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Dairy cattle slurry fertilization management in an intensive Mediterranean agricultural system to sustain soil quality while enhancing rapeseed nutritional value

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ABSTRACT

Animal excreta are commonly recycled as fertilizers, although attention should be given to environmental impacts. Legislation must also be adapted to new research findings. The framework of this study is an intensive fodder Mediterranean agricultural system affected by EU legislation on the protection of waters against nitrate pollution. This paper studies the effect of two N based dairy cattle slurry (DCS) rates (170 *vs.* 250 kg N ha^{-1} yr^{-1}) plus additional mineral N (up to 450kg N ha^{-1} divided between two crops), on different soil quality parameters. A control (no N applied) was included. The experiment, which lasted for 8 years, included forage maize followed by ryegrass, grain maize and rapeseed. In the whole period, the organic carbon inputs from the DCS treatments comprised C slurry inputs (14.8 or 21.9 Mg ha^{-1}) plus the C input difference in crop residues (8.3 Mg ha^{-1}) between DCS and the control treatment. In the 0-0.3 m soil depth, slurries significantly increased soil organic carbon (SOC) from by 2.3 or 2.7% yearly (*c.* 2.8 Mg C with 10 Mg C ha^{-1} input) mainly in its light fraction. The size of the microbial biomass increased by 5.1% yearly (*c.* 0.12 Mg C with 10 Mg C ha^{-1} input). A higher aggregate stability against slaking disruption was observed. Soil pH slightly decreased, P (Olsen) fertility increased (up to 10 mg P kg^{-1}) as did K availability (up to 140 mg K kg^{-1}) and Mn and Ni bioavailability. In rapeseed plants, seed Ca, S, Cu and Mn content increased as did K, S, Fe, Mn and Zn in the rest of the plant biomass. These changes were within acceptable concentration ranges. The higher N rate from DCS has proved useful for the circular nutrient economy, while improving soil physical and chemical quality and the sustainability of the agricultural system as a whole.

Keywords: aggregate stability; exchangeable cations; heavy metals; organic fertilizer; organic matter fractions; soil microbial biomass.

1. Introduction

The intensification of agricultural management practices in crops, including fodder crops for animal feed, linked to the need for improving the carrying capacity of the different productive areas (Searchinger et al., 2018, Wang et al., 2020), is a positive trend.

Intensification by double-annual cropping (or just by the reduction of the length of fallow periods between crops) can help to meet growing global demand while limiting. the environmental impacts associated with cropland expansion such as increased soil erosion and loss of wildlife habitat or carbon. However, some restrictions (i.e. production costs, limits to N management) explain why double-annual cropping only occupies 2-3% of total cropland in the USA (Borchers et al., 2014) or 9% in Europe (Estel et al., 2016). In Europe, maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) maintain their importance as they are more frequently grown as summer crops and also combined with different winter crops (i.e. winter rapeseed (*Brassica napus* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) or forage-ryegrass (*Lolium multiflorum*). In double forage cropping systems, linked to livestock activities, fertilization is provided by manures, slurries or other organic fertilizers (waste animal based) whether or not combined with mineral fertilizers (Huang et al., 2015; Ovejero et al., 2016; Demurtas et al., 2016; Perramon et al., 2016).

Fertilization is an important cost in intensive agricultural systems and organic fertilizers may be a cheaper source of nutrients. The positive impact of waste recycling on productivity is fully endorsed by economic panel data models (Aldieri et al., 2019). Organic fertilizers are relevant in the context of the European circular economy action plan (reduction of waste to a minimum) where the use of recycled nutrients is a priority in order to reduce the Union’s dependency on nutrients imported from third countries

(E.C., 2019). However, in Europe legislation also exists for the so-called "nitrate vulnerable areas" in which the maximum amount of organic fertilizer to be applied is based on N criteria or N plant demand (E.C., 1991) with the permitted maximum equivalent to 170 kg N ha^{-1}. The Directive aims to protect water quality across the European Union (EU) by preventing nitrates from agricultural sources from polluting ground and surface waters. The limit seems quite reasonable if only one crop is cultivated annually, but there is a considerable increase in how the needs for N change if a double-annual cropping system is introduced; consequently, this limit might prevent appropriate nutrient supply to plants as nutrient demand increases. The EU has allowed derogations of the Directive and in some regions it allows the application of higher N amounts from an organic origin, up to a maximum of 250 kg N ha^{-1} year^{-1} (i.e. E.C., 2011). However, there is controversy about other impacts than N leaching such as over soil quality and potential nutrient imbalances. Manures and organic wastes contain potentially toxic elements (Bloem et al., 2017) that might present soil fauna concerns, for instance, for the presence of some earthworm species and their role in soils (Valdez-Ibañez et al., 2019a; Valdez et al., 2020); besides, liquid manures may stimulate the mineralization of native soil C while raising the P soil content to very high levels (Angers et al., 2010).

In these double-annual cropping Mediterranean systems, where summer crops play a crucial role in N demand, studies have focused on agronomic and N cycle aspects (Ovejero et al., 2016; Perramon et al., 2016 and 2018) and to a lesser extent on soil quality effects such as on water-stable soil aggregates (Yagüe and Quílez, 2013) or on soil organic C concentrations (Chataway et al., 2011) which may help to guarantee sustainability in the medium-long term.

The use of higher rates of organic fertilizers in intensive systems might maintain or increase soil organic C (SOC) directly (OC slurry input) or through residue retention (Zhao et al., 2020) due to potentially higher yields. This fact is of special interest in areas with low SOC or from the C sequestration point of view. It also might reduce the negative impact of compaction (Mujdeci et al., 2017) related to the increase in traffic of machinery over the soil surface, as organic matter is one of principal aggregating agents in soils (Tisdall and Oades, 1982).

Organic fertilizers provide essential nutrients (macro and micro) other than N but the ratios of these nutrients differs from those needed by plants. Thus, as N rate increases it might lead to an accumulation of other nutrients in soil, e.g. P (Eghball and Power, 1999). The introduction of different crops in a rotation, with different rooting systems and P nutrient demand is of interest. Rapeseed has been recommended as a crop when organic wastes are applied because of its high N fertility demand (CRDC, 2009) and a high rhizosheath acid phosphatase activity in the rhizosphere which leads to a high P uptake efficiency (Nobile et al., 2019). Furthermore, used as an animal feed, it often produces measurable advantages in dairy rations (Moore and Kalscheur, 2017) in terms of feed efficiency and milk production compared with soybean (*Glycine max* L.), while saving on feed cost.

Organic fertilizers also contain trace elements from animal feed via impurities or supplements in trace elements which are often formulated in diets in safety amounts which can largely exceed requirements. The undigested minerals are excreted and can increase their concentrations in soil superficial layers. Soil acts as a trace element reservoir, which poses potential threats to the environment through their mobile forms or bioavailability in some plants (Veiga et al., 2012). In fact, Blanco-Penedo et al. (2009) in a study conducted in the NW of Spain found that Mn concentration in the soil explained up two-thirds of the variability in liver Mn concentrations in calves. The prolonged application of trace elements to the soil surface can lead to a reduction of soil buffering capacity and can cause permanent contamination of soil or groundwaters (He et al., 2005). Even though the application of organic matter (OM) is an effective strategy for heavy metal immobilization through complexation, thus reducing plant uptake (Putwattana et al., 2015), the risk of soil contamination by trace elements such as Cd, Cu or As must not be underestimated (Zhang et al., 2012).

