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Abbreviations: AN, ammonium nitrate; AGBM, aboveground dry matter; FS, slurry from fattening pigs; M, mineral fertilizer; OM, organic matter; PS, pig slurry; SMB, soil microbial biomass; SOM, soil organic matter; SS, swine slurry; WSA, water-stable aggregates; WSA_{MOD}, modified standard stability test for water-stable aggregates.

Pig slurry and mineral fertilization strategies' effects on soil quality: macroaggregate stability and organic matter fractions

Abstract

Applying pig slurry to the land as fertilizer at appropriate agronomic rates is important to close nutrient cycles and optimize the value of organic matter. However a long-term discussion has taken place about its effects on soil quality. In the north-east of Spain, eight fertilization strategies were evaluated on the soil quality parameters aggregate stability, soil organic matter (SOM) physical fractions and soil microbial biomass (SMB). Six strategies used different pig slurries (PS) which provided organic matter from 1.7 to 2.6 t $ha^{-1}yr^{-1}$, the rest (mineral N fertilization and a control) did not. Pig slurries were applied at sowing and/or at cereal tillering, as sidedressing. Field experiments were maintained for an 8-year period, in a silty loam soil devoted to a rainfed winter cereal. Soil samples were taken once, before the last sidedressing in 2011. Aggregate stability was quantified using the standard water-stable aggregate method but including a modification which meant that pre-wetting was avoided (WSA_{MOD}). When using the WSA_{MOD} method, we found a tendency for the percentage of water-stable aggregates to increase due to PS application (differences of up to 74% in the increment) and it was more marked the nearer they were measured to the application time (3 months vs 12 months). The strategies which include PS show a positive effect on the SOM amount, mainly in the 0.05-0.2 mm light fraction, which increased by up to 34% with every 10 t ha^{-1} organic C applied, and on SMB (up to 53% increment). There is a positive and significant linear relationship ($p<0.05$, $R^2=0.75$) between the SOM light fraction (%) and the water-stable aggregates soil content (%, WSA_{MOD}). Thus, the introduction of PS in fertilization strategies improves soil quality parameters. However, the soil quality benefits need to be balanced with any other potential environmental impact.

Keywords: biological soil fertility; field studies; organic fertilizer, physical soil fertility; dryland areas; soil organic carbon; soil structure.

1. Introduction

Good soil structure is a most desirable soil characteristic for sustaining agricultural productivity and for preserving environmental quality (Amézqueta, 1999). Stability of a soil's structure is its ability to retain a heterogeneous arrangement of solids (aggregates) and void space that exists at a given time when exposed to different external stresses. Letey et al. (2003) proposed soil organic matter (SOM) and water-stable aggregates as attributes of soil quality because they improve soil functions which have beneficial effects on crop development or on avoiding environmental pollution. To protect these functions it is necessary to evaluate and predict the behaviour of soils in time and space, under a wide range of agricultural land uses (Pla, 2010).

Gobin et al. (2011) in a recent EU report about SOM best management practices, constraints and trade-offs pointed out the importance of continually supplying C in the form of organic matter (OM) as a food source for microorganisms and to build up stable C in soil that contributes to soil aggregate formation. However, the stability of soil aggregates depends not only on the quantity of the OM added but also on the quality of this OM (Tisdall and Oades, 1982).

Soil aggregation has been conceptualized (Tisdall and Oades, 1982) as an hierarchical system of three aggregate levels: macroaggregates (>0.25 mm) which break down into microaggregates (20-250 μm) before aggregates <20 μm are released. The binding of microaggregates appear to be relatively permanent and the OM associated with them is stabilized against decomposition. Other researchers (Gale et al., 2000) showed that new microaggregates are formed within existing macroaggregates, where organic fragments become encrusted with clay particles and microbially produced mucilage.

In Mediterranean dryland agricultural systems, soils are characterized by their low SOM content and erratic rainfall (Beguería et al., 2011) enhances aggregate breakdown. One of the main mechanisms of aggregate breakdown is the compression of air entrapped inside aggregates during wetting (slaking). Slaking increases as the initial moisture content decreases from saturation (Le Bissonnais, 1996). Furthermore, in dryland agriculture areas, water is the primary factor controlling crop productivity, so any soil and crop management practices that can maintain a good structure, thus enhancing water storage in soil void spaces and its availability to plants, will increase yields (Bosch-Serra, 2010). Consequently, it is important to search for management practices which can improve aggregation and SOM content.

As management practice, in Spain, fertilization with pig (*Sus scrofa domesticus*) slurry (PS) is a feasible option for farmers, because slurry is easily available, minimizes cost inputs and allows the closing of the nutrient cycles as well as being a good environmental option. Pig slurry use has received attention as a nitrogen (N) fertilizer source, but not much attention has been paid to its OM content, mainly because it is low compared with that of farmyard manures. Pig slurry's C/N ratio ranges from 3.6 to 4.6 (Sánchez and Gónzalez, 2005; Yagüe et al., 2012) and for farmyard manure C/N can range from 13 to 33 (Probert et al., 2005). It is important to note that Spain produces yearly around 26 million pigs (Eurostat, 2011) and a great part of PS is applied to cereal crops. The Spanish area cropped with winter cereals is close to 5.5 million hectares; around 90% of this surface is under rainfed conditions (MARM, 2010).

Physical fractionation of SOM using density separation may help to understand soil organic matter dynamics based on turnover time. 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 OM adsorbed on mineral surfaces or sequestered within soil aggregates. The light fraction of SOM is sensitive to changes in management practices (Bremer et al., 1994) and it is considered to represent an early indicator for determining the long term impacts of management on soil quality (Leifeld and Kögel-Knabner, 2005). On the other hand, soil microbial biomass (SMB) is another component of SOM which reflects soil biological activity and its changes may also precede other changes in chemical and physical properties. Carter (1992), in the context of a humid climate, found a close correlation between soil microbial biomass and the mean weight diameter of aggregates and Cambardella (2007) remarked that aggregate formation occurs primarily in zones of high microbial activity because this is where the humic substances are being produced.

