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Linking organic matter chemistry with soil aggregate stability: Insight from <sup>13</sup>C NMR spectroscopy

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## Covering Letter

8<sup>th</sup> September, 2017

Dear Joshua Schimel,  
Chief Editor  
Soil Biology and Biochemistry

According with Associated Editor and the two reviewers' comments, we have revised the manuscript "Linking organic matter chemistry with soil aggregate stability: insight from <sup>13</sup>C NMR spectroscopy", Ref.: Ms. No. SBB12426.

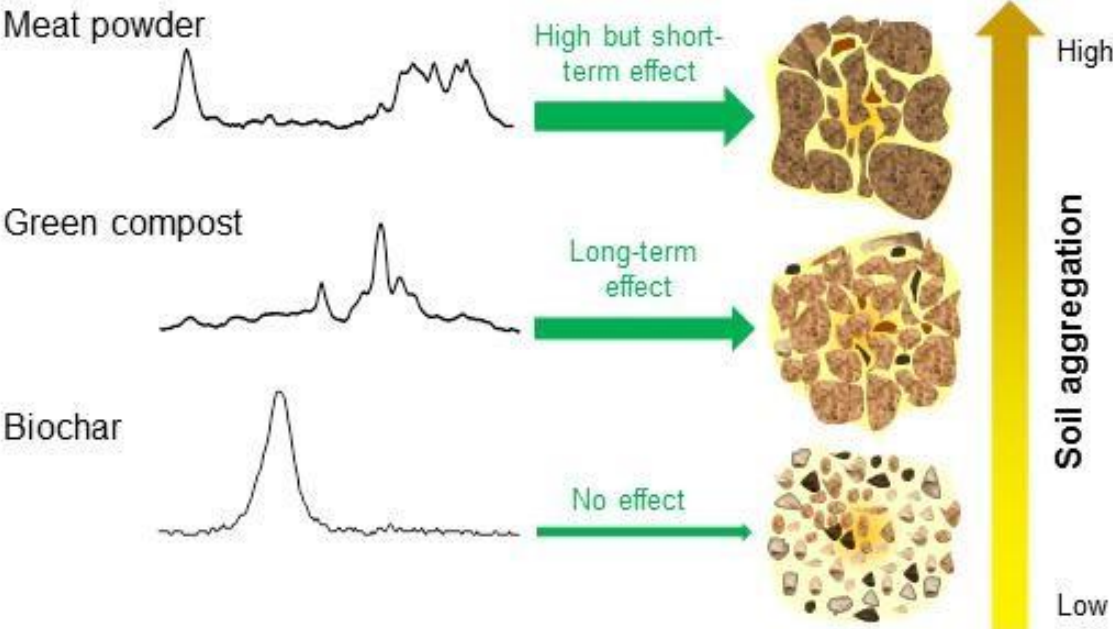
In the revised version, we have changed the text providing answers to all the numerated points raised by the referees. Please, find in attach the reviewer's comments with our point-by-point responses (reported in italic type and red font) and the revised manuscript with and without changes highlighted (track changes version).

Thanks are due to both reviewers for their comments that allowed us to greatly improve the manuscript. We also thank the Editor for giving us a chance to resubmit this manuscript.

Sincerely,  
Tushar C. Sarker (corresponding author) and all co-authors



**Graphical Abstract**



## \*Highlights (for review)

### Highlights

- Meat powder and alfalfa litter induce rapid soil aggregation.
- Biochar incorporation barely affects soil aggregation dynamics.
- Organic matter carbon distribution by  $^{13}\text{C}$  NMR explains soil aggregation patterns.
- Aggregation is positively associated to carbohydrate fractions of soil amendments.
- High content of aromatic carbons hamper soil aggregation.

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1 **Title:**

2 Linking organic matter chemistry with soil aggregate stability: insight from  $^{13}\text{C}$  NMR 3  
spectroscopy

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26



26 **ABSTRACT**

27 Soil aggregation is considered as a crucial process in agro-system sustainability due to the role in  
28 soil physical, chemical and biological dynamics. In this context, we tested the hypothesis that the  
29 initial chemical characteristics of organic matter (OM) are suitable to explain the variability of soil  
30 aggregation dynamics after organic amendment. All OM types were characterized by  $^{13}\text{C}$ CPMAS  
31 NMR and elemental chemical parameters to investigate the effects of amendment quality on soil  
32 aggregation. Manipulative experiments were carried out in controlled conditions, using three soil  
33 types (S1, S2 and S3), amended with ten chemically different OM (alfalfa litter, biochar, cellulose,  
34 glucose, green compost, maize litter, manure compost, meat powder, sawdust, and solid digestate),  
35 plus the untreated controls. Treatments were incubated in triplicate mesocosms for 300 days and  
36 sampled at 4 dates for measuring aggregation index (AI), based on water stability of soil aggregates  
37 (WSA). We found that meat powder and alfalfa litter induced a rapid initial increase of AI,  
38 exceeding that of the controls by one to two orders of magnitude, likely acting as a C source for  
39 microbes. Biochar incorporation in soil barely affected AI, with intermediate effects with other  
40 OM types. Considering C bond types corresponding to OM  $^{13}\text{C}$ CPMAS NMR spectral regions, *O*-  
41 alkyl C and di-*O*-alkyl C (carbohydrate fraction) was positively associated to AI, while *H*, *C*-  
42 substituted aromatic C and *O*-substituted aromatic C (aromatic fraction) showed a significant  
43 negative correlation with AI. This study demonstrates that OM chemical quality is a major  
44 controlling factor in soil aggregation process, with organic C quality defined by  $^{13}\text{C}$ -CPMAS NMR  
45 spectroscopy being more predictive of aggregation dynamics compared to classical elemental  
46 parameters. As such, this study provides a significant novel contribution to clarify the relationships  
47 between OM chemistry and soil aggregation.

48

49 **Key words:** Soil aggregation; Organic amendment;  $^{13}\text{C}$ -CPMAS NMR; Soil microbes; C/N  
50 ratio.

## 51 **1. Introduction**

52 Soil aggregation is an important ecosystem process leading to the formation and stabilization of  
53 soil structure, i.e. the resulting matrix of soil aggregates and pore spaces (Rillig et al., 2015).  
54 Aggregates can form by rearrangement, flocculation and cementation of mineral and organic  
55 particles (Bronick and Lal, 2005). Soil structure facilitates gas and water diffusion, promoting  
56 microbial function, root penetration and growth, and reducing soil susceptibility to erosion (Annabi  
57 et al., 2011; Six et al., 2004). Therefore, soil aggregation is considered as a crucial aspect of soil  
58 quality and a keystone for a sustainable management of agro-ecosystems.

59 The ecological factors that affects soil aggregation have been well documented and reviewed  
60 (Amezketta, 1999; Bronick and Lal, 2005; Six et al., 2004). The formation and stability of soil  
61 aggregates depends on several biological, chemical, and physical processes. Soil biotic community  
62 and biological mechanisms play a vital role in the stabilization of soil structure (Degens, 1997), as  
63 well as known destabilizing agents (Oades, 1993). Soil biota is involved in the aggregation process  
64 either by producing effective biological binding agents (e.g. polysaccharidic colloids by root  
65 exudates and microbial mucilages that promote the formation of aggregates), or by mechanically  
66 holding soil particles together, as shown for fungal hyphae, mycelium, and plant roots (Rillig et  
67 al., 2015). Among chemical mechanisms, organo-mineral interactions may lead to aggregations of  
68 clay particles and OM, which stabilize soil structure and C compounds within the aggregates  
69 (Oades, 1988). These organo-mineral interactions rely on cation bridges, where cations provide a  
70 link between negatively charged clay surface and negatively charged organic functional groups  
71 (Muneer and Oades, 1989). Moreover, organomineral interactions and mineralogical influences on  
72 soil aggregation revealed that the occlusion of iron oxyhydroxides provides a cementation of other  
73 minerals directly in contact with OM (Keil and Mayer, 2014), and iron oxyhydroxides act as  
74 binding agents in some aggregates, particularly in highly weathered soils.

75 Soil texture, clay mineralogy, cation content, and OM are considered the main abiotic  
76 determinants. OM can either directly act as a binding agent (Piccolo and Mbagwu, 1999; Spaccini  
77 et al., 2002) or indirectly promote soil microbial activity, thus enhance aggregate formation and  
78 stability maintenance (Murphy, 2015). In fact, a variety of organic compounds that promote  
79 aggregate stability can be produced by fungal and bacterial activity (Hendrix et al., 1990), or  
80 released during OM decomposition (Schmidt et al., 2011). The controlling role of OM on aggregate  
81 stability is especially important because its amount and properties can be modified through  
82 agronomic management. A wide range of non-humified organic materials are routinely used as soil  
83 amendments, including crop residues, composts, peats, and organic wastes from agro-industries.  
84 Most of the published studies on this matter have assessed the immediacy of organic amendment  
85 effects on soil aggregate stability, generally reporting a positive effect. However, beside successful  
86 applications of organic amendments (Abiven et al., 2007; Annabi et al., 2011; Six et al., 2004)  
87 inconsistencies about their ameliorating effect have also been reported (Albiach et al., 2001;  
88 Spaccini et al., 2002). On the other hand, the use of humified organic matter extracted from carbon-  
89 rich sources such as lignite or oxidized coal were proved not only to increase soil aggregate  
90 stability but also to reduce run-off erosion (Piccolo et al., 1997). The different results, thus call for  
91 further investigations to clarify the impact of different OM types on soil aggregate structure.

