RELATIONSHIPS BETWEEN SOIL MICROBIAL BIOMASS, AGGREGATE STABILITY AND AGGREGATE ASSOCIATED-C: A MECHANISTIC APPROACH

RELATIONS ENTRE LA BIOMASSE MICROBIENNE DU SOL, LA STABILITE DES AGREGATS ET LE C ASSOCIE AUX AGREGATS: UN APPROCHE MECANISTIQUE

RELAZIONI TRA LA BIOMASSA MICROBICA DEL SUOLO, LA STABILITÀ DEGLI AGGREGATI ED IL C ASSOCIATO AGLI AGGREGATI: UN APPROCCIO MECCANICISTICO

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Summary

For the identification of C pools involved in soil aggregation, a physically-based aggregate fractionation was proposed, and additional pretreatments were used in the measurement of the 1-2 mm aggregate stability in order to elucidate the relevance of the role of soil microorganisms with respect to the different aggregate breakdown mechanisms. The study was carried out on three clay loam Regosols, developed on calcareous shales, known history of organic cultivation. Our results showed that the soil C pool controlling the process of stabilisation of aggregates was related to the microbial community. We identified the resistance to fast wetting as the major mechanism of aggregate stability driven by microorganims. The plausible hypothesis is that organic farming promotes fungi growth, improving water repellency of soil aggregates by fungal hydrophobic substances. By contrast, we failed in the identification of C pools controlling the formation of aggregates, probably because of the disturbance of mechanical tillage which contributes to the breakdown of soil aggregates. The physically-based aggregate fractionation proposed in this study resulted useful in the mechanistically understanding of the role of microorganisms in soil aggregation and it might be suggested for studying the impact of management on C pools, aggregates properties and their relationships in agricultural soils.

Key-words: *organic C pools, microbial C, soil aggregates, fast wetting, water abrasion*

Résumé

Pour l'identification des pools de C impliqués dans le processus d'agrégation du sol, un fractionnement d'échantillon d'agrégats de sol basé sur un mécanisme physique a été proposé, et des prétraitements supplémentaires ont été utilisés pour évaluer l'indice de stabilité des agrégats de dimension comprise entre 1-2 mm afin de

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comprendre le rôle des microorganismes du sol en ce qui concerne les différents mécanismes de la rupture des agrégats. L'étude a été réalisée sur trois types de Régosols argileux, développés sur des schistes calcaires, ayant comme antécédents l'usage agricole selon les critères de l'agriculture biologique. Nos résultats ont montré que le pool de C contrôlant le processus de stabilisation des agrégats est lié à la communauté microbienne du sol. Nous avons identifié la résistance au mouillage rapide comme le principal mécanisme de stabilité des agrégats determiné par les microorganims. L'hypothèse plausible est que l'agriculture biologique favoriserait la croissance des champignons, améliorant ainsi l'aspect hydrophobe des agrégats du sol par apport au sol des substances hydrophobes d'origines fongiques. En revanche, nous n'avons pas réussi à identifier le pool de C contrôlant la formation d'agrégats, probablement à cause de la perturbation due au travail mécanique du sol et qui contribue à la destruction des agrégats. Le fractionnement des agrégats basé sur le mécanisme physique proposé dans cette étude a été utile dans la compréhension mécaniste du rôle des microorganismes dans l'agrégation du sol et il pourrait être suggéré pour l'étude de l'impact de la gestion des écosystèmes sur les pools de C et pour évaluer les propriétés des agrégats et de leurs relations dans les sols agricoles.
Mots clés: *pools de C organique, C microbien, agrégats de sol, mouillage rapide, abrasion de l'eau*

