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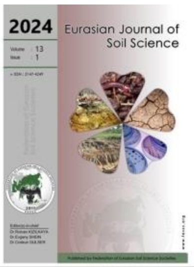

Aggregate stability and carbon and N dymamics in macroaggregate size fractions with different soil texture

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Abstract

Soil nutrient cycling, the distribution of soil aggregates, and their stability are directly influenced by soil texture. Different sizes of soil aggregates provide microhabitats for microorganisms and therefore influence soil carbon (C) and nitrogen (N) mineralization. The purpose of the present study was to assess the aggregate stability and dynamics of carbon and nitrogen in macroaggregate size fractions (1-8 mm) with different clay content from meadow soils. Surface soil samples (0-15 cm) were collected from 4- to 5-year-old forage crops. Four macroaggregate size classes were isolated by dry sieving and analyzed for their mass proportions: fine macroaggregates (FM) (less than 1 mm), medium-fine macroaggregates (MFM) (1-2 mm), medium-coarse macroaggregates (MCM) (2-4 mm), and large-coarse macroaggregates (LCM) (4-8 mm). The dry mean weight diameter (MWD), organic carbon (OC), total nitrogen (TN), carbon and nitrogen of microbial biomass (C-MB, N-MB) were determined. CO2 emission and net nitrogen mineralized (NM) were measured after 14 weeks of incubation. The amounts of FM were significantly lower than those of intermediate macroaggregates (MCM and MFM) and decreased markedly with increasing clay content within soil macroaggregates. In general, the amounts of macroaggregate size fractions were lowest in soils with high clay content. MWD exhibited a significant correlation with particle size distribution, OC, and MB-C. OC, TN, MB-C, and MB-N contents within macroaggregates increased with decreasing macroaggregate size and increasing clay content of macroaggregate fractions. The CO2 emission and NM content increased with increasing macroaggregate size, indicating higher organic C and N mineralization activity in larger macroaggregates. Mineralization of OC was lowest in macroaggregate fractions with the highest clay content. We conclude that clay content can increase the protection of microbial biomass in meadow soils. Small macroaggregates tend to contain more recalcitrant organic matter compared to larger macroaggregates.

Keywords: Soil aggregates, aggregate stability, soil texture, soil organic matter.

Introduction

The mineralization of organic carbon (C) and nitrogen (N) in soils has been reported to enhance soil fertility and thus can directly affect different levels of soil quality (Thomsen et al., 2012; Cai et al., 2016). Soil properties, soil aggregation, microbial biomass associated with aggregates, and agricultural management practices play key roles in the C and N dynamics (de Moraes Sá et al., 2014; Abbasi et al., 2015; Naresh et al., 2018) and the distribution of N and C among soil aggregate fractions (Bimüller et al., 2016; Xiao et al., 2017; Xie et al., 2017; Mitran et al., 2018). Gupta and Germida (2015) highlighted the role of soil fungal biomass in changing soil macroaggregates, carbon and nutrient flush following crop cultivation. Similarly, Ogunwole et

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al. (2014) indicated that soil organic carbon, aggregate-associated organic carbon, and aggregate size are important soil quality indicators. A better understanding of the mineralization of organic C and N in different particle-size fractions is necessary to improve the management of soil fertility and ultimately enhance agricultural sustainability (Cai et al., 2016; Ghimire et al., 2017; Xie et al., 2017).

It is generally known that the addition of organic materials/wastes contributes to enhancing the formation of aggregates in the soil. Awad et al. (2018) found that biochar and biopolymer amended with oyster shell powder increased the proportion of micro-aggregates (<0.25 mm) relative to the control soil, while the proportion of large macro-aggregates (1–2 mm) increased with the addition of polyacrylamide alone. Wang et al. (2011) showed that in a reddish paddy soil based on a long-term fertilization experiment, the application of organic materials decreased the proportion of small water-stable aggregates (WSA) (<1 mm) and increased the proportion of large WSA (>2 mm), resulting in an increase in the mean weight diameter of WSA, whereas the application of chemical fertilizer had little effect.