92 The first step in this direction was made more than 50 years ago by Monnier, (1965), who  
93 proposed a conceptual model describing aggregate stability dynamics after the organic amendment  
94 in soil with different quality. In particular, easily decomposable amendments (e.g. green manure)  
95 had an intense effect on aggregate stability at the very short term (week to month), which peaked  
96 at progressively later stages for lower quality materials (e.g. on a monthly scale for wheat straw).  
97 Differently, more recalcitrant amendments (e.g. decomposed manure) had a low initial effect  
98 progressively increasing over time. Later, a meta-analysis based on 48 empirical studies Abiven et  
99 al., (2009) qualitatively validated such conceptual model, but also indicated the need for effective

100 predictors of OM quality and aggregate stability, in order to properly translate the model into  
101 effective agronomic practices. Indeed, in the Monnier's model the amendment quality is largely  
102 assumed by simply naming the organic input (i.e. green manure *vs* straw *vs* decomposed manure),  
103 which is possibly advantageous for application purpose, but cannot provide accurate predictions  
104 of the effect magnitude and dynamics, in terms of aggregate stability. In this context, some studies  
105 used the well know C/N ratio as predictor of OM quality, but reported inconsistent relationships  
106 with aggregate stability (Martens and Frankenberger, 1992; Sonnleitner et al., 2003).

107         The limited effort in search of chemical indicators to consistently describe OM chemistry  
108 and predict aggregate stability is surprising, especially considering the recent advance in chemical  
109 analytic techniques. In this perspective, several throughput methods have been applied to directly  
110 characterize OM chemistry, including pyrolysis-gas chromatography/mass spectrometry (Huang  
111 et al., 1998), near infrared reflectance spectroscopy (Gillon et al., 1999), and <sup>13</sup>C-cross-polarization  
112 magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Kögel-  
113 Knabner, 2002). In detail, <sup>13</sup>C-CPMAS NMR has been proven useful to relate the characteristics  
114 of soil organic matter to soil aggregate stability (Piccolo et al., 2005; Spaccini et al., 2006) and  
115 provide a description of chemical composition of complex matrices, such as plant litter (Kögel-  
116 Knabner, 2002), and its relationships with decay rate (Bonanomi et al., 2013) and plant growth  
117 (Mazzoleni et al., 2015).

118         In this study, we combined a detailed OM characterization by <sup>13</sup>C-CPMAS NMR, in solid  
119 state with a manipulative experiment of soil incubation in mesocosms to investigate the link  
120 between OM carbon distribution and soil aggregation stability over a 1-year observation period. In  
121 detail, we tested the capability of 10 organic amendments, spanning over a wide range of chemical  
122 quality, to induce soil aggregation in three soil types with different texture. Specific aims of the  
123 study were to:

124 (i) assess the magnitude and timing of aggregation stability induced by different organic  
125 amendment types and describe their effects 1 year after OM incorporation in soils; (ii)  
126 explore the relationships between soil aggregation dynamics and organic amendment  
127 quality, as defined by  $^{13}\text{C}$ -CPMAS NMR spectroscopy and standard chemical parameters;  
128 (iii) identify the  $^{13}\text{C}$ -CPMAS NMR spectral regions and corresponding organic C types more  
129 predictive of organic amendment effects on soil aggregate stability.

130

## 131 **2. Materials and methods**

### 132 *2.1. Chemical characterization of organic amendments*

133 Ten types of organic amendment were selected, as representing a wide range of OM with different  
134 chemical composition and biochemical stability ([Table 1](#)). They were characterized for total C  
135 and N content by flash combustion of micro samples (5 mg of sample) in an Elemental Analyzer  
136 NA 1500 (Fison 1108 Elemental Analyzer, Thermo Fisher Scientific). All organic amendments  
137 were analyzed by solid state  $^{13}\text{C}$ -CPMAS NMR, thus allowing a comparative and detailed  
138 characterization of molecular properties. The NMR spectras were recorded on a Bruker AV-300  
139 instrument (Bruker Instrumental Inc, Billerica, MA, USA), equipped with a magic angle spinning  
140 (MAS) probe with wide-bore of 4 mm, using specific calibrated acquisition parameters:

141 2 s of recycle time;  $^1\text{H}$ -power for CP 92.16 W:  $^1\text{H}$   $90^\circ$  pulse 2.85  $\mu\text{s}$ ;  $^{13}\text{C}$  power for CP 150,4 W;  
142 1 ms of contact time; 20 ms of acquisition time; 2000 scans. Samples were packed in 4 mm  
143 zirconium rotors with Kel-F caps. The cross polarization pulse sequence was applied with a  
144 composite shaped “ramp” pulse on the  $^1\text{H}$  channel in order to account for the inhomogeneity of  
145 Hartmann-Hann condition at high rotor spin frequency. The Fourier transform was performed with  
146 4 k data point and an exponential apodization of 50 Hz of line broadening. Selection of spectral  
147 regions and identification of corresponding classes of organic C bonds were performed according  
148 to previous studies ([Bonanomi et al., 2013](#); [Kögel-Knabner, 2002](#); [Mathers et al.,](#)

149 [2007; Piccolo et al., 2005](#)). The following seven chemical shift regions representatives of main C  
150 types were considered: 0-45 ppm = alkyl C; 46-60 ppm = *N*-alkyl and methoxyl C; 61-90 ppm =  
151 *O*-alkyl C; 91-110 ppm = di-*O*-alkyl C; 111-140 ppm = *H*- and *C*- substituted aromatic C; 141-160  
152 ppm = *O*-substituted aromatic C (phenolic and *O*-aryl C); and 161-190 ppm = carboxyl C. The  
153 relative contribution of each region was determined by integration of MestreNova 6.2.0 software,  
154 Mestre-lab Research 2010 , and expressed as percentage of the total area.

155

## 156 2.2. Aggregation experiment

157 Three agricultural soils, hereafter named S1 (Capasso), S2 (Castel Volturno), and S3 (Torino) were  
158 selected for the soil incubation experiment, as showing different texture and nutrient availability,  
159 but similar organic matter content ([Table 2](#)). S1 soil (40°59'57.89" N, 14°19'00.89" E, elevation  
160 24 m a.s.l., mean annual temperature 15.9°C and rainfall 988 mm), is a Silandic and vitric andosols,  
161 dedicated to horticulture, has a loamy texture with slightly higher soil organic carbon content. S2  
162 soil (41°01'58" N, 13°56'36" E, elevation 3 m a.s.l., mean annual temperature  
163 17°C and rainfall 599 mm), is a Vertic xerofluvent ([USDA Soil Taxonomy System, Soil Survey  
164 Staff, 1999](#)) from the experimental farm of the Agricultural Department of University of Napoli,  
165 with a clay loam texture and maize as main crop. S3 soil (45°04'13" N, 7°41'12" E, elevation 245  
166 m a.s.l., mean annual temperature 11.5°C and rainfall 670 mm), from the LTE experimental sites  
167 of the Agricultural Universities of Torino, with long term maize cropping system, is a Typic  
168 ustifluent with a sandy loam texture ([Supplementary Fig. S1](#)). Soils were collected from the top  
169 layer (first 20 cm) of agricultural fields, sieved at 2 mm and oven air-dried at 30°C.

170 The aggregation experiment was carried out in mesocosms in laboratory condition. Plastic  
171 jars were filled with 200 g of dry soil and were incorporated with 4 g (2% w/w) of each dry OM  
172 type and thoroughly mixed. All OM types were previously dried and grounded at 2 mm.  
173 Mesocosms were kept in a growth chamber under controlled temperature (18±2°C night and

174 24±2°C day) and moisture content was monitored regularly and watered every seven days to field  
 175 capacity with distilled water. While criticizable when applied to standardize experimental  
 176 conditions for different soils, watering to field capacity was considered suitable in our experiment,  
 177 as applied to soil types with similar retention curves, as showed elsewhere (Bonanomi et al., 2017).

178 Both amended soil treatments (AT) and unamended controls (CT) were incubated for 10,  
 179 30, 100, and 300 days. The full experimental design included three soil types, ten organic  
 180 amendments and one control, triplicated for each of 4 incubation times, for a total of 396  
 181 experimental units. At each sampling date, the soil was collected, air dried and submitted to  
 182 assessment of soil aggregation stability.