Our hypothesis is: as Mediterranean systems become intensified by double-annual cropping, the N rates from fertilizers of organic origin allowed by EU legislation might be increased to 250 kg N ha^{-1} yr^{-1}. The measure proposed will cover crop demand to a greater extent, while avoiding negative soil impacts or changes in plant composition. Data will be provided by means of an experimental field where there were five years of double cropping plus three years during which a total of four crops were grown. The aim of this study was to assess the influence of dairy cattle slurry (DCS) applied during years at two N rates: 170 or 250 kg N ha^{-1}, on soil quality parameters and on the nutrient and heavy metal composition of rapeseed. Results could provide arguments to legislators to ensure that decisions on N thresholds are well informed. The soil quality parameters include: i) soil macro-aggregate stability against slaking, ii) soil organic carbon fractions including microbial biomass C, iii) other soil and plant chemical parameters: nutrients (N, P, K, Ca, Mg, Cu, Fe, Mn, Mo, Ni and Zn), Na and trace elements (As, Cd, Co, Cr, Pb and V).

2. Materials and Methods

A fertilizer experiment was established at the Mas Badia experimental station in Tallada d'Emporda (42º 03' 02''N, 03º 03' 37''E, 18 m a.s.l) in 2008 and was maintained until June 2016 when soil sampling was performed.

The area has a dry Mediterranean climate (Papadakis classification; MAPA, 1989). The historic temperature, precipitation and crop reference evapotranspiration ($ET_{0,}$ FAO Penman-Monteith equation) data for a period of 27 years (1989-2016) were obtained from an automatic meteorological station located in the experimental station (Fig. 1). In 2016, annual average temperature, precipitation and ET_0 were 15.3ºC, 519 mm and 996 mm, respectively. The dry period lasts from June to August. Soil at the study site was classified as Oxyaquic Xerofluvent (Soil Survey Staff, 2014). The soil is very deep (>1.20 m) and well drained, without stones. The surface layer (0-0.30m) has a loamy texture (Table S1) and a basic pH (1:2.5; w:v, 8.2); it is non-saline (1:5; w:v; 0.18 dS m^{-1}) and calcareous (13.7%, calcium carbonate equivalent).

[Figure 1]

A double-annual crop forage rotation: maize − ryegrass was established in 2008 and maintained until 2013 (Fig. S1). Forage maize was harvested when the interior of the kernel was of doughlike consistency. Ryegrass was harvested (two cuts) at maximum biomass before coming into ear. In the 2013-2016 period, after the maize harvest for forage in September 2013, the rotation was modified and it included: winter rapeseed – grain maize (short cycle) - grain maize (long cycle) – winter rapeseed, which means four crops in three years (Fig. S1). Maize was irrigated (sprinkler system) during the spring-summer period and the rest of the crops were not irrigated. Stubble of rapeseed and maize (grain) was incorporated by disc-harrowing into the soil but the rest (stalks) was removed. Main tillage before sowing was done with a mouldboard or a disc harrow (~0.30 m deep). The last changes in the crop rotation were related to local weather conditions (right climate conditions) and market tendencies (prices).

Fertilization was based on dairy cattle slurry (DCS) which was analyzed prior to application (Table 1).

[Table 1]

The fertilizer treatments (Table 2) were two doses of DCS complemented with mineral N fertilization (calcium ammonium nitrate, 27%) and a control (no NPK fertilization). The 170DCS rate equalled 170 kg N ha^{-1} yr^{-1} (~52 Mg DCS ha^{-1}) corresponding to the maximum legal dose according to nitrates Directive 91/676 (E.C., 1991) in vulnerable areas. The 250DCS rate equalled 250 kg N ha^{-1} yr^{-1} (~77 Mg DCS ha^{-1}) which was related to the N crop extraction potential. They were distributed between two successive crops, prior to spring and autumn sowings (170DCS: 100 and 70 kg N ha^{-1}; 250DCS: 150 and 100 kg N ha^{-1}, respectively). As the DCS rates provided between 38% and 55% of theoretical N plant needs, fertilization was supplemented with mineral fertilizer (calcium ammonium nitrate, 27% N) up to 450 kg N ha^{-1} for two crops in a rotation: 280 kg N ha^{-1} for 170DCS and 200 kg N ha^{-1} for 250DCS. The exception was the 2014-2015 cropping season where a summer crop (maize) was followed by a summer crop (maize) but the fertilization schedule for summer crops was maintained. Treatments were distributed according to a randomized block design with three replicates. An area of 40 m^2 (5 m x 8 m) was assigned to each replicate. A total of nine plots were set up.

[Table 2]

2.1. Soil and plant analysis

Eight months after the last slurry application (7th June 2016), each of the nine plots was sampled for soil and plant analysis. Soils were sampled from 0-0.1 m depth (a composite sample of 5 points plot^{-1} was obtained) and air dried. A soil sample fraction was sieved to 3.15 mm for aggregate stability evaluation and another one to 2 mm for chemical analysis.

In soil, aggregate stability was measured from aggregates remaining after a fast wetting of dry aggregates (stability against slaking) in four subsamples of each treatment.

Results were expressed as mean weight diameter index (MWD, mm) proposed by Le Bissonnais (1990) and adapted by Amézqueta et al. (1996):

$$\mathrm{MWD} = \sum_{i=1}^{n} \mathrm{W_i D_i} \tag{1}$$

where n corresponds to the number of aggregate size fractions considered in the analysis and D_i is the mean diameter of aggregates that potentially can stay in the *i* th and *i*+1 sieves. The five size classes were: < 3.15 mm to 2 mm < 2 mm to 1 mm; < 1 mm to 0.5 mm; < 0.5 mm to 0.25 mm; < 0.25 mm which were respectively associated to: D_1=2.575 mm, D_2=1.5 mm; D_2=0.75 mm; D_3=0.375 mm; D_4=0.125 mm. No detected > 3.15 mm. The W_i is the mass percentage of each fraction calculated as dry weight of aggregates in the ith size fraction (g) divided by the sum of total sieved soil dry weight fractions (g) including the smallest (< 0.25 mm) one.

Soil organic carbon (SOC) was divided into three physical sizes and into two density fractions following the procedure NF X 31-516 from AFNOR (2007). This means that five fractions were distinguished: ≤ 0.05 mm, > 0.05–0.2 mm (light), > 0.05–0.2 mm (heavy), > 0.2–2 mm (light), and > 0.2–2 mm (heavy). Organic carbon was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulfate (Yeomans and Bremner, 1988). The total SOC content was obtained as the sum of the different fractions. Carbon content of soil microbial biomass (SMB) was quantified by the fumigation-extraction method (AENOR, 2003) adopting the value of 0.38 as the the efficiency factor for SMB carbon extraction (Vance et al., 1987). All SMB analyses were done in triplicate for each composite sample.

Analytical procedures from MAPA (1994) were used for other chemical parameters: pH (potentiometry; 1:2.5 soil:distilled water), electrical conductivity at 25ºC (1:5 soil:distilled water), total N (Kjeldahl method), available P (Olsen method) and available K (ammonium acetate 1N, pH=7). Cation exchange capacity (CEC) and exchangeable cations were evaluated by extraction with ammonium acetate 1N (pH=7). The analysis of exchangeable cations (K^+, Na^+, Mg^{2+} and Ca^{2+}) followed Hendershot et al. (2008). The micronutrients Fe, Zn, Mn, Cu and Ni plus Cd were extracted with a DTPA (diethylenetriaminepentaacetic acid) solution (1:2, w:v) following Baker and Amacher (1982). From microwave digested samples (UNE-EN 16174:2012; AENOR 2012a) with aqua regia (3:1, v:v, HCl:HNO_3) the elements: P, K, Ca, Mg, Na, Fe, As, Cd, Co, Cr, Cu, Mo, Mn, Ni, Pb, V and Zn were quantified using inductively coupled plasma mass spectrometry (UNE-EN 16171; AENOR, 2016).