It is widely observed that organic matter (OM) biodegradation is faster in coarse- than in fine-textured soils (van Veen and Kuikman, 1990; Saggar et al., 1996), which was associated with faster OM turnover in soil macro-aggregates and lower organic C and N in coarse-textured soils (Six et al., 2000). Bossuyt et al. (2001) found that the formation of water-stable macroaggregates (>250 µm) was not significantly influenced by residue quality or bacterial activity; however, fungal activity positively influenced the stability of these macroaggregates. Ouyang et al. (2013) reported that biochar produced from dairy manure significantly improved macroaggregate formation in two contrasting textured soils (clay and sandy soil) at a ratio of 2% (w/w, dry weight). Cai et al. (2016) reported that in long-term experiments of manure application with or without chemical fertilization, OC and TN content significantly increased and C and N mineralization were enhanced in the three particle-size fractions. The amount of C and N mineralization followed the order 0.053 mm > 0.20-0.053 mm > 2.00-0.25 mm fraction, whereas the amount of OC and TN in different aggregate fractions followed the reverse order. In manure fertilization experiments, Hao et al. (2017) found that long-term manure use significantly increased the amount of coarse fractions and their associated OC and TN stocks in the soils. Also, P-fertilization increased the OC and TN in soil aggregates >2 mm and decreased the loss of N from chemical fertilizers in the legume-grain rotation system. Amellal et al. (2001) showed that the mineralization of organic substances in the larger sand- and silt-sized fractions (50–2000 µm and 2–50 µm, respectively) in petroleum hydrocarbon-contaminated soils was more significant than in the finer (clay-rich) aggregate size fractions (<2 µm). Six et al. (2000) also found that the C content of macroaggregates (0.250-2.00 mm and >2 mm fractions) was higher than that of microaggregates (<0.250 mm fractions) in soils dominated by illite and chlorite. However, they did not observe increased C concentrations with increasing aggregate size in soil with mixed mineralogy (Fe- and Al-oxides and kaolinite). There is little available information on the dynamics of C and N in different aggregate fractions of meadow soils. According to Naresh et al. (2018), there is limited consensus on the pattern of changes in soil OM mineralization and nutrient release across aggregates of different sizes. The present study was conducted with the hypothesis that (i) large-sized macroaggregates (8-4 mm) can mineralize more C and N than fine macroaggregates (<1 mm), (ii) soil fine fraction (clay+ silt) would increase soil aggregation and affect the C, N, and MB dynamics in different aggregate fraction sizes, and (iii) the distribution of microbial biomass carbon (MBC) and nitrogen (MBN) in different aggregate classes affects SOM mineralization in each aggregate size. The objectives of the present study were to quantify the relative rates of C and N mineralization in different particle-size fractions and to examine the effects of clay content and soil aggregation on C and N storage in aggregate-size classes.

Material and Methods

Soil sampling

The soils used in this study were collected from twelve dairy farms located in Sainte-Croix de Lotbinière, in eastern Quebec, Canada. A total of 34 plots spread over 380ha are cultivated in meadow crops. These farms are in transition from conventional management to a low input organic management system. Solid cattle manure is applied on the basis of 132 kg N ha^{-1} which is 20 Mg ha^{-1} on a wet basis. The soil series represents gleyed humo-ferric podzols, samples are taken from the soil layer from 0 to 15 cm. these soils were plowed every 4 to 5 years. Finally, only 14 samples were retained, the other soils are sandy and do not present the different categories of aggregates.

Aggregate-size fractionation

Undisturbed soil samples were taken under wet conditions in the field. Composite samples for each site were formed from nine wet field subsamples. The soils were sampled with a special spade. Special care was applied in order to maintain the natural structure of the soil samples. Parts of the samples showed signs of deformation due to the smoothing of the spade-cut surface during the process.

Aggregates were separated by dry sieving of moist soil (10 to 14%) as described by Mendes et al. (1999). Dry sieving is simple, rapid and valid method for aggregate separation when examining biologycally active N pools in aggregates (Sainju, 2006). Field-moist soils were stored at 4°C for 7 to 10 d until they reached a gravimetric water content of 10% (Sainju, 2006). Drying the soil samples at 4 °C reduces its impact on microbial communities and activities within aggregates (Mendes et al., 1999). Soil aggregates were separated by placing 200-g soil fragments (<8 mm, air dried at 4°C) on the top of a set of sieves in decreasing order of mesh sizes: 8.00, 4.00, 2.00, and 1.00 mm (Miller et al., 2009). The aggregates of 8.00-4.00 mm, 4.00-2.00 mm, 2.00-1.00 mm and < 1 mm were referred to as large-coarse macroaggregate (LCM), medium-coarse macroaggregate (MCM) (4-2 mm), medium-fine macroaggregate (MFM), (2-1 mm) and fine macroaggregates (FM) (<1 mm), respectively. After gentle manual crumbling, fragments such as roots and stones constituted a negligible share and were removed. Then the sieves were attached to a Tyler To-Tap sieve shaker and shaken at 200 oascillations/min for 3 min (Sainju, 2006). The aggregates (8.00-4.00, 4.00-2.00, 2.00-1.00, and <1.00 mm) retained and passed through the sieves were weighed (Mendes et al., 1999; Sainju, 2006), maintained at field-moist, and stored in plastic bags at 4 °C before experimentation.

