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Organic matter quality defined by ^{13}C NMR spectroscopy explains nitrogen mineralization and soil aggregation better than C/N ratio

Ph.D. dissertation by
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To my beloved parents

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Abstract

Soil organic matter (SOM) plays a key role in sustainable agricultural production by the improvement of physical, chemical and biological properties of soils. The decline in organic matter (OM) content of many soils is becoming a major process of soil infertility and degradation, particularly in European semi-arid Mediterranean regions. Organic amendments can increase SOM content, thus influence soil characteristics by the interdependent modification of biological, chemical and physical properties. Then, a better understanding of the impact of organic amendment on soil processes is required. The underlying hypothesis of this thesis is that the organic amendment can regulate soil processes which directly linked to its initial chemical characteristics.

However, identifying and defining OM quality based on molecular composition is operationally difficult. In fact, OM contain multiple types of biomolecules with different aqueous solubility, and, hence, the different susceptibility to microbial decomposition (e.g. peptides, carbohydrates, lipids, lignin, organic acids, and polyphenols). Generally, lignin/N and C/N ratios are extensively used as descriptors of OM quality, but those simple indicators are not always give reliable information about their potential of effects on soil functions. In this context, several chemical throughput methods, such as pyrolysis-gas chromatography/mass spectrometry, near infrared reflectance spectroscopy and nuclear magnetic resonance (NMR) spectroscopy have been utilized to characterize OM at the molecular level. In particular, solid-state ^{13}C-CPMAS NMR spectra were found useful to provide an overview of the total organic chemical composition of complex matrices of SOM. This thesis showed that the chemical quality of OM, defined by solid-state ^{13}C-CPMAS NMR spectra, can be an important indicator of soil functions, which explain nitrogen mineralization and soil aggregation process better than classical C/N and lignin/N ratio indices. Finally, modeling approach based on novel implementation of OM quality by ^{13}C-CPMAS NMR, to purposely overcome the limitations of C/N as a single OM quality indicator and to explore the relationship between OM quality and soil structural stability. This thesis provides evidence for the importance of OM, and in particular its chemical quality, which influences soil processes by inter-depended modification of soil physical, chemical and biological parameters.

Chapter-1

General Introduction

Tushar C. Sarker

1.1. Effect of organic amendment on soil processes

Soil is a fundamental non-renewable resource along with possibly accelerated degradation rates and exceedingly gradual formation and regeneration processes (Van-Camp et al. 2004). Soil fertility refers to the capability of soil to serve physical, chemical and biological requirements for the plant growth to maintain productivity, reproduction and quality, relevant to plant and soil type, land use and climatic conditions (Abbott & Murphy 2007). It is now well understood that the appropriate agricultural utilization of soil resources needs equal attention for biological, chemical and physical components of soil fertility, thus attaining a sustainable agricultural system. Soil organic matter (SOM) plays a key role in soil conservation and/or restoration by sustaining its fertility, and hence in sustainable agricultural production, due to the improvement of physical, chemical and biological properties of soils (Sequi 1989). Zhao et al. (2009) reported that, to develop soil fertility; efforts need to be made to enhance SOM content. Organic amendments can increase SOM content (Van-Camp et al. 2004) and thus influence soil characteristics by the interdependent modification of biological, chemical and physical properties (Fig. 1.1).

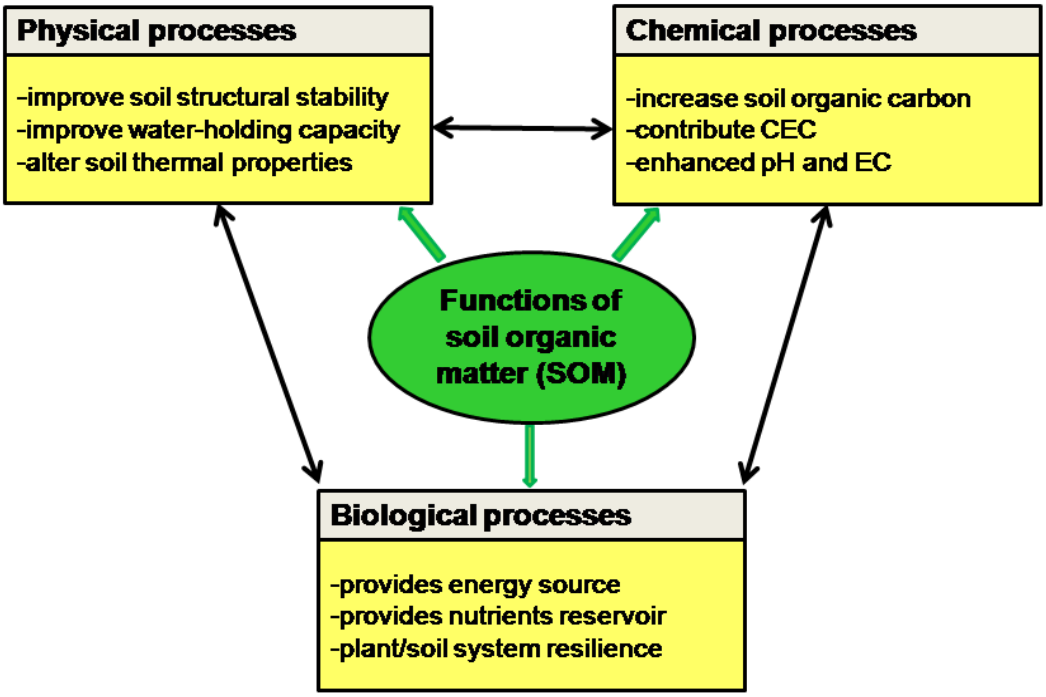

Figure 1.1. Functions of soil organic matter, interaction occur among different soil processes.

Over the past few decades many studies have reported evidence describing the effects of organic inputs on soil processes (biological, physical and chemical) (Abiven et al. 2007, 2009; Annabi et al. 2007, 2011; Garcia-Pausas & Paterson 2011; Krull et al. 2004; Murphy et al. 2011; Tejada et al. 2008). The influence of organic amendment on soil properties depends upon amount, type, size and dominant component of the added organic matter (OM) (Ros et al. 2003). A major review published by Diacono & Montemurro (2010) reporting the effect of organic amendment of soil fertility. Authors specially highlighted several key points such as (i) frequent addition of exogenous OM to cropland led to an increased soil biological functions. For instance, microbial biomass carbon and enzymatic activity increased up to 100% and 30% using high-rate compost and sludge addition, respectively; (ii) long-term organic incorporation enhanced soil organic carbon (SOC) content up to 90% compare to un-amended soil, and up to 100% compare to chemical fertilizer treatments; (iii) routine application of OM specially composted matters, increased soil physical fertility, by means of developing aggregate stability and decreasing soil bulk density.

1.1.1. Soil physical processes

Soil physical fertility refers to the soil structural stability and soil aggregation is a key process to maintain soil structural stability, which can be improved by means of proper management of organic amendments. This agronomic practice could improve soil pore space suitable for gas exchange, water retention, root growth and microbial activity (Van-Camp et al. 2004). OM is considered a vital factor affecting aggregation; indeed, the input of exogenous OM has been reported to improve soil aggregate stability and protect against the disruptive action of water in both laboratory and field experiments. A broad variability of improvements in aggregate stability has been observed that corresponds to the soil type and the quality of the amendments applied. Soils with high OM content is less prone to erosion processes compare to soils with low OM content; for instance, in arid and semi-arid areas (Durán Zuazo & Rodríguez Pleguezuelo 2008).

OM is an especially important factor controlling structural stability because its amount and properties can be modified trough agronomic management. A large variety of organic matter types including crop residues, composts, peats, organic wastes from agro-industries, and biochar are widely used as soil amendments. The link between OM addition, decomposition and stabilization, and soil aggregate dynamics intensively been studied and recognized (Abiven et al.

2007, 2009; Six et al. 2004; Tisdall & Oades 1982). Organic binding agents involved in stabilizing aggregates, classified into three main groups; i) transient, ii) temporary, and iii) persistent, based on the age and degradation of the OM (Tisdall & Oades 1982). Transient group is the most vital groups consisting mainly polysaccharides; microbial polysaccharides, originated by various OM are added to soil and polysaccharides associated with roots and the microbial biomass in the rhizosphere (Oades 1978). Temporary binding agents are roots and hyphae, particularly arbuscular mycorrhizal (AM) hyphae. This group persists for months or sometimes years and is affected by soil management practice (Tisdall & Oades 1979, 1980). Persistent binding groups are degraded, aromatic humic material associated with amorphous iron, aluminium and aluminosilicates and probably derived from temporary binding groups. Tisdall & Oades (1982) observed a significant but transient increase in aggregate stability when glucose was added to soil and a weaker but more persistent effect when the soil was enriched with cellulose. Transient and temporary effects of OM on aggregate stability were also due to the turnover of microbial products and cells while the persistent effects were due to humified compounds (Monnier 1965; Tisdall & Oades 1982).

Organic products stabilize soil structure by minimum two different ways; i) by increasing the inter particle cohesion within aggregates and ii) by enhancing their hydrophobicity, thus decreasing their breakdown by slaking. Particulate OM has distinct effects on the cohesion and soil hydrophobicity, relying on their intrinsic characteristics and associating decomposing microflora or exudates. During decomposition, microorganisms synthesized polysaccharides which are hydrophilic and tend to adsorb to mineral particles and increase their inter-particle cohesion (Chenu 1989). Humic substances are principally hydrophobic and increase the hydrophobicity of clays (Jouany 1991) and thus products with high humic substances content (e.g. manures or composts) are believe to increase aggregate hydrophobicity. Annabi et al. (2011) reported addition of immature municipal solid waste compost improved aggregate stability through an enhanced resistance to slaking, microbial activity and subsequently by increasing hydrophobicity, while mature green wastes and bio-waste compost improved aggregate stability by increasing inter particular cohesion. Tejada et al. (2008) found addition of green manure and compost increased structural stability increased 5.9% and 10.5% and bulk density decreased 6.1% and 13.5%, respectively compared to the control soil.