Globally, there have been few long-term studies relating aggregate stability and SOM fractions, and they were mainly focused on manure (Whalen and Chang, 2002; Wortmann and Shapiro, 2008; Annabi et al., 2011; Karami et al., 2012) rather than on slurry. In Mediterranean conditions, the major studies on soil aggregate stability have been focused on the study of the effects of tillage (Mrabet et al., 2001; Hernanz et al., 2002; Álvaro-Fuentes et al., 2007; Álvaro-Fuentes et al., 2008; Melero et al., 2011) or soil types (Amézqueta et al., 2003; Ramos et al., 2003).

Within a framework of actual field conditions, the objectives of this study were: (i) to evaluate soil macroaggregate stability in a soil where different fertilization strategies (mineral or/and pig slurry application) were maintained during seven growing seasons; (ii) to assess changes in soil organic matter physical fractions and the amount of soil microbial biomass linked to several fertilization strategies; and (iii) to relate macroaggregate stability properties to soil organic matter fractions.

2. Materials and methods

2.1. Soil and climate description

The experimental field (at depths of 0-30 cm), has a silty loam soil texture, an average organic matter content of 2%, no salinity is present and average calcium carbonate content is 30% (Table 1). The soil is classified as a Typic Xerofluvent (Soil Survey Staff, 1999).

The area has a semiarid climate with an annual average temperature of 12.6ºC and high summer temperatures (average of maxima is 30.7ºC). Average annual precipitation (for the period 2000-2010) is 436 mm with a maximum of 593 mm and a minimum of 292 mm. Most rain falls in April-May and September-October (Fig. 1).

2.2. Description of the fertilizer experiment

The experiment was established in 2002 in Oliola, Lleida, NE Spain. The altitude is 443 m and coordinates are 41° 52′ 29″ N, 1° 09′ 10″ E. The experimental field was cropped with cereal until 2011 with the exception of the 2007/08 growing period where the field was left fallow. In this manuscript we present data for the period 2002 until February 2011 (which means seven full cereal growing seasons and one fallow year). Straw was collected and packed after harvest (July) and removed from fields; stubble was buried at the end of the summer (September) through tillage based on disc harrowing (~15 cm). Fertilization was done at sowing around mid October and/or at cereal tillering (when new shoots are produced) in February.

Several fertilization strategies (with pig slurry or/and mineral fertilizer) were included in a broad fertilization experiment aiming to find out the best strategy for fertilizer recommendations as well as the long term effects on soil quality, productivity and environment. They were arranged according to a randomised design with three replications (blocks).

From them, seven fertilization strategies were selected plus a control (Table 2). Strategies were chosen according to maximum grain yields and the amount of OM applied (using similar intervals) from PS of different origins (fattening pigs and sows). Control treatment (0-0) and nitrogen mineral strategy (120 kg N ha^{-1}) only received phosphorus and potassium fertilization (96 kg P_2O_5 ha^{-1} yr^{-1} and 107 kg K_2O ha^{-1} yr^{-1}) at sowing, but in the control no nitrogen was applied and it was maintained throughout all growing seasons. At sowing (October), four strategies received 30 t ha^{-1} yr^{-1} of slurry from fattening pigs (FS) and the rest did not (Table 2). Slurry was buried as soon as possible (<24h) after application. At cereal tillering (February), plants received a complementary amount (sidedressing) of fertilizer. Two strategies received mineral fertilizer (M), another two received slurry from fattening pigs (FS) and the last ones received slurry from sows (SS). The range of OM applied (Table 2) in plots receiving slurry only at tillering (0-60FS, 0-90SS) was similar to the applied range in plots receiving slurry at sowing (30FS-0, 30FS-60M, 30FS-20FS, 30FS-60SS) .

2.3. Description of analytical methodologies

On the 23rd February 2011, soil sampling was done in the first 10 cm depth of the chosen strategies in each block, in order to corroborate the absence of significant differences between blocks in relevant soil characteristics (Table 3). The measured parameters were: pH by potentiometry in a 1:2.5 (w/v) soil: distilled water suspension, electrical conductimetry in 1:5 (w/v) soil: distilled water suspension and carbonates by the Bernard calcimeter method. On the same day, separately, soil samples from plots of the central block were taken at 0-10 cm depth with soil cores (7 cm in diameter with steel bores). For each plot of every strategy, four points were sampled and a composite sample was obtained. According to the requirements of different analytical methodologies, part of the composite sample was air-dried and stored and the rest was maintained refrigerated.

Sampling was done two days before fertilization sidedressing (SideD), which means that two strategies (0-60FS, 0-90SS) were sampled twelve months after the last slurry application (Table 2) and four strategies (30FS-0, 30FS-60M, 30FS-20FS, 30FS-60SS) after three months. This allowed us to evaluate the influence of soil sampling timing on the results of aggregate stability test.

2.3.1. Aggregate stability

Aggregate stability was measured in four subsamples of each treatment according to the standard stability test for water-stable aggregates (WSA) proposed by Kemper and Koch (1966), and later improved by Kemper and Rosenau (1986) but our method include a modification which was to avoid sample pre-wetting ($\mathrm{WSA_{MOD}}$). The standard WSA test is a single-sieve (0.25 mm) method and it includes wetting the air-dried sample (Wi) by vapour until saturation (slow wetting), wet-sieving, dispersing the “aggregate stable-mass” (Wsa) and discounting the “sand-mass” (Wsand). The water-stable aggregates (WSA) as a mass percentage is calculated by Eq. 1:

$$\mathrm{WSA\,(\%)} = \frac{\mathrm{Wsa\,(g) - Wsand\,(g)}}{\mathrm{Wi\,(g) - W\,sand(g)}} \cdot 100 \qquad \text{[Eq. 1]}$$

This procedure is based on the application of low energy stress. As slow pre-wetting avoids slaking of aggregates (destabilizing effect of entrapped air), the measure of stability is related to differential swelling (microcracking of aggregates). However, from the agronomic and environmental point of view, slow wetting (associated with gentle rainfall) is of little importance in semiarid Mediterranean areas.