Mean weight diameter

Subsamples of the aggregate fractions (8.00-4.00, 4.00-2.00, 2.00-1.00, and <1.00 mm) were oven-dried at 60°C for 48 hours and weighed for the fraction percentage measurement and soil texture determination. Particle size analysis was performed by mechanical dispersion of the aggregate fractions after OM removal. Particle-size analysis was completed through the adaptation of hydrometer method outlined by Kroetch and Wang (2008). Mean weight diameter (MWD) of aggregates was calculated (Hillel, 2004; Liu et al., 2019) as follows:

$$\mathrm{MWD} = \sum_{i=1}^{n} x_i w_i$$

where i is aggregate size (k = 1, 2, 3 indicate macro-aggregate, micro-aggregate and silt + clay-sized fraction); x_i (mm) is the mean diameter of any particular size range of aggregates separated by sieving; wi (%) is the weight of the aggregates in that size range as a fraction of the total dry weight of soil used. MWD is commonly used to express aggregate stability (Blair, 2010). High MWD values indicate lower erodability of soil (Ciric et al., 2012) and larger aggregate size fractions in the soil and, therefore, greater aggregate stability (Piccolo et al., 1997).

Organic C and total N in soil fractions

A sub-sample of each aggregate size fraction was ground to 0.15 mm for organic carbon (OC) and total N (TN) content measurement. The OC content was determined by the Walkley-Black potassium dichromate oxidation procedure (Allison, 1965) and TN was analyzed by Kjeldahl digestion method (Rutherford et al., 2008).

Microbial biomass C and N in soil fractions

Soil microbial biomass (SMB) C and N in different particle-size fractions (8.00-4.00, 4.00-2.00, 2.00-1.00, and <1.00 mm) were determined according to the method modified by Vance et al. (1987). This method was described by Sbih et al. (2012). Briefly, the amounts of C and N deriving from SMB were extracted with 0.5 M K_2SO_4 from non-fumigated and fumigated soil fractions and in the extracts were determined respectively by wet dichromate oxidization technique and standard Kjeldahl digestion method (Rutherford et al., 2008). The microbial biomass C (MB-C) and the microbial biomass N (MB-N) are determined according to Joergensen (1996) and Brookes et al. (1985) respectively. Data were expressed in mg kg^{-1} dry soil aggregate and represented the average of two replicates.

Organic C and N mineralization in soil fractions

Organic C and N mineralization in bulk soil and different particle-size fractions (8.00-4.00, 4.00-2.00, 2.00-1.00, and <1.00 mm) was determied according to Sbih et al. (2003). Briefly, two 50-g soil samples (oven-dried at 60°C, not ground) were moistened with demineralized water at 85% field capacity and placed in a 1-L polyethylene flask. Soil fractions were incubated in the flask at room temperature (25 ± 2°C) for 4 wk and the incubation periodes were 1, 2, 3 and 4wk soil moisture was maintained constant. In order to traped evolved CO_2 vials containing 1 M NaOH were introduced in flask, during incubation. At the end of each period, vials containing NaOH were sampled to measure the CO_2 content. Soil aggerates were then sampled (20 g) to measure mineralized N. The CO_2 evolved was determined by titration of excess NaOH and reported as C-CO_2 emision (mgC/ g aggregate- C) (Ross et al., 1995). NH_4^+ and NO_3^- were extracted with 2 M L^{-1} KCl (soil-solution ratio of 1:4) and determined by Autoanalyser. The amount of N mineralized was calculated as the difference between the final and initial extractable inorganic N concentrations, after adjustment soil from fresh dry weight. The net N mineralized (%) is calculated as N-min (%) = $[(NH_4^+\text{-N} + NO_3^-\text{-N})_f / \text{total } N_f] \times 100$, where, f represent either bulk soil or aggregate fraction (Xie et al., 2017).

Data Analysis

Statistical calculations were performed with the full data set of all soils. the relationships between the various parameters and the effect of clays on the aggregates and their sizes was evaluated by ANOVA (PROC GLM procedure of XLSTAT software). Mean separation was done with an *F*-protected LSD test, and the Pearson's correlation is used to find the relationship between the different parameters.

Results and Discussion

Aggregate size distribution

The amount of different macroaggregate size fractions is expressed as a percentage of total soil mass. In general, FM (<1mm) represented the lowest fraction whereas intermediate macroaggregates class (1-4 mm) represented the greatest proportion for all soils (Table 1). The same observation was reported by Xie et al. (2017) who examined the OC stock over 20 cm depth of aggregates, its mineralization under the effect of organic and inorganic inputs in intensive wheat-maize cultivation in northern China. The large-coarse macroaggregate size fraction >2 mm was the most abundant, suggesting that LCM (as determined by dry sieving) comprised the bulk of the macroaggregates within the soil studied.

Table 1. Amounts of different macroaggregate size fractions, expressed as percentage of total soil mass.