Riassunto

Per identificare le frazioni di C coinvolte nell'aggregazione del suolo, nel presente lavoro è proposto un frazionamento degli aggregati fisicamente basato, e pretrattamenti aggiuntivi sono suggeriti nella determinazione degli aggregati stabili di dimensioni di 1-2 mm al fine di chiarire l'importanza del ruolo dei microorganismi del suolo rispetto ai diversi meccanismi di rottura degli aggregati. Lo studio è stato condotto su tre Regosols franco argillosi, sviluppati su scisti calcarei, gestiti secondo i criteri dell'agricoltura biologica. Il risultati mostrano che la frazione di C coinvolto nel processo di stabilizzazione degli aggregati è quello relativo alla comunità microbica. La resistenza al fast wetting è il principale meccanismo della stabilità degli aggregati in cui i microrganismi sono coinvolti. L'ipotesi plausibile è che la gestione del suolo favorisca lo sviluppo dei funghi, migliorando l'idrorepellenza degli aggregati del suolo grazie alla presenza di sostanze idrofobiche fungine. Per contro, non siamo riusciti a riconoscere l'influenza delle diverse frazioni di C nella formazione degli aggregati, probabilmente a causa del disturbo della lavorazione meccanica dei suoli che contribuisce alla rottura degli aggregati. Il frazionamento degli aggregati su base fisica proposto in questo studio risulta utile nella comprensione meccanicistica del ruolo dei microrganismi nell'aggregazione del suolo e può essere suggerito per studiare l'impatto della gestione sui pools di C, sulle proprietà degli aggregati e sulle loro relazioni in terreni agricoli.
Parole chiave: *frazioni di C organico, C microbico, aggregati del suolo, inumidimento rapido, abrasione dell'acqua*

Introduction

The abundance and stability of the aggregates are critical for several soil functions, such as plant growth (Hamblin, 1985), resistance to erosion (Le Bissonnais and Arrouays, 1997; Barthès and Roose, 2002), soil organic matter turnover (Puget et al., 1999; Six et al., 2001), presence, activity and diversity of organisms, both mesofauna (Quénéhervé and Chotte, 1996) and microflora (Six et al., 2006). On the other hand, as regards to the latter, soil biota are strongly involved in stabilising aggregates. In particular, soil microorganisms stabilise soil aggregates in several ways as reviewed by Chenu and Cosentino (2011): fungi act mainly through mechanical enmeshments of soil particles, bacteria and fungi exude extracellular polysaccharides which bind the particles and increase inter-particle cohesion, and fungi increase water repellency of soil aggregates by exuding hydrophobic substances.

Several experiments investigating the role of microorganismis in soil aggregation were based on the physically separation of soil aggregates and the most common methods are based on sieving process. The major problem associated with these procedures is the degree of disaggregation of the soil before fractions are separated (Ladd et al., 1996). In the wet sieving methods, the compression of air entrapped in the pores during rapid rewetting of soils exerts a force on the solid mass that causes aggregate breakdown, but if the soil is rewetted slowly, the air can escape and so there is less force than when the soil is rapidly water saturated. It should be noted that some authors recommended more vigorous methods, such as using agitator beads, which are particularly useful for studying microaggregates in clay soils (e.g., Vertisols; Jocteur Monrozier et al., 1991), and there are a number of papers in the literature which show the effect of increasingly vigorous dispersion on the destruction of macroaggregates (Bossuyt et al., 2001). However, Ladd et al. (1996) observed that finer particles obtained by energetic dispersion procedures may contain artefacts, because of the disruption of organisms and consequent release of soluble materials that could readily associate with clay surface exposed for the first time to modern biological compounds.

The difficulties with all these procedures are both to standardise the methods and to compare the energy inputs with other methods (Chotte, 2005). Moreoer it should be taken into account that soils have different structural stabilities and react differently to these disaggregating procedures (Le Bissonnais and Arrouays, 1997). When exposed to water stress of wet sieving, soil aggregates undergone to breakdown caused by the wetting (slaking and swelling), the chemical (dispersion by water) and the shaking action (water abrasion) as a whole. The use of sample pretreatments has been thus suggested to discriminate the single aggregate breakdown mechanism (e.g. Hénin et al., 1958; Le Bissonnais, 1996). This allows to detect the relevance of different soil properties with respect to the aggregate breakdown mechanisms and to differently rank soils on the basis of their