Depends on above information, we can summarized that repeated applications of organic amendments can significantly increase soil physical fertility, mainly by improving aggregate stability, as well as by improving water holding capacity.

1.1.2. Soil chemical processes

Soil chemical properties such SOC content, cation exchange capacity (CEC), electric conductivity (EC) and soil pH are very important parameter for soil processes, which are significantly mediated by organic amendment. Most often SOC is reported as the most important indicator of soil quality and agronomic sustainability due to its impact on soil properties and serve a reservoir of soil nutrients (Kukal et al. 2009; Liu et al. 2006). Cropping, tillage practice, residues removal caused a net depletion of SOC, and to sustenance of SOC level a minimum of 2.9Mg C is required to be added per hectare per annum as inputs (Mandal et al. 2007). Krull et al. (2004) reported to enhance SOC, the input rate must exceed the rate of loss from decomposition and leaching processes and this could be achieve by the frequent addition of organic residues. Additions of labile organic products (e.g. green or animal manure) improved productivity primarily by increased nutrient availability, whereas stable organic products (e.g. biochar, sawdust) improved productivity by enhancing SOC (Kimetu et al. 2008). Increase in SOC followed by different OM addition has been well established (Kukal et al. 2009; Mandal et al. 2007; Zhang et al. 2015). In a 3 years long field experiment Montemurro et al. (2006) observed significant increased total SOC content 43.17% and 23.98%, respectively by the municipal solid waste compost and olive pomace compost application, at the end of the experimental period.

Soil pH is considered to be a vital aspect of soil health, as plant growth, microbial functions, solubility of metal ion, and clay dispersion are influenced by soil pH (Haynes & Naidu 1998). Addition of organic products can regulate soil pH values, as soil pH is correlated to the intrinsic chemical properties of the OM and soil properties. Tang & Yu (1999) mentioned that the direction and the magnitude of pH change depend on the concentration of organic anions in the organic products, initial soil pH and the decomposition degree of the organic products. The presence of particulate functional groups such as carboxylic, phenolic, acidic alcoholic, amine, and amide allows SOM to play as a buffer over a broad range of soil pH values (Krull et al. 2004). OM with very high concentration of organic anions and nitrogen content (such as

leguminous residues) will have a higher influence on pH in respect to OM with lower alkalinity and nitrogen content such as wheat stalk (Butterly et al. 2011; Xu & Coventry 2003). Literature reported incorporation of different OM at different rate can increase (Garcıa-Gil et al. 2004; Mkhabela & Warman 2005; Zhang et al. 2006) or decrease (Bastida et al. 2008; Ilay et al. 2013; Kavdir & Killi 2008) soil pH. Kavdir & Killi (2008) reported pH values decreased with increasing application rates, while Garcıa-Gil et al. (2004) observed application rate had no significant influence on pH.

EC of the soil solution is related to the dissolved solutes content of soil and is often used as a measurement of soil salt content (Brady & Weil 1996), which can be influenced by the organic amendment (Rayment & Lyons 2011). Increased EC value by the organic amendment in different soils was observed (Kavdir & Killi 2008; Zhang et al. 2006), and EC values was increased with increasing application rates (Bastida et al. 2008). High CEC value is due to the high negative charge of OM, and contributes to increase soil capability to retain plant nutrient and creating them available to plants (Garcıa-Gil et al. 2004; Kaur et al. 2008; Ros et al. 2006b). Application of fertilizer, manure and charcoal (especially high temp char) can improve the CEC of the soil. Evidences suggest that there is an adjacent relationship between soil CEC, buffer capacity and pH, and these parameters are influenced by SOC content. Nevertheless, below a threshold value of 2% SOC content, there appears to be less or no effect on CEC (Krull et al. 2004).

1.1.3. Soil biological processes

Soil biodiversity consists of soil microflora (e.g. bacteria, fungi) and soil fauna, which can be classified by size as microfauna (e.g. nematodes, protozoa), mesofauna (e.g. acarids, enchytraea), and macrofauna (e.g. earthworms, termites). Soil biota (flora and fauna) have been shown to be sensitive to organic amendments (Treonis et al. 2010) and the quantity and/or quality of OM regulate soil biotic community and associated function (Garcia-Pausas & Paterson 2011; Murphy et al. 2011). Addition of OM increased SOM which supply a major source of metabolic energy to continue biological processes (Krull et al. 2004). Several authors (Kaur et al. 2008; Ros et al. 2006a) found direct effect of OM input on soil microorganisms; for instance, soil biological properties such as microbial biomass C, basal respiration and some enzymatic activities, are dramatically enhanced by compost application. This phenomenon is frequent in the upper layers

of the soil because OM labile fraction density was high in top soil (Ros et al. 2006a, b; Tejada et al. 2006, 2009). Treonis et al. (2010) found positive effects of organic amendment on soil microorganisms and the responses to organic amendment was tend to be rapid near the soil surface (0–5 cm).

Addition of different OM in the soil caused increase soil temperature and aeration, provides optimum conditions for microorganisms activity and greater contact between them and the OM, which lead to higher decomposition rates (Coppens et al. 2007; Fontaine et al. 2007). OM provides energy for decomposers (e.g. soil microbes, fungi and earthworms), since microorganisms used organic carbon (OC) as main energy source. OC either assimilated into their tissues and released as metabolic products, or respired as carbon dioxide (CO_2) in the atmosphere. Moreover, the macronutrients (e.g. N, P and S) exist in the organic products chemical structures, are converted into inorganic forms by the help of microbes. Subsequently, they are either immobilized and used in the synthesis of new microbial tissues or mineralized and released into the plant available soil mineral nutrient pool (Baldock & Skjemstad 1999). According to general trend, the quality and quantity of OM applied to the soil are the vital factors in regulating the abundance and diversity of microbial species and the function of microbes involved in nutrient cycling. An exponential increase in the soil respiration rate, reflecting the growth of microbial biomass was observed by addition of two crop residues (straw and cotton) and two animal by-products (meat bone meal and blood meal) in Mediterranean agricultural soil (Cayuela et al. 2009), and applied at three rates (5, 10 and 20 mg/g on dry weight basis). They observed the amount of total extra CO_2 evolved, differed significantly ($P < 0.005$) among application doses: 5 > 10 > 20 mg/g and residue type: meat bone meal > blood meal > cotton cardings > wheat straw. In addition, authors reported all residues caused a significant increase in soil microbial biomass size and functions, being the intensity of the response related to their chemical properties. Enhanced microbiological and biochemical (microbial biomass C, basal respiration and different enzymatic activities) properties were reported by the addition of organic urban wastes (both fresh and composted) in a two years long field experiment in Mediterranean semiarid region (Ros et al. 2003). Moreover, Annabi et al. (2011) reported enhanced microbial activity by immature compost compare to mature one, enhanced microbial activity may be due to larger labile organic pool was presence in immature compost. We can

summarize that addition of exogenous organic matter to soil lead to an improvement in soil biological properties, which depends on the quantity and quality of OM applied.

1.2. Role of organic amendment on nutrient availability

Plants productivity directly influenced by nutrients availability, and SOM is an important source of nutrients for plants in general and crops in particular, organic amendments can improve the soil nutrient status, but nutrient availability often losses via leaching or depressed by microbial immobilization of nutrients during decomposition (Krull et al. 2004). Utilization of organic amendment enhances soil nutrient storage for crops, reduce nutrient leaching and thus improve crop production (Steiner et al. 2007).

With the exception of inorganic fertilizers, SOM offers the largest pool of macro-nutrients and addition of organic amendments have been shown to increase yields by increasing the nutrient status of the soil, and sustained nutrient availability (Baldock & Skjemstad 1999; Krull et al. 2004). In order to sustain high productivity, plants require large amounts of N, and it is renowned that microbes convert organic N into inorganic forms (NH_4^+ and NO_3^-) by mineralization (Zhang et al. 2006). Plants mineral N availability decreases with the increasing C/N of added substrate. OM with low C/N consists more N than microorganisms need, and the surplus N being added in the soil. Legumes are rich in N, and additions of N rich legumes in soil will cause mineralization and added to N pool. In contrast, addition of organic products like crop straw, wood powder with high C/N ratio, microbial population competes with plants for N, thus immobilized it (Amlinger et al. 2003), though contrast results was also reported in literatures (Berg & McClaugherty 2013; Hättenschwiler et al. 2011).

