvest in September. During each cropping season of the experimental period (2002-2012), tillage and fertilization management were maintained just the same. The stubble was left in the field and was incorporated in the soil, after each annual harvest, by disc-harrowing tillage. At the end of autumn or in early winter, tillage was done by a mouldboard plough (~ 25-30 cm depth).

Fertilization with dairy cattle manure and mineral fertilizer treatments were included in a broad experiment aimed at obtaining fertilizer assessments in terms of yield. The treatments were in a randomised complete block design with three replicates (blocks). From them, one inorganic fertilizer treatment and two organic amendment treatments were selected (Table II), plus a control (no N, no manure applied). Treatments were chosen according to historical maximum grain yields which were between 15 and 18 Mg ha^{-1} at 14% moisture content (Fig. 1), without significant differences between chosen treatments of dairy cattle manure (DCM) and mineral nitrogen fertilizer (MNF). Manure treatments were: DCM, applied only at sowing, at wet-weight rates of 30 and 60 Mg ha^{-1} yr^{-1} (named 30DCM-0 and 60DCM-0, respectively; Table II). The lowest DCM rate took into account the legislation in force at the start of the experiment, which established a maximum amount of 210 kg N ha^{-1} to be applied annually from organic sources. At this rate, the annual average amount of organic carbon applied in the period 2002-11was 2.4 Mg ha^{-1} and 3.3 Mg ha^{-1} in the 2012 cropping season (Table II). It can also be expressed as an average manure equivalent nutrient amount for the period 2002-2012 of 230±60 kg N ha^{-1} yr^{-1}, 76±33 kg P ha^{-1} yr^{-1} and 210±94 kg K ha^{-1} yr^{-1}. It was complemented with 27 kg P ha^{-1} yr^{-1} as calcium superphosphate and 75 kg K ha^{-1} yr^{-1} as potassium sulphate. When the manure rate was doubled (60DCM-0), no PK mineral fertilizer was added. Manures were incorporated by disc-harrowing just before sowing.

The MNF treatment consisted of 300 kg N ha^{-1} (named 0-300MNF) applied as calcium ammonium nitrate (27% N) at the V6-V8 Zadock’s development stage (late May).

Mineral fertilizer and control (0-0) treatments received, at sowing time, 55 kg P ha^{-1} yr^{-1} as calcium superphosphate and 150 kg K ha^{-1} yr^{-1} as potassium sulphate. The control was maintained throughout all growing seasons.

Sampling and analysis of manures and soil properties

In every cropping season, at manure application time, a composite sample of manure applied was analysed (Table II). Organic matter was analysed according to the total volatile solids methodology which in our case includes ashing at 550ºC for 6 h in a muffle furnace. The loss of weight equals the total volatile solids (Chatterjee et al., 2009). The carbon content was obtained by dividing by a coefficient of 1.82 according to the molecule that is considered to be representative of the organic matter present in manures.

Soil samplings were run for each fertilization treatment in each block after the last harvest, which means eight-nine months after the last manure application (28th March 2012) and incorporation (2nd April 2012). To assess aggregate stability and organic carbon fractions, samples were taken on 12 November 2012. In each plot, four individual points were sampled (0-0.10 m depth) and a composite soil sample was obtained. The three composite samples for each treatment (from the three blocks of the experiment) were air-dried, stored and sieved (2 mm) just before the different analytical procedures were carried out. Two days later (14 November) sampling was done (0-0.20 m depth) in the same plots to evaluate earthworm abundance. Finally, in December 12th, undisturbed soil samples (0-0.05 m depth) were taken in order to study porosity through image analyses of soil thin sections.

Water-stable aggregates (WSA_{MOD}) without sample pre-wetting and mean weight diameter of aggregates (MWD_{FW})

Aggregate stability was evaluated according two methods: the standard single-sieve technique in distilled water and the multiple-sieve technique in ethanol.

Each composite sample (one per plot) was divided into four subsamples and all of them were evaluated for aggregate stability, which means 12 analyses for each treatment as treatments were replicated in three blocks. The standard stability test for water-stable aggregates was modified; in that sample pre-wetting (WSA_{MOD}) was avoided, because slaking decreases as the initial water content increases (Truman et al., 1990). In the WSA_{MOD} test, four grams of 1-2 mm air-dried sample were directly placed on a 0.25 mm opening sieve and transferred to a Yoder apparatus for disaggregation following the procedure of Kemper & Rosenau (1986). Soil remaining on the sieve was oven-dried (105ºC, 24h) and weighed to give the “aggregate stable-mass” which was expressed as a percentage of total mass. The mass of sand in both parameters was previously discounted.

The resistance to slaking was also quantified as the mean weight diameter of aggregates remaining after fast wetting (MWD_{FW}). The methodology followed Le Bissonnais (1990) as modified by Amézqueta et al. (1996). The disrupting mechanism was applied to 4 g of air-dry, 1-2 mm diameter aggregates. Aggregates were placed in 0.25 mm opening sieves and gently immersed for 10 minutes in 100 mL of deionized water. The sieves were transferred to the modified Yoder apparatus and disaggregation consisted of mechanically moving the sieves immersed in ethanol (95%) up and down, 10 times over a distance of 1.3 cm. The fraction >0.25 mm was oven dried (105ºC, 24h) and dry sieved for 1 minute on a column of four 6.5 diameter sieves with hole openings of 2.0, 1.0, 0.5, and 0.25 mm using a standard mechanical sieve shaker.