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physically-based aggregate behaviour (Amezketa et al., 1996; Le Bissonnais and Arrouays, 1997). For instance, Oades and Waters (1991) observed that in an Alfisol and a Mollisol larger aggregates were progressively broken down into smaller aggregate using increasing intensity of aggregate disruption (i.e., from slow to fast wetting, and further shaking) allowing to illustrate the concept of aggregate hierarchy driven by organic cements, conversely to what occurred for Oxisol, where clay particles act as main cementing agent.
In this study an approach to fractionation of soil aggregate attempting to isolate fractions that were physically meaningful is proposed, in order to identify the C pools controlling the processes of formation and stabilisation of aggregates and to mechanistically understand how soil microorganisms improve soil stability and how they were related to C associated to stable aggregates. The sampling site is agrarian and since 2004 had known history of organic cultivation, and neither nutrients nor organic matter inputs were supplied. The soils were characterised by a weak evolution degree (i.e., Regosols; IUSS Working Group WRB, 2007) and developed on calcareous shales. As such, the study site is able to provide a natural baseline for C pools content and aggregate properties in similar soils and parent materials.

Materials and Methods

Study area

Three clay loam Regosols (A, B and C) were selected from Monte Pastore (44°22' N, 11°08'E; Bologna province, northern Italy). The soils were representative of the most common soil types of agricultural land in the northern Italian Appennine. They developed on shales and calcilutites (E-R Ambiente, Servizio geologico sismico e dei suoli, 2006). The sampling sites were chosen on a moderate slope (15%). The area has a temperate warm subcontinental climate, the mean annual rainfall is 820 mm and the mean temperatures ranges from 10 to 15°C. The soil moisture and temperature regime were ustic and mesic respectively. The main characteristics of soil profiles were reported in Guidi et al. (2013).
In the study area, since 2004 crops rotation was practised with certified organic wheat, peas, barley and clover. Each year, soil structure was disturbed by tillage with mouldboard plough, working to 20 cm depth, for the control of weeds.
In the early autumn 2010 after tillage of fields, two pits in each site were dug and soil samples were taken from the 0-20 cm topsoil of each pit.

Aggregate fractionation

The bulk soil (BS) samples collected from the 0-20 cm topsoil were air-dried to constant weight and dry-sieved to pass a 2-mm screen (<2 mm). Then, the 1-2 mm aggregates (AG) were separated from the fine earth fraction by dry sieving to 1 mm and weighted. A weighted aliquot of AG was placed in 0.2-mm sieve and wetted by immersion in water for 10 min (i.e., water saturation), then wet sieved at 60 cycles min^{-1} (i.e., water abrasion). At the end of wet sieving, the aggregates

remaining in the sieves were oven dried at 40°C, weighted and their amount was expressed as the percentage of AG (MA). This separated fraction represented the macroaggregates stable to water stress (i.e., water saturation and abrasion).

Measurement of the 1-2 mm aggregate stability to the different breakdown mechanisms

The wet stability of the 1-2 mm aggregates to different aggregate breakdown mechanism was determined by Yoder's (1936) wet sieving procedure (Kemper and Rosenau, 1986) using pretreatments as a way to elucidate the mechanisms and processes involved in aggregate breakdown (Hénin et al., 1958; Le Bissonnais, 1996).
The aggregate stability to water saturation and abrasion (WAS_t) was determined as follows

$$WAS_t = \frac{MA - coarse\,sand}{100 - corse\,sand} \cdot 100$$

WAS_t, MA and coarse sand were expressed as percentage. The coarse sand fraction (>0.2-mm) was determined on 1-2 mm aggregate after H_2O_2 oxidation.
To test the resistance to water saturation, the aggregate stability to fast wetting (WAS_{fw}) was determined on 10 g of 1-2 mm aggregates in a 0.2-mm sieve, gently immersed in water for 10 min, and weighting the dried aggregates (at 40 °C) remaining in the sieves. The WAS_{fw} index was calculated as

$$WAS_{fw} = \frac{weight\ of\ retained\ materials - weight\ coarse\,sand}{10\ g\ of\ aggregates - weight\ corse\,sand} \cdot 100$$

