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Linking organic matter chemistry with soil aggregate stability: Insight from ¹³C NMR spectroscopy

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Covering Letter

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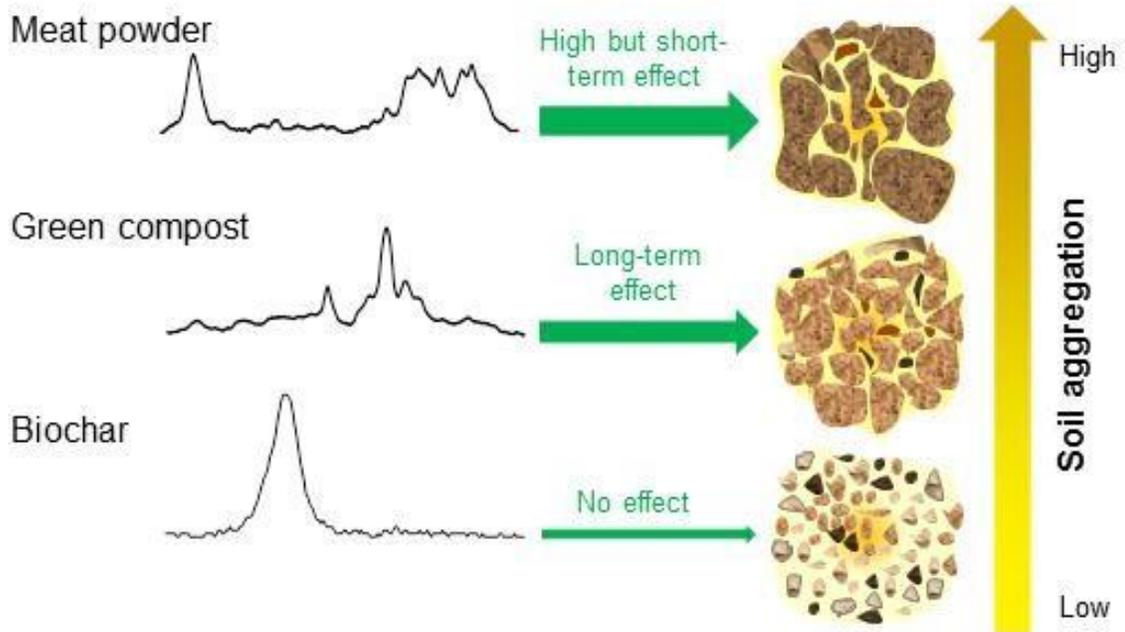
According with Associated Editor and the two reviewers' comments, we have revised the manuscript "Linking organic matter chemistry with soil aggregate stability: insight from ¹³C NMR spectroscopy", Ref.: Ms. No. SBB12426.

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}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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2 Linking organic matter chemistry with soil aggregate stability: insight from ^{13}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}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}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}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}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 ¹³C-cross-polarization
112 magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Kögel-
113 Knabner, 2002). In detail, ¹³C-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 ¹³C-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}C -CPMAS NMR spectroscopy and standard chemical parameters;
128 (iii) identify the ^{13}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}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; ^1H -power for CP 92.16 W: ^1H 90° pulse 2.85 μs ; ^{13}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 ^1H 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}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}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}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}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}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 ^{13}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 ¹³C-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 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}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 ¹³C 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 α -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 ¹³C-
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, ¹³C-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

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- 618

Table 1**Table**

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

Organic matters	Nitrogen	Carbon	C/N
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.

Parameter	S1	S2	S3
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 ⁻³	1.19	1.16	1.15
Electrical conductivity, dS m ⁻¹	0.61	0.28	0.18
pH	7.72	8.7	8.1
Organic carbon, g kg ⁻¹	13.14	10.5	10.4
Total nitrogen, g kg ⁻¹	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](#).