In order to expose aggregates to a more disruptive breakdown mechanism (slaking owing to the fast wetting of dry aggregates), in the modified test ($\mathrm{WSA_{MOD}}$), four grams of 1-2 mm air-dried sample (Wi) were directly placed on a 0.25 mm opening sieve and transferred to a Yoder apparatus for disaggregation without pre-wetting (no vapour saturation wetting). The rest of the procedure was similar to the standard WSA test.

Other different stability tests were used (data not shown) but were discarded because the associated gentle treatments (i.e. disaggregation measurement in ethanol) prevented us from finding differences between fertilization strategies.

2.3.2. Soil organic matter physical fractionation

The carbon content of the SOM physical fractions was analysed in five fraction sizes: <0.05 mm, 0.05-0.2 mm (light), 0.05-0.2 mm (heavy), 0.2-2 mm (light), 0.2-2 mm (heavy) for each fertilizer strategy according to the procedure NF X 31-516 established by AFNOR (2007). Total oxidizable organic carbon was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate (Yeomans and Bremner, 1988).

2.3.3. Soil microbial biomass

Carbon content of soil microbial biomass (SMB) was determined by the fumigation-extraction method UNE 77310-2 (AENOR, 2003). All analyses were done in triplicate for each composite sample.

2.4. Statistical analysis

In the statistical analysis significance is indicated by probability (p) levels. Values of p, higher than 0.05, are considered non-significant (NS). According to Chew's (1976) specifications, as we were just interested in comparing strategies, when the analysis of variance (ANOVA) indicated differences in the responses to strategies, we computed Duncan's Multiple Range Test (DMRT) for comparing all possible pairs of means at the 0.05 probability level. If the regression between variables was significant and the size of the coefficient of determination (R^2) was 0.75 or higher, fit was considered acceptable. The statistical analysis was performed using the statistical package SAS V8 (SAS Institute, 1999-2001).

3. Results and discussion

In relation to aggregate stability, if only the initial SOM content (Table 1) is taken into account, our soil can be classified as unstable under wet conditions (Carter, 1992). Nevertheless, the presence of carbonates can promote water-stable microaggregates formation within macroaggregates or stabilize macroaggregates formed from fresh organic matter decomposition (Fernández-Ugalde et al., 2011).

3.1. Effect of fertilization strategies on aggregate stability

Water-stable aggregates, as a mass percentage, were in the interval between 21 to 37% (Table 4). After the slaking occurrence in a dry soil, and allowing for our clay content (260 g kg^{-1}), it is expected to find around a 20% (w/w) of soil aggregates >200 µm (Le Bissonnais, 1996). This value corresponds to WSA average value from the control treatment (21.18%; > 250 µm) and it is explained by the resistance of clay bonds between skeleton grains. As the amount of light OM in the system increases (Fig.2), WSA tend to increase (Table 4) although the differences are more visible when applications are closer to sampling date (i.e. when it was three months ago compared with one year ago). This stability increment can be explained because OM may impart hydrophobic characteristics.

These results are important since the destabilizing effect of entrapped air (slaking) of macroaggregates occurs mainly in the surface layers. In our environmental conditions soils are dry with limited amounts of cover for several months (i.e. July to February) as winter cereal is usually sown by the end of October-early November and harvested into late June. Summer time is characterised by rather few rainfall events but with heavy rainstorms. Furthermore, wetting rate interacts with soil texture and the slaking effect is stronger for silty clay soils (with similar characteristics to our soil) in comparison with loam or sandy loam soils (Dickson et al., 1991).

3.2. Effect of fertilization strategies on soil organic matter physical fractions and soil microbial biomass

The total soil organic carbon content obtained as a sum of different fractions ranged from 1.37 to 1.90 g C 100 g soil^{-1} (Fig. 2). These values (due to processing limitations, data of 30FS-20FS are not shown) were positively associated to total oxidizable organic carbon obtained by dichromate and a significant relationship ($r=0.91$, $p<0.01$; data not shown) was found.

Total organic carbon did not show significant differences associated with fertilization strategies, probably due to the low amount of added OM (Table 2). Strategies of common PS agronomic recommendations (30FS-0 and 0-90SS), according to N criteria, show the tendency to increase soil organic carbon content (0.46 g C 100 g soil^{-1}; Fig. 2) which means, in the medium term (7 growing seasons plus a fallow year), an increment of up to 0.8% SOM compared with the optimum mineral fertilization (0-120M). The SOM increment vs. mineral fertilization is mainly associated with the 0.05-0.2 mm light SOM fraction (Fig.2) which increased by up to 34% (in 0-90SS strategy) with every 10 t ha^{-1} organic C applied. This trend is higher than that reported in literature from UK (Bhogal et al., 2009) and it is of great importance in dryland Mediterranean agricultural systems as it can be linked to the sustainable fertility of the system.

In the control treatment (0-0), the highest relative percentage of soil organic carbon content obtained in the fraction lower than 0.05 mm size can be observed . This

SOM fraction is the oldest, the most stable carbon and for agricultural soils in temperate areas, could be more than 50 years old. The predominance of the oldest fraction or the reduction of the 0.05-0.2 mm SOM light fraction cannot be observed in the mineral treatment (0-120M) because it produced higher biomass (47%) than the control during this period of seven growing seasons (Table 2), which means that more roots and a higher amount of stubble have remained in the field.