Rapeseed shoots were sampled from a surface of 0.5 m^2 (1 m x 0.5 m) $plot^{-1}$ and they were divided for further analysis in two parts: seeds and the rest of shoot biomass (RS) which included pods plus branches. Seed harvest was performed the 20th of June 2016 in 12 m^2 (8 m x 1.5 m) $plot^{-1}$. Samples from the two rapeseed shoot fractions were prepared for analysis following UNE-EN ISO 6498:2012 (AENOR, 2012b). They were digested in a microwave with nitric acid and hydrogen peroxide (3:2:2, v:v:v, HNO_3: H_2O_2: H_2O) following UNE-EN 13805:2015 (AENOR, 2015). Major nutrients (P, K, S, Mg), micronutrients (Mn, Fe, Co, Cu, Zn, Mo) and heavy metals (Co, Cd) were quantified using inductively coupled plasma mass spectrometry (UNE-EN 15763:2010; AENOR, 2010). Total N was just analyzed in seeds following a Kjeldahl method (Chang, 2003). The seed oil content at harvest was analyzed using low-resolution pulsed nuclear magnetic resonance (NMR) spectrometry (ISO 8292-2:2008, ISO, 2008) with a Bruker's Minispec equipment.

2.2. Carbon balance calculations

Topsoil C inputs (0-0.3 m) included C from slurries (Table 2) and C from stubble and plant roots. The effects of such C inputs on SOC were considered jointly following Rasmussen et al. (1980) and Bhogal et al. (2009).

The C input from plants was assumed to be proportional to yield biomass (quantified as dry matter). Average yield biomasses in DCS treatments was similar, without statistical differences. In the control plots, yield biomass averaged 40% of those in DCS treatments. As the DCS effect on SOC was compared to the control, in slurry treatments only 60% of the total crop C return to soil (stubble plus roots, Table S2) was attributed to the effect of slurry addition, which means 8.3 Mg C ha^{-1} for the full period and for every DCS treatment.

Stubble biomass was calculated from the harvest index ratio (Unkovich et al., 2010) thus, the ratio between grain/seed yield biomass to shoot biomass. Shoot corresponds to total plant above-ground dry matter (stalks and leaves/pods and branches plus grains/seeds). It was assumed that stubble was equivalent to 5% and 10% of plant biomass (excluding grain/seed yields) for grain maize and rapeseed respectively (Table S2). Carbon from stubble was adopted from Redin (2018) and Williams et al. (2013). Stubble was assumed to be zero for forage crops.

Root biomass and its C input were estimated (Table S2) from root:plant above-ground ratios (Anderson, 1988; Gan et al., 2009; Crush et al., 2010), C in roots (Redin, 2018) and root fraction from 0 to 0.30 m depth (Gan et al., 2009).

The relative mean annual SOC storage rates (%) were calculated as the difference between the SOC from the DCS treatments and the control (ΔSOC-content), and they were divided by the SOC content in the control and the time elapsed since the implementation of the experiment (8 years). A similar calculation was performed for SMB changes. The DCS retention coefficient (Mg C with 10 Mg C ha^{-1}) has been calculated taking into account the SOC changes in soil (ΔSOC-content) per unit of C added via the control plot used as reference (ΔC-input).

2.3. Statistical analysis

The statistical analyses were done using the SAS statistical package v9.4 (SAS Institute, 2002–2012). Data were subjected to appropriate analysis of variance (ANOVA) according to the experimental field design. When the ANOVA detected significant differences, separation of means was performed according to Duncan's multiple-range test (P=0.05).

3. Results

3.1. Aggregate stability

The DCS application significantly improved the resistance to soil physical degradation by slaking (P = 0.0006) compared with the control, despite the relatively low OC inputs applied from manures (14-22 Mg OC ha^{-1}) from the start of the experiment in 2008. The mean weight diameter after a fast wetting was 748, 1110 and 1152 µm for the control, 170DCS and 250DCS treatments, respectively. The addition of DCS favours (in relative terms) aggregation (Fig. 2) at the highest size diameters from 2000 to 3150 µm, while the ones <250 µm are reduced (without significant changes in the intermediate sizes).

[Figure 2]

3.2. Soil organic carbon fractions and microbial biomass

It is assumed that SOC in the control was equivalent to the initial concentration in soil, as in this SOC fraction analysis procedure the total sum of the five fractions tends to give lower figures than in a single (and total) sample analysis. The total SOC content ranged from 9.2 (control) to 11.2 g C kg^{-1} in 250 DCS (Fig. 3). The latter, for a 0.30 m depth, and assuming for a soil loamy texture a bulk density of 1350 kg m^{-3} (Trueba et al., 1999), equals an equivalent of 37.3 Mg SOC ha^{-1}, 44.1 Mg SOC ha^{-1} and 45.4 Mg SOC ha^{-1} for the control, 170DCS and 250DCS, respectively. The SOC build-up showed a significant increase in the SOC fraction lower than 0.05 mm but, in relative terms, increases were important in light SOC fractions (Fig. 3).

[Figure 3]

The soil microbial biomass was significantly higher (40% up) in DCS treatments than in the control (Fig. 4) without differences between DCS rates.

[Figure 4]

3.3. Soil nutrients and heavy metal contents

The addition of DCS slightly decreased soil pH and maintained negligible salinity although the slight increase in the EC (Table 3). Total N, available P and exchangeable K also increased with DCS addition. The total N increase did not result in quantitative changes in the C:N soil ratio (Table 3, Fig. 3). No significant differences in exchangeable cations (rather than K) or cation exchange capacity were found when comparing DCS treatments with the control (Table 3). Exchangeable potassium equalled 72, 109 and 140 mg K kg^{-1} for the control, 170DCS and 250DCS, respectively.

[Table 3]

Furthermore, the soil heavy metal content was not affected and it can be considered for As, Cd, Co, Cr, Pb, V, Cd, Cu, Fe and Zn, similar to the soil background. The exceptions were Mn and Ni extracted with DTPA which increased with DCS rates but without significant changes in total Mn or Ni soil content (Table 4).

[Table 4]

3.4. Plant nutrients and heavy metals contents

The classical negative relationship between N and oil seed content was observed (Table 5, Eq. 1)

$$\text{Rapeseed oil (\%)} = 62.01 - 0.5469\ \text{N (g kg}^{-1}\text{)};\ R^2 = 0.99. \qquad (1)$$

In rapeseed, DCS application decreased the P and Ca seed content but increased N and S seed concentration. It also increased K and S concentration in the rest of the plant biomass (Table 5). Related to micronutrients, as the DCS rate increased, Cu seed concentration decreased and Mn increased, while Fe and Zn increased in the rest of the biomass (Table 5). No differences were found in the rest of the analysed components, including Cd and Co heavy metals.