183

### 184 2.3. Assessment of soil aggregate stability

185 Water stability of soil aggregates (WSA) was assessed according to Kemper and Rosenau, (1986).  
 186 Twenty grams of air dried soil were sieved at 4.75 mm and put in the highest of a sequence of three  
 187 sieves of 1.00, 0.50, and 0.25 mm mesh size. The soil was pre-soaked in distilled water for 30 min,  
 188 and then the nest of sieves and their contents were oscillated vertically in water 20 times using a 4  
 189 cm amplitude at the rate of one oscillation per second. After wetsieving, the resistant soil materials  
 190 on each sieve, including unstable aggregates (< 0.25 mm), were recovered, dried in the oven at  
 191 50°C for 48 h and weighted afterwards. Aggregate stability was expressed as Aggregation Index  
 192 (AI), calculated as the sum of the soil dry mass fractions remaining on each sieve after sieving,  
 193 multiplied by the mean diameter of the adjacent meshes

194 (Spaccini et al., 2004):

$$195 \quad AI = \sum_{i=1}^n X_i W_i$$

196 (1)

197 where  $X_i$  is the mean diameter of the  $i^{th}$  sieve size class (i.e.  $X_1 > 1.0$ ;  $1.0 > X_2 > 0.5$ ;  $0.5 > X_3 >$

198 0.25;  $X_d < 0.25$  mm) and  $W_i$  is the dry mass proportion of the total aggregates in the  $i^{th}$  fraction.  
199 Higher AI values indicate higher proportions of macroaggregates in the sample and therefore,  
200 higher stability.

#### 201 202 2.4. Data analysis

203 In order to explore the chemical variability of the organic amendments, a data matrix of signals  
204 recorded in their  $^{13}\text{C}$ -CPMAS NMR spectra was submitted to Cluster Analysis using the complete  
205 linkage as linking rule and Pearson's correlation coefficient as a similarity measure. Then, a  
206 Principal Component Analysis (PCA) was carried out on a data matrix of the reference spectral  
207 regions in the organic materials. In the PCA, the content of C and N of organic amendments, as  
208 well as C/N ratio, were included as supplementary variables (i.e. plotted in the multivariate space  
209 but not used to calculate the principal components), following the approach suggested by [Legendre](#)  
210 [and Legendre, \(1998\)](#).

211 Data from the manipulative experiment were submitted to Generalized Linear Modelling  
212 (GLM), considering main and interactive effects of soil type (S, three levels), organic amendment  
213 (OM, ten levels) and incubation time (treated as a continuous covariate) on soil aggregation index  
214 (AI). Pair-wise differences were tested using Tukey's HSD post-hoc test.

215 To address the relationships between the OM carbon distribution and AI recorded at different  
216 incubation times, simple linear correlation analysis was separately tested between AI and each OM  
217 chemical descriptor, including elemental chemical parameters (i.e. N and C content, C/N ratio) and  
218 regions of the  $^{13}\text{C}$ -CPMAS NMR spectra selected from reference literature ([Kögel-Knabner, 2002](#);  
219 [Mathers et al., 2007](#); [Piccolo et al., 2005](#)). Correlation was tested for statistical significance  
220 controlling for multiple comparisons, according to Bonferroni's correction.

221

### 222 3. Results



### 223 3.1. Organic matter initial biochemistry

224 The organic amendments showed a wide range of chemical quality in terms of element content  
225 (Table 1), and organic C molecular composition as assessed by  $^{13}\text{C}$ -CPMAS NMR data (Fig.  
226 1A). Considering C bond types and corresponding reference regions along the NMR spectrum, the  
227 alkyl-C (0-45 ppm) and the methoxyl and *N*-alkyl C (46-60 ppm) fractions showed highest peaks  
228 in meat powder, followed by green compost and alfalfa litter, and less pronounced content in  
229 manure compost, solid digestate, sawdust and maize litter, being substantially absent in biochar,  
230 glucose and cellulose (Fig. 1A). The *O*-alkyl-C (61-90 ppm) and the di-*O*-alkyl-C (91-110 ppm)  
231 regions, mainly associated with sugars and polysaccharides, respectively, were abundant in  
232 glucose and maize litter, followed by other amendments, while in biochar such molecular types  
233 were not detected (Fig. 1A). The *H*- and *C*-substituted aromatic C (111-140 ppm) fraction was the  
234 most abundant in biochar, but showed much lower content in all other materials. The *O*-substituted  
235 aromatic C fraction (141-160 ppm) showed minor or no peaks in all the materials, whereas  
236 carboxylic C (161-190 ppm) were most abundant in meat powder and, to a lesser extent, in alfalfa  
237 litter (Fig. 1A).

238 The dendrogram from cluster analysis (Fig. 1B) allowed a comparison among the organic  
239 amendments in terms of spectral signals and corresponding C bond types. Biochar and meat  
240 powder were clearly different from all other OM types, as well as one another (Fig. 1B). Glucose  
241 also showed dissimilarity from all other OM, while similarities were observed between alfalfa litter  
242 and green compost, and between manure compost and solid digestate (Fig. 1B). PCA provided a  
243 satisfactory ordination of the  $^{13}\text{C}$ -CPMAS NMR spectral regions across OM types (Fig. 1C), with  
244 the first two eigenvalues accounting for 91.6% (54.4, 37.2 %) of the total variance. The loading  
245 vectors of amendment quality parameters (i.e. relative abundance of each  $^{13}\text{C}$ -NMR region  
246 measured in each sample and how they relate to the PC axes) and the factorial scores of the 10  
247 organic materials pointed out that biochar is characterized by aromatic C, meat powder by alkyl C,

248 *N*-alkyl C and carboxyl C, cellulose and maize litter by the *O*-alkyl C and di*O*-alkyl C. Other  
249 organic amendments, placed close to the origin in the PC space biplot, showed spectral differences  
250 of lower magnitude (Fig. 1C).

251

### 252 3.2. Aggregation dynamics of soil amended with OM

253 In the soil aggregation experiment, all treatment factors (i.e. type of soil and amendment,  
254 incubation time) significantly affected aggregate stability, by either main or interactive effects  
255 (Table 3 and Supplementary Tables S1-S3). In general, the application of OM enhanced soil  
256 aggregation; however, AI onset timing and magnitude were highly variable among the tested  
257 conditions (Fig. 2). Incubation time greatly affected the aggregate stability of amended soil  
258 samples, with a significant first order effect on AI (Table 3 and Supplementary Tables S1-S3).  
259 However, time-dependent effects were highly variable depending on amendment and soil types,  
260 as shown by the significant interactions in the GLM results (Table 3 and Supplementary Tables  
261 S1-S3).

262 Initially, all amendment types except biochar caused an increase in soil aggregation. In  
263 particular, we observed a rapid and steep AI increase after the first 10 days of soil incubation with  
264 meat powder and alfalfa litter treatments (Fig. 2), generally decreasing at later stages. Similar  
265 trends, but with a lower effect magnitude persisting up to 30 days of incubation, were found for  
266 cellulose-rich amendments, such as maize litter and cellulose. In the case of sawdust, an initial AI  
267 increase, with different onset timing in different soils, was followed by a decrease and then by an  
268 increase at a later stage. Samples incubated with glucose showed soil typedependent response. Soil  
269 treatments with other amendments showed lower levels of aggregate stability, with a relative  
270 maximum after 100 days of incubation with green compost, and slight, but progressively increasing  
271 trends in the cases of manure compost and solid digestate in all tested soils (Fig. 2). In contrast,  
272 the addition of biochar barely affected soil AI compared to the control (Fig. 2).

273 The relative contribution of different aggregate size fractions varied among OM types during  
274 the incubation period, depending on soil type with the highest size aggregates generally  
275 predominating in soils incubated with highly aggregating amendments, mostly in the soil S2  
276 (Supplementary Fig. S2 ).

277  
278 *3.3. Relationships between AI and organic matter chemistry*  
279 Soil aggregate stability, as assessed by AI, was variably associated to the chemical quality of  
280 organic amendments, greatly depending on incubation time, but also on soil types (Fig. 3A, B).  
281 Concerning N content and C/N ratio, such parameters showed a general pattern of not significant  
282 correlation with AI (Fig. 3A), with some exceptions at early incubation stage. Specifically, the  
283 initial N content of OM was positively associated to AI of the S1 soil after 10 days of incubation,  
284 and for the S2 soil after 10 and 30 days, but not for the S3 soil (Fig. 3A). In the case of the C/N  
285 ratio of the organic amendments, significant positive correlation scores were observed only at  
286 intermediate (100 days), and late (300 days) stage for soils S2 and S3, respectively, whereas, no  
287 significant correlations were observed for S1 soil throughout the incubation period (Fig. 3A).