The aggregate stability for each treatment was expressed as MWD (µm). It was calculated as Eq. [1]:

$$\mathrm{MWD}_{\mathrm{FW}} = \sum_{i=1}^{n} Wi \times Di \times 10^{3} \qquad \text{Eq. [1]}$$

where *n* corresponds to the number of aggregate size fractions considered in the analysis (in this case four size class: <0.25 mm; ≥0.25 mm to 0.5 mm; ≥0.5 mm to 1mm; ≥1 mm to 2 mm), Di is the mean diameter of aggregates that potentially can stay in the *i*th and *i*+1 th sieves which were: D_1=0.125 mm; D_2=0.375 mm; D_3=0.75 mm; D_4=1.5 mm; and Wi is the mass percentage of each fraction (dry weight of aggregates in the ith size fraction (g) divided by the sum of total sieved soil dry weight fractions (g)).

Soil organic carbon fractions

For each sample, five soil density and particle size fractions were obtained according to the procedure NF X 31-516 established by AFNOR (2007). According to it, the soil particle fraction sizes obtained were: <0.05 mm, ≥0.05-0.2 mm and ≥0.2-2 mm. The two upper size fractions were divided by flotation into two density fractions each: light (the fraction that floats in water) and heavy (the rest). The light fraction was analysed following the total volatile solids method. In the heavy fractions and in the smallest size one (<0.05 mm), as they are linked to the soil mineral components, the oxidizable SOC was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate following the Walkley-Black method (MAPA, 1994).The same method was used for all initial soil composite samples as it is the routine method used in agronomic soil laboratories.

Earthworm abundance

Earthworms were sampled from an excavated hole in field conditions. A template was used to define a 0.25 m x 0.25 m area in two randomly chosen locations in each plot, and samples were obtained from the defined area in a 0-0.20 m depth (Baker & Lee, 1993; Smith et al., 2008). No chemical expellant was used. Earthworms in each sample were removed by hand-sorting. Abundance was measured for intact and fragmented earthworms.

Soil porosity

The undisturbed samples were dried at room temperature and impregnated with polyester resin with a fluorescent dye (Uvitex©). One vertical thin section (5 cm wide, 13 cm long) was made from each block, according to the procedures of Stoops (2003). From each thin section two images (42 x 31.5 mm each) were obtained under two light conditions: parallel polarisers and crossed polarisers plus a third image under an ultraviolet incident light. They were processed with ImageJ (Rasband, 2008) to obtain digital binary images from which the porosity, associated with pores with an apparent diameter >25µm (the minimum threshold allowed by the established procedure) was analysed. Pore-size distribution analysis of each image was based on an open mathematic algorithm: the Quantim4 library (Vogel, 2008). The area occupied by pores was divided in four intervals according to the pores' apparent diameter: 25-65 µm; 65-100 µm, 100-200 µm, 200-400 µm and >400 µm. Shape descriptors used followed Ferreira & Rasband (2012): Circularity, with a maximum value of 1 indicating a perfect circle (4π pore area / pore perimeter^2); Aspect ratio, or the ratio of the particle's fitted ellipse (major axis / minor axis); and Solidity (area of the pore / convex area of the pore).

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, with the exception of the earthworm abundance where the DMRT was done at the 0.10 probability level.

RESULTS

In all treatments, the WSA_{MOD} and MWD_{FW} values were in the interval between 25.3 to 35.0% and 244 to 325 µm respectively (Table III). No significant differences were found between the MNF treatment and the control (Table III). However, resistance to slaking, evaluated as WSA_{MOD} and MWD_{FW}, was significantly improved by long-term manure addition, with no differences between the two manure rates (Table III).

The SOC content obtained by dichromate oxidation in the bulk soil ranged from 7.3 to 14.6 g C kg $soil^{-1}$ (Table IV). Although dichromate oxidation does not recover all the organic carbon (Skjemstad & Taylor, 1999), in both manure treatments it was significantly higher than those from the MNF treatment or the control. The more stable carbon (size<0.05mm) was also significantly improved with the manure addition at the highest rate (Table III). The sum of total light SOC (from 0.05 up to 2 mm; Table IV) was positively and significantly ($p<0.001$) correlated with the aggregate stability (Figs. 2a and 2b), but the best adjustment was obtained with WSA_{MOD} ($R^2= 0.80$).

The abundance of earthworms (Fig. 3) increased with DCM rates and in the 60DCM-0 treatment was significantly higher ($p<0.10$) than the control (0-0) or the MNF.

Porosity (apparent diameter >25 µm) accounted for 17.11% to 22.63% of the thin section area (Fig. 4), without significant differences between fertilizer treatments and the control, nor for the different pore sizes with the exception of the upper class (> 400 µm) which was lower in the control plots. Shape differences (Table V) were present at pore ranges of 100-200µm (Solidity) and 200-400µm (Circularity and Solidity). MNF plots were more solid than in the control and in the 30DCM plots, as well as more circular. When looking at all sizes, pore circularity tended to decrease as pore size increased (Table V). The opposite trend occurred in the aspect ratio.