[Table 5]

4. Discussion

The mean DCS retention coefficient (from 2008 to 2013) was equivalent to *c*. 2.8 Mg C with 10 Mg C ha^{-1} input. These values, with less than one third of the OC applied remaining in the soil yearly, are consistent with the results of other authors such as Bhogal et al. (2009). The relative mean annual SOC storage increased annually by an average of 2.5% whereas the light fraction increased by about 4.2% and the microbial biomass C by 5.1%. The light fraction of SOC is a labile source of soil C (unprotected C) and it is strongly influenced by factors related to the recent history of OM addition (Gosling et al., 2013). Thus, it is an early and sensitive indicator of changes in OM status associated with fertilization practice (Leifeld and Kögel-Knabner, 2005; Bhogal et al., 2009; Yagüe et al., 2012, 2016) and an important nutrient source. There are indicators that it can become saturated (Six et al., 2002) but little information is available on the mechanics of the asymptotic relationship with C inputs (Beare et al., 2014). Besides, these labile organic C components such as polysaccharides are recognised to act as transient binding agents, which can explain the higher MWD as the

DCS dose increases. This positive effect has also been detected when dairy cattle manure (Yagüe et al., 2016) or pig slurry (Domingo-Olivé et al., 2016) have been annually applied at sowing for a period of 11 or 12 years, respectively. Labile organic matter is abundant in macroaggregates but not in microaggregates (Cambardella and Elliott, 1993; Six et al., 1998). Thus, this mass aggregate distribution is followed by a tendency to increase macroporosity (Valdez-Ibañez et al., 2019b). These results also agree with Hernandez et al. (2007) who suggested that slurry addition induces a reactivation of soil microbial growth.

Rapeseed responded to the lowest DCS rate applied (Table 2) as according to Soper (1971) it responds well up to 200 kg N ha^{-1}. Besides, oil decreased linearly with N seed content (Eq. 1) as other authors have found (Hao et al., 2004) but not with the total N applied, which indicates that the higher amount of mineral N applied as a complement in 170DCS treatment was highly available, more than N from excreta. However values were close to the 41.5% oil average from Australian fields (CRDC, 2009). In plant seeds, the N content (protein content) increased with DCS followed by an opposite and significant P decline (Table 5), despite the soil content build-up in both nutrients (Table 3). These results draw an opposite trend to Cadot et al. (2018) who reported, in a neutral soil pH, a decrease of protein concentration and an increase in P with repeated P applications. Our disagreement with Cadot et al. (2018) findings can be explained because our soil P (Olsen) levels (Table 3) are below or close to 10 mg P kg^{-1} which means that rapeseed might still respond to P applications (Soper, 1971). In addition, our yields with DCS are 50% higher than Cadot et al. (2018) which means, in our case, a higher P demand that might not have been fully satisfied from our calcareous soil (Table S1). Slurries favoured P availability (Table 3) but levels were still low for the loam texture soil (Rodríguez-Martín et al., 2009). Additional constraining factors such as the low precipitation during the rapeseed growing period (Fig. 1) could slow P soil transport to the roots by the diffusion process which, in turn, could minimize P absorption efficiency.

As soil total N increases, there could be higher N availability by mineralization at the higher DCS rate. In fact, the mineral N in soil has been positively related to the N content of the unprotected SOC (Six et al., 2002). The subsequent higher risk of nitrate leaching may be controlled by a reduction of the mineral N fertilizer complement applied, as other authors established in a similar agricultural system (Perramon et al., 2018)

Exchangeable soil K^+ increased with DCS (Table 3) and followed a smooth tendency to increase CEC due to the addition of organic matter. However, differences between control and both DCS treatments were just in RS (Table 5). Our findings are in agreement with Grant and Bailey (1993) in the sense that a small amount of K is removed in the seed and with Soper (1971) who suggested a critical level of 100 mg kg^{-1} exchangeable K^+ be used to distinguish between sufficient and deficient soils for rapeseed production This threshold value closely matched K^+ exchangeable content of 109 mg K kg^{-1} soil in the 170DCS treatment (Table 3).

In this calcareous soil, well above deficiency levels (CCC, 2020) rapeseed accumulated high amounts of Ca in RS (Table 5) As a dicotyledonous plant, it has a high demand for Ca (>4.9 g kg^{-1}) which doubles P and S ones but, usually, only 10% of plant Ca ends up in the seeds (CCC, 2020). Differences in seed Ca content might be attributed to some translocation delays due to lack of water (Ho and White, 2005) as Ca is relatively slow moving within the canola plant (GRDC, 2015). Magnesium does not show differences probably because the soil is well provided with this element (Table 3).

Rapeseed is highly S demanding and DCS satisfies this demand (Table 5), allowing adequate S concentrations in plant tissues which must be higher than 2 g kg^{-1}, but below 10 g kg^{-1} for a satisfactory supply (Jones, 1986). The increase in S concentrations as N increases is of interest as it helps to complete protein synthesis (Finlayson et al., 1970) avoiding the increase in free amino acids.

The micronutrients Zn and Cu and their bioavailability did not differ from the control. Our total and Zn extractable with DTPA concentrations were in the low ranges described by Kabata-Pendias et al. (1989). The DTPA-extractable soil Cu (Table 4) was much higher than the minimum level of 0.35 mg Cu kg^{-1} established from the rapeseed growth curves in sandy-sandy loam soils (Karamanos et al., 1986). The Cu straw concentration (1.3-1.5 mg kg^{-1}) nearby doubled from Karamanos et al., (1986) values, that confirms the natural availability of Cu. Changes in seed Cu content (Table 5) can be attributed to a dilution effect. It cannot be attributed to an Mn:Cu antagonism which is diagnosed when the ratio in RS is higher than 15 (Karamanos et al., 1989); according to our values the maximum figure in RS is 12 (250DCS, Table 5). The concentrations of Mn (in seeds and in RS) and Zn (in RS) increased with DCS (Table 5). These positive responses are justified as concentrations were in the lowest value of ranges found by other authors (Karamanos et al., 1986) with values around 32-37 mg kg^{-1} for Mn and 12-16 mg kg^{-1} for Zn. However, Fe (Table 5) was in a similar range pointed out (38- 43 mg kg^{-1}) by Karamanos et al. (1986) and even higher for 250DCS (Table 7, 60 mg kg^{-1}). The higher Mn bioavailability could be related to the slightly decrease in soil pH. The higher Ni availability is of interest as the values are also in the lowest figures (Feigin et al., 1991) and because Ni is also required by animals. By contrast, the Cd and Pb soil concentrations were lower than the common soil values (Kabata-Pendias and Dudka,

1991) and so were those for Cd in plants (Kabata-Pendias and Dudka, 1991; E.C., 2002).

Furthermore, the concentrations of elements Cd, Co, Cu, Fe, Mn and Zn in RS (Table 5) were within the lowest levels of normal concentrations in plant foliage used in diets of domestic livestock (Chaney, 1989).

Our data on soil quality advocate the use of the highest DCS dose as the fodder crop production is intensified. The achievement of high yields linked to soil quality maintenance/improvement are in line with the European Commission's intentions (E.C., 2020a) as regards the implementation of a fair, healthy and environmentally-friendly food system. Attention should be given to the improvement of soil structure (through better aggregate stability) by DCS applications, as this soil property is linked to the reduced risk of greenhouse gas emissions (Ball et al., 2013), one of the main targets in the EU climate action (E.C., 2020b).

Our results help to solve some of the existing policy-practice gaps in farm slurry management (i.e. nutrient management) pointed out by different authors (Micha et al., 2020). These balanced results guarantee the productive sustainability of the system into the future. In a long term further research is needed in terms of the saturation value of the SOC light fraction and on the mechanisms that affect SOC stabilization.

5. Conclusions

The annual average increase of 2.5% in SOC was *c.* 70% based on the OC from slurries and *c.* 30% to the increase in root and stubble biomass. The benefit of moving the DCS dose from 170 kg N ha^{-1} to 250 kg N ha^{-1} was observed by a positive trend for SOC content, mainly for its light C fraction. Besides, SMB grew by 40% with slurry applications (end of the 8 CS).