Soil microbial biomass shows significant differences ($p<0.01$) between treatments with the lowest value in the control (0-0) and a tendency to increase when slurry has been added (Fig. 3). This fact can be firstly explained by PS addition, which induces a reactivation of soil microbial growth and activity (Hernández et al., 2007) associated with the decomposition of the labile fraction of its carbon content (Rochette et al., 2000) and secondly, by a higher stubble incorporation (Table 2) which also stimulates microbial activity (Consentino et al. 2006).

3.3. Relation between stability of aggregates and soil organic matter fractions

We observed a significant relationship (Fig. 4) between SOM light fraction (from 0.05 to 2 mm) and WSA (discarding 0-90SS treatment) which agrees with the recognition of the role of the light fraction of SOM in the formation and stability of soil structure, especially in the stabilization of soil macroaggregates (Miller and Jastrow, 1990; Kay, 1998).

From the relationship (Fig. 4) it can be observed that treatments which received sow slurry at tillering achieve lower values of stability (WSA) for a given value of SOM light fraction (0.05-2 mm). This aspect requires further research on the nature of this fraction as it may also contain a different portion of microbial biomass and some humified organic matter associated with the slurry type.

Although soil microbial biomass can represent a significant proportion of the SOM light fraction (Gregorich and Janzen, 1996), in our case it has not been possible to establish a relationship with aggregate stability, probably because its influence is less clear in clayey soils (Kiem and Kandeler, 1997; De Gryze et al., 2005) such as ours (Table 1).

4. Conclusions

The results of this study showed that pig slurry applied over soils increased soil aggregate stability, when using a test method (WSA_{MOD}) which allowed us to assess the destabilizing effect of entrapped air after a fast wetting of dry aggregates. The use of the WSA_{MOD} test is recommended for such evaluation since it compares most realistically the field conditions experienced in dryland agricultural systems.

Slurry applications within the ranges of common agronomic recommendations lead to an increase of soil organic matter. This fact is mainly associated with the rise of its light fraction (from 0.05 to 2 mm) which has a positive and significant linear relationship with water-aggregate stability values when the WSA_{MOD} test is used.

The use of slurry in these dryland agricultural systems, at agronomic rates, can be recommended as it has a positive impact on soil quality parameters: aggregate stability, light soil organic matter and soil microbial biomass.

Further research is needed to evaluate these parameters, and other ones not taken into account in this research, over longer periods and in the framework of different agricultural systems in order to balance with any other potential environmental impact such as excessive nutrient accumulation.

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Figures legends

Fig. 1. Mean monthly precipitation, reference evapotranspiration (ET_0) and air temperature for the historic period: 2000-2010.

Fig. 2. Distribution of soil organic matter (SOM) physical fractions as a percentage of total soil oxidizable organic matter (0-10 cm depth) for different fertilization strategies: the ones which only include mineral fertilization (Mineral), the ones with slurry applied twelve months before sampling (S 12-mo) or the ones with slurry applied three months before sampling (S 3-mo). Strategy acronyms are described in Table 2.

Fig. 3. Distribution of soil microbial biomass for each fertilization strategy: the ones which only include mineral fertilization (Mineral), the ones with slurry applied twelve months before sampling (S 12-mo) or the ones with slurry applied three months before sampling (S 3-mo). Strategy acronyms are described in Table 2. Within columns, any two means having a common letter are not significantly different according to Duncan's Multiple Range Test at the 5% level of significance. Bars represent the standard error of three replicates.

Fig. 4. Relationship between soil organic matter (SOM) light fraction (0.05-2 mm) and water-stable aggregates (%, w/w) measured according to the WSA_{MOD} test (0-90SS strategy, light square, is not included). Dark square is 30FS-60SS strategy. Bars represent the standard error of three replicates.

Table 1. Selected soil physical and chemical characteristics at the experimental site (0-30 cm). Samples were obtained at the start of the fertilization experiment (October 2002).

Parameter	**Value**
Particle size distribution (g kg^{-1})	
Sand (2000 <Ø< 50 µm)	131
Silt (50 <Ø< 2 µm)	609
Clay (Ø < 2 µm)	260
pH (1:2.5)	8.2
Electrical conductimetry (1:5; dS m^{-1})	0.18
Organic matter (g kg^{-1})	20.1
CEC[†] (cmol^{+} kg^{-1})	11.1
Carbonates (g kg^{-1})	300

[†] CEC: cation exchange capacity

Table 2. Description of annual fertilization strategies including averages of the total nitrogen (TN), organic N (Norg) and organic matter (OM) applied. Fertilizers (mineral or from different slurry sources) were anually applied at presowing (PreS) or/and tillering as sidedressing (SideD). Data on accumulated (7 cereal growing seasons) aboveground biomass (AGBM) and grain yield (0 % humidity) are presented

	Annual fertilizer treatment		**2002 to 2010 (7 growing seasons)**			**Presowing fertilization in 2011**			**(7 growing seasons)**	
Strategy			**TN**	**Norg**	**OM**	**TN**	**Norg**	**OM**	**AGBM**	**Grain yield**
	PreS	**SideD**	------------------------ kg ha^{-1} yr^{-1} (± SD[¶]) --------------------------						--------- kg ha^{-1} --------	
0-0	0	0	0	0	0	0	0	0	21768	18373
0-120M[†]	0	120M	120	0	0	30	0	0	31958	21550
0-60FS [‡]	0	60FS	405±76	130±70	2622±784	0	0	0	37553	20897
0-90SS [§]	0	90SS	175±102	61±48	1764±1228	0	0	0	34849	24800
30FS-0	30FS	0	194±48	59±15	1681±240	170±1	64±2	1854±10	27961	21153
30FS-60M	30FS	60M	254±48	59±15	1681±240	170±1	64±2	1854±10	33174	23640
30FS-20FS	30FS	20FS	320±33	102±25	2491±254	170±1	64±2	1854±10	35408	22363
30FS-60SS	30FS	60SS	279±44	82±17	2107±273	170±1	64±2	1854±10	34557	22329

[†] M: mineral fertilizer applied as ammonium nitrate (AN). Numbers indicate the applied rate: 60 kg AN ha^{-1} yr^{-1} or 120 kg AN ha^{-1} yr^{-1}.