DISCUSSION

Soil aggregate stability

The significant increase of aggregate resistance against the slaking disaggregating effect, in DCM treatments (Table III), was in agreement with findings of other authors about improvement in aggregate stability associated with long term cattle manure applications (Aoyama et al., 1999; Tripathi et al., 2014). Slaking is associated with a lack of organic bonding between particles (Ashman et al., 2003). These bonding agents can be temporary and transient, but in soils with low OM content (<10 g kg^{-1}) transient binding agents such as polysaccharides are the most important (Tisdall & Oades, 1982). The introduction of animal residues can stimulate microbial activity (Hernández et al., 2007) and consequently, the production of polysaccharides. The increase in aggregate stability due to manure addition has a supplementary value, because the particular soil's clay content is very low (Table I). The importance of mineral components (lithology) on soil aggregate stability was pointed out by Cerdà (1996). Clay content and SOC associated with aggregates are the principal determinants of water stable aggregation (Boix-Fayos et al., 2001). Thus, low clay content aggregates are more vulnerable to disruptive forces compared with high clay content ones (Edwards & Bremner, 1967; Lehrsch et al., 1991). Aggregate stability improvements by manure addition would also result in better protection against water erosion, as aggregation increases infiltration and reduces runoff (Bronick & Lal, 2005; Arjmand Sajjadi & Mahmoodabadi, 2015).

Soil organic carbon

The carbon balance (manure *vs.* mineral fertilization) results in a positive value (Table IV) for low rate manure (30DCM-0) which represents approximately 6.7 Mg C ha^{-1} , in the first 10 cm depth (as average value a bulk density of 1350 kg m^{-3} has been adopted). The increase of SOC content in manure treatments was mainly associated with the light SOC fraction, which increased by 107% or by 282% for 30DCM-0 and 60DCM-0 treatments respectively, when compared with MNF fertilization. Differences in the light fraction of SOC can be explained because it is a labile source of soil C and it is strongly influenced by factors related to the recent history of organic matter addition (Gosling et al., 2013). Thus, it is sensitive to changes in management practices (Gregorich et al., 1997). Our results corroborate Leifeld & Kögel-Knabner (2005) statements saying that the light SOC fraction is an early indicator of future (long-term) impacts of management on soil quality. The importance of the SOC light fraction as a sensitive indicator of changes in OM status associated with fertilization practices was also observed by Bhogal et al. (2009) on annual manure additions (>7 years). Changes in the SOC light fraction linked to manure applications controlled the magnitude of changes in aggregate stability (Fig. 2a and 2b) although a small fraction could be associated with other temporary stabilizing materials, e.g. stover incorporation and roots, as can be seen for the mineral fertilizer treatment (Table IV). This is the key point of our research which distinguishes it from the works of others authors (Whalen & Chang, 2002; Hou et al., 2012; Tripathi et al., 2014; Gelaw et al., 2015; Mikha et al., 2015) where soil aggregation was related to total SOC. It also reinforces the warning of Pulido Moncada et al. (2015) when using the SOC as an estimator of aggregate stability, that specific fractions of the SOC can be the stabilizing agent as, in our case, the light fraction despite the increment of the most stable SOC fraction at the highest manure rate (Table IV). The light fraction is considered to be the decomposing plant and manure part with a rapid turnover and low specific density (Whitbread, 2003), that stimulates polysaccharide production (transient binding agents) by microbial activity. The continued polysaccharide production will result in increments in the aggregate stability (Tisdall & Oades, 1982).

Total porosity, size classes and shape parameters

Fertilization, whatever is the nature of the fertilizers (minerals or manures), enhances the presence of pores (Fig. 4) in the upper size class (>400µm) without modifying their shape (Table V). This result is in accordance with Allison (1973) in the sense that aggregation improvement extends the volume of large pores. This fact is particularly relevant in this agricultural system as these pores favor aeration and soil water infiltration (Pagliai et al., 2004) and, as a consequence, the runoff coefficient and the sediment transport capacity of water is reduced (Dosskey et al., 2007). Compared with the results of Miller et al. (2002), no increase of macropores and mesopores (100-1000 µm) was observed as manure rates increased, although the tendency existed (Fig. 4).

The solidity parameter gives information about the irregularity, tortuosity and roughness of a pore. Also, circular pores are smooth pores, which tend to seal when soil is wet (Pagliai & Vignozzi, 2002). Then, the moderately irregular pores have walls which increase water retention capacity and superficial contact by capillarity. This behavior, enhanced by manure application at an agronomic rate (30DCM), is an environmentally positive aspect of manure use, despite its attenuation at a higher rate (60DCM).