288 When initial OM chemical quality was molecularly defined by <sup>13</sup>C-CPMAS NMR  
289 reference regions, we observed an outstanding pattern of association with AI for specific C bond  
290 types. In particular, we found a trend of significant negative correlation for aromatic C types  
291 (corresponding to spectral regions at 111-140 and 141-160 ppm) generally consistent throughout  
292 the incubation period and in all tested soils (Fig. 3B), with only some minor exceptions of  
293 nonsignificant correlation scores for the S2 soil.

294 On the other hand, positive associations with AI were recorded for carboxylic C (161-190  
295 ppm), di-O-alkyl-C (91–110 ppm), O-alkyl-C (61–90 ppm) and alkyl C (0-45 ppm), but with  
296 different patterns of statistical significance for different incubation periods and soil types. In detail,  
297 carboxylic C and alkyl C were positively associated to aggregate stability in two soils at early

298 incubation period (i.e. 10 and 30 days for the S1 and S2 soils, [Fig. 3B](#)). Differently, di-*O*-alkyl-C  
299 and *O*-alkyl-C were positively associated to aggregate stability at medium-to-late stage, showing  
300 significant correlation scores with AI after 300 days of incubation of S2 and S3 soils, and after 100  
301 and 300 days in all soil types, respectively ([Fig. 3B](#)).

302 Finally, the *N*-alkyl and methoxyl C (46-69 ppm) fraction was unrelated to soil aggregate  
303 stability, independent of incubation period and soil type ([Fig. 3B](#)).

304

#### 305 **4. Discussion**

306 Our experiment, based on three soil types with different texture, ten organic materials  
307 representing a broad range of biochemical quality, and an incubation period of sufficient duration  
308 to appreciate aggregate stability dynamics, demonstrated that all treatments largely and variably  
309 affected the soil AI magnitude, onset timing, and persistence. As general trends, we observed that  
310 OM with high decomposability rapidly induces large, but not persistent aggregation stability, while  
311 cellulose-rich OM initially causes less, but more persistent aggregates stability. Differently, stable  
312 OM is less capable to enhance AI when incorporated into the soil. Finally, by defining OM quality  
313 with <sup>13</sup>C-CPMAS NMR, our results provide a significant novel contribution towards a full  
314 understanding of the relationships between OM biochemistry and AI.

315

##### 316 *4.1. Organic amendment and soil aggregation dynamics*

317 Improvements in aggregate stability following organic amendment of different soil types have  
318 been previously reported ([Abiven et al., 2007](#); [Annabi et al., 2011](#); [Spaccini et al., 2002, 2004](#)).  
319 We found an intense initial effect on aggregation with meat powder, glucose and alfalfa litter  
320 followed by a rapid decrease. These results are consistent with previous findings by [Abiven et al.,](#)  
321 [\(2007\)](#) on soil amendment with the labile residues of cauliflower. Such pattern, consistent among  
322 labile organic materials, can be related to the initial dominance of bacteria within the microbial

323 decomposer community, whose activity is favored by the high concentration of soluble C and the  
324 high C/N ratio in less mature organic residues (Eiland et al., 2001). The resultant bacterial by-  
325 products (extra-cellular polysaccharides) have been shown to aid the formation of soil aggregates  
326 (Alami et al., 2000). This support the hypothesis of a rapid, microbially induced improvement in  
327 aggregate stability following addition of fresh organic residues rich in labile polysaccharides  
328 (Abiven et al., 2007). Tisdall and Oades, (1982) had already observed a significant but transient  
329 increase in aggregate stability after glucose addition to the soil, related to the rapid decomposition  
330 of glues. Degens and Sparling, (1996) observed that addition of glucose at two concentration rates  
331 to soil aggregates of different size, did not affect the water-stable aggregation (MWD) of the largest  
332 size class (> 2 mm), while MWD of the smallest size class (<  
333 0.25 mm) increased for both treatment levels and high glucose concentration also enhanced MWD  
334 of aggregate class of intermediate size. Later, Piccolo and Mbagwu, (1999) confirmed that while  
335 carbohydrates had only a transient effect on aggregate stability, this property was persistent for  
336 longer time due to hydrophobic materials, including humified substances.

337 In our experiment, maize litter, cellulose and sawdust had an intermediate effect on  
338 aggregation, compared to other amendments. These results fits well with the molecular  
339 composition of these organic materials, which are richer in hemicellulose- and cellulose-like  
340 fractions, but poorer than other OM in soluble fractions, thus being less susceptible to microbial  
341 attack and more persistent. This is consistent with earlier studies by Tisdall and Oades, (1982),  
342 reporting weaker but more persistent aggregation effect when the soil was enriched with cellulose.  
343 More recently, Clark et al., (2007) observed that amending a sodic clay soil with sawdust reduced  
344 aggregate formation, as a result of a decrease in microbial activity and residue breakdown,  
345 producing less bacterial byproducts.

346 In our experiment, manure compost, green compost and solid digestate produced a slight but  
347 progressive AI increase, and still persisting 300 days after the addition to soil samples. This is

348 consistent with the well-known positive effect of compost applications on soil aggregate stability  
349 in the long run (Annabi et al., 2011; Tejada et al., 2009). Such effect is generally linked to the low  
350 decomposition rates of the amending materials, providing a continuous release of nutrients capable  
351 to sustain microbial populations for long time periods (Murphy et al., 2007). The persistence of  
352 aggregation stability after compost addition decisively depends on the type and maturity of the  
353 composted residues. For instance, Tejada et al., (2009) observed late positive effects over a 4-years  
354 soil amendment experiment, with highest soil structural stability produced by non-leguminous  
355 plant compost, as related to its highest content of humic acids ( $63.6 \text{ g kg}^{-1}$ ) involved in clay-organic  
356 complex formation. As far as compost maturity is concerned, Annabi et al., (2011) reported that  
357 the addition of immature compost, compared to mature compost, had a more intense but transient  
358 effect on soil aggregation stability. Transient and temporary effects were ascribed to by-products  
359 of cells lysis and microbial turnover, while persistent effects were attributed to humified  
360 compounds in the mature compost (Monnier, 1965; Tisdall and Oades, 1982).

361 Finally, we found that biochar addition did not affect aggregation throughout the incubation  
362 period. Our finding is consistent with recent evidence by Zhang et al., (2015), reporting that neither  
363 soil aggregation nor aggregate stability was significantly affected by biochar amendments. Two  
364 possible reasons lie, the low content of carboxyl groups in biochar, which may limit soil particles  
365 binding, and biochar is recalcitrance to oxidation, which could inhibit its degradation by microbes.  
366 Indeed, all other factors being equal, biochar is both chemically unusual and energetically less  
367 advantageous to mineralize than most other organic C compounds in the soil ecosystem (Lehmann  
368 et al., 2015). However, there is little and often conflicting evidence about biochar effects on  
369 aggregate stability. In contrast to our findings, Herath et al., (2013) reported that biochar addition  
370 can enhance soil aggregation, with different effect magnitude depending on soil and biochar types.  
371 Accordingly, a recent meta-analysis by Omondi et al., (2016) showed that the biochar addition can  
372 effectively improve soil physical properties, in particular aggregate stability by 8%, and that effects

373 can vary with biochar type and soil texture. About this latter observation, [Blanco-Canqui, \(2017\)](#)  
374 confirmed that biochar is mostly effective on the aggregation of soil with medium to fine texture.  
375 On the other hand, it is worth mentioning that in our experiment we used only one type of biochar  
376 with high aromatic C content, while biochar chemistry can be variable in relation to the organic  
377 source and the pyrolytic conditions. Then, further studies testing different soil and biochar types  
378 are needed to clarify the impact of this material on soil aggregation.

379

#### 380 *4.2. Linking organic matter chemistry with soil aggregation*

381 In our experiment, soil aggregation dynamics were highly variable over the 300 days of incubation.  
382 The underlying biological, chemical, and physical processes cannot satisfactorily be explained by  
383 the limited information on organic matter N content and C/N ratio. We observed very poor  
384 correlations between AI and such parameters, especially considering the whole incubation period  
385 ([Fig. 3A](#)). Though often used indicators of OM chemical characteristics, N content and C/N ratio  
386 were also previously tested as predictors of soil aggregation or aggregate stability, showing no  
387 particular relationships with these dependent variables ([Sonnleitner et al., 2003](#)). Differently, we  
388 found that OM quality defined by  $^{13}\text{C}$ -CPMAS NMR spectra significantly explained the observed  
389 AI dynamics. NMR data provide an improved definition of OM chemical quality, which improves  
390 the predictability of OM effects on soil aggregation, compared to classic information on C, N, and  
391 C/N ratio. Our PCA analysis showed that C, N and C/N ratio are less capable to capture the range  
392 of chemical quality of the organic amendments compared to NMR spectral regions, resulting in  
393 lower correlation magnitude with the PC axes. In addition, and most important, we found that the  
394 correlation magnitude between AI and OM quality parameters, as well as the occurrence of  
395 significant correlation scores, was generally higher for NMR regions and lower, and not consistent  
396 across different soil types, for C, N and C/N ratio ([Fig. 3A, B](#)).