[‡] FS: slurry from fattening pigs. Numbers indicate the average theoretical applied rate: 20 t ha^{-1} yr^{-1}, 30 t ha^{-1} yr^{-1} or 60 t ha^{-1} yr^{-1}.

[§] SS: slurry from sows. Numbers indicate the average theoretical applied rate: 60 t ha^{-1} yr^{-1} or 90 t ha^{-1} yr^{-1}.

[¶] SD: standard deviation.

Table 3. Average values (n=3) of pH, electrical conductivity (EC) and carbonates in soil sampling (0-10 cm)

Strategy	**pH[†] (1:2.5)**	**EC (1:5; dS m^{-1})**	**Carbonates (%)**
0-0	8.30	0.20	29.7
0-120M	8.27	0.21	31.0
0-60FS	8.20	0.23	30.0
0-90SS	8.27	0.22	30.3
30FS-0	8.30	0.20	29.3
30FS-60M	8.27	0.20	29.0
30FS-20FS	8.27	0.20	28.7
30FS-60SS	8.27	0.20	28.7
Treatment	NS	NS	NS

[†] pH (1:2.5) in relation, 1 soil and 2.5 distilled water (w/v); EC (1:5), electrical conductivity in relation, 1 soil and 5 distilled water (w/v).

Non significant (NS): $p > 0.05$;

Table 4. Aggregate stability average values, from four replicated measurements, obtained for each methodology and fertilization strategy.

Strategy[†]	**$\mathrm{MWD_{SW}}$**[‡]	**$\mathrm{MWD_{FW}}$**	**$\mathrm{MWD_{MB}}$**	**WSA**[§]**$_{\mathrm{ST}}$**	**$\mathrm{WSA_{MOD}}$**
		µm		**%**	
0-0	970 (10.31)[¶]	567 (1.75) BC	912 (1.10) BC	70.5 (9.93)	21.18 (4.25) C
0-120M	842 (10.71)	630 (3.17) AB	920 (1.09) BC	56.3 (18.65)	23.11 (15.58) C
0-60FS	865 (13.95)	645 (6.25) AB	852 (1.18) D	52.3 (13.77)	23.88 (7.96) BC
0-90SS	957 (1.04)	652 (4.62) A	850 (1.18) D	51.3 (23.98)	23.82 (8.82) BC
30FS-0	927 (13.98)	427 (4.65) D	960 (1.04) A	76.5 (7.45)	36.86 (16.28) A
30FS-60M	892 (12.36)	495 (0.00) D	895 (1.12) C	65.3 (12.40)	33.03 (14.23) AB
30FS-20FS	760 (1.32)	450 (4.44) D	907 (1.10) C	66.7 (10.49)	31.14 (13.49) AB
30FS-60SS	980 (6.12)	542 (7.41) C	950 (2.11) AB	72.7 (13.07)	32.34 (15.15) AB
Treatment	NS	S	S	NS	S

[†] M: mineral fertilizer applied as ammonium nitrate (AN);numbers indicate the applied rate; 60 kg AN ha^{-1} yr^{-1} or 120 kg AN ha^{-1} yr^{-1}. FS: slurry from fattening pigs; numbers indicate the average theoretical applied rate; 20 t ha^{-1} yr^{-1}, 30 t ha^{-1} yr^{-1} or 60 t ha^{-1} yr^{-1}. SS: slurry from sows; numbers indicate the average theoretical applied rate: 60 t ha^{-1} yr^{-1} or 90 t ha^{-1} yr^{-1}.

[‡] MWD: Mean weight diameter (SW: slow-wetting procedure; FW: fast-wetting procedure; MB: mechanical breakdown after pre-wetting).

[§] WSA: Water aggregate stability (ST: Standard method by Kemper and Rosenau; MOD: Modified method from Kemper and Rosenau description without pre-wetting).

[¶] Numbers in brackets are the coefficients of variation (%).

Non significant (NS): $p > 0.05$; Significant (S): $p<0.05$. Within columns, means followed by the same letter are not significantly different according to Duncan Multiple Range Test (0.05).

Table 5. Linear correlation coefficients (r) between aggregate stability tests

	MWD_{FW}[†]	**MWD_{SW}**	**MWS_{MB}**	**WSA_{ST}**‡	**WSA_{MOD}**
MWD_{FW}	-	0.20NS	0.65NS	0.85**	0.82*
MWD_{SW}	-	-	0.20NS	0.23NS	0.11NS
MWS_{MB}	-	-	-	0.15NS	0.58NS
WSA_{ST}	-	-	-	-	0.74*

[†] MWD: Mean weight diameter (SW: slow-wetting procedure; FW: fast-wetting procedure; MB: mechanical breakdown after pre-wetting).

‡WSA: Water aggregate stability (ST: Standard method by Kemper and Rosenau; MOD: Modified method from Kemper and Rosenau description without pre-wetting).

Levels of significance: ** $p<0.01$; * $p<0.05$; NS $p>0.05$.