Earthworms

Earthworms' abundance could have been underestimated in terms of anecic species as they can easily escape to deeper soil layers. For these ecotypes other different expulsion techniques are available (Valckx et al., 2011). Nevertheless, some authors say (Bartlett et al., 2006, 2010) that behavioral expulsion techniques overestimate anecic species in comparison to endogeic species. Besides, Murchie et al. (2015) found that the species which increased with the application of cattle slurry were epigeic earthworms; and to a lesser extent (just one species) endogeic earthworms, while anecic species were not affected. Thus, the hand sorting technique was a sensible option. Earthworm abundance tended to increase with manure rates (Fig. 3) but had no influence over macroporosity differences between fertilization treatments (mineral or manures), only when they were compared with the control (no N). As organic debris was similar in plots that received N fertilization, and the contribution of earthworms is the physical mixing of soil minerals, water, microbes and residual matter, this fact could have been the reason for the absence of significant differences in soil macroporosity between all the fertilized plots (Fig. 4). Furthermore, the excretion from the gut releases organic materials which begin to form a structural fabric within the soil (Lee & Foster, 1991). Stable aggregates in soil are linked to the burrowing actions of earthworms (Ketterings et al., 1997). Earthworms enhance the litter-derived C transfer into the soil profile (Novara et al., 2015) and the incorporation of crop-derived C into macroaggregates and

more importantly into microaggregates formed within macroaggregates (Pulleman et al., 2005). The dominant mechanism for enhanced aggregate stability from excreted pellets is the generation of bonds of clay-polyvalent cation-organic matter linkages. Polysaccharides as well as other organic polymers are involved in the bonds described, and the differences between them (more or less anionic groups) will depend on the ingested materials (Shipitalo & Protz, 1989). At this point, the added manure could set up some differences in aggregation in comparison with to the mineral fertilized plot which just received plant residues. As a consequence, the relationship between the presence of the light organic matter fraction (greater amount in manured treatments; Table IV) and aggregate stability (Fig. 2) could be enhanced by the activity of earthworms.

Management of the agricultural system

Manure application is a fertilization option in which a consideration of overall sustainability must be included: yield productivity, soil quality and overall nutrient management. In this intensively managed Mediterranean agricultural system, maize yields (15 to 18 Mg ha^{-1}) are considered high, whatever the fertilization option is (mineral or/and manures). Thus, the preservation of soil quality, as a decision factor when evaluating fertilization strategies, can be easily accepted by farmers.

Considering the yields attained (Table II) and an average maize nutrient extraction for each 1000 kg of grain and hectare of close to 28-30 kg N, 4.4-5.3 kg P and 19-21 kg K (Betrán-Aso, 2010), at the lowest DCM rates there could be a constraint on N availability depending upon mineralization values (i.e. residual effect). The applied phosphorus is close to crop needs, which justifies the addition of mineral P. This is a key point as surface application of manure often results in very high P concentrations at the soil surface (Andraski & Bundy, 2003), and P can be lost by sediment transport losses. Nevertheless, application of dairy manure with high solid content (210-280 g kg^{-1}) reduces sediment and particulate P losses in runoff (Yagüe et al., 2011). Our manure applications, with high dry matter content (304±68 g kg^{-1}), could also help in avoiding the transport of sediments and the consequent P runoff because they increase aggregate stability related to the usual slaking phenomena in Mediterranean conditions. For potassium, as the residues are incorporated, there is considerable K recycling, but also a need for complementary mineral fertilization. Furthermore, DCM tends to increase earthworm abundance and the solidity of some pores (200 to 400 µm) related with water infiltration which will result in a better water management efficiency.

CONCLUSIONS

Dairy cattle manure applied annually to maize favoured soil aggregate stability against the destabilizing effect of slaking on dry aggregates. These results were independent of the measurement procedures: WSA_{MOD} or MWD_{FW}. The improvement in aggregate stability was associated with increments in the SOC content, mainly in the light organic matter fraction. For a period of eleven years, the increment of SOC in manured plots: 30 or 60 Mg ha^{-1} applied, ranged from 3.9–4.9 g C kg $soil^{-1}$ to 6.7–9.1 g C kg $soil^{-1}$ respectively, when compared with mineral fertilization. The earthworm abundance increased with manure rates although it was not translated into an increment of the areas occupied by pores with an apparent diameter >25µm. Changes in porosity by any fertilization treatment consisted in the increase of the upper fraction (>400µm). In the interval from 100 up to 400 µm, the 30DCM treatment maintained a lower circularity and pore solidity than MNF. These changes in pore shape could be translated as a way to facilitate water infiltration and to avoid surface sealing. The annual use of manure in these agricultural systems, at low rates such as 30 Mg ha^{-1}, can be recommended because of the positive impacts on soil quality parameters and the achievement of high yields. Applying higher annual manure rates such as 60 Mg ha^{-1} is a more risky option for a long term sustainable management strategy (high nutrient addition in relation to the crop's needs), as there may be groundwater water quality concerns (e.g. leaching of nitrates).

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Figure legends:

Figure 1. Average grain yield (14% moisture content) of the period from 2001 to 2012 for each fertilization treatment: 0-0 or the control (no N, no manure applied); 0-300MNF: mineral N fertilizer (calcium ammonium nitrate, 27% N) applied at rate of 300 kg N ha^{-1} $yr^{-1}$when six-eight leaves were visible; 30DCM-0 and 60DCM-0: dairy cattle manure applied at wet-weight rate of 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing. Mean values of abundance followed by a different letter are significantly different at the 0.05 probability level based on the Duncan Multiple Range Test. Bars represent the standard error of three replicates.