397 In particular, *O*-alkyl and di-*O*-alkyl C fractions were positively associated to aggregate  
398 stability at medium-to-long term, indicating a promoting effect on soil structure. Accordingly,  
399 [Kavdır et al., \(2005\)](#) found that the carbohydrate content of organic amendments was positively  
400 correlated to the stability of soil aggregates, and [Tisdall and Oades, \(1982\)](#) mentioned a generally  
401 greater aggregate stability associated with OM content, also showing highest correlation  
402 magnitude with labile organic matter pools, such as microbial C biomass or extractable  
403 carbohydrates ([Haynes and Beare, 1996](#)).

404 Interestingly, significant positive correlations with AI were also observed for aliphatic and  
405 carboxyl C fractions, but limited to short term observations. Such result substantially depends on  
406 the very high content of these molecular fractions in some organic amendments, firstly meat  
407 powder and, to a lesser extent, alfalfa litter. For soil samples incubated with such materials, the  
408 rapid decrease of AI after initial peaking suggests a rapid but transient burst of microbial activity,  
409 producing short-lived polysaccharide compounds, rapidly subjected to chemical or biological  
410 breakdown during microbial turnover. Further support to such hypothesis comes from the rapid  
411 initial aggregation onset observed, with differences in magnitude for different OM and soil types,  
412 for all amendments rich in easily degradable carbohydrates and proteinaceous compounds (i.e.  
413 glucose, meat powder, and alfalfa litter). Consistently, previous authors showed that these types of  
414 organic materials enhance microbial growth ([Leifeld and Kögel-Knabner, 2005](#)) and that  
415 preferential microbial consumption of carbohydrates or alkyl-rich compounds can alter soil  
416 structure ([Chevallier et al., 2010](#)).

417 Previous experiments with crop residues amendment to soil also showed a positive  
418 association between aggregate stability and the initial protein content of the organic materials  
419 ([Martens, 2000](#)). In our data, protein signals cannot be univoquely identified, as being possibly  
420 distributed in different <sup>13</sup>C NMR spectral regions according to the specific C bond types of  
421 aminoacidic side chains. *N*-alkyl and methoxyl C fractions could be used as a proxy, since



422 including diagnostic signals (56-59 ppm) of amino acidic  $\alpha$ -carbons (Breitmaier and Voelter,  
423 1987). However, this region also includes mobile methoxyl groups in lignin moieties (Baldock et  
424 al., 1990; Knicker, 2000). As a likely consequence, we observed positive, but not statistically  
425 significant correlation scores between *N*-alkyl and methoxyl C content in the organic materials and  
426 soil aggregate stability.

427 Finally, we found negative associations between AI and NMR spectral regions  
428 corresponding to aromatic C fractions, substantially independent from incubation time and soil  
429 type. These results are related to the generalized null effect on aggregation observed in samples  
430 amended with biochar. The most likely explanation for such effect may be the change of the *O*alkyl  
431 C to aromatic C during pyrolysis observed by Czimczik et al., (2002), and soil enriched with  
432 aromatic C by the biochar application. The principle chemical difference between biochar and  
433 other organic matter is the much larger proportion of aromatic C and, specifically, the occurrence  
434 of fused aromatic C structures, in contrast to other aromatic structures. This means that  
435 microorganisms will not be able to readily utilize the C as an energy source or the N and possibly  
436 other nutrients contained in the C structure (Lehmann et al., 2015). However, biochar  
437 mineralization would actually require adapted microbial communities, capable to produce the  
438 required, energetically costly enzymatic tools (Schimel and Weintraub, 2003). In absence of  
439 evidence on both aspects, biochar suffers a substantial inertness to biological and chemical  
440 reactions, at least at the time scale corresponding to our observation periods (Kuzyakov et al.,  
441 2014). Hence, our hypothesis is that organic materials rich in aromatic C are unable to induce soil  
442 aggregation as not sufficiently supporting microbial growth. Such hypothesis is also consistent  
443 with other phenomenological observations of dissolved aromatic compounds decisively  
444 contributing to persistent mineral-bound carbon pool (Kramer et al., 2012), and of soil microbe  
445 inhibition by the presence of recalcitrant and/or fungitoxic aromatic compounds (Incerti et al.,  
446 2013).

447

**448 5. Conclusion**

449 Proper, sustainable management of OM additions to soils may increase aggregate stability and thus  
450 reduce soil erosion problems. With this in mind, it is necessary to select the quality and timing of  
451 OM additions in order to correctly produce the expected increases in aggregate stability over time.  
452 Noteworthy, we found that meat powder, glucose, and alfalfa litter often induces a rapid initial  
453 increase of AI followed by rapid decrease, likely acting as a prompt C source for microbes. An  
454 opposite response was founded for biochar, barely affecting AI when incorporated into the soil.  
455 We also found a positive effect on aggregation by some cellulose rich organic matters such as  
456 maize litter, cellulose, and sawdust. Moreover, some composted OM showed initially low but  
457 persistent and in some cases progressively increasing effect on soil aggregation. The use of  $^{13}\text{C}$ -  
458 CPMAS NMR provides an improved definition of OM biochemical quality, helping to explain the  
459 variable effects of different amendments on aggregation dynamics. In detail,  $^{13}\text{C}$ -CPMAS NMR  
460 revealed that the spectral regions roughly corresponding to carbohydrates (*O*-alkyl and di-*O*-alkyl  
461 C) and aromatic C fractions are crucial to predict amendment effects on aggregate stability, with a  
462 consistent improvement, compared to classical elemental chemical parameters such as C, N  
463 content and C/N ratio. Finally, as a major novel contribution, our study is the first attempt to linking  
464 litter biochemistry with dynamics of soil aggregation. Nevertheless, our experiment could be  
465 expanded to the field condition on others soil types, using a wider range of OM types to evaluate  
466 the generality of our present findings.

467

**468 References**

469 Abiven, S., Menasseri, S., Angers, D.A., Leterme, P., 2007. Dynamics of aggregate stability and  
470 biological binding agents during decomposition of organic materials. *European Journal of Soil*  
471 *Science* 58, 239-247.

- 472 Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil  
473 aggregate stability-A literature analysis. *Soil Biology and Biochemistry* 41, 1-12.
- 474 Alami, Y., Achouak, W., Marol, C., Heulin, T., 2000. Rhizosphere soil aggregation and plant  
475 growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. Strain isolated  
476 from sunflower roots. *Applied and Environmental Microbiology* 66, 3393-3398. Albiach, R.,  
477 Canet, R., Pomares, F., Ingelmo, F., 2001. Organic matter components and aggregate stability after  
478 the application of different amendments to a horticultural soil. *Bioresource Technology* 76, 125-  
479 129.
- 480 Amezketa, E., 1999. Soil aggregate stability: a review. *Journal of Sustainable Agriculture* 14, 83-  
481 151.
- 482 Annabi, M., Le Bissonnais, Y., Le Villio-Poitrenaud, M., Houot, S., 2011. Improvement of soil  
483 aggregate stability by repeated applications of organic amendments to a cultivated silty loam soil.  
484 *Agriculture, Ecosystems & Environment* 144, 382-389.
- 485 Baldock, J.A., Oades, J.M., Vassallo, A.M., Wilson, M.A., 1990. Significance of microbial activity  
486 in soils as demonstrated by solid-state carbon-13 NMR. *Environmental Science & Technology* 24,  
487 527-530.
- 488 Blanco-Canqui, H., 2017. Biochar and Soil Physical Properties. *Soil Science Society of America*  
489 *Journal* 84, 687-711.
- 490 Bonanomi, G., Chirico, G.B., Palladino, M., Gaglione, S.A., Crispo, D.G., Lazzaro, U., Sica, B.,  
491 Cesarano, G., Ippolito, F., Sarker, T.C., 2017. Combined application of photo-selective mulching  
492 films and beneficial microbes affects crop yield and irrigation water productivity in intensive  
493 farming systems. *Agricultural Water Management* 184, 104-113.
- 494 Bonanomi, G., Incerti, G., Giannino, F., Mingo, A., Lanzotti, V., Mazzoleni, S., 2013. Litter  
495 quality assessed by solid state  $^{13}\text{C}$  NMR spectroscopy predicts decay rate better than C/N and  
496 Lignin/N ratios. *Soil Biology and Biochemistry* 56, 40-48.