The weight of coarse sand fraction (>0.2-mm) was determined as described above. An aliquot of retained aggregates was stored for further analysis.
To test the wet cohesion independently from the breakdown due to water saturation and thus determine the wet aggregate stability to water abrasion, 10 g of 1-2 mm aggregates were placed in sieves and gently immersed in 95% solution of ethanol for 10 min (Le Bissonnais, 1996) and then wet sieved for 10 min at 60 cycles min^{-1} . The material remaining in the sieve was oven dried, weighted and the stability index related to the abrasion (WAS_{ab}) was calculated as follows

$$WAS_{ab} = \frac{weight\ of\ retained\ materials - weight\ of\ coarse\,sand}{10\ g\ of\ aggregates - weight\ of\ coarse\,sand} \cdot 100$$

The weight of coarse sand fraction (>0.2-mm) was determined as described above.

Determination of microbial biomass of 1-2 mm aggregates

On the 1-2 mm aggregates (AG), the microbial biomass C (MBC) and N (MBN) were determined using the fumigation extraction method (Vance et al., 1987;
Brookes et al., 1985) carried out on soil samples previously conditioned by incubation for one week at 60% of their water holding capacity in a thermostat at

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20°C. The organic C and N extracted by 0.5M K_2SO_4 from $CHCl_3$-fumigated and non-fumigated samples were measured by a TOC-TN analyzer (Shimadzu, TOC-V/CPN). For the calculation of MBC and MBN, a k_{EC} = 0.45 (Jenkinson et al. 2004) and k_{EN} = 0.54 (Brookes et al. 1985; Jörgensen and Müller 1996) were used, respectively. The C extracted by 0.5M K_2SO_4 from non-fumigated soil samples was used to estimate the labile C pool (Badalucco et al., 1992).

Soil and aggregate associated- C and N analysis

On the bulk soil (BS), 1-2 aggregates (AG), aggregates stable to fast wetting and water abrasion (MA) and aggregates retained on the sieve after water immersion alone, the amount of the total organic C and total N were determined by dry combustion (EA-1110 Thermo Scientific Lab.).

Statistical analysis

All statistical analyses were carried out using SPSS software package (SPSS Inc., Chicago, IL). The correlations were estimated using Pearson's or Spearmann's coefficients depending on the linearity of dependence evaluated by visual inspection of the data. After the one-way analysis of variance, differences among sites were estimated using the Duncan's test. A probability level of 0.05 was always used as threshold for significance.

Results

Aggregate distribution and aggregate stability

In all sites the 1-2 mm aggregates (AG) constituted the 40% of the 0-20 cm bulk soil. The 60% and 81% of AG was stable to water stress (MA%, i.e., aggregate stable to water saturation and abrasion) in site A and B-C, respectively (Figure 1). Similarly to MA, the corresponding wet aggregate stability index (WAS_t) followed the trend A<C=B, as a significant lower WAS_t value in site A than C and B was observed (57 and 79-81%, respectively; Table 1). The lowest resistance of aggregates from site A to water saturation and abrasion was also confirmed by both the fast wetting test and the ethanol pretreatment (WAS_{fw} and WAS_{ab}, respectively; Table 1).

Microbial biomass and labile C

In the 1-2 mm aggregates from soil samples collected at 0-20 cm depth, the microbial biomass C (MBC) varied from 519 to 894 mg C kg^{-1} (Table 2). The amount of microbial biomass N (MBN) was similar between sites (99-107 mg N kg^{-1}; Table 2). The amount of labile C (i.e., C extractable in K_2SO_4) was 173-205 mg C kg^{-1}. No differences were found between sites.