Among others, N is one of the most important nutrients for plants growth and productivity. Increased soil mineral N content followed by organic amendment is reported by many authors (Barbarick & Ippolito 2007; Burgos et al. 2006; Eghball 2002; Hartl & Erhart 2005). Plant available potassium (K) and phosphorus (P) content of soil was also increased by organic amendment (Hartl et al. 2003; Steiner et al. 2007). After five years field application of biowaste compost from organic household waste and yard trimmings, Hartl et al. (2003) observed significant increase of soil potassium content on an average by 26%. Enhanced K may be due to the large fraction of woody plant material and kitchen refuse was existed in the compost raw material. Zhang et al. (2006) reported application of co-compost, derived from biosolids-

municipal solid waste increased soil P and K of arable land, and the addition rate were 50, 100 and 200 t/ha and the addition frequency was once in 4 years. The P concentrations by 50, 100 and 200 t/ha treatment were 32.9, 53.7 and 86.0 mg/kg, respectively at 0-15 cm depth. These values were higher ($p < 0.0001$) in respect to control (7.2 mg/kg). Similarly, K concentration in soil was increased along with the rate increment of compost application at the depth of 0-15 cm. And the values were 103.9, 125.5 and 168.0 and 75.6 mg/kg by 50, 100 and 200 t/ha and the control treatment, respectively. Moreover, significant increased ($p< 0.05$) in P and K contents were observer by the application of chicken manure compared to all other treatments (Steiner et al. 2007).

As we mention earlier plant nutrient availability is often limited by leaching, ammonium volatilization or microbial immobilization. Nutrients leaching from agricultural soil caused deplete soil fertility, accelerate soil acidification, and adversely affect the quality of surface and groundwater. Nutrients leaching significantly reduced by organic amendment and improved the plant nutrients use efficiency in agriculture (Clough et al. 2013; Torstensson et al. 2006). Torstensson et al. (2006) compare nutrient leaching between conventional system (mineral fertilizers and pesticides were used) and organic farming system. Authors observed potassium-leaching decreased on an average 16 kg/ha/yr by organic systems, in respect to conventional system (27 kg/ha/yr) over the 6 year period. Moreover, addition of biochar to a typical Midwestern agricultural soil substantially decreased nutrient leaching, and authors stated that biochar application to the soil could be an potential management alternative for reducing nutrient leaching from agriculture crop field (Laird et al. 2010).

1.3. Link between OM chemical quality and soil processes

The intrinsic initial biochemical characteristics of OM are major factors driving its decomposition rate (Bonanomi et al. 2013; Cornwell et al. 2008), hence directly or indirectly affecting interconnecting ecological processes, including nutrient dynamics (Bonanomi et al. 2010, 2014; Gartner & Cardon 2004) and suitability for microbial feeding (Incerti et al. 2013; Voříšková & Baldrian 2013). Moreover, few authors reported functions of OM on soil properties is better predict by initial biochemical characteristics of the added organic products (Abiven et al. 2009; Flavel & Murphy 2006; Schmidt et al. 2011). So, better understanding of OM chemical quality is required. Defined OM quality in terms of chemical composition (Swift et al. 1979) is

operationally complex because OM consist of different organic compounds with particular susceptibility to decomposition (e.g. cellulose, organic acids, amino acids, simple sugars, lignin, tannins, humic substances) (Rovira & Vallejo 2007). During the last decades, a significant effort has been made to find out effective indicators of OM quality, which is capable to serve reliable predictions of its effects on soil processes. In this perspective, several chemical throughput methods are currently available and have been applied to collect direct information on the characteristics of organic matter, including pyrolysis-gas chromatography/mass spectrometry (Huang et al. 1998), near infrared reflectance spectroscopy (Gillon et al. 1999) and ^{13}C-cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Keiluweit et al. 2015; Kögel-Knabner 2002).

Recently, several authors attempt to linked between OM initial chemical characteristics defining by ^{13}C-CPMAS NMR and soil aggregation (Abiven et al. 2009; Martens 2000), soil water repellency (Cesarano et al. 2016), OM microbial decomposition (Bonanomi et al. 2013; Heal et al. 1997; Kögel-Knabner 2002), C and N mineralization (Flavel & Murphy 2006; Rowell et al. 2001) processes. Carbon complexity impacts on the microbial SOM decomposition rate (Kögel-Knabner 2002), and thus regulates amendment stability and N release (Bernal et al. 1998), as easily degradable forms of carbon are preferentially utilized by microbial populations. Recently, in a literature analysis Abiven et al. (2009) reported the effect of initial biochemical characteristics of the organic products on decomposition and thus effect soil aggregation. The joint impacts of the biochemical composition and quantity of OM added to soils affect the rate and stability of aggregation, and the rate of aggregate turnover (Bronick & Lal 2005). In a study about influence of plant litter on soil water repellency (SWR), Cesarano et al. (2016) defined litter quality with ^{13}C-CPMAS NMR, and authors reported that biochemical quality of plant litter is a major controlling factor of SWR. Rowell et al. (2001) observed net N mineralization rate was best predicted by a model incorporating the initial organic N concentration and the proportion of phenolic C determined from solid-state ^{13}C-NMR spectroscopy. In contrast, Flavel & Murphy (2006) reported no significant relationship between either ^{13}C-NMR spectral groupings, or their ratios, and the CO_2 evolved or gross N mineralized from the amendments.

1.4. Modeling approach

Modeling OM decomposition and functions, is fundamental to understand biogeochemical cycling in terrestrial ecosystems. Current models use C/N or lignin/N ratios to describe susceptibility to decomposition, or implement separate C pools decaying with different rates, disregarding biomolecular transformations and interactions and their effect on decomposition dynamics. The main examples are biogeochemical models simulating carbon dynamics and other ecosystem processes, such as CENTURY (Parton et al. 1994), ROTHAMSTED (RothC) (Coleman & Jenkinson 1996), LPJ (Sitch et al. 2003), and the Biome- (Hunt 1977) and Forest- (Running & Gower 1991) BGC (Bio Geochemical Cycles) models. Other models, specifically designed for application to agro-ecosystems, include DNDC (Li et al. 2003), DayCent (Del Grosso et al. 2001) and CANDY (Franko et al. 1995).

Although widely used, research has found that simple indicators such as lignin/N or C/N are not always reliable indicator of decomposition rates. For instance, Berg & McClaugherty (2013) suggested that the use of C/N ratio to predict decay rate throughout the decomposition process should be avoided, because, molecular composition of OM is known to progressively change as a function of the relative susceptibility of biomolecules to breakdown, with rapid mineralization of labile sugars, and selective preservation of less degradable lipids, lignin, and polyphenols (Davidson & Janssens 2006; Rovira & Vallejo 2007). Berg & Matzner (1997) attempted to capture such compositional changes in a three-phase model, whereby labile biomolecules and mineralized nutrients (i.e. N and P) controlled decay rate up to 30–40 % of mass loss, while lignin became progressively more important from then onwards. The model has been successfully tested on a range of biomasses including hardwoods and coniferous plants (Berg & McClaugherty 2013). More recently, Incerti et al. (2017) developed a new model called OMDY (Organic Matter DYnamics); a new model of organic matter decomposition based on biomolecular content as assessed by ^{13}C-CPMAS NMR spectroscopy.

1.5. General objectives

The general objective of this thesis was to investigate the effect of organic amendment on key soil processes. Specifically, I aim to determine the relationship between amendment chemical quality and key soil processes, such as nitrogen mineralization and soil aggregation. Finally, we developed a new model that represents OM aggregation in relation to OM chemical quality.

Addressing this broad objective was approached by sequentially addressing the following more specific research objectives:

1) to assess the nitrogen mineralization capability of different OM types in soil, and explore the relationships between soil mineral N release and OM chemical quality defined by ^{13}C-CPMAS NMR spectroscopy and classical elemental parameters. Also, compare the capability of classical C/N ratio and NMR spectral C type as a predictor of mineral N release dynamics (*Chapter 2*);
2) to investigate the capability of different OM types to induce soil aggregation, and relate aggregation process with OM quality parameters defined by ^{13}C-CPMAS NMR spectra and traditional elemental analyzer (*Chapter 3*), and;
3) to develop a new model based on novel implementation of OM quality defined by ^{13}C-CPMAS NMR spectroscopy, to purposely overcome the limitations of using C/N as a single OM quality indicator and to explore the relationship between OM quality and soil aggregation (*Chapter 4*).

Chapter – 2

Predicting nitrogen mineralization from organic amendments: beyond C/N ratio with a ^{13}C NMR approach

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In collaboration with Giuliano Bonanomi, Stefano Mazzoleni

Abstract

The processes N mineralization and immobilization, which occur in soil during microbial decomposition of organic matter (OM), are important for N dynamics in soil systems. In this context, we test the hypothesis that the initial chemical quality of OM characterized by ^{13}C-CPMAS NMR spectra explains the variability of N release dynamics better than traditional C/N ratio index after organic input. OM was characterized by traditional elemental parameters and ^{13}C-CPMAS NMR spectra to investigate the effects of organic matter initial chemical quality on N dynamics. Incubation experiment was carried out in laboratory condition, using three soil types (S1, S2 and S3), nine organic substrates (alfalfa litter, biochar derived from wood, cellulose, grass litter, fish meal, glucose, meat powder and wood powder), replicated 3 times for each of 5 incubation times, for a total of 405 microcosms. We found that highly proteinaceous organic matter such as meat powder and fish meal driven a rapid initial N mineralization followed by alfalfa litter and humus, while organic materials like wood powder, cellulose, biochar, glucose and grass litter immobilized N when incorporated into the soil, depending on soil type and incubation time. Considering ^{13}C-CPMAS NMR spectral regions, the carboxyl C (161-190 ppm), N-alkyl and methoxyl C (46-60 ppm) and alkyl C (0-45 ppm) regions had a significant positive correlation with N mineralization, while the di-*O*-alkyl C (61-90 ppm) and *O*-alkyl C (91-110 pmm) had a significant negative correlation with N mineralization. Our study suggests that biochemical quality of organic matter defining by ^{13}C-CPMAS NMR is capable to predict N release patterns better than the traditional C/N ratio index. These results serve as a unique contribution towards a full understanding the correlation between organic matter initial chemistry and N dynamics in soil.