	SS	d.f.	MS	F	p
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

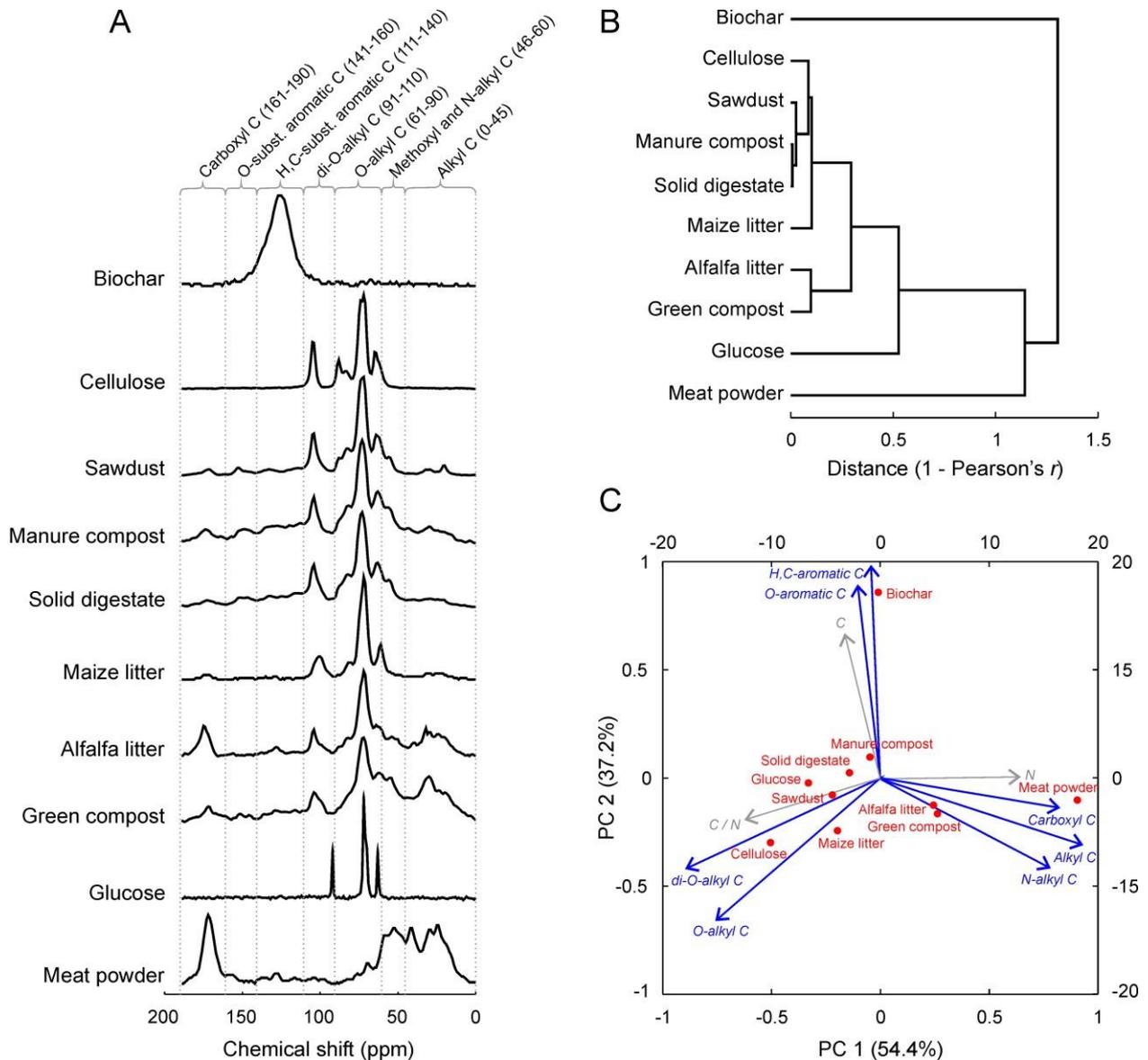


Fig. 1. Chemical differences among organic materials used for soil amendment. (A) ^{13}C -CPMAS NMR spectra of the materials. Reference spectral regions and corresponding C types are reported on top of the panels, with chemical shift ranges indicated in brackets and by vertical dotted lines. (B) Dendrogram 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.