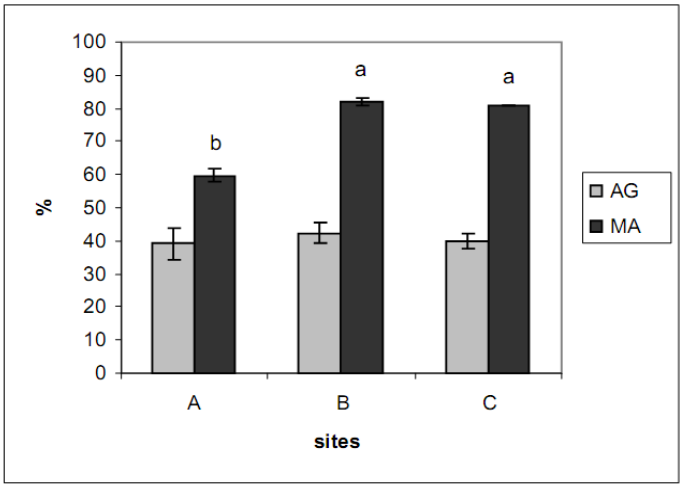

Figure 1

The percentage of 1-2 mm aggregates (AG) and of 1-2 mm aggregates stable to water stress (MA). The letters show the significant differences at p level <0.05 (Duncan's test) between sites.

Table 1 - *Wet 1-2 mm aggregate stability (WAS) to different breakdown mechanisms: fast wetting (fw), water abrasion (ab) and total (i.e., fast wetting and water abrasion; t). Numbers in italics showed the standard deviation. The letters show the significant differences at p level <0.05 (Duncan's test) between sites*

Site	WAS_{fw}		WAS_{ab}		WAS_t[§]	
		%		%		%
A	[b]83.4	*5.4*	[b]73.9	*3.1*	[b]57.3	*2.3*
B	[a]97.6	*2.2*	[a]83.3	*1.3*	[a]80.9	*0.9*
C	[a]95.6	*1.1*	[a]83.9	*1.0*	[a]79.5	*0.1*

[§]Data published in Guidi et al. (2013)

Table 2 - *Microbial biomass C (MBC) and N (MBN), and K_2SO_4 extractable C in the 1-2 mm aggregates. Numbers in italics showed the standard deviation.*

Site	MBC		MBN		K_2SO_4 extractable-C	
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
A	519	*161*	105	*2*	205	*9*
B	894	*21*	107	*3*	173	*2*
C	640	*165*	99	*11*	176	*33*

Total organic C and N

The amount of total organic C (TOC) of the bulk soil (BS) was 13.2, 14.4 and 18.1 g C kg^{-1} in A, C and B sites, respectively, and it was significant lower in the A site than in B and C ones (Table 3). Although no significant differences between sites were observed in the amount of C associated to 1-2 mm aggregate (AG), the AG of site A had lower C content than site B and C (Table 3). This trend was also observed in the amount of C associated to stable aggregates, even if the C content

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in the stable aggregates was always lower than in AG, especially in MA.
On the average the N amount in the whole soil was 2.1 g kg^{-1} (Table 3). The amount of N associated to both aggregates and stable aggregates slightly decreased, in particular in MA as already observed for TOC. Significant difference between sites was observed nor for total N of the whole soil neither for aggregate associated-N. The TOC/TN ratio was on the average 7.4 and it was similar among sites and aggregate fractions.

Table 3 - *Content of total organic C (TOC), total N (TN) and TOC/TN ratio in the bulk soil (BS), in aggregates (AG) , in aggregates resistant to fast wetting and to fast wetting and water abrasion (MA). Numbers in italics showed the standard deviation. The letters show the significant differences at p level <0.05 (Duncan's test) between sites*

Site	Bulk soil (BS)						Aggregates (AG)					
	TOC§		TN§		TOC/TN		TOC§		TN§		TOC/TN	
	g kg^{-1}						g kg^{-1}					
A	[b]13.2	*1.0*	2.0	*0.2*	6.8	*0.2*	12.3	*0.1*	1.8	*0.1*	7.0	*0.3*
B	[a]18.1	*0.6*	2.5	*0.2*	7.4	*0.9*	17.6	*1.3*	2.3	*0.0*	7.6	*0.6*
C	[ab]14.4	*2.1*	1.8	*0.1*	8.0	*0.6*	15.2	*3.8*	2.0	*0.0*	7.6	*1.9*