2.1. Introduction

Nitrogen (N) is a fundamental element for life, presence in the basic building blocks of life. Though N is abundant (78%) of earth's atmosphere but most living organisms cannot use atmospheric N_2 as a source of N (Deenik 2006; IRRI 2009), since too much energy is required to break down the triple bond between the atoms of N within N_2. More than 95% of N in soil exists in organic matter (OM) and it is an essential nutrient taken up in large quantity by plants.

When OM are added to soil microorganism population decompose OM and convert the organic form of nitrogen into plants available inorganic forms as ammonia (NH_4^+) and nitrate (NO_3^-), this process is known as N mineralization, whereas N immobilization is the conversion of inorganic N into organic N (Alexander 1977), and both processes occur simultaneously in soil. According to the classical concept, OM with low C/N ratios tend to exhibit net N mineralization (Mondini et al. 2008; Van Kessel et al. 2000), while OM with high C/N ratios exhibit net N immobilization (Li et al. 2013; Mohanty et al. 2010). Briefly, a low C/N ratio (less than about 30) means sufficient N is supplied through the decomposition of the OM to meet the N needs of the decomposing organisms. As a result, there will be a net release and build up of inorganic N in soil (mineralization). When the C/N ratio of added OM is high (30 or more), microorganisms will require more N from soil (in the form of NH_4^+ or NO_3^-) to decompose the C present in the OM. This N will be immobilized (unavailable to plants) until these microorganisms die and the N is released (IRRI 2009) (Fig. 2.1). This means that organic amendments can either be a source of plant available mineral N or can be causes micorbial immobilization (Ambus et al. 2002; Gabrielle et al. 2004).

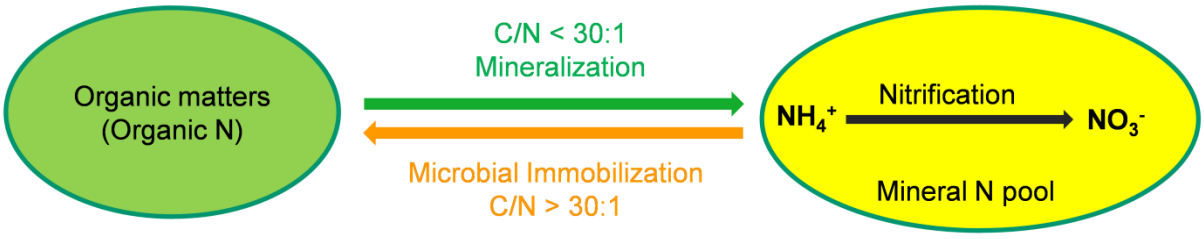

Figure 2.1. Processes constituting the N mineralization-immobilization turnover depending on classical C/N ratio index.

Wide range of OM types including crop residues, animal residues, composts, peats, organic wastes from agro-industries, and biochar are widely used as soil amendments. In general animal residues (Cayuela et al. 2009; Mondini et al. 2008) and legumes are (Fosu et al. 2007) are very high N content, thus low C/N ratios (1-20) and incorporation of these matters to soil will raise microbial community thus driven fast decomposition and mineralization. The N availability from OM depends on the amount of N mineralized or immobilized during decomposition. Then, a better understanding of the impact of different OM types on soil N dynamics is required. However, previous studies demonstrated that the decomposition and nutrient release rates of OM are often regulated by environmental factors and biochemical composition of OM and their interaction (Abiven et al. 2005; Mohanty et al. 2013). The chemical quality like initial N concentration, C/N ratio, lignin, polyphenols, lignin/N ratio are considered useful indicators that control decomposition and N release (Nakhone & Tabatabai 2008; Vahdat et al. 2011), where the C/N ratio is often considered as single most predictive parameter (Flavel & Murphy 2006; Hadas et al. 2004; Nicolardot et al. 2001). However, it has not been clearly established which of these variables correlate best with N mineralization, as contrasting results have been reported in the literature. For instance, Berg & McClaugherty (2013) suggested that the use of C/N ratio to predict decay rate throughout the decomposition process should be avoided, because, irrespectively of its initial value, it progressively decreases as C is lost through respiration, while N is immobilized in the microbial biomass (Bonanomi et al. 2010). More recently, Hättenschwiler et al. (2011) revisited the commonly held view that N and lignin control the rate of plant litter decomposition, and indicated that, at least in tropical ecosystems, non-lignin plant carbon molecules at low concentration play the major role. In line with this, a recent study based on a controlled experiment of the decomposition of 64 litter types, showed a weak association between C/N ratio and decay rates (Bonanomi et al. 2013). Moreover, the litter molecular composition is known to progressively change as a function of the relative susceptibility of biomolecules to breakdown, with rapid mineralization of labile sugars, and selective preservation of less degradable lipids, lignin, and polyphenols (Davidson & Janssens 2006; Rovira & Vallejo 2007). Moreover, Rowell et al. (2001) reported total C/N ratio will not necessarily give an adequate indication of an amendment's potential of N supply, such as N release dynamics of composted matters (Ambus et al. 2002). Since, C/N ratio cannot explain all differences in N mineralization; we proposed a hypothesis that OM quality characterized by ^{13}C-CPMAS NMR

spectra that predicts the N mineralization or immobilization process better than classical C/N ratio index.

In this perspective, several chemical throughput methods are currently available and have been applied to collect direct information on the characteristics of organic matter, including pyrolysis-gas chromatography/mass spectrometry (Huang et al. 1998), near infrared reflectance spectroscopy (Gillon et al. 1999) and ^{13}C-cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Keiluweit et al. 2015; Kögel-Knabner 2002). In detail, ^{13}C-CPMAS NMR has been proven useful to provide a description of the total organic chemical composition of complex matrices, such as plant litter (Kögel-Knabner 2002), and its relationships with litter decay rate (Bonanomi et al. 2013).

In this study, we used a detailed OM characterization by ^{13}C-CPMAS NMR in solid state (Kögel-Knabner 2002), coupled with microcosms incubation experiment in laboratory condition, to investigate the effects of OM chemistry on soil N mineralization process. In detail, we evaluated the capability of 9 organic amendment types, spanning a wide range of biochemical quality, to mineralize nitrogen in 3 soil type with different texture. Specific aims of the study were to:

(1) assess N mineralization capability of different OM types depending on incubation time;
(2) explore the relationships between soil mineral N release and OM biochemical quality, as defined by ^{13}C-CPMAS NMR spectroscopy and classical elemental analyzer; and
(3) investigate the capability of traditional C/N ratio and NMR spectral regions as a predictor of N release dynamics and compare two parameter with each other.

2.2. Materials and methods

2.2.1. Organic matter collection and chemical characterization

Nine organic amendment types (Fig. 2.2A) representing a wide range of organic matter chemistry were selected, i) Alfalfa litter; ii) biochar-wood; iii) cellulose; iv) litter from the perennial grass *Dactylis glomerata*; v) fish meal; vi) glucose; vii) humus; viii) meat powder; and ix) wood powder.

Organic amendments were characterized for total C and N content by flash combustion of micro samples (5 mg of sample) in an Elemental Analyser NA 1500 (Fison 1108 Elemental

Analyzer, Thermo Fisher Scientific). Moreover, all amendments were characterized by ^{13}C-CPMAS NMR in solid state under the same condition, thus allowing a quantitative comparison among NMR spectra. A spectrometer (BrukerAV-300, Bruker Instrumental Inc, Billerica, MA, USA) equipped with a magic angle spinning (MAS) probe with wide-bore of 4 mm was used, set up with MAS of 13,000 Hz of rotor spin, a recycle time of1 s, a contact time of 1 ms, an acquisition time of 20 ms, and 2000 scans (for details see Bonanomi et al. 2013). Selection of spectral regions and identification of corresponding classes of C-types were performed according to previous studies (Bonanomi et al. 2015; Kögel-Knabner 2002; Li et al. 2015; Mathers et al. 2007; Pane et al. 2011). The following seven regions and 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.

2.2.2. Nitrogen mineralization experiment

Three soils showing contrasting texture, nutrient availability and organic matter content were selected to represent a range of soil types (S1: Botanical garden, S2: Capasso and S3: Concilio; see Supplementary Table 2.S1). Soils were collected from the top layer (first 20 cm), sieved at 2 mm and were oven air-dried at 25-30°C.

Nitrogen mineralization experiments were carried out with 3 different soil types and 9 different OM types in microcosms at laboratory condition (Fig. 2.2). Plastic jars were filled with 100 g of dry soil and were incorporated homogeneously with 3 % (w/w) of each dry organic matter type. The soil with added organic material (amended treatment, AT) or without (control treatment, CT) were replicated 3 times for each of 5 incubation days (3, 10, 30, 100 and 300 days). Microcosms were kept in a growth chamber under controlled temperature (18±2°C night and 24±2°C day) and watered every seven days to field capacity with distilled water. The full experimental design entailed three soil types, nine organic substrates, replicated 3 times for each of 5 incubation times, for a total of 405 experimental units (Fig. 2.2). At each harvesting time, the soil was collected, air dried and stored until analyses.