Figure 2. Relationship between soil organic carbon (SOC) light fraction and aggregate stability, (a) applying the modified water aggregate stability test (without pre-wetting, WSA_{MOD}) or (b) according to the mean weight diameter test after fast wetting (MWD_{FW}). Bars represent the standard error of four replicates, *** $p<0.001$.

Figure 3. Earthworms abundance in each fertilization treatment: 0-0 or the control (no N, no manure applied); 0-300MNF: mineral N fertilizer (calcium ammonium nitrate, 27% N) applied when six-eight leaves are visible and at rate of 300 kg N ha^{-1} yr^{-1}; 30DCM-0 and 60DCM-0: dairy cattle manure applied at wet-weight rate of 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing. Mean values of abundance followed by a different letter are significantly different at the 0.10 probability level based on the Duncan Multiple Range Test. Bars represent the standard error of three replicates.

Figure 4. Average values (n=6) of the porosity area (apparent diameter > 25 µm) and its distribution for each fertilization treatment maintained in during a period of 11 years in a maize crop. The fertilization treatments were: 0-0 or the control (no N, no manure applied); 0-300MNF: mineral N fertilizer (calcium ammonium nitrate, 27% N) applied

at rate of 300 kg N ha^{-1} yr^{-1} when six-eight leaves were visible, and; 30DCM-0 and 60DCM-0: dairy cattle manure applied at wet-weight rate of 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing. Mean values of abundance followed by a different letter were the only ones with significant difference at the 0.05 probability level based on the Duncan Multiple Range Test. Analysis were done with the values of transformed data ($x^{0.5}$). Bars represent the standard error of three replicates.

Table I. Selected soil physical and chemical characteristics of the experimental site. Samples (0-0.30 m) were obtained at the start of the fertilization experiment (October 2002).

Parameter	**Value**
Particle size distribution (g kg^{-1})	
Sand (2000 <Ø[‡]< 50 μm)	497
Silt (50 <Ø< 2 μm)	435
Clay (Ø < 2 μm)	68
pH (water; 1:2.5[†])	8.4
Electrical conductivity (1:5[†]; dS m^{-1}, 25ºC)	0.19
Organic matter (g kg^{-1})	13.0
Total N (g kg^{-1})	0.8
Phosphorus (mg P kg^{-1}; Olsen)	23
Potassium (mg K kg^{-1}; Ammonium acetate)	84

[†]Relation; soil: distilled water.

[‡] Ø: particle apparent diameter.

Table II. Description of annual fertilization treatments with averages of the organic carbon (C) applied. Fertilizers (mineral or from different dairy cattle manure rates) were annually applied at sowing or when the crop had six-eight visible leaves (V6-V8).

	Annual slurry fertilizer treatment		**2002 to 2011 (10 yr)**	**Fertilization at sowing in 2012**
Treatment[†]	**Sowing**	**V6-V8**	---------- Mg C ha^{-1} yr^{-1} --------	
0-0	0	0	0	0
0-300MNF	0	300	0	0
30DCM-0	30	0	2.4 (±0.6)	3.3
60DCM-0	60	0	4.8 (±1.2)	6.6
Significance				

Numbers in brackets are the standard deviation.
** Significance at the 0.01 probability.
[†]MNF: mineral nitrogen fertilizer (calcium ammonium nitrate, 27% N), applied when the crop has six-eight leaves. Number behind indicates the applied rate of 300 kg N ha^{-1} yr^{-1}.
DCM: dairy cattle manure. Numbers indicate the average theoretical applied wet-weight rate: 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing.
[‡]Mean values in a column followed by different letter are significantly different at the 0.05 probability level based on the Duncan Multiple Range Test.

Table III. Average values (n=12) of the mass percentage of water-stable aggregates (WSA_{MOD})[‡] and of the mean weight diameter after a fast wetting (MWD_{FW})[§], associated with different fertilization practices maintained during a period of 11 years in a maize crop.

Treatment[†]	WSA_{MOD} (%)[¶]	MWD_{FW} (μm)[¶]
0-0	25.31 (4.76) B	244.03 (8.70) B
0-300MNF	25.26 (6.08) B	247.28 (4.46) B
30DCM-0	34.25 (14.12) A	324.95 (16.16) A
60DCM-0	35.03 (1.24) A	309.65 (13.21) A
Significance	*	**

Numbers in brackets are coefficients of variation (%).
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
[†]MNF: mineral nitrogen fertilizer applied as calcium ammonium nitrate (27%, N); the number behind indicates the applied rate of 300 kg N ha^{-1} yr^{-1} at six-eight visible leaves. DCM: dairy cattle manure; numbers indicate the average theoretical applied rate of 30 Mg ha^{-1} yr^{-1} or 60 Mg ha^{-1} yr^{-1} at sowing.
[‡]WSA_{MOD}: Water aggregate stability from Kemper and Rosenau (1986) method and its modification without pre-wetting.
[§]MWD_{FW}: mean weight diameter after fast-wetting, according to Le Bissonnais (1990) and modified by Amézqueta et al. (1996).
[¶]Mean values in a column followed by different letter are significantly different at the 0.05 probability level based on the Duncan Multiple Range Test.

