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Linking organic matter chemistry with soil aggregate stability: Insight from  $^{13}\text{C}$  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.

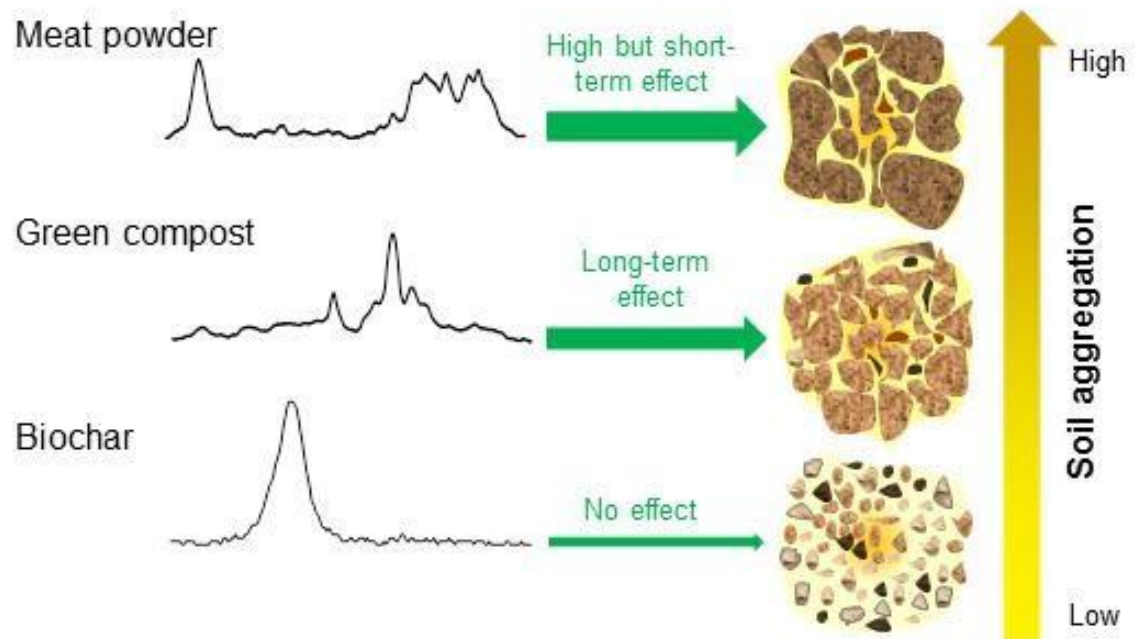
In the revised version, we have changed the text providing answers to all the numerated points raised by the referees. Please, find in attach the reviewer's comments with our point-bypoint responses (reported in italic type and red font) and the revised manuscript with and without changes highlighted (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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2    Linking organic matter chemistry with soil aggregate stability: insight from  $^{13}\text{C}$  NMR 3  
spectroscopy

4

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## ABSTRACT

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

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

## 1. Introduction

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

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

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

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

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

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

In this study, we combined a detailed OM characterization by  $^{13}\text{C}$ -CPMAS NMR, in solid state with a manipulative experiment of soil incubation in mesocosms to investigate the link between OM carbon distribution and soil aggregation stability over a 1-year observation period. In detail, we tested the capability of 10 organic amendments, spanning over a wide range of chemical quality, to induce soil aggregation in three soil types with different texture. Specific aims of the study were to:

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

## 2. Materials and methods

### 2.1. Chemical characterization of organic amendments

Ten types of organic amendment were selected, as representing a wide range of OM with different chemical composition and biochemical stability ([Table 1](#)). They were characterized for total C and N content by flash combustion of micro samples (5 mg of sample) in an Elemental Analyzer NA 1500 (Fison 1108 Elemental Analyzer, Thermo Fisher Scientific). All organic amendments were analyzed by solid state  $^{13}\text{C}$ -CPMAS NMR, thus allowing a comparative and detailed characterization of molecular properties. The NMR spectras were recorded on a Bruker AV-300 instrument (Bruker Instrumental Inc, Billerica, MA, USA), equipped with a magic angle spinning (MAS) probe with wide-bore of 4 mm, using specific calibrated acquisition parameters: 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; 1 ms of contact time; 20 ms of acquisition time; 2000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross polarization pulse sequence was applied with a composite shaped “ramp” pulse on the  $^1\text{H}$  channel in order to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The Fourier transform was performed with 4 k data point and an exponential apodization of 50 Hz of line broadening. Selection of spectral regions and identification of corresponding classes of organic C bonds were performed according to previous studies ([Bonanomi et al., 2013](#); [Kögel-Knabner, 2002](#); [Mathers et al.,](#)

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

## 2.2. Aggregation experiment

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

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

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

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

### 2.3. Assessment of soil aggregate stability

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

(Spaccini et al., 2004):

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

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

0.25;  $X_4 < 0.25$  mm) and  $W_i$  is the dry mass proportion of the total aggregates in the  $i^{th}$  fraction. Higher AI values indicate higher proportions of macroaggregates in the sample and therefore, higher stability.