	Clay content (g kg^{-1})											
Macroaggregate size fractions	150	-	200	(n=7)	201	-	300	(n=5)	301	-	400	(n=2)
<8-4 mm range mean	20.8	-	28.8	(25.3) bc	30.2	-	35.9	(32.9) a	36.6	-	38.6	(37.6) a
<4-2 mm range mean	28.5	-	41.0	(33.5) a	30.2	-	42.0	(35.3) a	27.8	-	33.7	(30.8) b
<2-1 mm range mean	17.6	-	26.2	(19.7) c	13.9	-	22.1	(18.8) b	18.5	-	25.2	(21.8) c
<1 mm range mean	12.1	-	26.5	(21.5) c	8.2	-	17.4	(13.0) c	9.3	-	10.3	(9.8) d
LSD (0.05)			3.5		4.23						5.7	

Different letters in each column indicate significant differences between different treatments n: number of soil samples; Means with the same letter are not significantly different at $P<0.05$ according to the LS means test.

In general, the amounts of macroaggregate size fractions were lowest in soils with high clay content (Table 1). The amount of FM (<1mm) decreased markedly with increasing clay content within soil aggregates (Table 1). These results are in agreement with the findings of Kristiansen et al. (2006), who also found that the proportion of soil recovered in aggregates> 2 mm increased with increasing clay content of the whole soil, while those <1 mm decreased. The results of the correlation analysis (Figure 1) showed that the amounts of clay+silt-sized particles within macroaggregate fractions were positively correlated with the mass proprotion of LCM (<8-4 mm) ($r=0.75$; $P\leq0.01$) and negatively correlated with the mass proportion of FM (<1 mm) ($r=0.90$; $P\leq0.001$).

Dry mean weight diameter

The dry MWD of macroaggregate size fractions varied between 2.80 and 3.70 mm (Figure 2). It increases as the proportion of small particles increases. The clay particles affect the mean value of MWD in the following order: 2.98 mm for soils with 150-200 g clay particules kg^{-1} soil < 3.40 mm for soils with 201-300 g clay particules kg^{-1} soil < 3.58 mm for soils with 301-400 g clay particules kg^{-1} soil. MWD exhibited significant correlation with particle size distribution. From figure 2, it is notable that the values of dry. Contrasting correlations between MWD and soil texture components, indeed, MWD were positively correlated with clay content ($r = 0.88$; $P \leq 0.01$) and negatively correlated with sand content ($r = -0.79$; $P \leq 0.01$). When the amounts of clay+silt-sized particles were considred, the correlation became more strong ($r=0.90$; $P<0.001$), suggesting that clay+silt-sized particles promotes the formation of stable aggregates in meadow soils. Khaledian et al. (2013) examined the effect of soil texture on soil permanganate organic carbon in somes soils fron deforested areas in Northern Iran. They concluded that clay and silt particles could provide some degree of physical protection for oxidizable C groups in lignin. In addition, positive correlations were obtained between MWD and soil OC ($r=0.66$; $P<0.05$) and MB-C ($r=0.64$; $P<0.05$). Different experiments have demonstrated that aggregate stability increases when clay and organic matter accumulation increase, confirming the role of these soil properties in soil aggregation (Norton et al., 2006; Nweke and Nnabude, 2015). Aziz and Karim (2016) monitored in the climatological region of Iraqi Kurdistan region with different uses of agricultural land certain soil properties in relation to soil aggregates. They obtained a positive correlation between aggregate stability

(MWD) and clay content and organic matter content of soils. Khaledian et al. (2013) obtained very highly positive correlations (*P*<0.001) between stable carbon and MWD. These relationships are in agreement with the fact that organic matter and clay content of soils acts as cementing and binding agent (Kemper and Koch, 1966; Kemper and Rosenau, 1986; Golchin et al., 1997; Seybold and Herrik, 2001). The stabilization of clay-organic complexes is the main mechanism of soil preservation, this is due to the soil clay content which seems to be the determining factor in the chemical stabilization of this complex (Blanco-Moure et al., 2016).

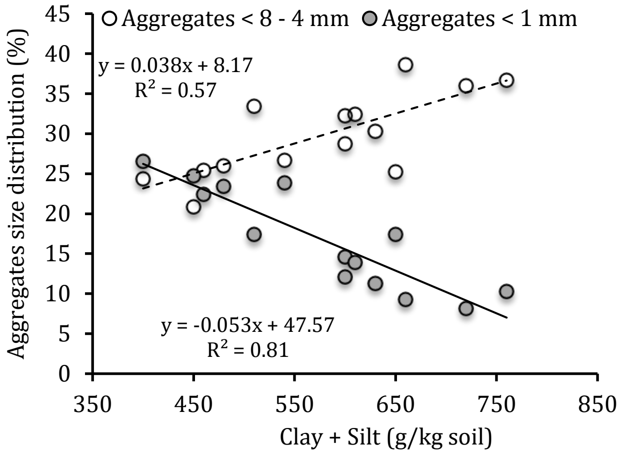

Figure 1. Mass proportion of two macroaggregate size fractions with different clay +silt contents