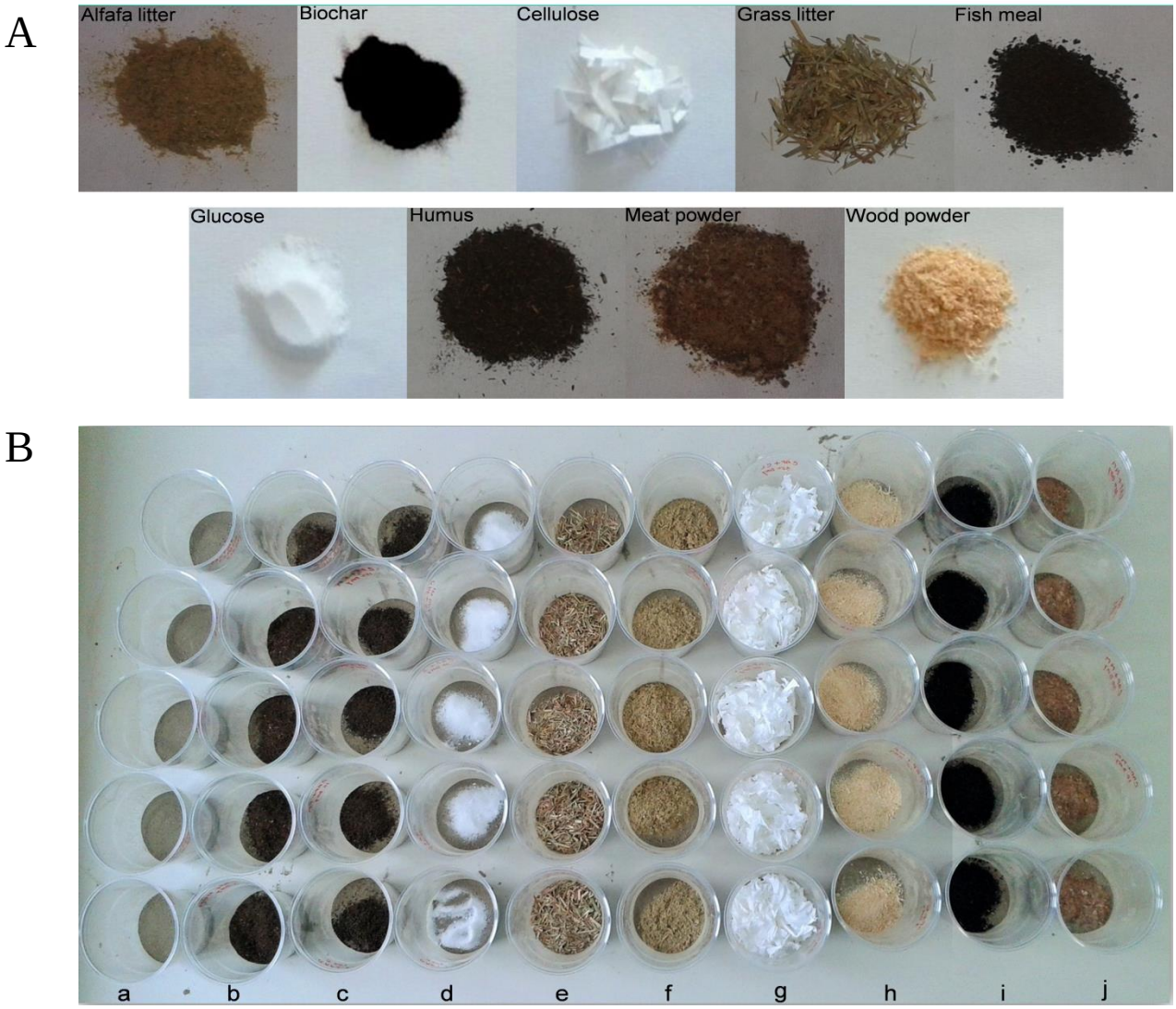

Figure 2.2. Microcosms incubation experiment using three soil types (S1, S2 and S3) and nine organic matter types. (A) showing nine different organic materials (alfalfa litter, biochar, cellulose, fish meal, glucose, grass litter, humus, meat powder, and wood powder) used for soil amendment; and (B) showing experiment set up mentioning treatments combinations used. Treatment as follows: a) control (without OM); b) soil + humus; c) soil + fish meal; d) soil + glucose; e) soil + grass litter; f) soil + alfalfa litter; g) soil + cellulose; h) soil + wood powder; i) soil + biochar; and j) soil + meat powder.

2.2.3. Soil chemical analysis

Electrical conductivity (EC) and pH of soil were determined by standard methods (Sparks 1996). After experiment pot soils were sieved through a 2 mm sieve and then mixed with distilled water

at 1:2.5 ratios and then set on electronic shaker and the EC was determined using a BASIC 30, CRISON conductimeter. Soil pH was determined in same water-soil mixture by using a BASIC 30, CRISON pH-meter.

NO_3^- and NH_4^+ content of incubated soil were measured by the help of the DR 3900 Spectrophotometer (Hach, Loveland, CO, USA) using Barcode Program. Samples were prepared using 1g of dry pulverized soil were taken in 2ml eppendorf tube and added 1 ml distilled water, then eppendorf tubes were set on an electric orbital shaker (SI50, UK) for 30 min. After 30 minutes of shaking, eppendorf tubes were centrifuged for 10 minutes at 13000 rpm with a centrifuge machine (MSE, MSB 010.CX2.5, UK). Thus samples were ready for measurement.

The kits LCK 340: assay range (5-35 mg/l) and LCK 303: assay range (2-47 mg/l) was used to assess NO_3^- and NH_4^+, respectively. For NO_3^- measurement, pipetted 0.2ml of supernatant sample in the cuvette then added 1ml of solution A in the cuvette and shake firmly. Waited 15 min. and thoroughly clean the outside of the cuvette and evaluated. The same procedure was also followed to measure NH_4^+ except adding solution A. Measurement was conducted for all 3 experiments, all sampling dates (at 3, 10, 30, 100 and 300 days from OM application) and triplicates were performed for each activity assay.

2.2.4. Statistical analysis

Data from the microcosms experiment were statistically evaluated by univariate Analysis of Variance (ANOVA), considering main and interactive effects of soil type (S: S1, S2 and S3), organic matter type used for soil amendment (OM: alfalfa litter, biochar, cellulose, grass litter, fish meal, glucose, humus, meat powder, wood powder) and incubation time (T: 3, 10, 30, 100 and 300 days) on soil NH_4^+, NO_3^-, pH and EC.

To address the relationships of organic amendment and mineral N (NH_4^+ and NO_3^-) release at different incubation time with organic matter biochemistry, different approaches were considered. First, simple linear correlation analysis was separately tested between mineral N (NH_4^+ and NO_3^-) release in soil and each organic matter chemical parameter, including both elemental chemical parameters (i.e. N content and C/N ratio) and regions of the ^{13}C-CPMAS NMR spectra selected from reference literature (Kögel-Knabner 2002; Mathers et al. 2007). In a more detailed analysis, correlation was extensively tested between mineral N (NH_4^+ and NO_3^-) release in the tested soil amended with the 9 amendment types and ^{13}C NMR data recorded for

the same organic materials at each resonance signal (n = 190), providing a fine-resolution profile of the variation in C types in the tested organic material associated with the effect on N mineralization. This analysis allows identifying restricted ^{13}C-CPMAS NMR spectral regions showing significant correlation with N mineralization. The correlation was tested for statistical significance controlling for multiple comparisons, according to Bonferroni's correction at $p< 0.01$. Dendrogram of organic materials obtained by hierarchical cluster analysis (HCA) as a successive chemometric approach applied to a data matrix of signals recorded in the ^{13}C-CPMAS NMR spectra.

2.3. Results

2.3.1. Organic matter initial biochemistry

Nine organic amendment types representing a wide range of OM chemistry in term of C, N content and C/N ratio, characterized by elemental analyzer are presented in Table 2.1. ^{13}C-CPMAS NMR spectra showed remarkable differences in organic C components among OM types in respect to spectral regions and major chemical shifts observed (Fig. 2.3A). The alkyl-C (0-45 ppm) region, characteristic of lipid and waxes, but also amino acids, and the methoxyl and N-alkyl C (46-60 ppm) region, which represents protein and peptide; these two spectral regions are abundant in meat powder and humus followed by fish meal, and alfalfa litter (Fig. 2.3A). These two regions are less pronounced in wood powder and grass litter; while both regions are not present in biochar, glucose and cellulose. The O-alkyl-C (61-90 ppm) and the di-O-alkyl-C (91-110 ppm) region, mainly associated with carbohydrates and polysaccharides, are dominated in glucose followed by wood powder, cellulose, grass litter, while in humus and biochar these regions are less abundant (Fig. 2.3A). The most pronounced difference observed in the H- and C-substituted aromatic C (111-140 ppm) and O-substituted aromatic C (141-160 ppm) regions, which represents more recalcitrant aromatic C fractions, are highly present in the biochar followed by humus, while are not abundant in all other OM. Finally, carboxyl C (161-190 ppm) region is abundant in meat powder, alfalfa litter, fish meal followed by humus, while this region is absent in glucose and cellulose (Fig. 2.3A).