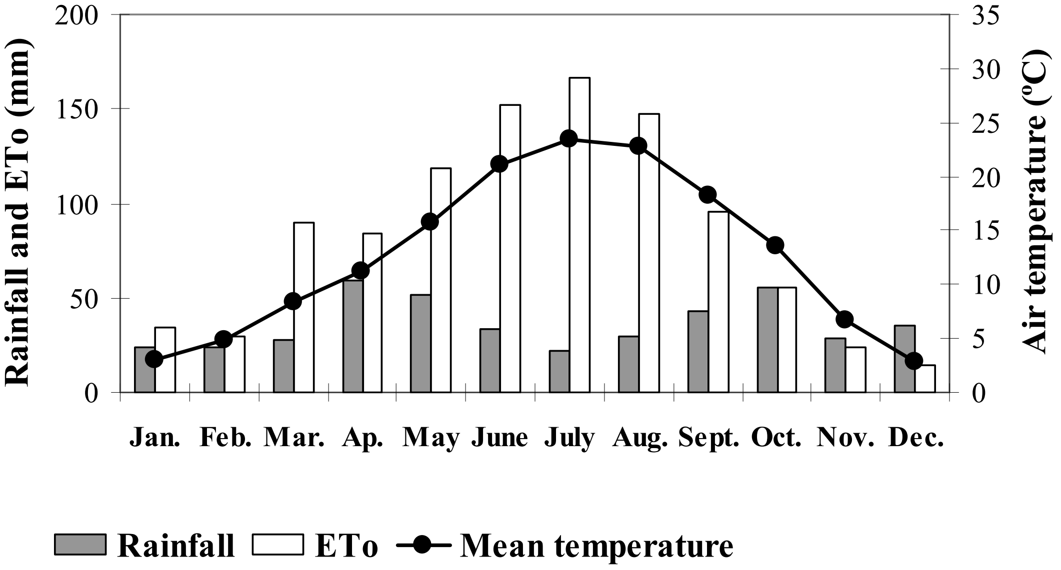

Figure 1. Monthly rainfall distribution, reference evapotranspiration (ET_0) and mean air temperature for the 2000-2010 period

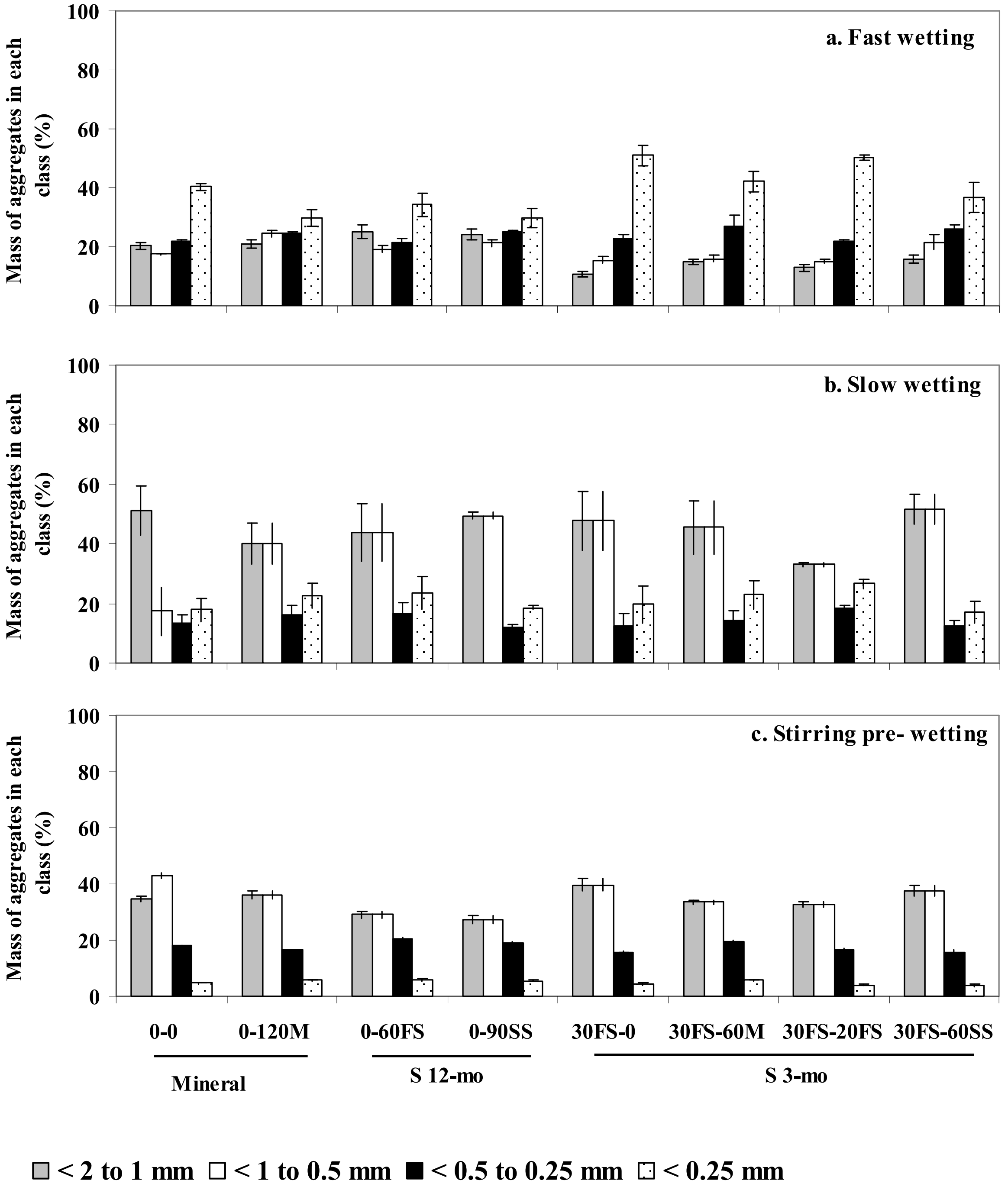

Figure 2. Size distribution of aggregates after fractionation in four classes (2 to 1 mm; <1 to 0.5 mm; < 0.5 to 0.25 mm; < 0.25 mm) for three methods: a) fast wetting, b) slow wetting, and and c) stirring after pre-wetting (mechanical breakdown) and for different fertilization strategies: the ones which only include mineral fertilization (Mineral), the ones with slurry applied twelve months before sampling (S 12-mo) or the ones with slurry applied three months before sampling (S 3-mo). Strategy acronyms are described in Table 1. Bars represent the standard error of four replicates.