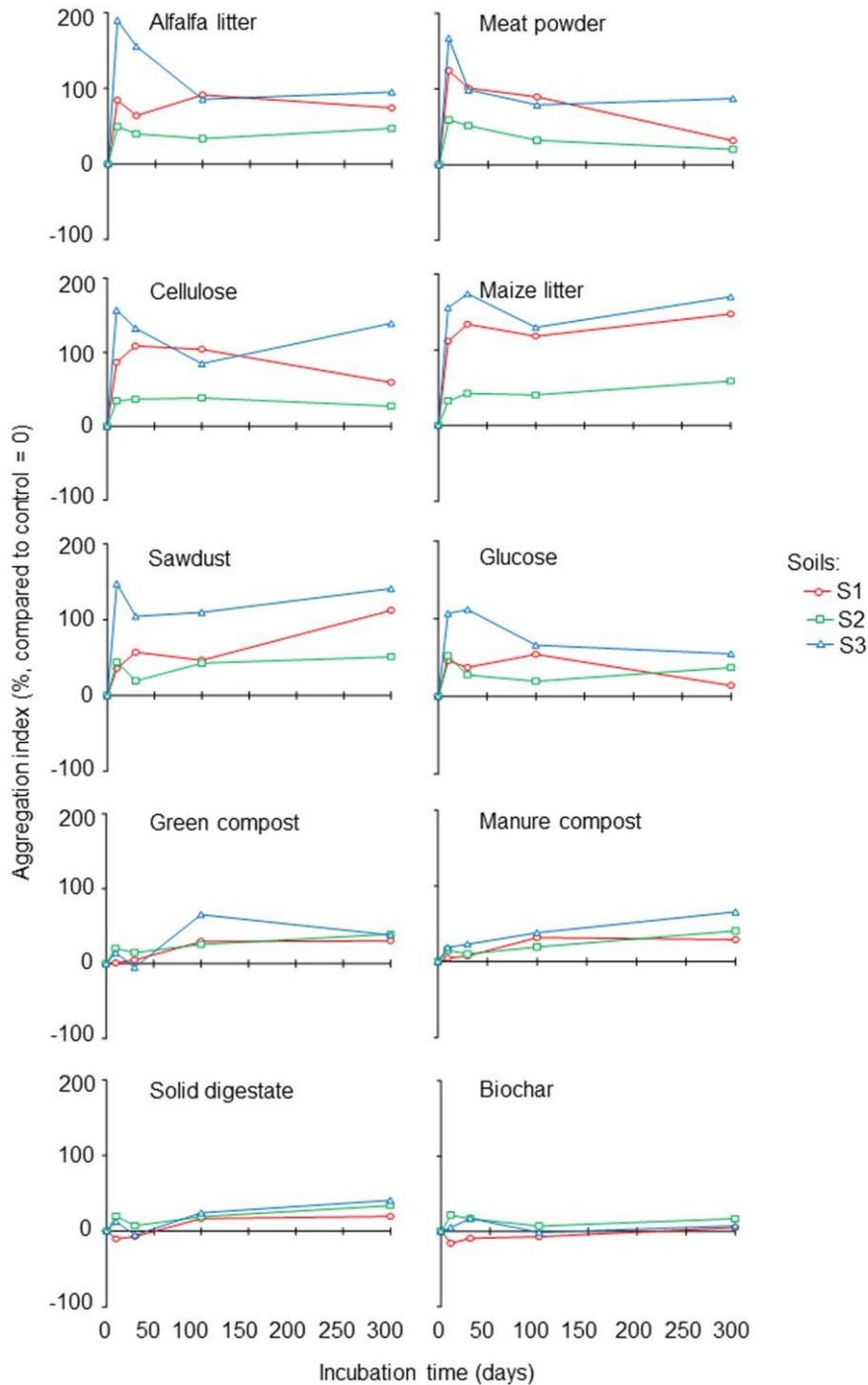


Fig. 2. Results of the manipulative experiment on soil aggregate stability after organic amendment. Data refer to 300-days dynamics of aggregation index (% , compared to control = 0) assessed in three different soils amended with 10 different organic materials (alfalfa litter, biochar, cellulose, glucose,

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](#).