- 497 Breitmaier, E., Voelter, W., 1987. Carbon-13 NMR spectroscopy: VCH Verlagsgesellschaft, 3rd  
498 ed. Weinheim, Germany, pp. 515.
- 499 Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124, 3-22.
- 500 Chevallier, T., Woignier, T., Toucet, J., Blanchart, E., 2010. Organic carbon stabilization in the  
501 fractal pore structure of Andosols. *Geoderma* 159, 182-188.
- 502 Clark, G.J., Dodgshun, N., Sale, P.W.G., Tang, C., 2007. Changes in chemical and biological  
503 properties of a sodic clay subsoil with addition of organic amendments. *Soil Biology and*  
504 *Biochemistry* 39, 2806-2817.
- 505 Czimczik, C.I., Preston, C.M., Schmidt, M.W.I., Werner, R.A., Schulze, E.-D., 2002. Effects of  
506 charring on mass, organic carbon, and stable carbon isotope composition of wood. *Organic*  
507 *Geochemistry* 33, 1207-1223.
- 508 Degens, B., Sparling, G., 1996. Changes in aggregation do not correspond with changes in labile  
509 organic C fractions in soil amended with <sup>14</sup>C-glucose. *Soil Biology and Biochemistry* 28, 453-462.
- 510 Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms  
511 and the factors affecting these: a review. *Soil Research* 35, 431-460.
- 512 Eiland, F., Klamer, M., Lind, A.M., Leth, M., Bååth, E., 2001. Influence of initial C/N ratio on  
513 chemical and microbial composition during long term composting of straw. *Microbial Ecology* 41,  
514 272-280.
- 515 Gillon, D., Joffre, R., Ibrahima, A., 1999. Can litter decomposability be predicted by near infrared  
516 reflectance spectroscopy? *Ecology* 80, 175-186.
- 517 Haynes, R.J., Beare, M.H., 1996. Aggregation and organic matter storage in meso-thermal, humid  
518 soils. *Structure and organic matter storage in agricultural soils*, CRC Press, Inc. p 213-262. Boca  
519 Raton, Florida 33431.
- 520 Hendrix, P.F., Crossley Jr, D.A., Blair, J.M., Coleman, D.C., 1990. Soil biota as components of  
521 sustainable agroecosystems. In: Edwards, C.A., Lal, Rattan, Madden, Patrick, Miller, Robert H.,

- 522 House, Gar (Eds.), Sustainable Agricultural Systems. Soil and Water Conservation Society, IA,  
523 pp. 637–654.
- 524 Herath, H., Camps-Arbestain, M., Hedley, M., 2013. Effect of biochar on soil physical properties  
525 in two contrasting soils: an Alfisol and an Andisol. *Geoderma* 209, 188-197.
- 526 Huang, Y., Stankiewicz, B.A., Eglinton, G., Snape, C.E., Evans, B., Latter, P.M., Ineson, P., 1998.  
527 monitoring Biomacromolecular degradation of *Calluna Vulgaris* in a 23year field experiment using  
528 solid state  $^{13}\text{C}$ -NMR and pyrolysis-GC/MS. *Soil Biology and Biochemistry* 30, 1517-1528.
- 529 Incerti, G., Capodilupo, M., Senatore, M., Termolino, P., Scala, F., Mazzoleni, S., Bonanomi, G.,  
530 2013. Biochemical changes assessed by  $^{13}\text{C}$ -CPMAS NMR spectroscopy control fungal growth  
531 on water extracts of decaying plant litter. *Mycoscience* 54, 449-457.
- 532 Kavdır, Y., Ekinci, H., Yüksel, O., Mermut, A.R., 2005. Soil aggregate stability and  $^{13}\text{C}$   
533 CP/MAS-NMR assessment of organic matter in soils influenced by forest wildfires in Çanakkale,  
534 Turkey. *Geoderma* 129, 219-229.
- 535 Keil, R.G., Mayer, L.M., 2014. 12.12-Mineral matrices and organic matter. In: Holland, H.D.,  
536 Turekian, K.K. (Eds.), *Treatise on Geochemistry*, second ed. Elsevier, Oxford, pp. 337–359.
- 537 Kemper, D.W., Rosenau, R.C., 1986. Aggregate stability and size distribution. In: Klute A (Ed)  
538 *Methods of Soil Analysis, Part 1* (pp 425–442). ASA and SSSA, Madison, WI.
- 539 Knicker, H., 2000. Biogenic nitrogen in soils as revealed by solid-state carbon-13 and nitrogen15  
540 nuclear magnetic resonance spectroscopy. *Journal of Environmental Quality* 29, 715-723. Kögel-
- 541 Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as  
542 inputs to soil organic matter. *Soil Biology and Biochemistry* 34, 139-162.
- 543 Kramer, M.G., Sanderman, J., Chadwick, O.A., Chorover, J., Vitousek, P.M., 2012. Long-term  
544 carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Global*  
545 *Change Biology* 18, 2594-2605.

- 546 Kuzyakov, Y., Bogomolova, I., Glaser, B., 2014. Biochar stability in soil: decomposition during  
547 eight years and transformation as assessed by compound-specific  $^{14}\text{C}$  analysis. *Soil Biology and*  
548 *Biochemistry* 70, 229-236.
- 549 Legendre, P., Legendre, L., 1998. Numerical ecology: second English edition. Developments in  
550 environmental modelling, Elsevier, Amsterdam, The Netherlands, 20.
- 551 Lehmann, J., Abiven, S., Kleber, M., Pan, G., Singh, B.P., Sohi, S.P., Zimmerman, A.R., Lehmann,  
552 J., Joseph, S., 2015. Persistence of biochar in soil. *Biochar for Environmental*  
553 *Management: Science, Technology and Implementation*. Chapter 10, p. 233-280, 2nd edi.  
554 Routledge, 711 Third Avenue, New York, NY 10017.
- 555 Leifeld, J., Kögel-Knabner, I., 2005. Soil organic matter fractions as early indicators for carbon  
556 stock changes under different land-use? *Geoderma* 124, 143-155.
- 557 Martens, D.A., 2000. Plant residue biochemistry regulates soil carbon cycling and carbon  
558 sequestration. *Soil Biology and Biochemistry* 32, 361-369.
- 559 Martens, D.A., Frankenberger, W.T., 1992. Modification of infiltration rates in an organic-amended  
560 irrigated. *Agronomy Journal* 84, 707-717.
- 561 Mathers, N.J., Jalota, R.K., Dalal, R.C., Boyd, S.E., 2007.  $^{13}\text{C}$ -NMR analysis of decomposing  
562 litter and fine roots in the semi-arid Mulga Lands of southern Queensland. *Soil Biology and*  
563 *Biochemistry* 39, 993-1006.
- 564 Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M.L., Termolino, P., Mingo, A., Senatore, M.,  
565 Giannino, F., Cartenì, F., Rietkerk, M., 2015. Inhibitory and toxic effects of extracellular self-DNA  
566 in litter: a mechanism for negative plant-soil feedbacks? *New Phytologist* 205, 1195-1210.
- 567 Monnier, G., 1965. Action des matières organiques sur la stabilité structurale des sols. *Thèse*  
568 *de la faculté des sciences de Paris*, pp.140.
- 569 Muneer, M., Oades, J.M., 1989. The role of Ca-organic interactions in soil aggregate stability. III.  
570 Mechanisms and models. *Soil Research* 27, 411-423.

- 571 Murphy, B.W., 2015. Impact of soil organic matter on soil properties-a review with emphasis on  
572 Australian soils. *Soil Research* 53, 605-635.
- 573 Murphy, K.M., Campbell, K.G., Lyon, S.R., Jones, S.S., 2007. Evidence of varietal adaptation to  
574 organic farming systems. *Field Crops Research* 102, 172-177.
- 575 Oades, J.M., 1988. The retention of organic matter in soils. *Biogeochemistry* 5, 35-70.
- 576 Oades, J.M., 1993. The role of biology in the formation, stabilization and degradation of soil  
577 structure. *Geoderma* 56, 377-400.
- 578 Omondi, M.O., Xia, X., Nahayo, A., Liu, X., Korai, P.K., Pan, G., 2016. Quantification of biochar  
579 effects on soil hydrological properties using meta-analysis of literature data. *Geoderma* 274, 28-  
580 34.
- 581 Piccolo, A., Conte, P., Spaccini, R., Mbagwu, J.S.C., 2005. Influence of land use on the  
582 characteristics of humic substances in some tropical soils of Nigeria. *European Journal of Soil*  
583 *Science* 56, 343-352.
- 584 Piccolo, A., Mbagwu, J.S.C., 1999. Role of hydrophobic components of soil organic matter in soil  
585 aggregate stability. *Soil Science Society of America Journal* 63, 1801-1810.
- 586 Piccolo, A., Pietramellara, G., Mbagwu, J.S.C., 1997. Use of humic substances as soil conditioners  
587 to increase aggregate stability. *Geoderma* 75, 267-277.
- 588 Rillig, M.C., Aguilar-Trigueros, C.A., Bergmann, J., Verbruggen, E., Veresoglou, S.D.,  
589 Lehmann, A., 2015. Plant root and mycorrhizal fungal traits for understanding soil aggregation.  
590 *New Phytologist* 205, 1385-1388.
- 591 Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon  
592 and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* 35, 549-563.
- 593 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber,  
594 M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., 2011. Persistence of soil organic matter as  
595 an ecosystem property. *Nature* 478, 49-56.