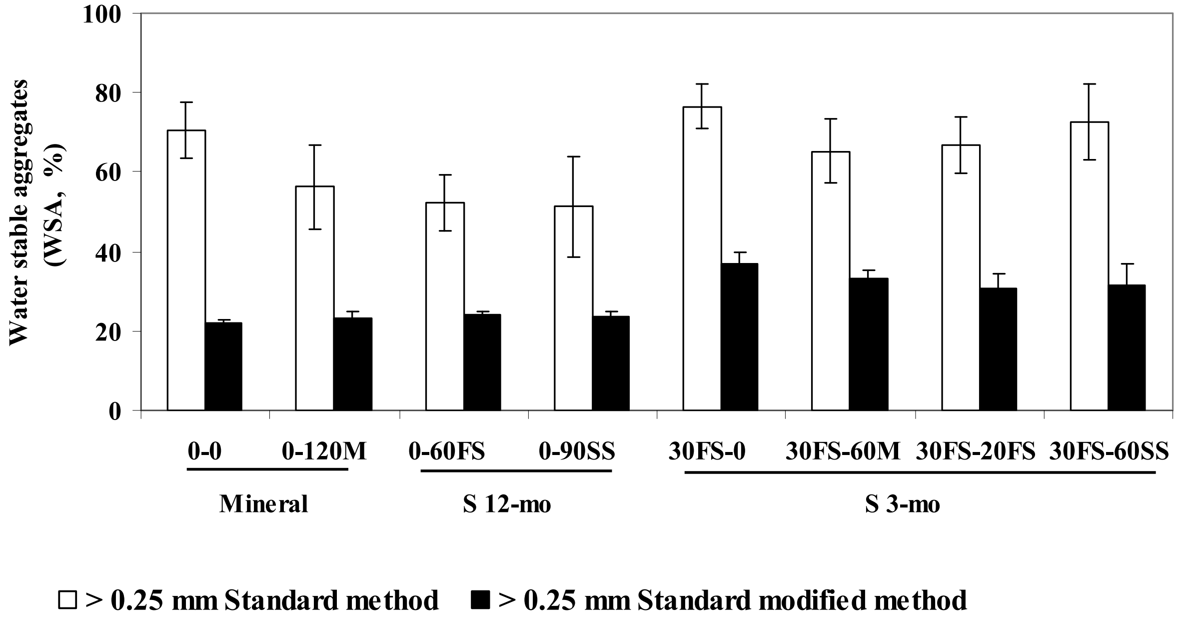

Figure 3. Water stable aggregates (>0.25 mm) measured by the standard method (white bars) described by Kemper and Rosenau (1986) and by the modified method (black bars) without vapour pre-wetting for different fertilization strategies: the ones which only include mineral fertilization (Mineral), the ones with slurry applied twelve months before sampling (S 12-mo) or the ones with slurry applied three months before sampling (S 3-mo). Strategy acronyms are described in Table 1. Bars indicate standard error of the four replicates.