Table IV. Average values (n=3) of organic soil carbon from a bulk soil and from different physical sizes and density soil fractions, associated to different fertilization practices maintained during a period of 11 years in a maize crop. Light fraction was analysed according to the total volatile solids methodology. The oxidizable organic carbon by dichromate oxidation was analysed in the remaining samples.

	Fractions (mm)[‡]					**Total oxidizableC (dichromate)[‡]**
	0.2-2		**0.05-0.2**		**<0.05**	
Treatment[†]	**Heavy**	**Light**	**Heavy**	**Light**		
	-- g C kg soil^{-1}					------------------------------
0-0	0.01	0.61C	0.59	2.33C	4.77B	7.27C
0-300MNF	0.01	0.93BC	0.64	2.56C	5.23B	7.90C
30DCM-0	0.01	1.96AB	1.24	5.28B	5.87B	11.80B
60DCM-0	0.01	2.17A	0.57	7.67A	8.07A	14.63A
Significance	NS	*	NS	***	*	***

NS: Not significant (p>0.05).
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
[†]MNF: mineral nitrogen fertilizer applied as calcium ammonium nitrate (27% N), the number behind indicates the applied rate of 300 kg N ha^{-1} yr^{-1}. DCM: dairy cattle manure, numbers behind indicate the average theoretical applied rate: 30 Mg ha^{-1} yr^{-1} or 60 Mg ha^{-1} yr^{-1}.
[‡]Mean values in a column followed by different letter are significantly different at the 0.05 probability level based on the Duncan Multiple Range Test.

Table V. Average values (n=6) of different shape porosity parameters: Circularity (Circ.), Aspect Ratio (AR), and Solidity (S), for each fertilization treatment[†] and pore sizes.

Pore size	***Shape parameters***[‡]		
25-65 µm	**Circ.**	**AR**	**S**
0-0	0.947	1.497	0.876
0-300MNF	0.959	1.478	0.872
30DCM-0	0.906	1.703	0.856
60DCM-0	0.938	1.538	0.874
Significance	NS	NS	NS
65-100 µm	**Circ.**	**AR**	**S**
0-0	0.848	1.657	0.839
0-300MN[†]	0.874	1.593	0.855
30DCM-0	0.787	1.790	0.817
60DCM-0	0.810	1.767	0.841
Significance	NS	NS	NS
100-200 µm	**Circ.**	**AR**	**S**
0-0	0.688	1.772	0.808B
0-300MNF	0.735	1.888	0.845A
30DCM-0	0.643	1.935	0.787B
60DCM-0	0.645	1.972	0.820AB
Significance	NS	NS	S (0.02)
200-400 µm	**Circ.**	**AR**	**S**
0-0	0.477AB	2.088	0.734B
0-300MNF	0.556A	1.937	0.809A
30DCM-0	0.451B	2.083	0.721B
60DCM-0	0.449B	2.093	0.762AB
Significance	S (0.04)	NS	S (0.03)
> 400 µm	**Circ.**	**AR**	**S**
0-0	0.300	2.215	0.644
0-300MNF	0.354	2.120	0.747
30DCM-0	0.263	2.235	0.624
60DCM-0	0.266	2.110	0.686
Significance	NS	NS	NS

[†]MNF: mineral nitrogen fertilizer applied as calcium ammonium nitrate (27%); the number behind indicate the applied rate of 300 kg N ha^{-1} yr^{-1}.DCM: dairy cattle manure; numbers behind indicate the applied rate of 30 Mg ha^{-1} yr^{-1} or 60 Mg ha^{-1} yr^{-1}.
[‡]Mean values in a column followed by different letter are significantly different at the 0.05 probability level based on the Duncan Multiple Range Test.