- 596 Six, J., Bossuyt, H., Degryze, S., Deneff, K., 2004. A history of research on the link between (micro)  
597 aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research* 79, 7-  
598 31.
- 599 Sonnleitner, R., Lorbeer, E., Schinner, F., 2003. Effects of straw, vegetable oil and whey on  
600 physical and microbiological properties of a chernozem. *Applied Soil Ecology* 22, 195-204.
- 601 Spaccini, R., Mbagwu, J.S.C., Conte, P., Piccolo, A., 2006. Changes of humic substances  
602 characteristics from forested to cultivated soils in Ethiopia. *Geoderma* 132, 9-19.
- 603 Spaccini, R., Mbagwu, J.S.C., Igwe, C.A., Conte, P., Piccolo, A., 2004. Carbohydrates and  
604 aggregation in lowland soils of Nigeria as influenced by organic inputs. *Soil and Tillage Research*  
605 75, 161-172.
- 606 Spaccini, R., Piccolo, A., Mbagwu, J.S.C., Zena Teshale, A., Igwe, C.A., 2002. Influence of the  
607 addition of organic residues on carbohydrate content and structural stability of some highland soils  
608 in Ethiopia. *Soil Use and management* 18, 404-411.
- 609 Tejada, M., Hernandez, M.T., Garcia, C., 2009. Soil restoration using composted plant residues:  
610 Effects on soil properties. *Soil and Tillage Research* 102, 109-117.
- 611 Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *European*  
612 *Journal of Soil Science* 33, 141-163.
- 613 USDA, 1999. Soil taxonomy-a basic system of soil classification for making and interpreting soil  
614 surveys, 2nd edi., United States Department of Agriculture–NRCS, Agriculture Handbook,  
615 number 436, Washington.
- 616 Zhang, Q., Du, Z., Lou, Y., He, X., 2015. A one-year short-term biochar application improved  
617 carbon accumulation in large macroaggregate fractions. *Catena* 127, 26-31.
- 618



**Table 1****Table**

Initial C, N content and C/N ratio of 10 different organic materials used in the soil aggregation experiment.

<b>Organic matters</b>	<b>Nitrogen</b>	<b>Carbon</b>	<b>C/N</b>
Alfalfa litter	3.93	38.29	9.73
Biochar	0.50	74.57	149.14
Cellulose	0.10	50.10	500.13
Glucose	0.00	43.05	-
Green compost	1.52	31.12	20.39
Maize litter	0.49	40.38	82.40
Manure compost	2.07	34.3	17.15
Meat powder	8.26	43.88	5.31
Sawdust	0.11	49.88	453.45
Solid digestate	1.91	43.8	22.93

**Table 2**

Physical and chemical properties (mean values of three replicates) of S1, S2 and S3 soil types at the beginning of the experimental activity.

<b>Parameter</b>	<b>S1</b>	<b>S2</b>	<b>S3</b>
Soil type	Silandic and vitric andosols	Vertic xerofluvent	Typic ustifluvent
Sand, %	45.60	51.3	62.4
Silt, %	46.42	24.4	30.2
Clay, %	7.98	24.3	7.4
Bulk density, g cm <sup>-3</sup>	1.19	1.16	1.15
Electrical conductivity, dS m <sup>-1</sup>	0.61	0.28	0.18
pH	7.72	8.7	8.1
Organic carbon, g kg <sup>-1</sup>	13.14	10.5	10.4
Total nitrogen, g kg <sup>-1</sup>	1.91	1.3	1.04
C/N ratio	7.38	8.07	10.0

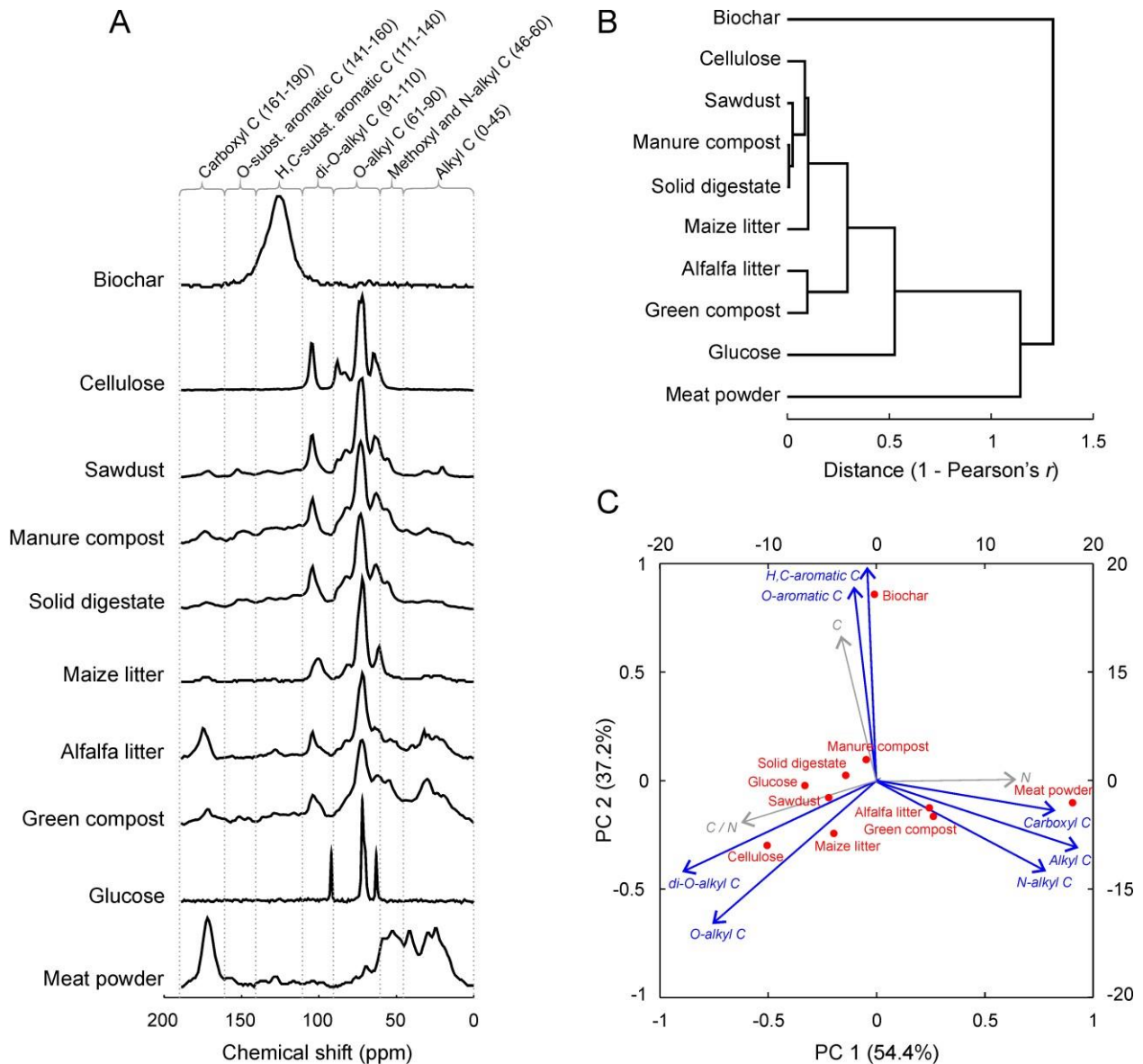
Summary of the Generalized Linear Modelling (GLM) testing for main and interactive effects of soil type (S, three levels: S1, S2 and S3), organic matter type used for soil amendment (OM, ten levels: alfalfa litter, biochar, cellulose, glucose, green compost, maize litter, manure compost, meat powder, sawdust, solid digestate) and incubation time (treated as a continuous covariate) on soil aggregation

**Table 3**

index (AI). Results of post-hoc testing for pair-wise AI differences between treatment combinations are in [Supplementary Tables S1-S3](#).