Slurry boosted soil aggregate stability against slaking and it improved P, K, Mn and Ni soil bioavailability. In the three latter parameters, bioavailability also increased with the DCS dose.

In rapeseed, slurry augmented S and Mn seed content. The highest DCS rate increased Fe, S, Mn and Zn content in the rest of the plant biomass. Thus, the 250DCS rate might also contribute to the reduction of supplemental trace elements in animal diets.

As the N crop extraction potential grew up through the reduction of fallow periods between crops, the increase of the DCS dose up to an equivalent of 250 kg N ha^{-1} improved soil quality, rapeseed nutrient value and enhanced the circular economy of nutrients in dairy cattle rearing farms.

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Appendix A. Supplementary data

The following tables are the supplementary data to this article: Table S1 and S2 and Figure S1.

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Table 1

Chemical parameter averages (±standard deviation) of the applied dairy cattle slurries (n=15) from 2008 to 2013 and from 2013 to 2016 cropping seasons.

Parameter[a]	**2008 - 2013**	**2013 - 2016**
pH (1:5; potentiometry)	8.3±0.6	8.5±0.2
Electrical Conductivity (dS m^{-1}, 1:5 conductimetry)[a]	4.8±3.0	4.1±1.2
Dry matter (%, gravimetry 105ºC)	8.7±1.6	9.0±3.4
Organic matter (% DM, ignition 550ºC)[b]	77.0±5.2	72.6±2.9
Organic- N (% DM, Kjeldahl method)	2.1±0.2	2.0±0.3
Ammonium-N (% DM, titrimetric method)	1.9±0.4	1.6±0.7
Total N (% dm)	4.0±0.4	3.6±0.9
Phosphorus (P, % DM, ICP)[c]	1.2±2.0	0.6±0.2
Potassium (K, % DM, ICP)	4.1±1.0	5.0±0.2

[a] Soil: distilled water; [b] DM: dry matter basis; the organic and the ammonium N were determined according to methods 4500-NH_3B-C from APHA (2012); [c] ICP: inductively coupled plasma atomic emission spectrometry (U.S.EPA, 2014).

Table 2

Crop fertilization treatments according to N application rates with dairy cattle slurry (DCS) before sowing and with mineral N fertilizer[a] at topdressing (TopD) and total C applied for the whole period (2008 - 2016) from DCS.

	Double annual forage cropping: Maize - Ryegrass (May 2008 - September 2013)				**Rotation: Rapeseed-Maize short cycle - Maize long cycle - Rapeseed (September 2013 - June 2016)**				**Total C from DCS (2008 - 2016) Mg ha^{-1}**
N rate	**Maize**		**ryegrass**		**Maize**		**Rapeseed**		
	Sowing	**TopD**	**Sowing**	**TopD**	**Sowing**	**TopD**	**Sowing**	**TopD**	
Control	0	0	0	0	0	0	0	0	0
170DCS	100DCS	200	70DCS	80	100DCS	200	70DCS	80	14.8
250DCS	150DCS	150	100DCS	50	150DCS	150	100DCS	50	21.9

[a] Mineral N fertilizer was calcium ammonium nitrate (27% N).

Table 3

Average values[a] (n=3) of different chemical soil parameters in 2016 according to N application rates with dairy cattle slurry.

N rate[b]	**pH (1:2.5)**	**EC (1:5)**[c] **(dS m^{-1})**	**Total-N (g kg^{-1})**	**P (Olsen) (mg kg^{-1})**	**CEC**[d]	**K$^+$**	**Mg^{2+}**	**Na$^+$**	**Ca^{2+}**
					----------------- (cmolc$^+$ kg^{-1}) ---------------------				
Control	8.3 A	0.20 B	1.2 B	6.7 B	7.89	0.18 C	1.77	0.11	5.83
170DCS	8.1 B	0.23 AB	1.4 A	9.7 A	7.96	0.28 B	1.74	0.19	5.75
250DCS	8.0 B	0.26 A	1.5 A	10.1 A	8.00	0.36 A	1.80	0.16	5.68
P value	0.0051	0.039	0.01	0.004	NS	0.005	NS	NS	NS

[a] Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range test (P=0.05); NS: not significant (P>0.05). [b] Control, no N applied; DCS, daily cattle slurry applied at 170 kg N ha^{-1} (170DCS) or 250 kg N ha^{-1} (250 DCS). [c] EC: Electrical conductivity. [d] CEC: Cation exchange capacity.

Table 4

Average[a] values (n=3) of nutrients and other elements in soil extracted with aqua regia (3:1, v/v, HCl:HNO_3) or with diethylenetriaminepentaacetic acid (DTPA) solution (1:2, w:v) in 2016 and according to different N application rates with dairy cattle slurry (DCS).

Nutrients extracted with aqua regia										
N rate[b]	P	K	Ca	Mg	Cu	Fe	Mn	Mo	Ni	Zn
	(mg kg^{-1} soil)									
Control	554	4962	43480	5387	15.3	19326	315	0.40	14.2	54.4
170DCS	581	5019	43606	5352	15.8	19412	321	0.43	14.3	58.0
250DCS	602	5122	44567	5564	16.0	20064	334	0.41	14.5	49.9
P value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Elements extracted with aqua regia							
N rate	As	Cd	Co	Cr	Na	Pb	V
	(mg kg^{-1} soil)						
Control	14.4	0.28	5.9	18.4	237	22.6	30.2
170DCS	14.9	0.30	6.0	18.3	229	22.1	30.1
250DCS	15.6	0.28	6.1	18.7	232	22.2	30.8
P value	NS	NS	NS	NS	NS	NS	NS

Elements extracted with DTPA						
N rate	Cd	Cu	Fe	Mn	Ni	Zn
	(mg kg^{-1} soil)					
Control	0.075	2.57	10.19	10.84 C	0.23 C	1.06
170DCS	0.078	2.47	10.84	12.60 B	0.25 B	1.30
250DCS	0.082	2.56	10.34	13.81 A	0.28 A	1.77
P value	NS	NS	NS	0.0002	0.0015	NS

[a] Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range test (P=0.05); NS: not significant (P>0.05). [b] Control, no N applied; DCS, daily cattle slurry applied at 170 kg N ha^{-1} (170DCS) or 250 kg N ha^{-1} (250 DCS).

Table 5

Average values (n=3) of raw oil in seeds, macronutrients, micronutrients and heavy metal extracted by nitric acid and hydrogen peroxide (3:2:2, v:v:v, HNO_3: H_2O_2: H_2O) in grain and in the rest of rapeseed shoot (RS)[a] in 2016 harvest.

	Oil	**N**	**P**		**K**		**Ca**		**Mg**		**S**	
N rate[b]	**Seed**	**Seed**	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**
	(%)	----------	----------	----------	----------	----------	(g kg^{-1})	----------	----------	----------	----------	----------
Control	45.8A	29.8B	6.57A	0.53	6.57	7.06B	5.23A	11.73	2.97	1.73	3.39B	2.15B
170DCS	40.1B	40.3A	5.21B	0.82	6.69	11.52A	4.46C	14.85	2.95	2.08	4.69A	3.23AB
250DCS	41.8B	36.8A	5.06B	0.86	6.48	12.17A	4.73B	15.45	2.91	2.04	4.41A	3.87A
P value	0.01	0.007	0.007	NS	NS	0.05	0.0009	NS	NS	NS	0.004	0.05

	Cd		**Co**		**Cu**		**Fe**		**Mn**		**Zn**	
N rate[b]	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**	**Seed**	**RS**
	----------	----------	----------	----------	----------	----------	----------	(μg kg^{-1})	----------	----------	----------	----------
Control	53	278	45	42	4575A	2499	77629	35659B	26375C	10862B	39836	6837C
170DCS	48	311	34	57	3986B	2419	103048	46252AB	29193B	23762B	40974	8750B
250DCS	43	366	28	62	4009B	2736	70736	60336A	30613A	32010A	41138	12314A
P value	NS	NS	NS	NS	0.0014	NS	NS	0.02	0.0014	0.0077	NS	0.0028

[a] Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range test (P=0.05); NS: not significant (P>0.05). [b] Control, no N applied; DCS, daily cattle slurry applied at 170 kg N ha^{-1} (170DCS) or 250 kg N ha^{-1} (250 DCS).