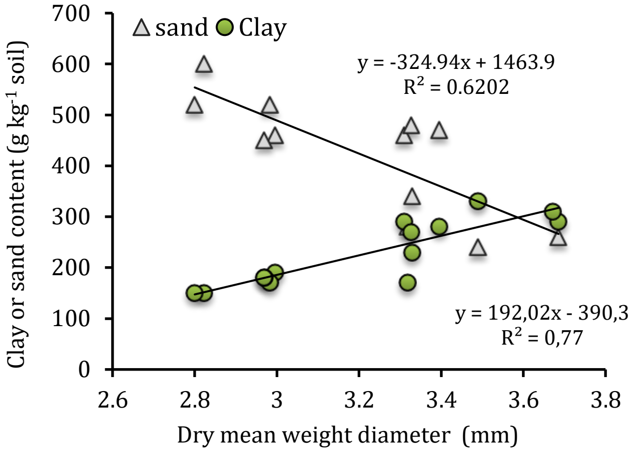

Figure 2. Values of dry mean weight diameter of soils with different sand and clay contents

Soil macroaggregate sizes markedly affected soil OC stock (Figure 3) and consequently, the distribution of microbial biomass C (Figure 4) and mineralization of OC (Figure 5) within soil macroaggregates.

Total oganic carbon

The OC content within soil macroaggregates increased with decreasing macroaggregate size from LCM (8-4 mm) to FM (<1 mm) (Figure 3). The OC content varied between 34.9 to 42.1 g kg^{-1} in soil fractions with lower clay content (150-200 g kg^{-1}) and from 37.6 to 56.4 g kg^{-1} in soil fractions with highest clay content (301-400 g kg^{-1}). Thus, the mean OC content in LCM class with highest clay content was 7.7% higher than in LCM with lowest clay content. However, the OC content of FM fraction with highest clay content was 55% higher than in FM fraction with lowest clay content. These results indicate that the OC is more concentrated or sequestered in the finest soil aggregate fraction of meadow soils. Clay particles provide a greater ability to chemically stabilize SOM due to the greater reactive surface area of clay particles and also to physically protect SOM by incorporation into aggregates (Feller and Beare, 1997; Six et al., 2002). This implies that as the clay content increases, the proportion of small pores increases, therefore decomposing microbes are excluded which stabilizes the OC (Mtambanengwe et al., 2004; Strong et al., 2004).

Microbial biomass carbon

The content of MB-C within soil macroaggregates increased as follows: FM (<1 mm) > MFM (2-1 mm) > (CMM) (4-2 mm) > LCM (8-4 mm) (Figure 4). These results indicate that MB-C is more concentrated in the finer fraction of soil macroaggregates. This is probably due to the fact that fine macroaggregates can have a high-water holding capacity and become a preferential microhabitat for MB. Seech and Beauchamp (1988), Miller and Dick (1995) reported that C-MB is mainly concentrated in microaggregates and as FM is a set of micraggregates that is why they contain more C-MB. The MB-C content within soil macroaggregates increased with increasing soil clay content (Figure 4). Microbial biomass C content was significantly correlated to OC content (r=0.70; *P*<0.05), and strongly correlated to soil clay+silt content (r=0.889; *P*<0.001). These results suggest that MB-C is concentrated mostly in the smallest macroaggregate sizes and the finest textured meadow soils. When investigated the relationship between soil clay content and soil organic matter turnover Müller and Höper (2004) found strong relationship between clay content and soil microbial biomass-C and concluded that clay particles might render some degree of protection for microbial biomass. Particles less than 2 µm (clay) are generally considered as protective microhabitats and provide protection for microbial biomass against desiccation (Heijnen and van Veen, 1991). Sainju et al. (2009) found greater MB-C at 0 to 5 cm or greater MB-C and MB-N at 5 to 20 cm in the <0.25 mm than in the 4.75 to 2.00-mm size class of soils from different cropping sequences. They concluded that microaggregates can protect microbial biomass from predators.

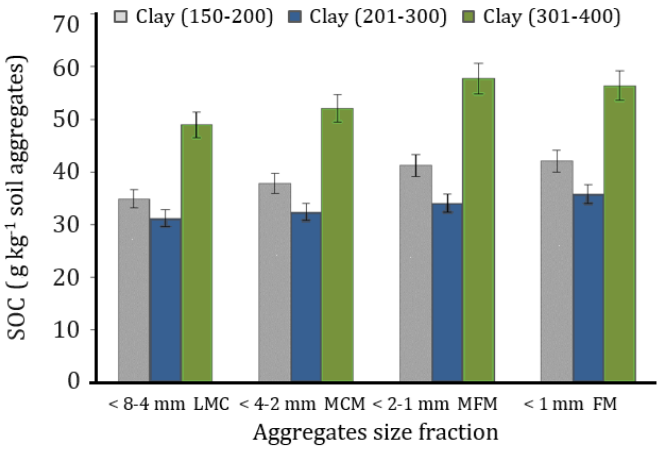

Figure 3. Organic carbon (OC) content of macroaggregate fractions with different clay contents.
(Lower case letters in Figure represent differences between same aggregates at different clay level are significantly different at P <0.05 by least squares means test. Uppercase letters represent differences between the same clay level in different soil aggregates are significantly different at P <0.05 by least square means test).