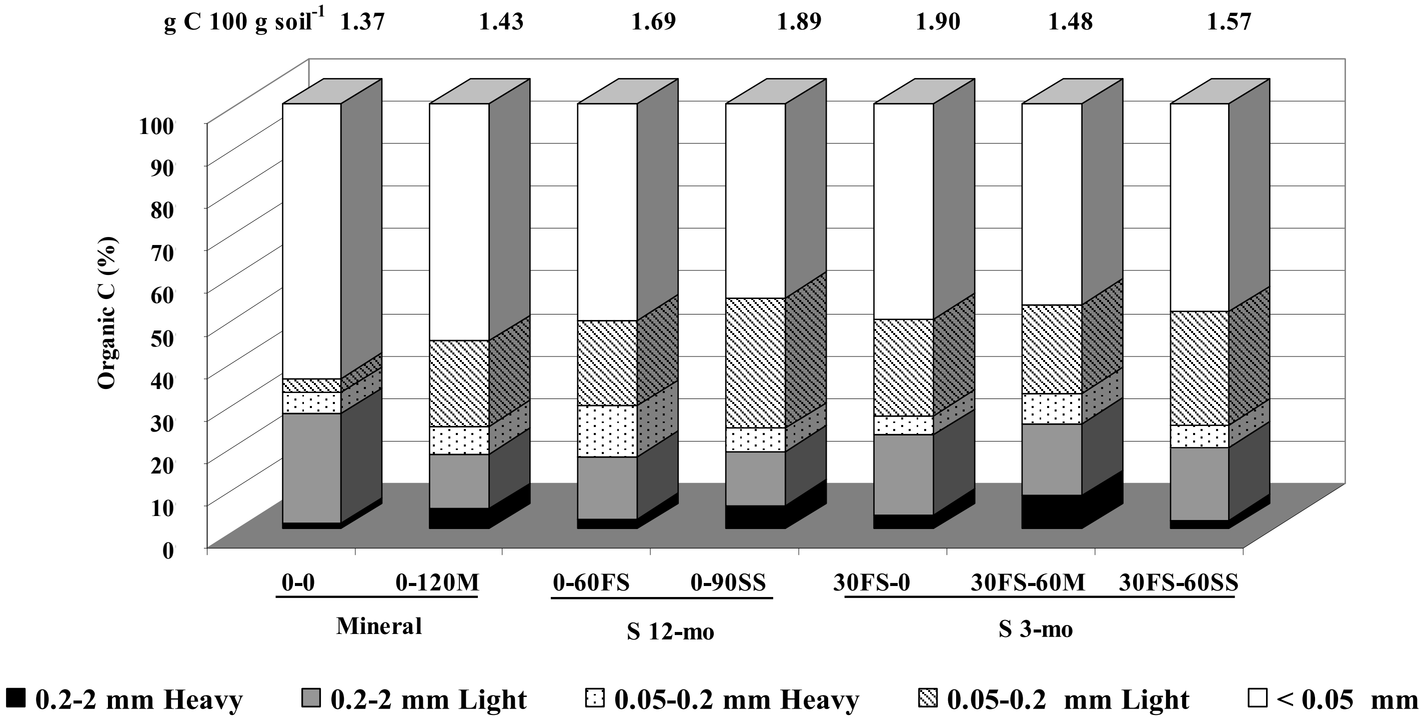

Figure 4. Distribution of organic carbon fractions as a percentage of total carbon content for different fertilization strategies: the ones which only include mineral fertilization (Mineral), the ones with slurry applied twelve months before sampling (S 12-mo) or the ones with slurry applied three months before sampling (S 3-mo). Strategy acronyms are described in Table 1.

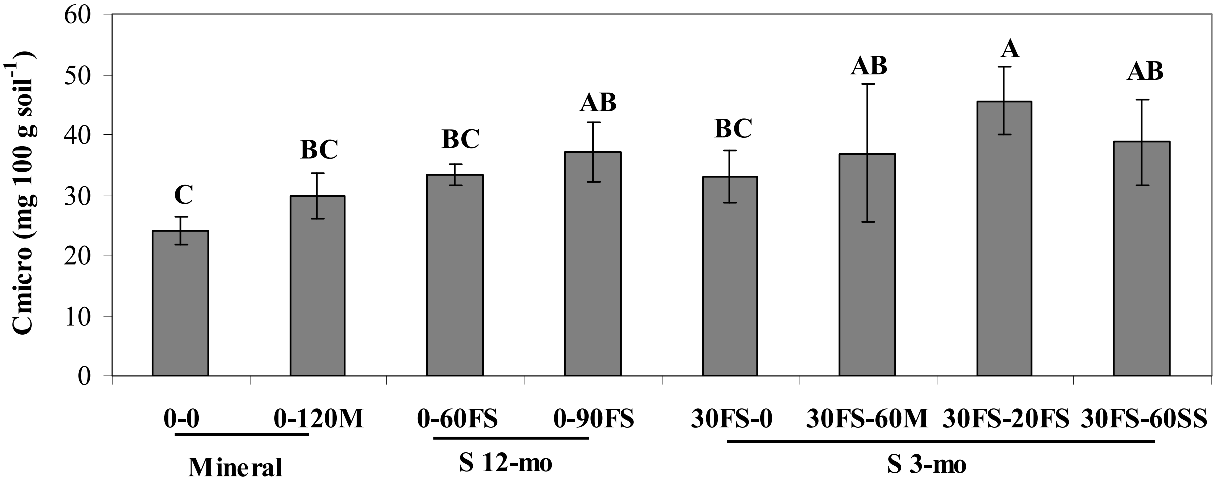

Figure 5. Distribution of microbial biomass carbon content for each fertilization strategy: the ones which only include mineral fertilization (Mineral), the ones with slurry applied twelve months before sampling (S 12-mo) or the ones with slurry applied three months before sampling (S 3-mo). Strategy acronyms are described in Table 1. Within columns, means followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p<0.05$). Bars represent the standard error of three replicates.

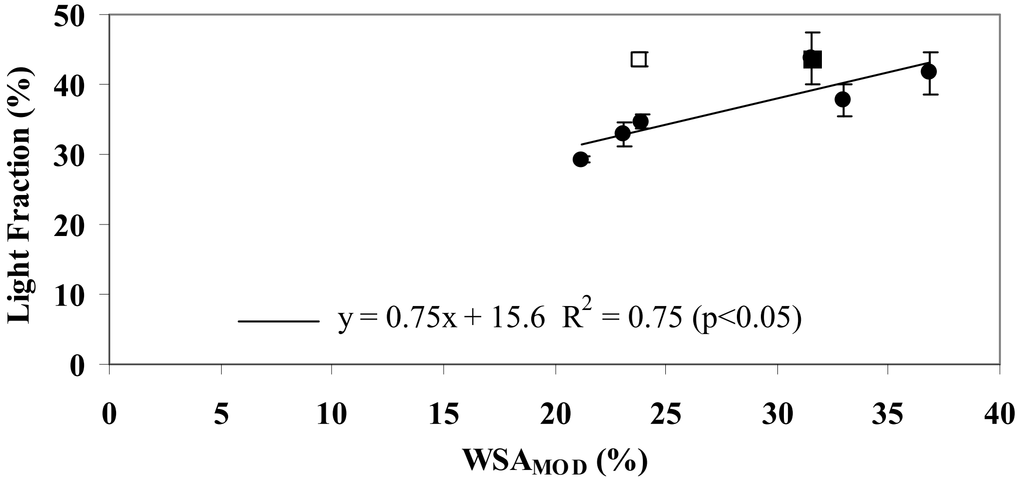

Figure 6. Relationship between light soil organic matter fraction (0.05-2mm) and water aggregate stability (WSA_{MOD}) by fast wetting (0-90SS strategy, square light, is not included). Square dark is 30 FS-60SS strategy. Bars represent the standard error of three replicates.