Appendix A

Table S1
Physical and chemical average characteristics of the soil (±standard deviation)[a].

	Depth (m)			
Parameter	**0-0.30**	**0.30-0.60**	**0.60-0.90**	**0.90-1.20**
Particle size distribution (g kg^{-1})[b]				
Sand (2000 <Ø< 50 μm)	485	492	423	630
Silt (50 <Ø< 2 μm)	404	408	473	305
Clay (Ø < 2 μm)	110	99	104	65
pH (water; 1:2.5; potentiometry)[c]	8.2±0.1	8.2±0.1	8.3±0.1	8.4±0.1
Electrical conductivity (1:5; dS m^{-1}, 25ºC)[c]	0.18±0.01	0.19±0.01	0.19±0.01	0.18±0.02
Organic matter (g kg^{-1}; Walkey and Black)	18±1.1	9±1.5	6±0.5	3±0.5
C/N	9.1±0.4	8.3±0.7	7.9±0.7	8.4±3.6
Calcium carbonate equivalent (%)	13.7±1.7	14.5±1.3	17.3±1.4	16.2±2.7

[a] Soil samples (n=15) were obtained at the start of the fertilization experiment. [b] Ø: particle apparent diameter. [c] Soil: distilled water.

Table S2

Average of grain/seed yield for DCS treatments and for the rest of shoot (RS) which includes the averaged fraction (stubble) left on soil at each harvest, and from the 2008 – 2016 period. The associated C root and C stubble inputs in soil (0-0.3 m depth) are included. Adopted harvest index (HI) as yield to shoot biomass, root: shoot biomass ratio and the root biomass fraction are also included.

Crop	Biomass			Harvests	HI	Root:shoot	Root fraction	Root (0-0.3m)	C roots	C stubble	C root input	C stubble input	Total C input
	RS	Stubble	Grain										
	-- (Mg DM ha^{-1} yr^{-1}) --			(Number)			(0-0.3m)	(Mg DM ha^{-1} yr^{-1})	----- (g kg^{-1}) ---		-- (Mg ha^{-1} yr^{-1}) --		(Mg ha^{-1})
Ryegrass (2 cuts cycle^{-1})	10.0	-	-	5	-	0.19[d]	0.70[g]	1.34	458[d]	-	0.62	-	3.1
Maize forage	21.0	-	-	6	-	0.21[c]	0.47[c]	2.10	481[d]	-	1.01	-	6.1
Maize grain (short cycle)	7.9	0.40	8.6	1	0.52[b]	0.18[c]	0.47[c]	1.42	481[d]	464[c]	0.68	0.19	0.9
Maize grain (long cycle)	11.9	0.60	12.9	1	0.52[b]	0.18[c]	0.47[c]	2.13	481[d]	464[d]	1.02	0.28	1.3
Rapeseed	6.9[b]	0.69	2.7	2	0.28[b]	0.29[f]	0.73[f]	2.04	452[d]	479[e]	0.92	0.33	2.5

[a] Stubble was equivalent to 5% and 10% of RS biomass for maize and rapeseed respectively. [b] Unkovich et al. (2010); [c] Anderson (1988); [d] Redin (2018); [e] Williams et al. (2013); [f] Gan et al. (2009); [g] Crush et al. (2010).

Legend figures

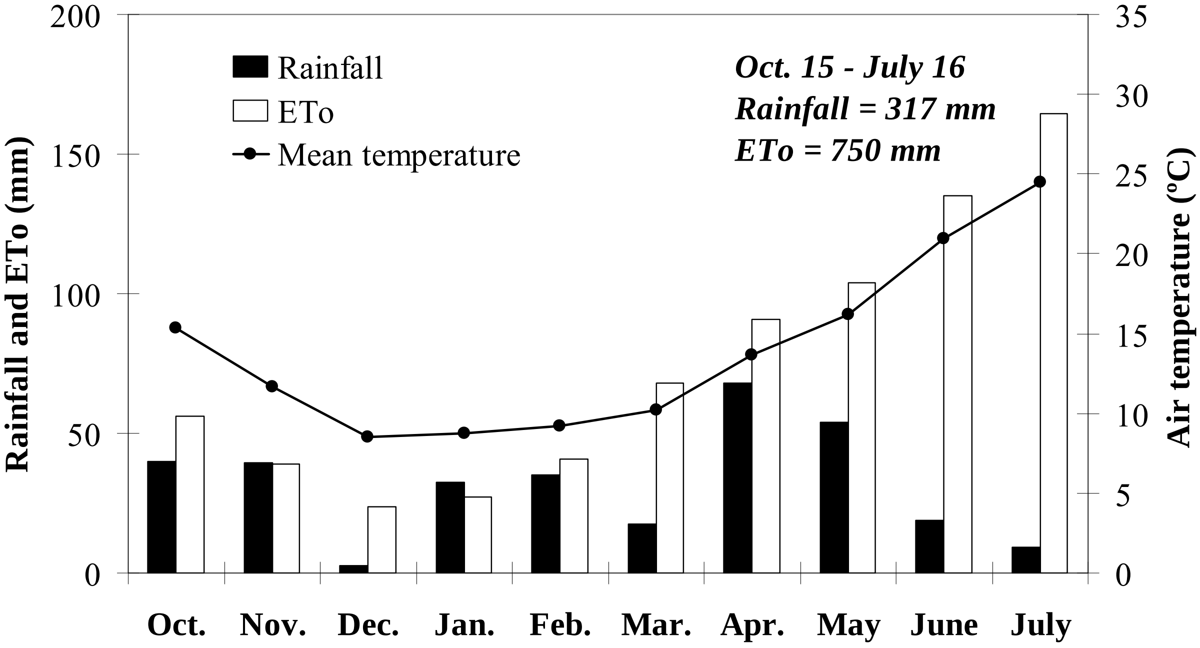
Figure 1. Monthly rainfall and mean air temperature and crop reference evapotranspiration (ET_0, FAO Penman-Monteith equation) for the last rapeseed growing period (October 2015 – July 2016) in the experimental field.

Figure 2. Mass aggregate distribution in five diameter ranges after slaking disturbance and for each fertilization strategy: control, no N applied; daily cattle slurry applied at 170 kg N ha^{-1} (170DCS) or 250 kg N ha^{-1} (250 DCS).

Figure 3: Distribution of soil organic carbon in three apparent diameter sizes (≤0.05mm, >0.05–0.2mm, >0.2–2mm) and in two density fractions: light (LF) and heavy (HF) for each fertilization strategy: control, no N applied; daily cattle slurry applied at 170 kg N ha^{-1} (170DCS) or 250 kg N ha^{-1} (250 DCS). Within columns, means of total C and C in each fraction followed by the same letter are not significantly different according to the Duncan's Multiple Range test (P>0.05).

Figure 4. Soil microbial biomass (SMB) for each fertilization strategy: control, no N applied; daily cattle slurry applied at 170 kg N ha^{-1} (170DCS) or 250 kg N ha^{-1} (250DCS). Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range test (P>0.05).