Site	Aggregates resistant to fast wetting						Aggregates resistant to fast wetting and water abrasion (MA)					
	TOC		TN		TOC/TN		TOC		TN		TOC/TN	
	g kg^{-1}						g kg^{-1}					
A	9.9	*0.0*	1.4	*0.3*	7.0	*0.6*	8.1	*0.0*	1.2	*0.1*	6.7	*0.5*
B	16.9	*0.1*	2.2	*0.6*	7.6	*2.0*	16.1	*1.6*	2.2	*0.2*	7.3	*0.1*
C	14.0	*0.0*	1.9	*2.1*	7.4	*0.5*	11.7	*0.4*	1.6	*0.1*	7.3	*0.1*

§Data published in Guidi *et al.* (2013)

Relationships between microbial biomass and aggregate properties

Of the C pools measured in this study, MBC was found to be positive correlated to the amount of stable aggregates (MA) (Table 4).

Table 4 - *Spearman's correlation coefficients (r_s) between measures of C pools in the 1-2 mm aggregates (AG) and indexes of soil aggregation. In each row, the correlation coefficient is given with the p-value below it in italic.*

C pools in AG	Aggregate properties				
	% of AG	% of MA	WAS_{fw}	WAS_{ab}	WAS_t
TOC	-0.143	0.671	0.614	0.657	0.671
	ns	*ns*	*ns*	*ns*	*ns*
MBC	0.086	0.829	0.771	0.257	0.839
	ns	*0.042*	*0.042*	*ns*	*0.041*
K_2SO_4 extractable-C	0.429	0.600	0.714	-0.371	0.611
	ns	*ns*	*ns*	*ns*	*ns*

MBC was also positively related to the aggregate stability index to the water saturation resistance (WAS_{fw}) but not to the water abrasion one (WAS_{ab}). Moreover, MBC was positively related to all aggregate associated-C and N pools (Table 5). However, according to the results of our stepwise regression analysis, TOC in 1-2 mm aggregates (AG) rather than MBC was selected as better predictor of the amounts of C in stable aggregates to fast wetting (r^2=0.98, p<0.01; data not shown) and in stable aggregates to fast wetting and water abrasion (MA; r^2=0.71, p<0.05; data not shown), respectively. Instead MBN was not related to any C and N pools.

Table 5 - *Pearson's correlation coefficients (r) between measures of microbial biomass and aggregate associated-C and N. In each row, the correlation coefficient is given with the p-value below it in italic.*

Microbial biomass	**Aggregate associated-C and N**					
	aggregates (AG)		aggregates resistant to fast wetting		aggregates resistant to fast wetting and water abrasion (MA)	
	TOC	TN	TOC	TN	TOC	TN
MBC	0.862	0.894	0.856	0.823	0.839	0.807
	0.027	*0.016*	*0.030*	*0.044*	*0.037*	*0.049*
MBN	0.462	0.111	0.425	0.094	0.131	0.182
	ns	*ns*	*ns*	*ns*	*ns*	*ns*

Discussion

The physically-based aggregate fractionation proposed in this work (1-2 mm aggregate separated by dry sieving, and further separation of aggregates stable to water stress by wet sieving after 10 min of rapid immersion in water) was helpful in the identification of the C pools involved in the aggregation of the studied Regosols. Furthermore, the additional pretreatments (rapid water immersion for 10 min to test the aggregate resistance to water saturation and ethanol immersion for 10 min followed by wet sieving for 10 min to test the wet cohesion) used in the measurement of the 1-2 mm aggregate stability allowed to elucidate the relevance of the role of soil microorganisms with respect to the different aggregate breakdown mechanisms. Indeed, according to the proposed procedure, of the C pools measured only the microbial biomass C seemed involved in stabilising aggregates improving resistance to stress caused by rapid immersion in water.