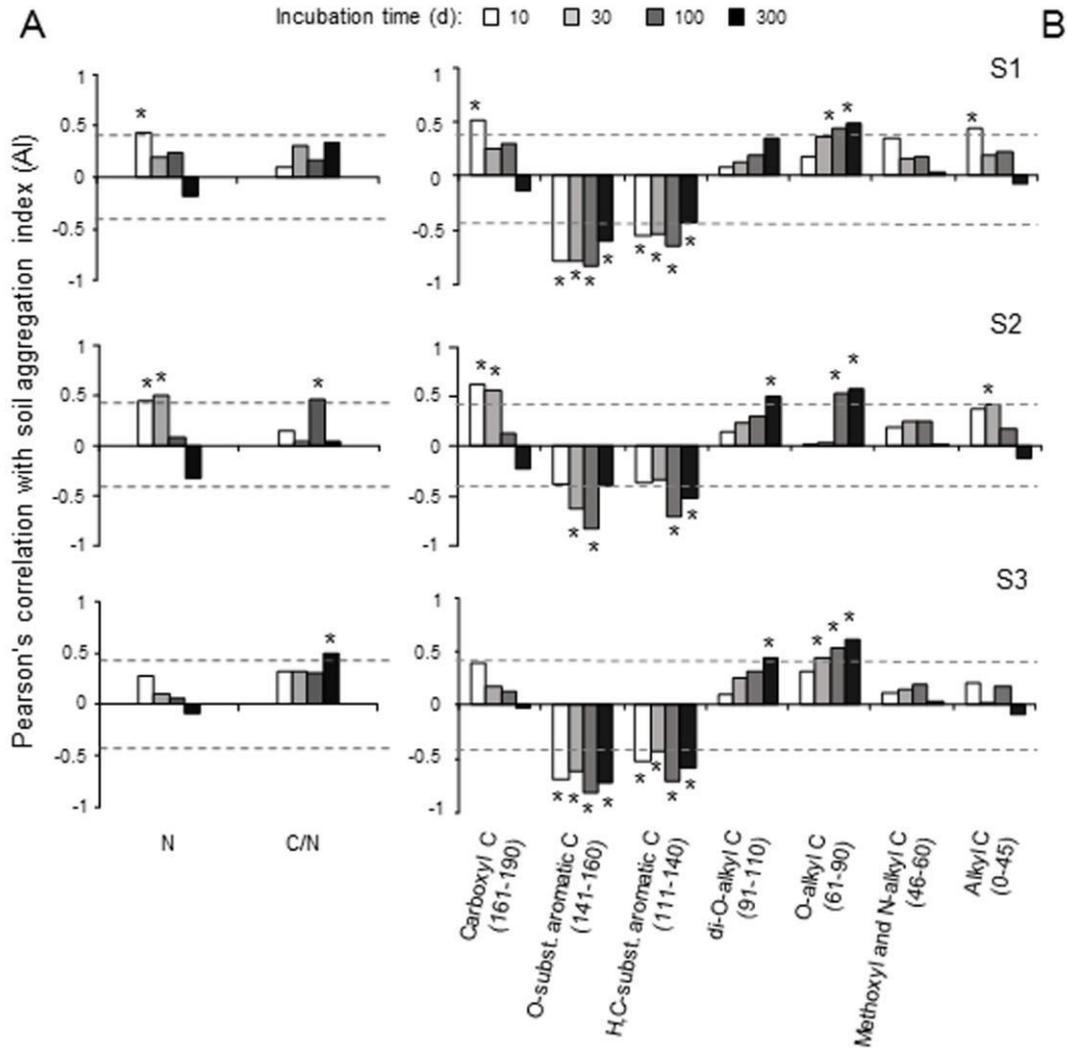


Fig. 3. Relationships between aggregate stability (AI) in soil amended with organic materials and chemical quality of the same amendments. Data refer to correlation (Pearson's r) between AI and either element chemical parameters (A) or organic C types corresponding to reference ^{13}C -CPMAS NMR spectral 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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