Figure S1. Scheme of crop distribution from 2008 to 2016 cropping seasons.



200
150
100
50
0
Rainfall and ETo (mm)
■ Rainfall
☐ ETo
Mean temperature
Oct. 15 - July 16
Rainfall = 317 mm
ETo = 750 mm
35
30
25
20
15
10
5
0
Air temperature (ºC)
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July

Fig. 1

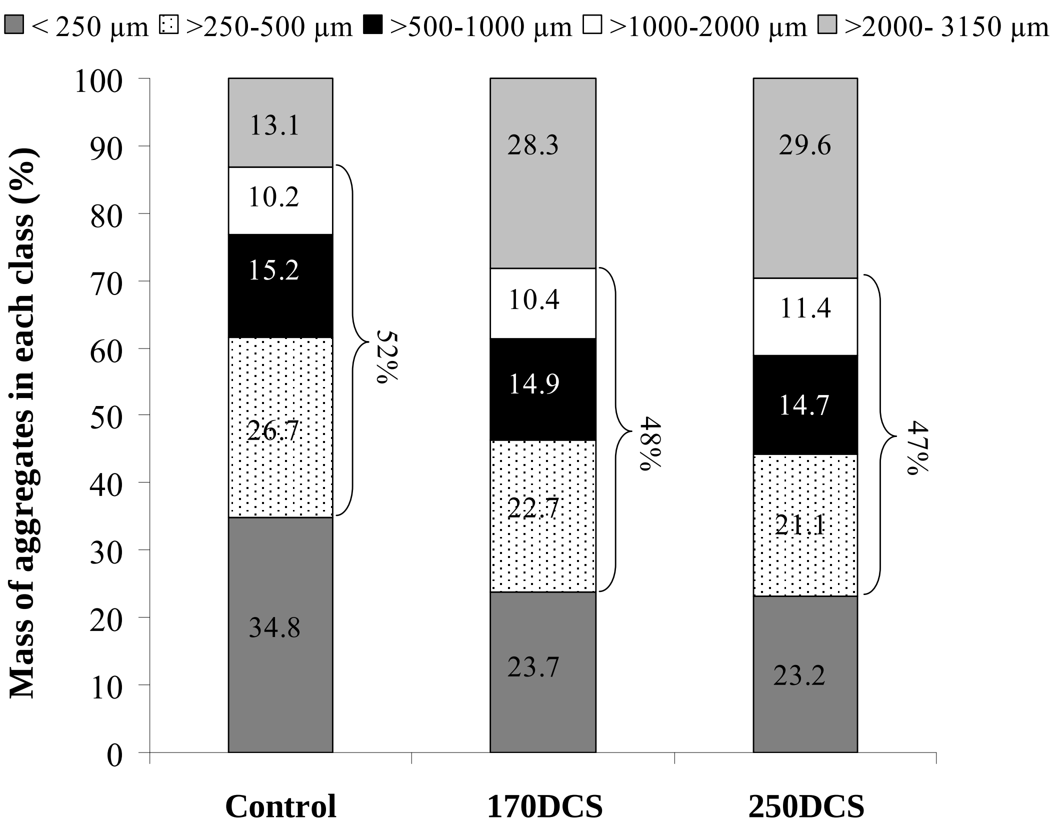
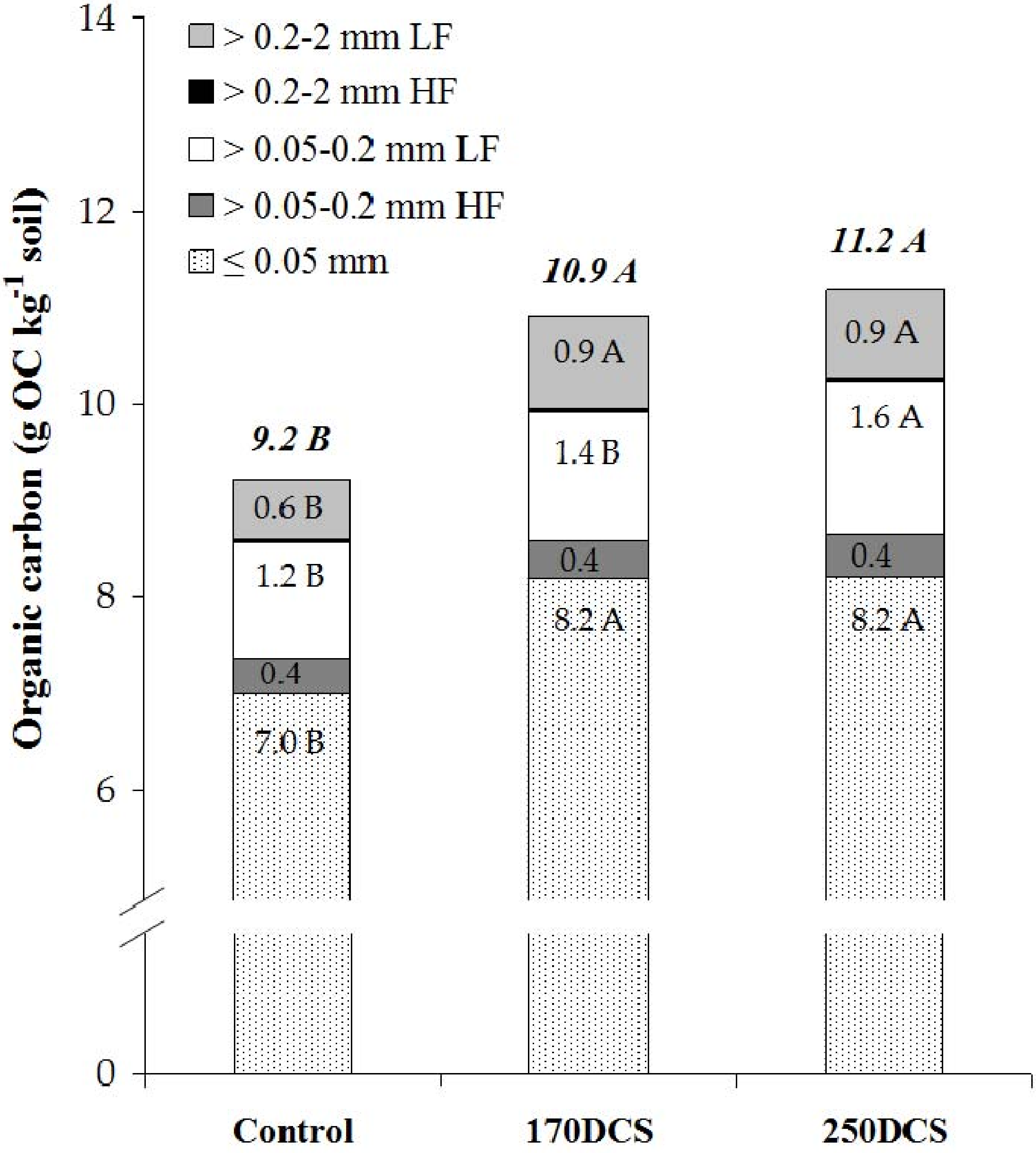
■ < 250 μm ⬚ >250-500 μm ■ >500-1000 μm □ >1000-2000 μm ■ >2000- 3150 μm
Mass of aggregates in each class (%)
100
90
80
70
60
50
40
30
20
10
0
13.1
10.2
15.2
26.7
34.8
52%
28.3
10.4
14.9
22.7
23.7
48%
29.6
11.4
14.7
21.1
23.2
47%
Control
170DCS
250DCS