The TOC content seemed to influence nor the 1-2 mm aggregate formation, neither their stabilisation. In all sites the 1-2 mm aggregates amounted to 40% of the bulk soil independently from the TOC content, and the amount of water stable 1-2 mm aggregates was not related to TOC value. Moreover, the lack of correlation between the wet aggregate stability indexes and the TOC allowed to detect the ineffectiveness of the TOC against the breakdown caused by both fast wetting and water abrasion. Organic matter is undoubtedly one of the main binding agent in soil, and relationships between TOC and aggregate size distribution and aggregate stability have been often reported (Six et al., 2004; Bronick and Lal, 2005).

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However, it is well kwon that the use of not excessive tillage is recommended for sustainable agriculture because of its negative effect on soil aggregation and TOC (Lal, 2008). Thus, it was possible that we did not found any correlation between TOC and aggregates properties because of the physical disturbance of tillage operations carried out each year for control of weeds.

No effects on soil aggregation were also found for the labile organic C fraction (i.e., K_2SO_4-extractable C), and microbial biomass C was not related to the amount of 1-2 mm aggregates. However, the positive correlation between MA and MBC supports the hypothesis that microbial growth is an important factor affecting soil aggregate stability, even if it is well known that tillage system also impact microbial community. In fact, as summarised by Six et al. (2006), in tillage systems a decline of the effect of microorganisms on soil aggregation can occur because of the inhibition of fungal growth and activity due to declined establishment and maintenance of extensive hyphal networks, or because of the changes in soil moisture that may differently influence bacteria and fungi either by directly affecting survival and growth or indirectly by shifting substrate availability. In our study tillage operation had probably inhibit the involvement of microbial biomass in aggregate formation of soils, but our results showed that microorganisms effect on aggregate stabilisation was preserved. This could be due to several raisons. Firstly, organic farming practices promote higher total microbial biomass than conventionally managed soils and shifts the community structure towards a more fungal dominated community (Six et al., 2006); therefore, it is reasonable to suppose that in our soils fungi dominated over bacteria. Secondly, previous studied showed that increased resistance to slaking and mechanical breakdown is related to fungal activity enhancing both the internal cohesion of aggregates and hydrophobicity of aggregates (Cosentino et al., 2006). In our soils the positive effect of MBC on soil aggregate stability was significant for fast wetting stress, but not for mechanical cohesion. Therefore, it seemed that organic management had promote fungal activity improving aggregate stability to fast wetting probably because of water repellency of soil aggregates by fungal hydrophobic substances (Chenu and Cosentino, 2011).

The amount of microbial biomass C was directly related to the C concentration in the 1-2 mm aggregates and, as consequence, to the C concentration in the stable aggregates. Thus, MBC appeared as an efficient stabilising agents, but it was not selectively preserved in stable aggregates. As regards to MBN, it was not related to any C and N pools in 1-2 mm aggregates. In accordance with these results, Hurisso et al. (2013) underlined the role of MBN in microaggregates (<250 μm) and MBC in macroaggregates (>250 μm), thus supporting that microbial growth is likely one of the major factor affecting macroaggregates in our soils.

Conclusions

In our Regosols the C pool controlling the process of stabilisation of aggregates was related to the microbial community. We identified the resistance to fast wetting as the major mechanism of aggregate stability driven by microorganisms.

The plausible hypothesis is that organic farming promotes fungi growth, improving water repellency of soil aggregates by fungal hydrophobic substances. By contrast, we failed in the identification of C pools controlling the formation of aggregates, probably because of the disturbance of mechanical tillage which contributes to the breakdown of soil aggregates.
The physically-based aggregate fractionation proposed in this study resulted useful in the mechanistically understanding of the role of microorganisms in soil aggregation and it might be suggested for studying the impact of management on C pools, aggregates properties and their relationships in agricultural soils.

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