#### 2.4. Data analysis

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

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

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

### 3. Results



### 3.1. Organic matter initial biochemistry

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

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

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

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

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

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

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

### 3.3. Relationships between AI and organic matter chemistry

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

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

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

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

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

## 4. Discussion

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

### 4.1. Organic amendment and soil aggregation dynamics

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

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

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

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

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

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

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

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

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

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

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

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



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

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

## 5. Conclusion

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

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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.

| <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.

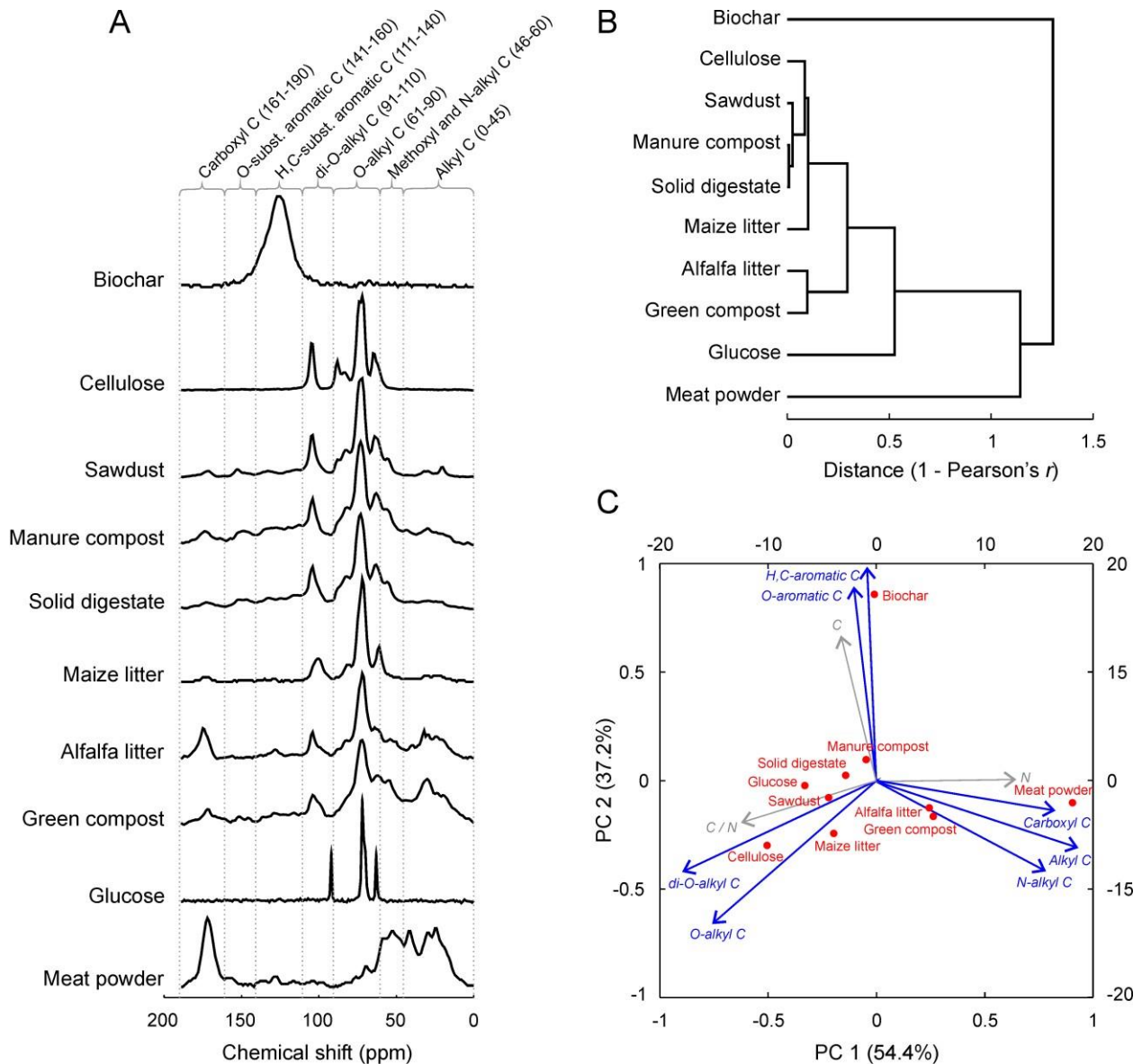
| Parameter                                   | S1                              | S2                 | S3                |
|---|---------------------------------|--------------------|-------------------|
| Soil type                                   | Silandic and<br>vitric andosols | Vertic xerofluvent | 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**