Fig. 2



Organic carbon (g OC kg^{-1} soil)
14
12
10
8
6
0
> 0.2-2 mm LF
> 0.2-2 mm HF
> 0.05-0.2 mm LF
> 0.05-0.2 mm HF
≤ 0.05 mm
9.2 B
0.6 B
1.2 B
0.4
7.0 B
10.9 A
0.9 A
1.4 B
0.4
8.2 A
11.2 A
0.9 A
1.6 A
0.4
8.2 A
Control
170DCS
250DCS

Fig. 3

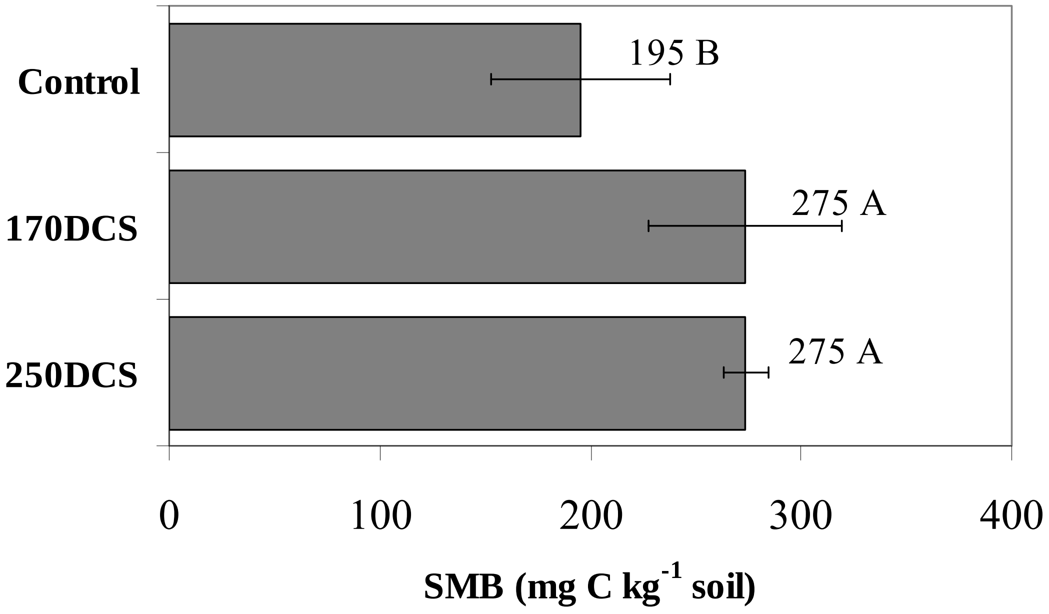

Control
170DCS
250DCS
195 B
275 A
275 A
0
100
200
300
400
SMB (mg C kg^{-1} soil)

Fig. 4

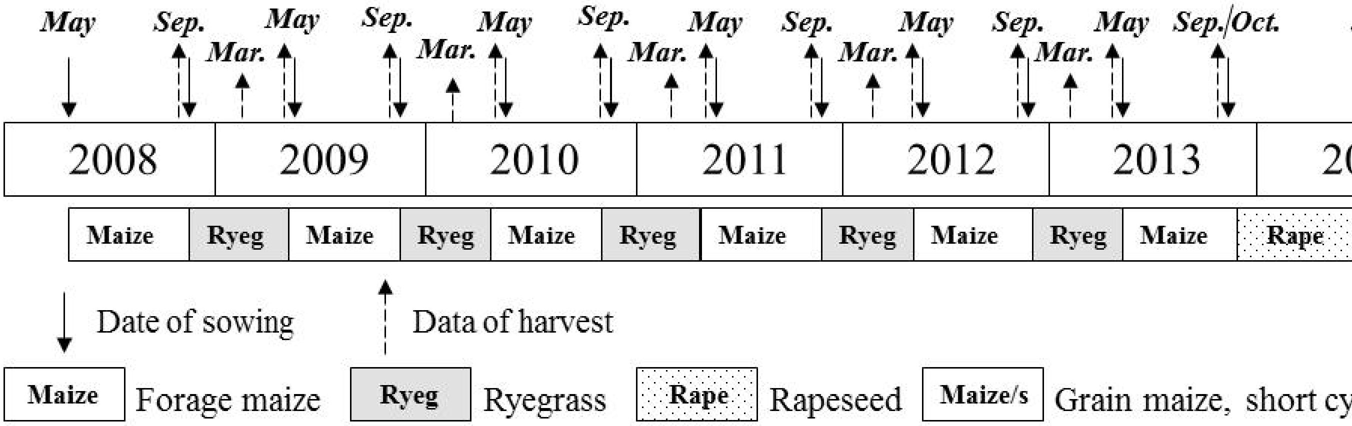

May
Sep.
Mar.
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep./Oct.
2008
2009
2010
2011
2012
2013
Maize
Ryeg
Maize
Ryeg
Maize
Ryeg
Maize
Ryeg
Maize
Ryeg
Maize
Rape
Date of sowing
Data of harvest
Maize
Forage maize
Ryeg
Ryegrass
Rape
Rapeseed
Maize/s
Grain maize, short cy

Fig. S1

Appendix A

Table S1

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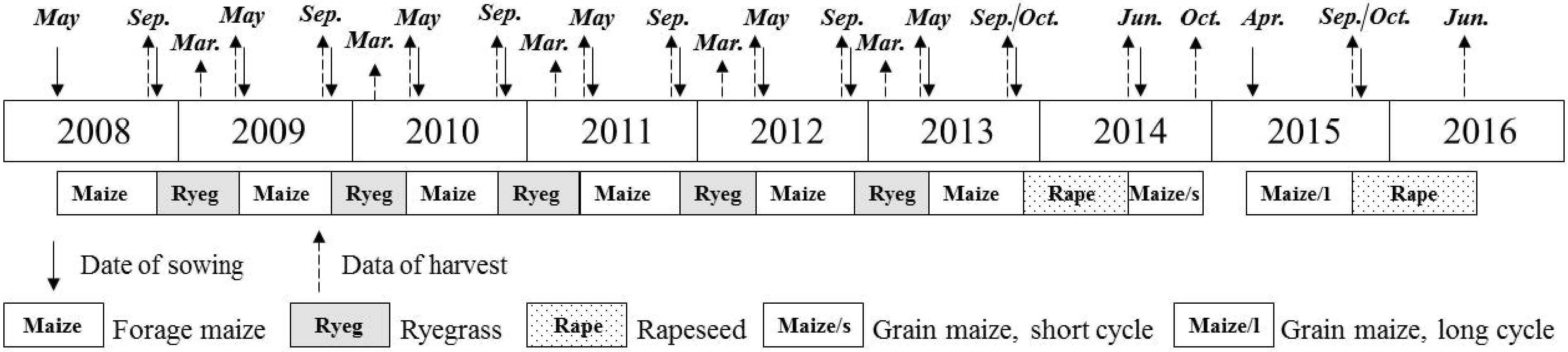
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep.
Mar.
May
Sep./Oct.
Jun.
Oct.
Apr.
Sep./Oct.
Jun.
2008
2009
2010
2011
2012
2013
2014
2015
2016
Maize
Ryeg
Maize
Ryeg
Maize
Ryeg
Maize
Ryeg
Maize
Ryeg
Maize
Rape
Maize/s
Maize/l
Rape
Date of sowing
Data of harvest
Maize
Forage maize
Ryeg
Ryegrass
Rape
Rapeseed
Maize/s
Grain maize, short cycle
Maize/l
Grain maize, long cycle

Fig. S1