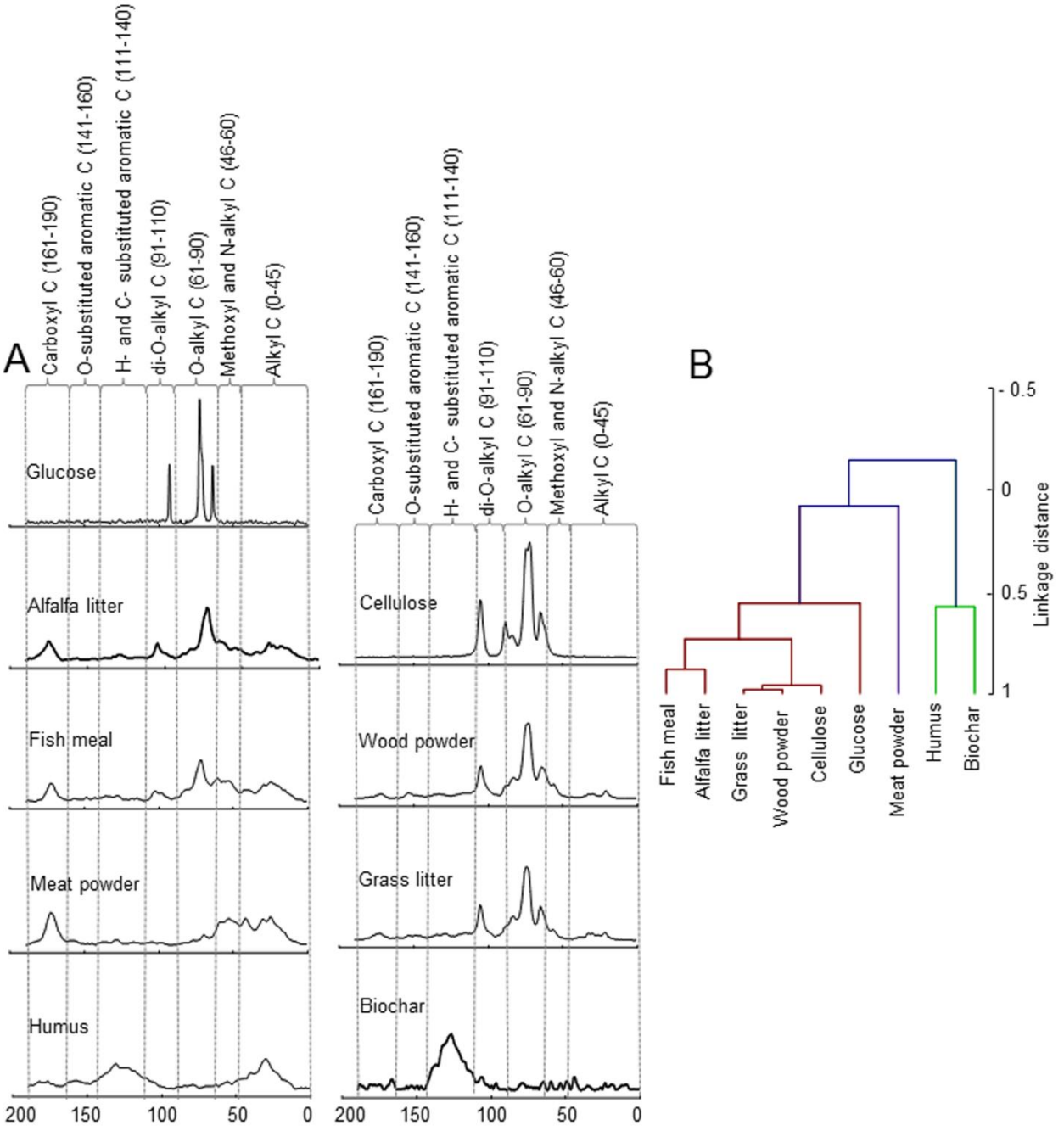

Figure 2.3. Chemical differences among organic materials used for soil amendment. (A) ^{13}C-CPMAS NMR spectra of the materials. Reference spectral regions and corresponding C types are reported on top of the panels, with chemical shift ranges indicated in brackets and by vertical dotted lines. (B) Dendrogram of organic materials, obtained by hierarchical cluster analysis (HCA) applied to a data matrix of on 1-ppm wide signals recorded in the ^{13}C-CPMAS NMR spectra.

Hierarchical clustering analysis provides a similarity relationship among used OM types in term of signals recorded in the ^{13}C-CPMAS NMR spectra (Fig. 2.3B). Dendogram showed 3 clear similarity groups; first group consist biochar and humus, second group contain only meat powder, while third group content all other OM types (Fig. 2.3B). In the 3rd group, fish meal and alfalfa litter are similar, so does grass litter, wood powder and cellulose, while glucose is separated from the others (Fig. 2.3B).

Table 2.1. Initial C, N content and the C/N ratio of nine different organic materials used in the N mineralization experiment.

Organic matters	Nitrogen	Carbon	C/N ratio
Alfalfa litter	3.93	38.29	9.73
Biochar (wood)	0.50	74.57	149.14
Cellulose	0.10	50	500
Fish meal	6.06	74.19	12.24
Glucose	0.00	43.0	-
Grass litter	1.19	45.34	38.10
Humus	2.4	35.9	14.96
Meat powder	8.26	43.88	5.31
Wood powder	0.11	49.88	453.45

2.3.2. Effect of organic amendment on soil mineral N forms

In the mesocosm incubation experiment, all amending treatments combinations (i.e. soil type, organic matter type, and incubation time) markedly influenced soil mineral N **(**NH_4^+ and NO_3^-) forms. However, occurrence and magnitude of mineral N dynamics were highly variable among OM types in combination with main and interactive effect of soil types and incubation days (Table 2.2). Among amendment types, the two OM derived from animal remains (e.g. meat powder and fish meal) showed the massive mineral N release, which was superior over control and all other amendment types (Fig. 2.4 and Fig. 2.5). Specifically, the strong NH4+ release was observed with meat powder and fish meal, followed by alfalfa litter, while larger NO_3^- release with fish meal and meat powder followed by alfalfa litter in tested soils (Fig. 2.4). Among soil types mineral N content was highest in S3 followed by S2 and S3 (Fig. 2.4). Control treatment

showed relatively constant mineral N release in tested soils (Fig. 2.4). Moreover, lower level of N release observed from the humus in all tested soils. In contrast, strong immobilization was observed with cellulose, grass litter and wood powder, while immobilization was intermediate with glucose (Fig. 2.5). Finally, interesting results were observed with biochar, caused very litter (S1 and S2 soil) or no (S3 soil) N immobilization in respect to control (Fig. 2.5).

Table 2.2. Summary of the ANOVA testing for main and interactive effects of soil type (S: S1, S2 and S3), organic matter type used for soil amendment (OM: alfalfa litter, biochar, cellulose, fish meal, glucose, grass litter, humus, meat powder, wood powder) and incubation time (T: 3, 10, 30, 100 and 300 days) on soil NH_4^+, NO_3^-, pH and EC.

	SS	d. f.	MS	F	P
NH_4^+					
Soil type (S)	1.7	2	0.87	0.17	0.842
Organic matter (OM)	496414.3	9	55157.15	10859.84	<0.001
Incubation time (T)	45034.8	4	11258.70	2216.71	<0.001
S × OM	2263.1	18	125.73	24.75	<0.001
S × T	3103.5	8	387.93	76.38	<0.001
OM × T	255235.3	36	7089.87	1395.92	<0.001
S × OM × T	24326.2	72	337.86	66.52	<0.001
Error	1523.7	300	5.08		
NO_3^-					
Soil type (S)	2759530	1	2759530	754.3312	<0.001
Organic matter (OM)	156721	2	78361	21.4203	<0.001
Incubation time (T)	1865669	9	207297	56.6655	<0.001
S × OM	921408	4	230352	62.9679	<0.001
S × T	204585	18	11366	3.1069	<0.001
OM × T	148311	8	18539	5.0677	<0.001
S × OM × T	1456520	36	40459	11.0596	<0.001
Error	1097474	300	3658		
pH					
Soil type (S)	8.97	2	4.49	37.5	<0.001

Organic matter (OM)	10.23	9	1.14	9.5	<0.001
Incubation time (T)	14.39	4	3.60	30.1	<0.001
S × OM	1.74	18	0.10	0.8	0.692
S × T	2.05	8	0.26	2.1	0.031
OM × T	10.02	36	0.28	2.3	<0.001
S × OM × T	5.15	72	0.07	0.6	0.994
Error	35.85	300	0.12		
EC					
Soil type (S)	25945646	2	12972823	2082.1	<0.001
Organic matter (OM)	77247590	9	8583066	1377.6	<0.001
Incubation time (T)	11151575	4	2787894	447.5	<0.001
S × OM	6797739	18	377652	60.6	<0.001
S × T	14154488	8	1769311	284.0	<0.001
OM × T	30620449	36	850568	136.5	<0.001
S × OM × T	15413441	72	214076	34.4	<0.001
Error	1869162	300	6231		

Incubation time of amended soil samples greatly affected inorganic N release dynamics, with both main and interactive effects in combination with amendment type, and soil type (Table 2.2). In other words, over the incubation period (300 days), soil treated with OM showed variable response dynamics according to the OM types, with differences in times of mineral N release. The NH_4^+ was the predominant form of mineral N during the initial 30 days, after which $NO_3^-$was dominated, with only very small amounts of NH_4^+ detected after 100 days. In details, initially we found increased NH_4^+ release (except humus) particularly with two animals remaining (e.g. meat powder, fish meal) with peaks at initial 10 days in S3 and S2 soil, while in case of S1 soil, the peak NH_4^+ release was observed at 30 days of incubation followed by rapid decreased and remain close to control during the rest of the incubation period (Fig. 2.4A). Considering NO_3^-, peak NO_3^- content observed at 30 days in S2 and S3 soil, while at S1 soil, peak NO_3^- content observed at 100 days of incubation (Fig. 2.4B). In case of humas, the initial N release was slower, or cause initial short term immobilization in S1 soil, and highest N release observed at the end of incubation time (300 days). Among immobilizing OM types, glucose

showed rapid initial but short-term immobilization followed by increased N content and remain close to control throughout the rest of the incubation period (Fig. 2.5), while biochar caused mild immobilization at intermediate period (30 to 100 days). In contrast, cellulose, grass litter and wood powder showed slower and lower initial but longer-term immobilization. With those OM types, immobilization increased gradually and progressively during the incubation period (Fig. 2.5).