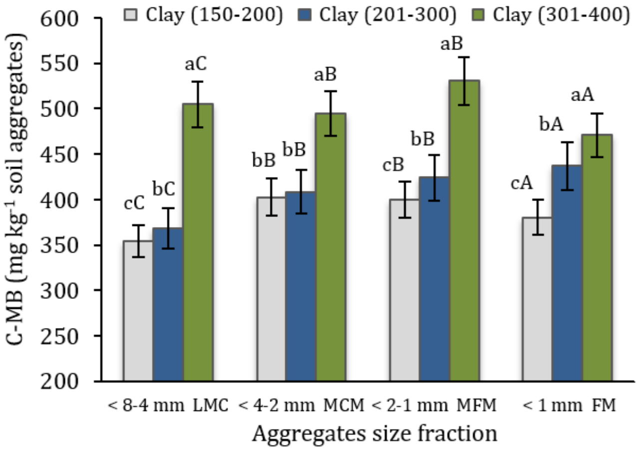

Figure 4. Microbial biomass carbon (C-MB) content of macroaggregate fractions with different clay contents
(Lower case letters in Figure represent differences between same aggregates at different clay level are significantly different at P <0.05 by least squares means test. Uppercase letters represent differences between the same clay level in different soil aggregates are significantly different at P <0.05 by least square means test).

Mineralization of organic carbon

Because microorganisms related to C and N dynamics could be heterogeneously distributed throughout pores of various sizes and within aggregates (Watteau and Villemin, 2018), it is expected that OC mineralization will vary among soil macroaggregates. The CO_2 emission during the 28-d incubation (Figure 5) was expressed as mg C g^{-1} aggregate C content.

As we can see from figure 5, the C-CO_2 emission increased with increasing macroaggregate size as follows: LCM (8-4 mm) > (MC) (4-2 mm) > (MFM (2-1 mm) > FM (<1 mm). These results indicate a higher organic C mineralization activity in coarse macroaggregates. Several studies have shown that OM decomposition is faster in coarse-textured soils than in fine-textured soils (vanVeen and Kuikman, 1990; Strong et al., 2004) because clay soils are better able to protect OM from decomposition (Mtambanengwe et al., 2004). Fernández et al. (2010) found highest CO_2 production at 15 days of soil incubation in the 1-4 mm aggregate fraction, however, the aggregates <1, >4 mm and undisturbed soil, their respiration was 59, 56 and 72% lower respectively, compared with the 1-4 mm class. Under three contrasting land uses of the topsoil from Dermosol sites (Acrisols in the FAO soil classification), Rabbi et al. (2014) show that the cumulative C mineralization (C-CO_2 gkg^{-1} aggregate) of the < 53 µm fraction was 28% lower than that of macroaggregates (250–2000 µm) and microaggregates (250–53 µm). However, because of the insignificant difference in C mineralization, slow soil OC pool sizes and mean residence time of slow soil OC pool between macro-aggregates and micro-aggregates, they concluded that soil OC mineralization rate and thus the protection of soil OC was similar in both macroaggregates and microaggregates. In another study, Rabbi et al. (2016) investigated the influence of pore geometry on the OC decomposition rate and bacterial diversity in both macroaggregates (250–2000 µm) and microaggregates (53–250 µm) using field samples. They found that the OC decomposition rate constant was similar in aggregate size ranges. In general, the C-CO_2 emission increased with decreasing clay content of soil macroaggregate fractions (Figure 5). Protection of OM within stable aggregates is potentially stabilized by a high clay content (Six et al., 2002). In general, OM compounds could be stabilized by interaction with soil clay fraction, as soil organic compounds binds chemically to clay minerals or Fe/Al oxides (Saidy et al., 2012; Torres-Sallan et al., 2017; Singh et al., 2018). Chen et al. (2018) concluded that organic C-mineral associations in soils could retard the decomposition of biochemically labile organic compounds like carbohydrates and peptides. Several studies reported that the stabilization of soil OC is influenced by the type of phyllosilcates present in soils (Goh, 2004; Gartzia-Bengoetxea et al., 2017; Singh et al., 2018). Meena et al. (2017) reported that smectic clays are more potent in accumulation and sequestration of SOC in black cotton soils of India. Jones and Singh (2014) highlighted the potential of mineral surfaces in influencing the stability of OM in organo-mineral interactions.