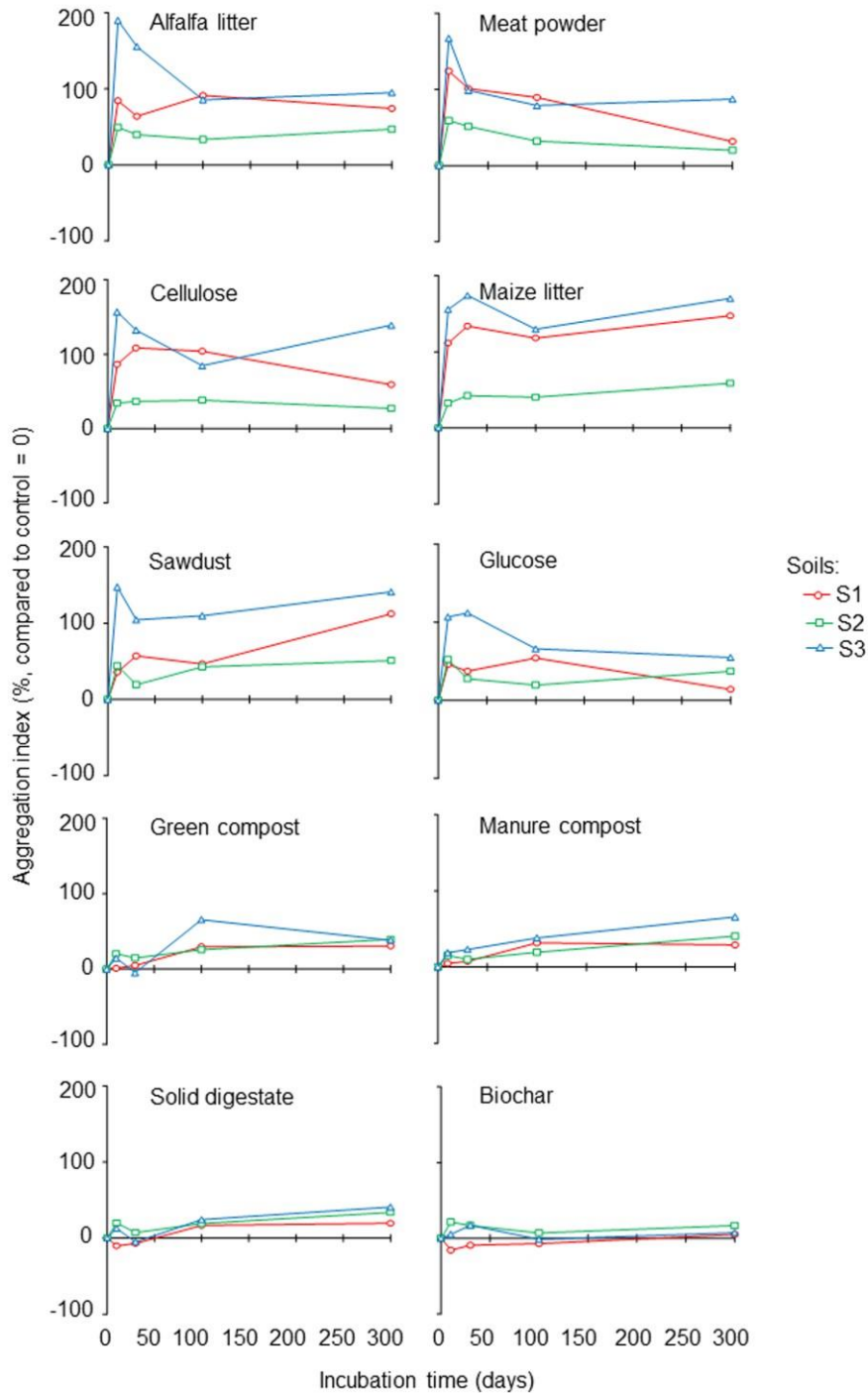
	<b>SS</b>	<b>d.f.</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Soil type (S)	3.901	2	1.951	177.72	< 0.00001
Organic matter (OM)	10.426	9	1.159	105.56	< 0.00001
Incubation time (T)	0.754	1	0.754	68.73	< 0.00001
S × OM	2.031	18	0.113	10.28	< 0.00001
S × T	0.199	2	0.099	9.06	0.00015
OM × T	1.936	9	0.215	19.60	< 0.00001
S × OM × T	0.477	18	0.027	2.41	< 0.00124
Error	3.292	300	0.011		

Figure



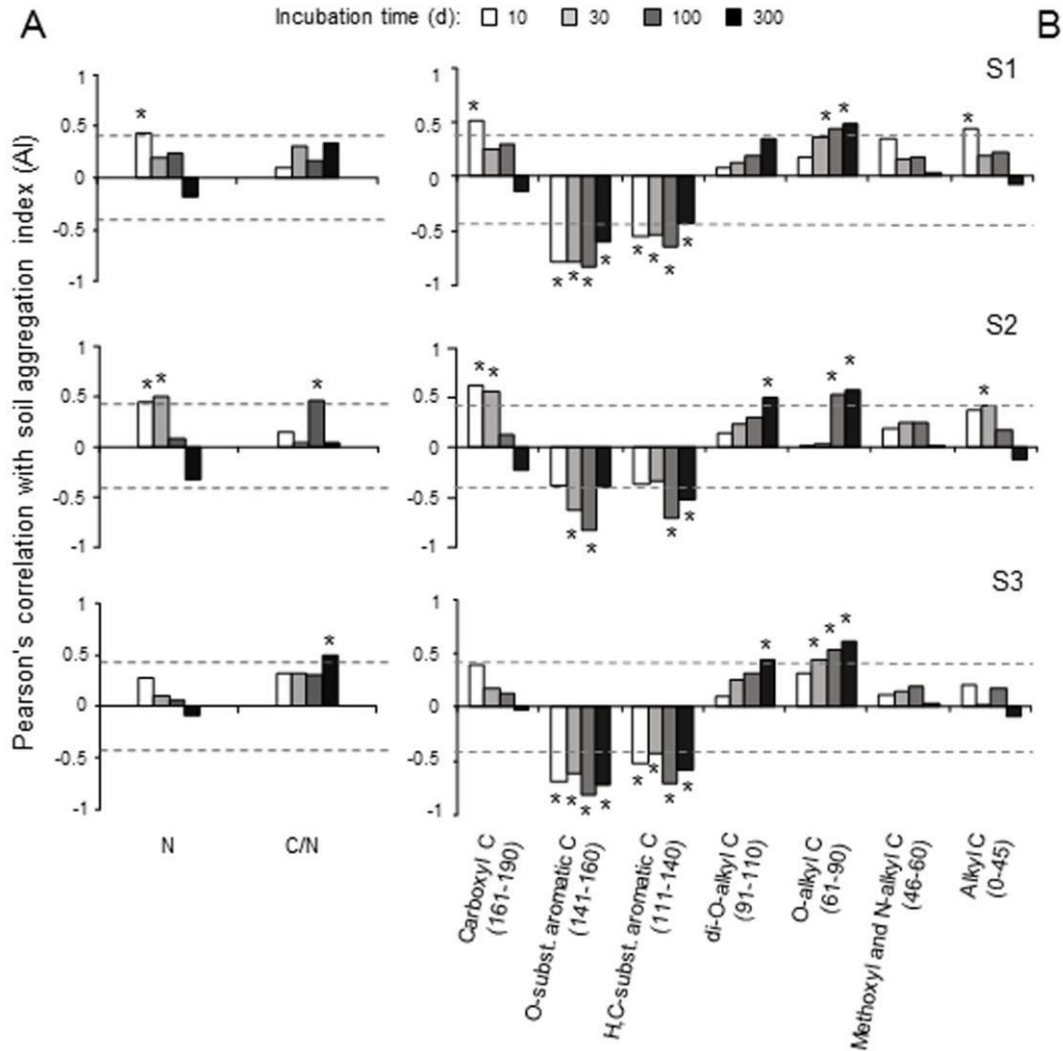
**Fig. 1.** Chemical differences among organic materials used for soil amendment. (A)  $^{13}\text{C}$ -CPMAS NMR spectra of the materials. Reference spectral regions and corresponding C types are reported on top of the panels, with chemical shift ranges indicated in brackets and by vertical dotted lines. (B) Dendrogram of organic materials based on spectral data. (C) PCA biplot of the reference spectral regions in the organic materials. Data refer to loading vectors of the spectral regions (blue arrows,

bottom x and left y axes) and factorial scores of the organic materials (red circles, top x and right y axis). Loading vectors of C/N ratio and C and N content of the organic materials (grey arrows), plotted as supplementary variables following Legendre and Legendre (1998), are also shown for comparison.



**Fig. 2.** Results of the manipulative experiment on soil aggregate stability after organic amendment. Data refer to 300-days dynamics of aggregation index (% , compared to control = 0) assessed in three different soils amended with 10 different organic materials (alfalfa litter, biochar, cellulose, glucose,

green compost, maize litter, manure compost, meat powder, sawdust, solid digestate). Deviation bars are omitted to improve readability. For detailed statistics see [Supplementary Tables S1-S3](#).



**Fig. 3.** Relationships between aggregate stability (AI) in soil amended with organic materials and chemical quality of the same amendments. Data refer to correlation (Pearson's  $r$ ) between AI and either element chemical parameters (A) or organic C types corresponding to reference  $^{13}\text{C}$ -CPMAS NMR spectral regions (B), separately calculated on 30 datapoints (i.e. 10 organic materials  $\times$  3 replicates) across three soil types and four sampling dates. Dashed lines indicate threshold values of statistical significance for  $r$ , while asterisks indicate significant  $r$  values.

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