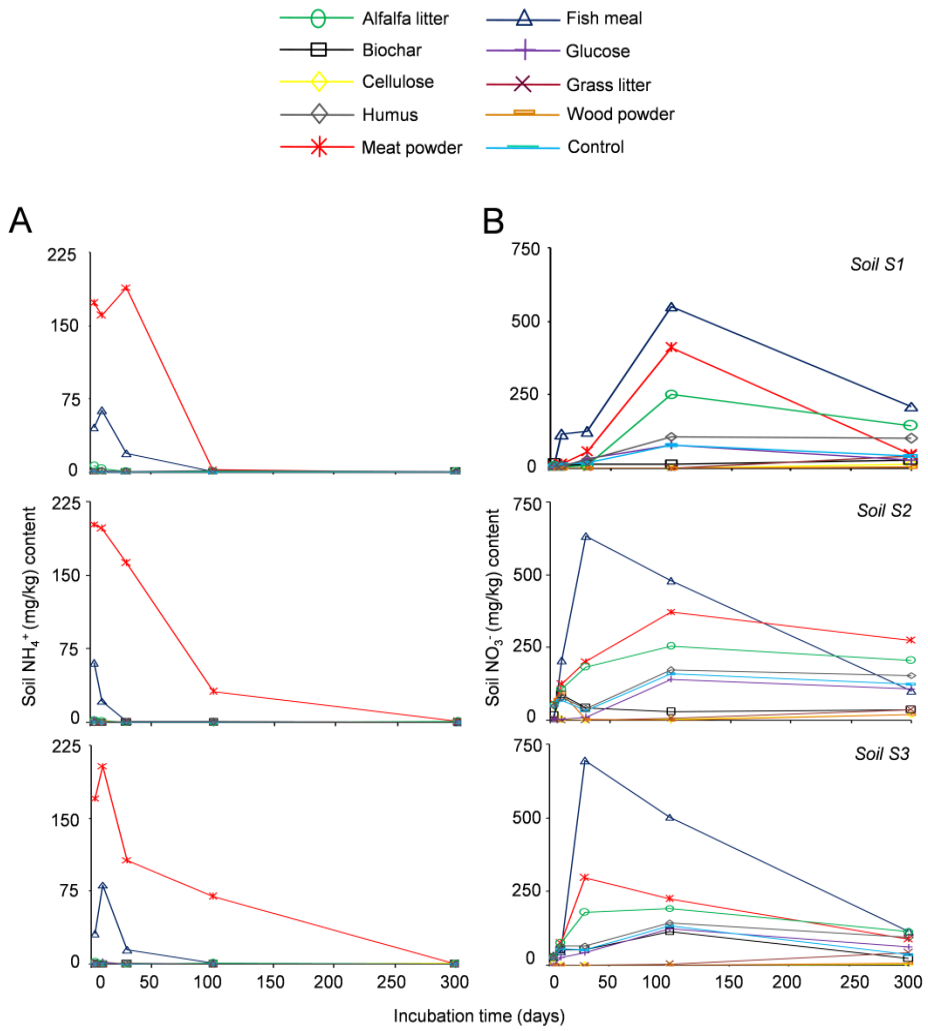

Figure 2.4. Mineral N content (NH_4^+ and NO_3^-) in three soil types (S1, S2 and S3), amendment with nine organic matter types (alfalfa litter, biochar, cellulose, fish meal, glucose, grass litter, humus, meat powder, and wood powder) during 300 days of incubation. (A) showing NH_4^+ content, and (B) showing NO_3^- content along with incubation time.

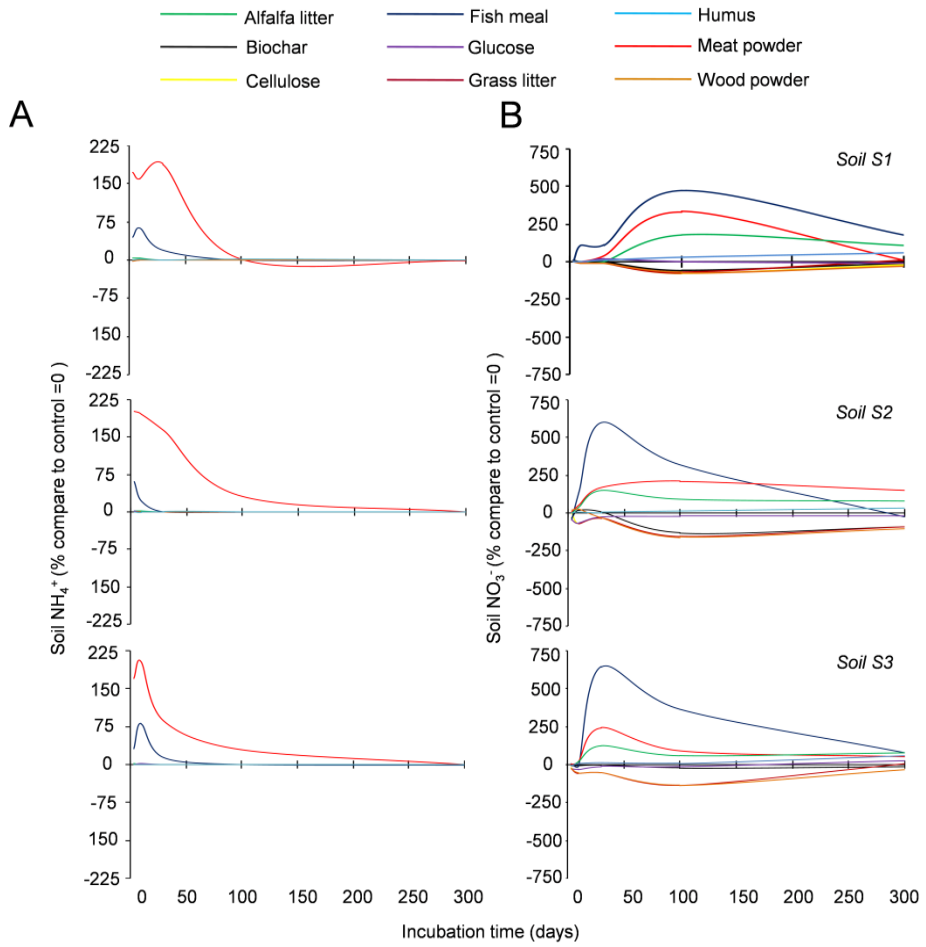

Figure 2.5. Soil mineral N content (% in respect to control = 0), in three soil types (S1, S2 and S3), amendment with nine organic matter types (alfalfa litter, biochar, cellulose, fish meal, glucose, *g*rass litter, humus, meat powder, and wood powder) during 300 days of incubation. (A) showing NH_4^+ content percentage, and (B) showing NO_3^- content percentage.

2.3.3. pH and EC dynamics

In our incubation experiment, all amending treatments combinations (i.e. soil type, OM type, and incubation time) markedly influenced soil pH and EC content with main and interactive effect (Table 2.2). Initially, all amendment types showed decrease pH value up to 30 days, followed by slowly increasing with the incubation time progress in all tested soils. In case of meat powder, after a peak of pH value, decreased consistently throughout the incubation period. Among OM types highest pH values 7.97 was observed with humus amendment at the end of the incubation (at 300 days) in Capasso soil (Fig. 2.6A). Contrast behaviors observed considering EC dynamics,

showed decreasing trend with incubation time (Fig. 2.6B). All amendment types showed high EC values at the middle stage of incubation (30 to 100 days) followed by slowly decreased. Meat powder and fish meal showed highest EC values 4410 µS/cm and 4330 µS/cm, respectively, in S2 soil, which was superior over control and all other amendment types (Fig. 2.6B).