Dynamic of nitrogen within soil macroaggregates

Total nitrogen and microbial biomass nitrogen

Figure 6 shows TN associated with different sizes of macroaggregates. The distribution of TN followed the same pattern as OC among the four macroaggregate classes. The size of macroaggregates markedly affected TN stock within soil macroaggregates. The content of TN increased with decreasing macroaggregate size fractions as follows: LCM (8-4 mm) < MCM (4-2 mm) < MFM (2-1 mm) < FM (1 mm) (Figure 6), indicating that smaller-size macroaggregate fraction was the main pool of total N.

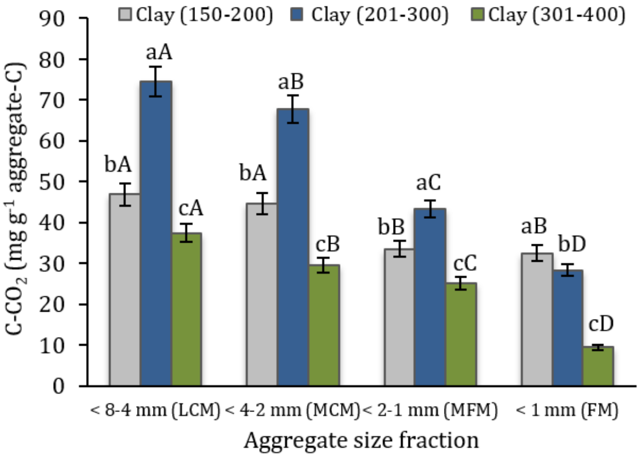

Figure 5. C-CO$_2$ emission from incubated macroaggregate fractions with different clay content.
(Lower case letters in Figure represent differences between same aggregates at different clay level are significantly different at P <0.05 by least squares means test. Uppercase letters represent differences between the same clay level in different soil aggregates are significantly different at P <0.05 by least square means test).

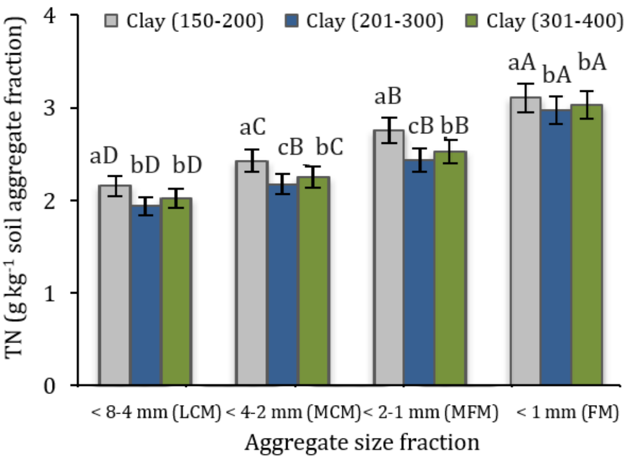

Figure 6. Total nitrogen (TN) content of macroaggregate fractions with different clay contents
(Lower case letters in Figure represent differences between same aggregates at different clay level are significantly different at P <0.05 by least squares means test. Uppercase letters represent differences between the same clay level in different soil aggregates are significantly different at P <0.05 by least square means test).

The amount of TN varied from 2.3 g kg^{-1} to 3.3 g kg^{-1} in macroaggregates with 150-200 g clay particules kg^{-1} soil and from 3.0 g kg^{-1} to 4.4 g kg^{-1} in macroaggregates with 301-400 g clay particules kg^{-1} soil. On average, the amount of TN in <8-4 mm size fraction in macroaggregates with 301-400 g clay particules kg^{-1} soil was 43% higher than that in macroaggregates with 150-200 g clay particules kg^{-1} soil. These results indicate that N is more concentrated or sequestered in the finest soil macroaggregate fraction.

The distribution of MB-N among soil aggregates showed the same pattern as the macroaggregate total N (Figure 7). This observation is in accord with the findings of Egan et al. (2018) who reported that long-term grazing and inorganic nutrient fertilization could contribute to greater N and C pools of smaller soil fractions. Several studies found that soil management practices can alter the aggregate associated organic C and N fractions with different clay contents (Sodhi et al., 2009; Tripathi et al., 2014).

Organic nitrogen mineralization

In general, N mineralization increased with increasing aggregate size, with the <4-2 mm aggregate size mineralizing more N, compared to smaller sized aggregates (<2–1 and <1 mm). The amount of N-mineralzed increased with increasing aggregate size as follows (Figure 8): LCM and MCM (4-2 mm) > MFM (2-1 mm) > FM (<1 mm), suggesting that larger macroaggregate fractions contain a larger proportion of readily mineralizable organic N than smaller macroaggregate fractions in meadow soils. Similarly, Muruganandama et al. (2008) found that N mineralization enzymes increased with increasing aggregate size of soils. They found that the potential activities of NAG, l-glutaminase, and arylamidase were significantly greater ($P < 0.05$) in the intermediate (0.5–1 mm) aggregate size than in other size fractions (<0.25, 0.25–0.5, 1–2, and 2–4 mm). In general, an increasingly greater proportion of the organic N within soil aggregate fractions was mineralized as the mineral particle size decreased, except for the small macro-aggregate (<1 mm) (Figure 8), which released less than 3 mg N g^{-1} aggregate N after 28 d of incubation. This indicates that in the small macro-aggregate fraction, OM was not available for microbial degradation and was therefore physically protected against decomposition (Hassink et al., 1993; Bimüller et al., 2014).