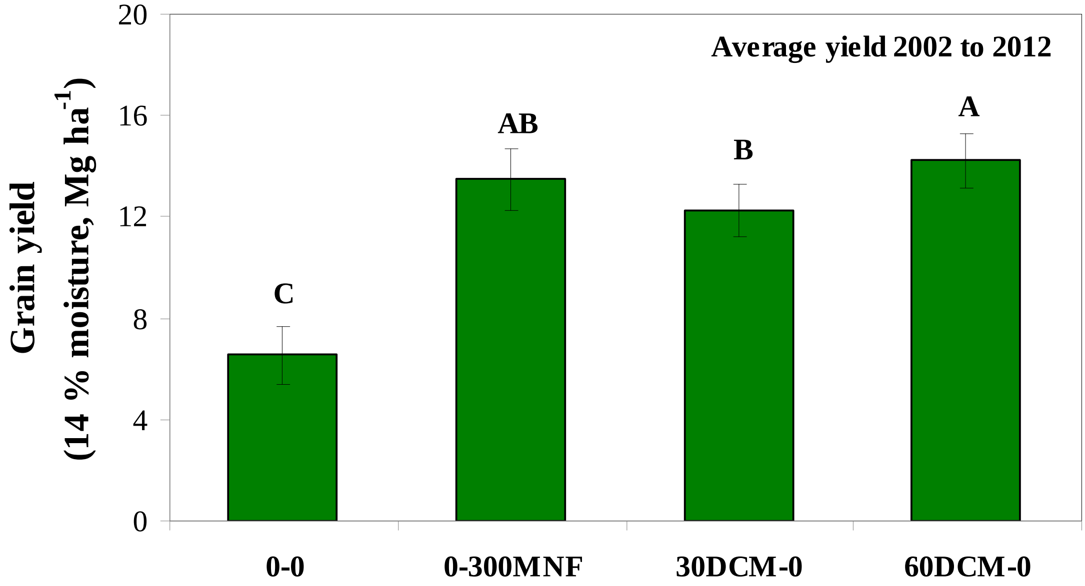

Figure 1. Average grain yield (14% moisture content) of the period from 2001 to 2012 for each fertilization treatment: 0-0 or the control (no N, no manure applied); 0-300MNF: mineral N fertilizer (calcium ammonium nitrate, 27% N) applied at rate of 300 kg N ha^{-1} yr^{-1}when six-eight leaves were visible; 30DCM-0 and 60DCM-0: dairy cattle manure applied at wet-weight rate of 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing. Mean values of abundance followed by a different letter are significantly different at the 0.05 probability level based on the Duncan Multiple Range Test. Bars represent the standard error of three replicates.

a)

b)

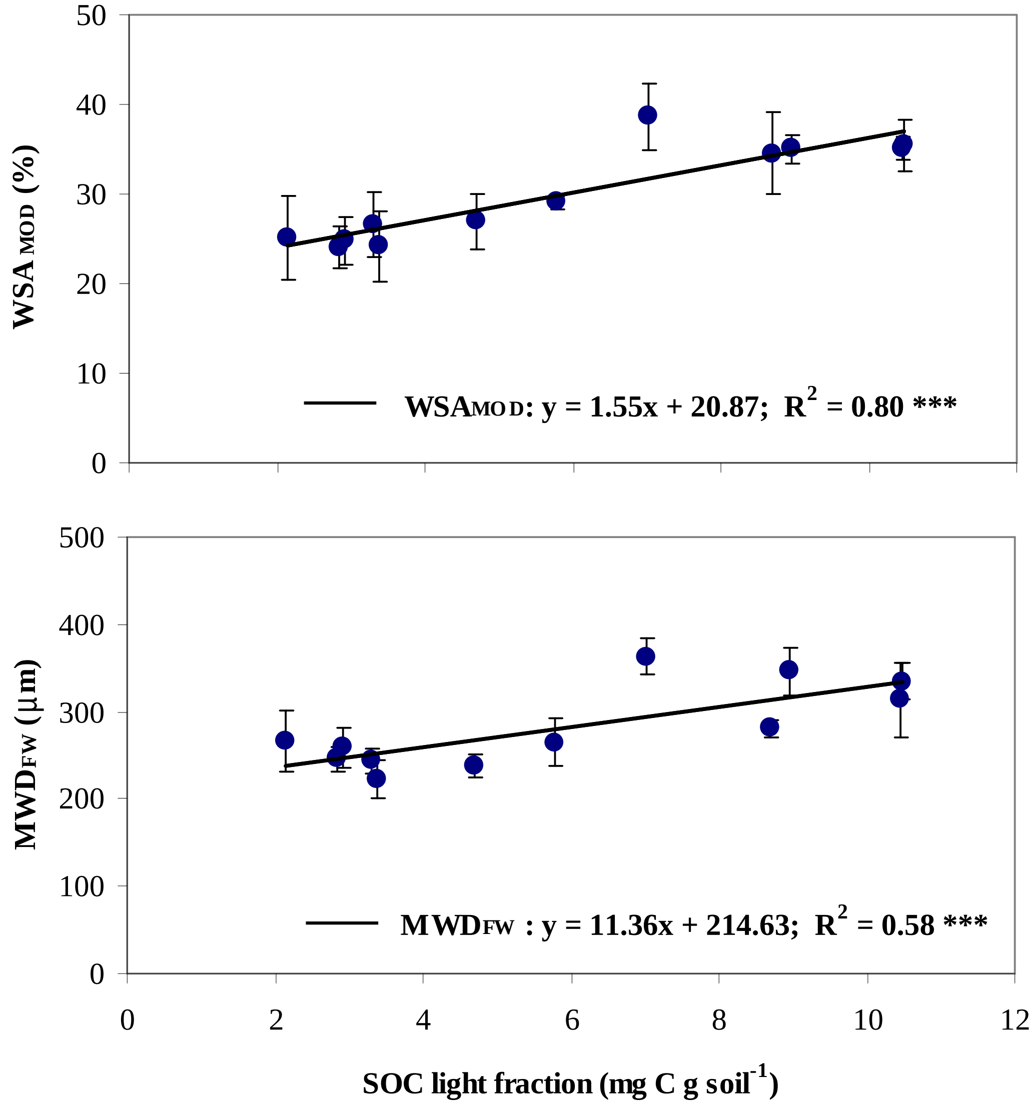

Figure 2. Relationship betweensoil organic carbon (SOC) light fractionand aggregate stability, (a) applying the modified water aggregate stability test(without pre-wetting, WSA_{MOD}) or (b) according to the mean weight diameter test after fast wetting (MWD_{FW}). Bars represent the standard error of four replicates, *** p<0.001.