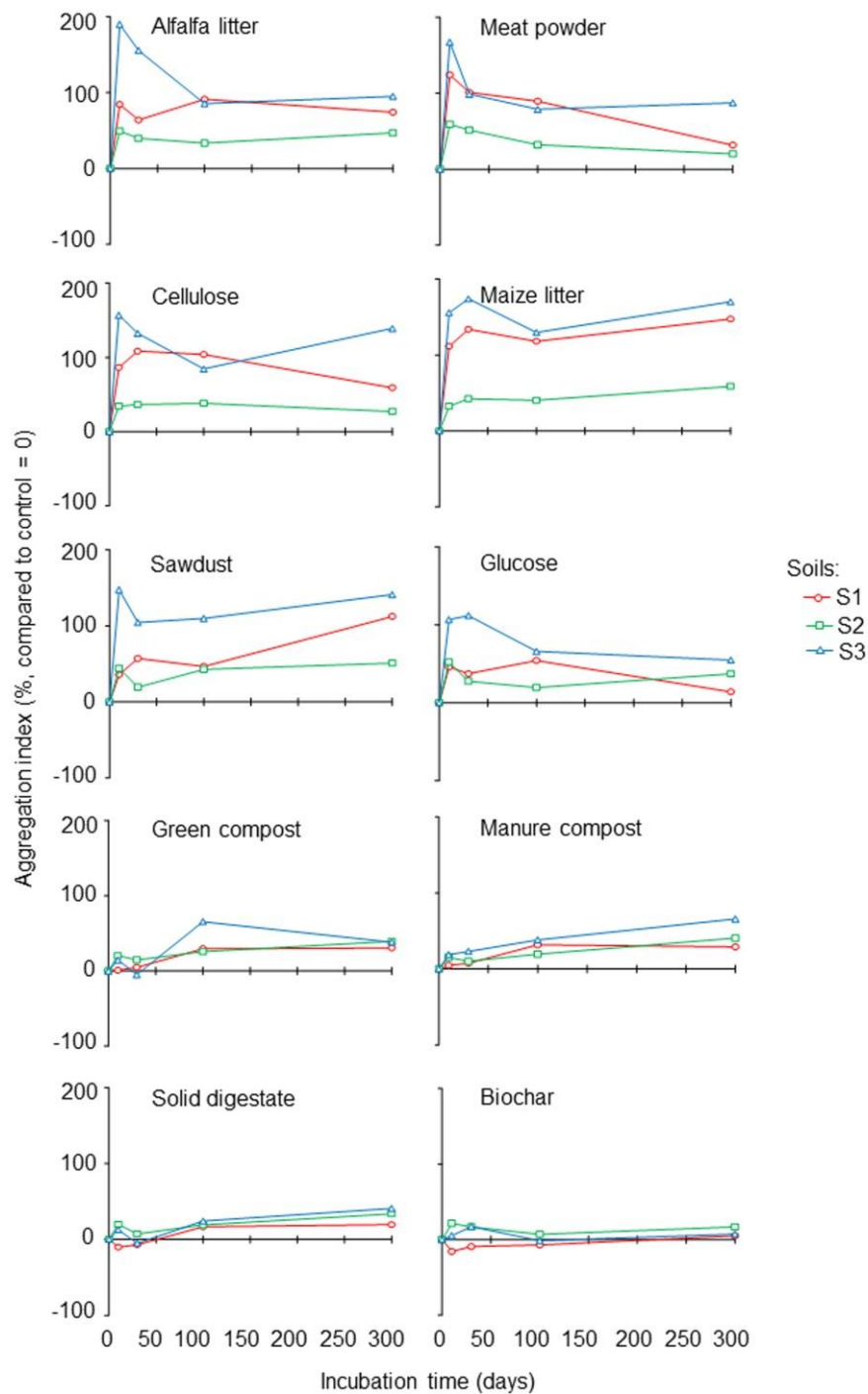
index (AI). Results of post-hoc testing for pair-wise AI differences between treatment combinations are in [Supplementary Tables S1-S3](#).