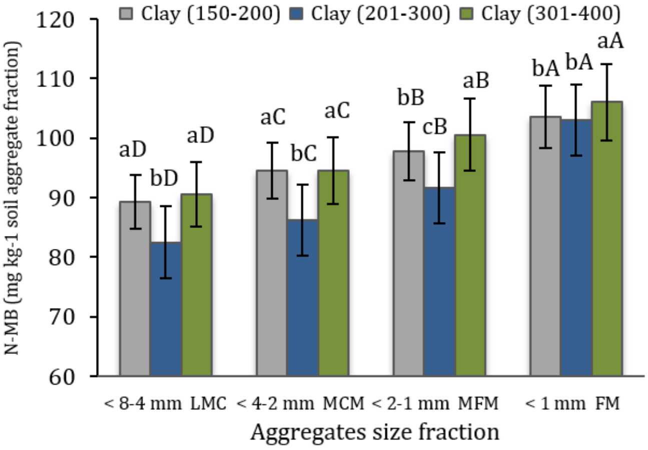

Figure 7. Microbial biomass nitrogen (N-MB) content of macroaggregate with different clay contents (Lower case letters in Figure represent differences between same aggregates at different clay level are significantly different at P <0.05 by least squares means test. Uppercase letters represent differences between the same clay level in different soil aggregates are significantly different at P <0.05 by least square means test).

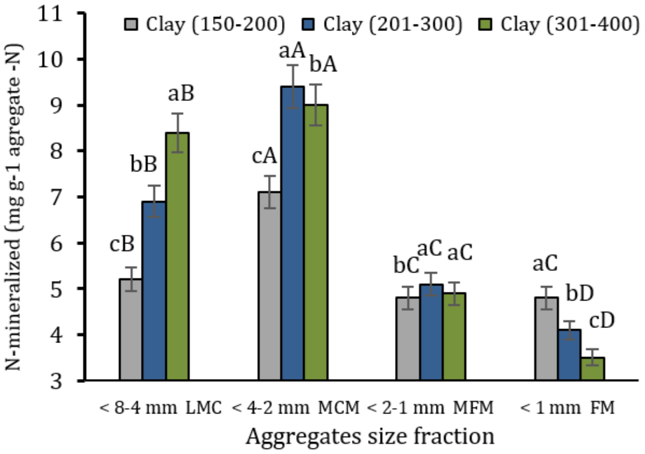

Figure 8. Nitrogen mineralization within macroaggregate fractions with different clay contents (Lower case letters in Figureure represent differences between same aggregates at different clay level are significantly different at P <0.05 by least squares means test. Uppercase letters represent differences between the same clay level in different soil aggregates are significantly different at P <0.05 by least square means test).

Nitrogen mineralization pattern in soil aggregate size fractions seems to be similar to CO_2 emission pattern. Indeed, a significant positive correlation was found between N-min and CO_2 emission (r=0.66, *P*<0.001). Unlike what we have seen with C-CO_2 emission, the C/N of both soil OM and microbial biomass are very highly correlated (r=0.62, *P*<0.001) to N-min at aggregates size <8-4 mm. Also, C-MB and N-MB were highly correlatd to N-min (r =0.84, *P*<0.001; r =0.67, *P*<0.001 repectively). However, these parameters are less correlated in the other aggregate sizes. It seems that LCM are more accessible to microbial and contain a larger proportion of readily mineralizable organic N than smaller aggregates. In contrast to our findings, other researchers have reported higher N mineralization in small aggregates (Craswell et al., 1970; Cameron and Posner, 1979).

Conclusion

Soil aggregates of different sizes provide diverse microhabitats for microorganisms and therefore influence soil activities. The clay content of soil aggregate fractions affects the mean weight diameter of macroaggregate size fractions. Generally, a greater proportion of the organic carbon (C) and organic nitrogen (N) within soil aggregate fractions is mineralized as the mineral particle size decreases. Clay content can increase the protection of microbial biomass in meadow soils. This study suggests that the mean weight diameter (MWD) should be considered in routine soil testing because it determines the particle size distribution of aggregates and essentially measures the stability of macroaggregates, maintaining high stability. Aggregates in soils are important for sustainable soil maintenance, as they are essential for preserving agricultural production, reducing soil erosion and degradation, and reducing environmental pollution. A well-aggregated soil creates favorable conditions for biological activity, resulting in an increase in the bioavailability of nutrients, thus improving plant growth conditions.

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