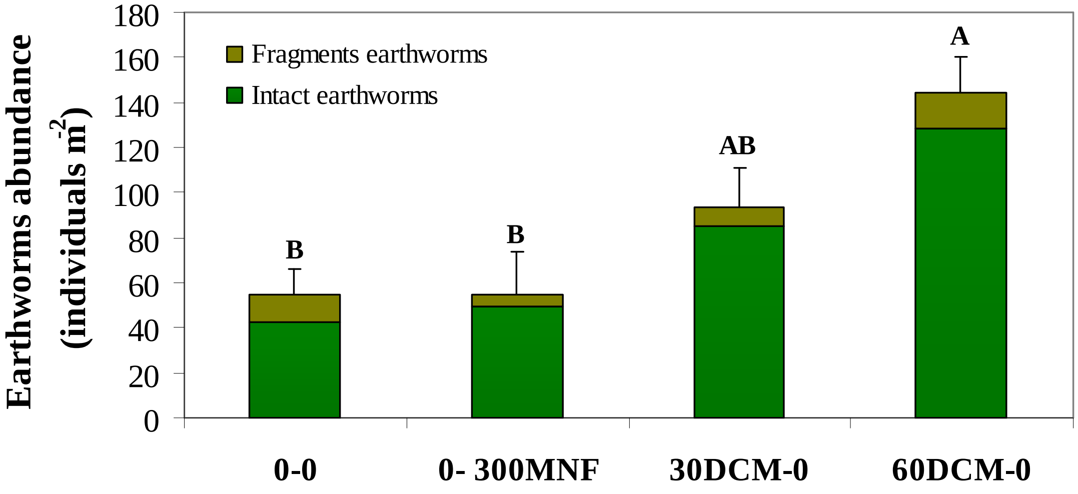

Figure 3. Earthworms abundance in each fertilization treatment: 0-0 or the control (no N, no manure applied); 0-300MNF: mineral N fertilizer (calcium ammonium nitrate, 27% N) applied when six-eight leaves are visible and at rate of 300 kg N ha^{-1} yr^{-1}; 30DCM-0 and 60DCM-0: dairy cattle manure applied at wet-weight rate of 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing. Mean values of abundance followed by a different letter are significantly different at the 0.10 probability level based on the Duncan Multiple Range Test. Bars represent the standard error of three replicates.

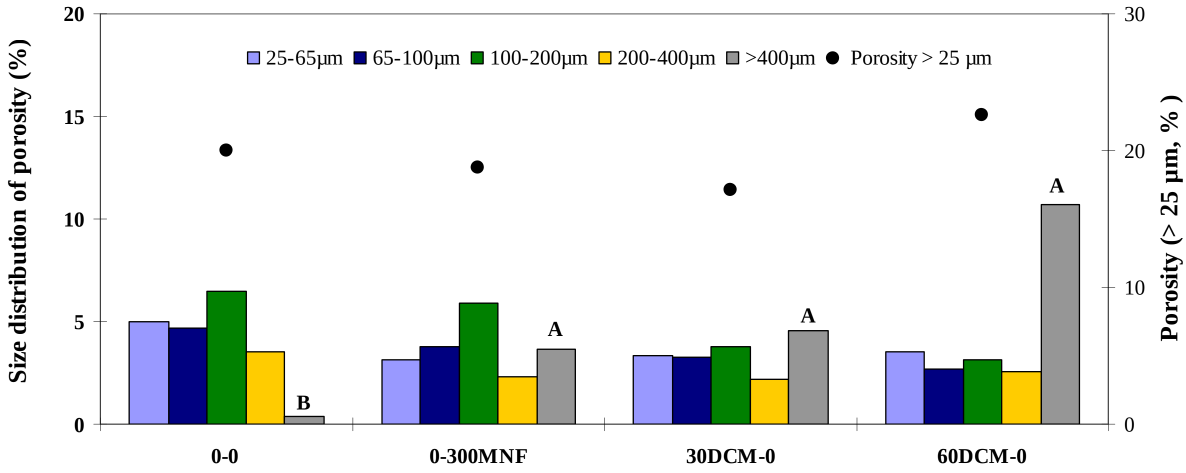

Figure 4. Average values (n=6) of the porosity area (apparent diameter > 25 µm) and its distribution for each fertilization treatment maintained in during a period of 11 years in a maize crop. The fertilization treatments were: 0-0 or the control (no N, no manure applied); 0-300MNF: mineral N fertilizer (calcium ammonium nitrate, 27% N) applied at rate of 300 kg N ha^{-1} yr^{-1} when six-eight leaves were visible, and; 30DCM-0 and 60DCM-0: dairy cattle manure applied at wet-weight rate of 30 Mg ha^{-1} yr^{-1}, or 60 Mg ha^{-1} yr^{-1} at sowing. Mean values of abundance followed by a different letter were the only ones with significant difference at the 0.05 probability level based on the Duncan Multiple Range Test. Analysis were done with the values of transformed data ($x^{0.5}$). Bars represent the standard error of three replicates.