|                     | 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 |        |           |



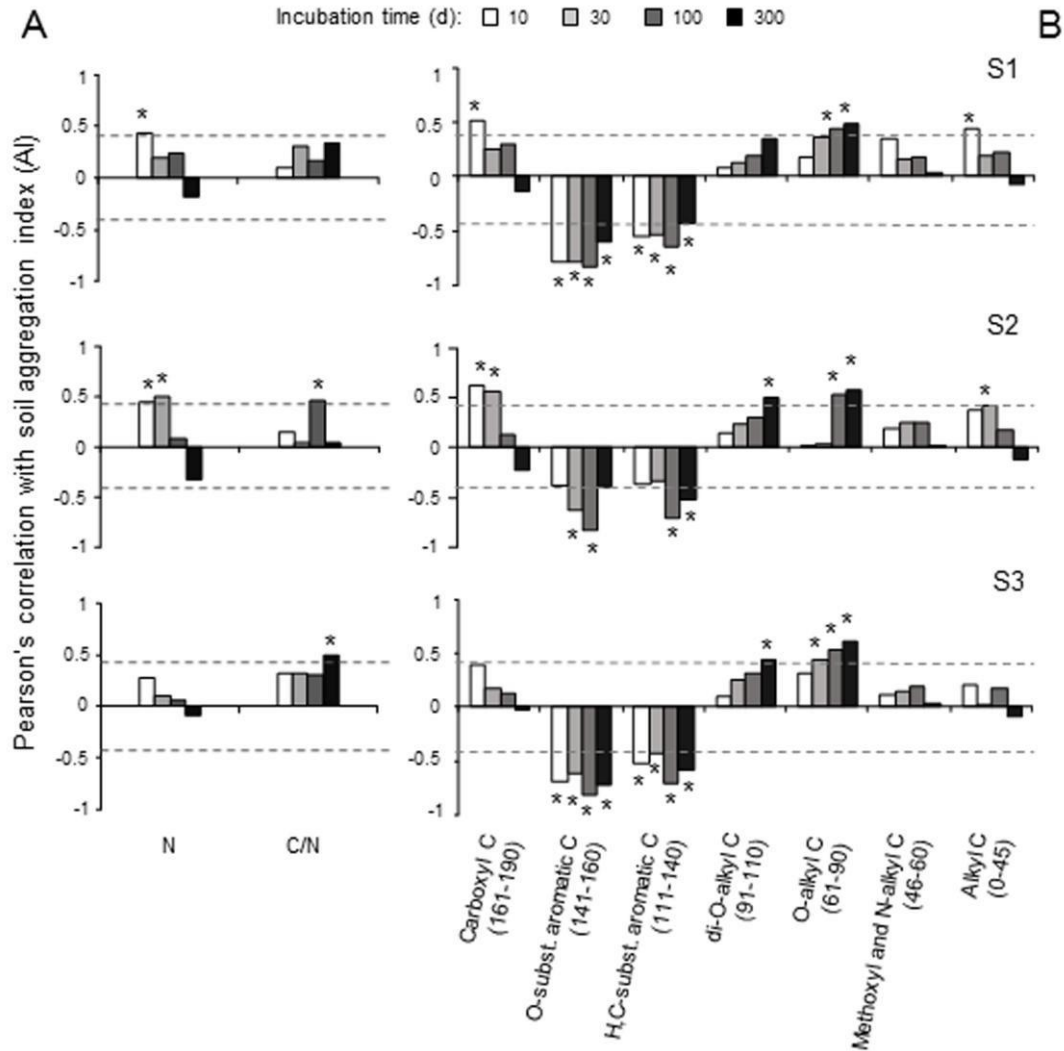
**Fig. 1.** Chemical differences among organic materials used for soil amendment. (A)  $^{13}\text{C}$ -CPMAS NMR spectra of the materials. Reference spectral regions and corresponding C types are reported on top of the panels, with chemical shift ranges indicated in brackets and by vertical dotted lines.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