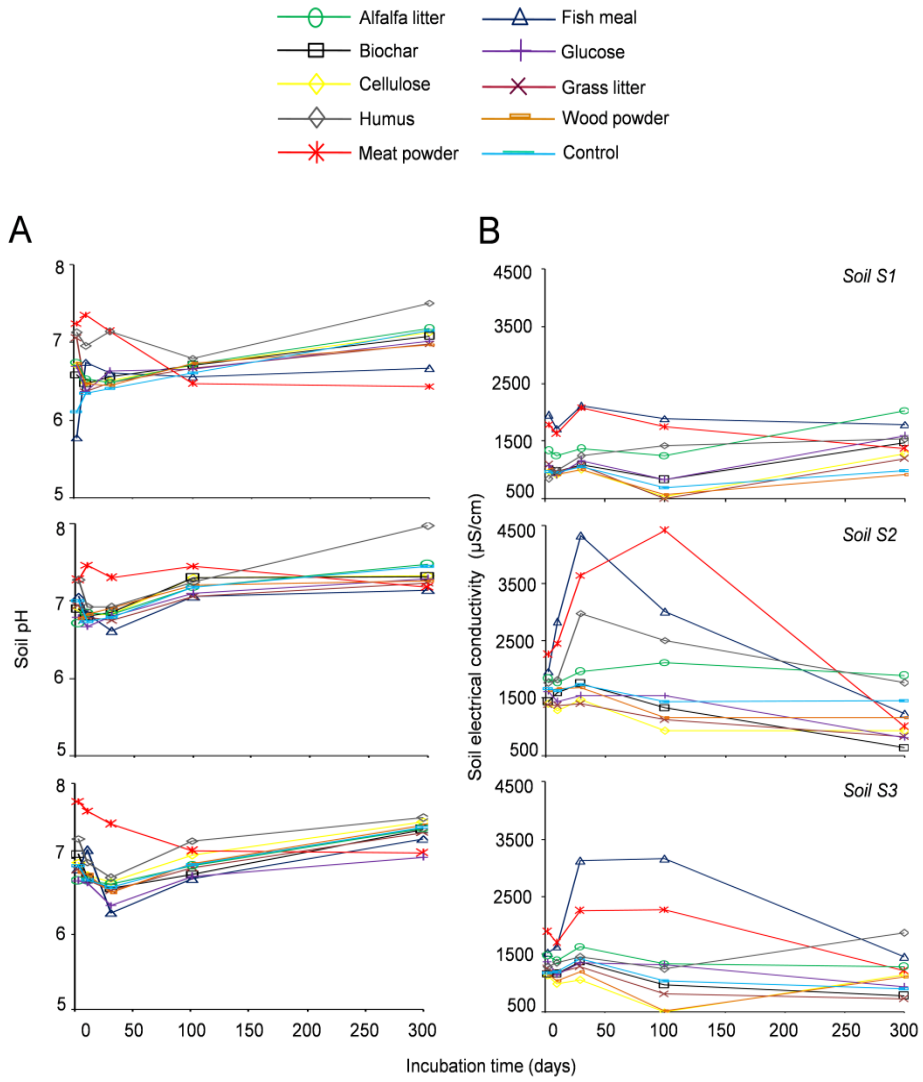

Figure 2.6. pH and electrical conductivity (EC) in three soil types (S1, S2 and S3), amendment with nine organic matter types (alfalfa litter, biochar, cellulose, fish meal, glucose, grass litter, humus, meat powder, and wood powder) during 300 days of incubation. (A) showing soil pH, and (B) showing soil EC.

2.3.4. Correlation between organic matter chemistry and N dynamics

2.3.4.1. Correlation between N release and OM classical elemental quality parameter

In general, both mineral N pools (NH_4^+ and NO_3^-) showed positive correlations with OM initial N content, while negatively correlated with the OM traditional C/N ratio (Fig. 2.7 and 2.8). Specifically, NH_4^+ release showed a decreasing trend of positive correlations with initial N content in all tested soils (Fig. 2.7). The correlation was significant ($p < 0.01$) throughout the incubation period in S2 soil, while in S1 and S3 soil significant positive correlation was observed up to initial 100 days of incubation (Fig. 2.7). Thereafter, NH_4^+ release showed both negative and positive correlations with C/N ratio in tested soil (Fig. 2.7). In details, significant ($p < 0.01$) negative correlation was observed at 100 and 300 days in Si and S2 soil, respectively. Surprisingly, in S3 soil there was a significant ($p < 0.01$) positive correlation between NH_4^+ release and C/N ratio at the end of the incubation period (Fig. 2.7).

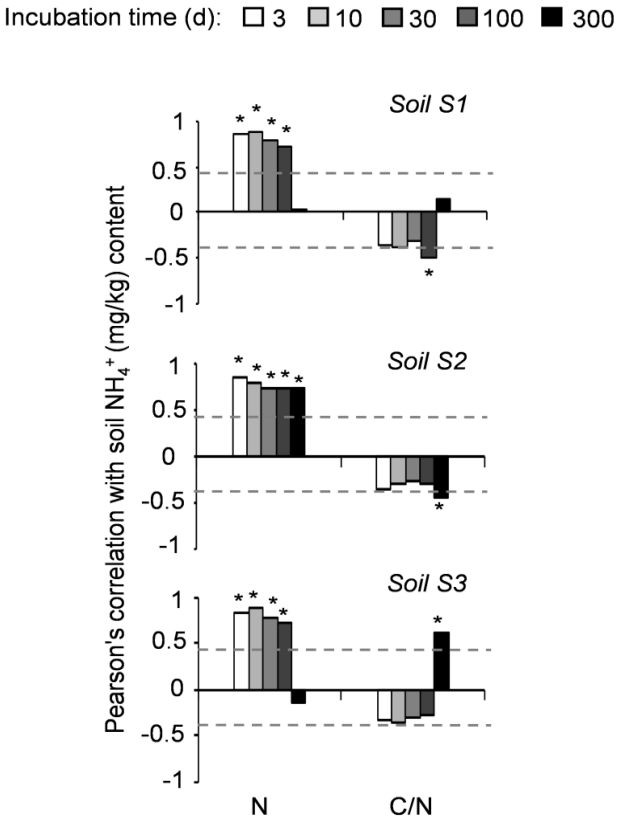

Figure 2.7. Relationships between NH_4^+ observed in three different soils amended with nine different organic materials at five different incubation times, and molecular composition of the

organic materials. Data for each bar refer to the correlation (Pearson's *r*) between NH_4^+ release values observed at a given incubation time in a given soil, and elemental N content, C/N ratio, detected in the organic materials used for soil amendments (N = 9 organic materials × 3 replicates). Dashed lines indicate threshold values of statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). Asterisks indicate significant *r* values.

Considering NO_3^-, increasing trend of positive correlation was observed with N content in all tested soil throughout the incubation period and the correlation was statistically significant ($p < 0.01$) in all tested conditions (Fig. 2.8). In contrast, NO_3^- content showed negative correlation with organic matter C/N ratio in all tested soils. Briefly, statistically significant ($p < 0.01$) negative correlation was observed in S3 soil throughout the incubation period, while in S1 and S2 soil significant ($p < 0.01$) negative correlation found at different times during the incubation period (Fig. 2.8).

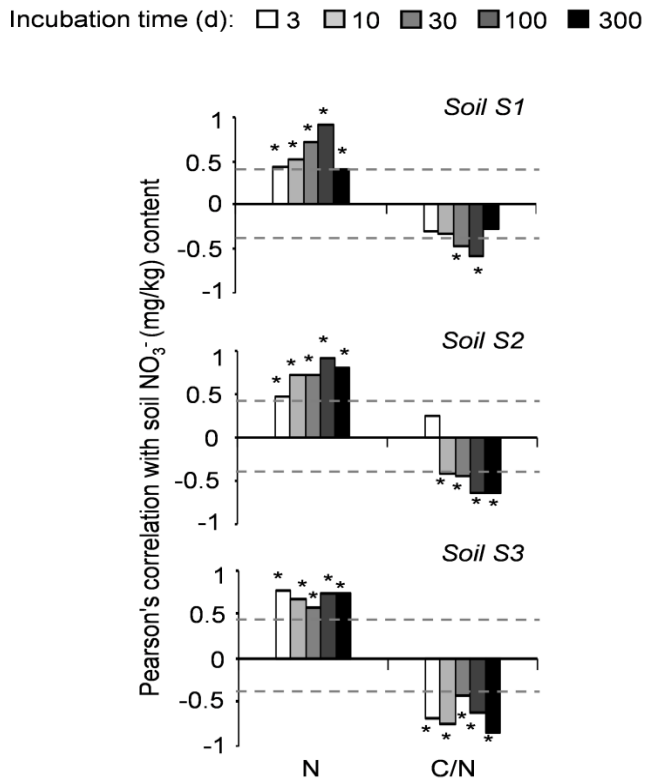

Figure 2.8. Relationships between NO_3^- released in three different soils amended with nine different organic materials at five different incubation times, and molecular composition of the

organic materials. Data for each bar refer to correlation (Pearson's *r*) between NO_3^- release values observed at a given incubation time in a given soil, and elemental N, C/N ratio, detected in the organic materials used for soil amendments (N = 9 organic materials × 3 replicates). Dashed lines indicate threshold values of statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). Asterisks indicate significant *r* values.

2.3.4.2. Correlation between N release and selected C types from ^{13}C-CPMAS NMR spectra

Considering OM chemical quality from ^{13}C-CPMAS NMR spectra, the mineral N release was differently correlated with NMR spectral regions depending on soil types and incubation time (Fig. 2.9 and 2.10). Specifically, NH_4^+ showed decreasing trend of positive correlation with carboxyl C in all three soils with progression of incubation time (Fig. 2.9). Positive correlation was statistically significant ($p < 0.01$) throughout the incubation period in S2 soil, while in S1 and S3 soil significant positive correlation observed until 100 days of incubation (Fig. 2.9). No significant correlations were observed with O-substituted aromatic C (141–160 ppm) and H, C-substituted aromatic C (111-140 ppm) regions to NH_4^+ release in both S1 and S2 soil, while in S3 soil both regions showed significant ($p < 0.01$) negative correlation at 300 days (Fig. 2.9). The di-O-alkyl C (91–110 ppm) and O-alkyl C (61–90 ppm) regions showed a negative correlation when related to NH_4^+ release in all three soils (Fig. 2.9). Briefly, di-O-alkyl C (91–110 ppm) region showed significant negative ($p < 0.01$) correlation throughout the incubation period in S2 and S3, whereas in S1 soil significant negative ($p < 0.01$) correlation found up to 100 days. The O-alkyl C (61–90 ppm) had significant negative ($p < 0.01$) correlation up to 100 days in S1 and S2, while in S3 soil significant negative ($p < 0.01$) correlation found at 3, 10 and 300 days. Finally, the N-alkyl and methoxyl C (46-60 ppm) and the alkyl C (0-45 ppm) regions had decreasing trend of positive correlation with NH_4^+ release in all three soils. In details, both regions had significant ($p < 0.01$) positive correlation throughout the incubation period in S2 soil, while in S1 and S3 significant ($p < 0.01$) positive correlation found up to 100 days of incubation.

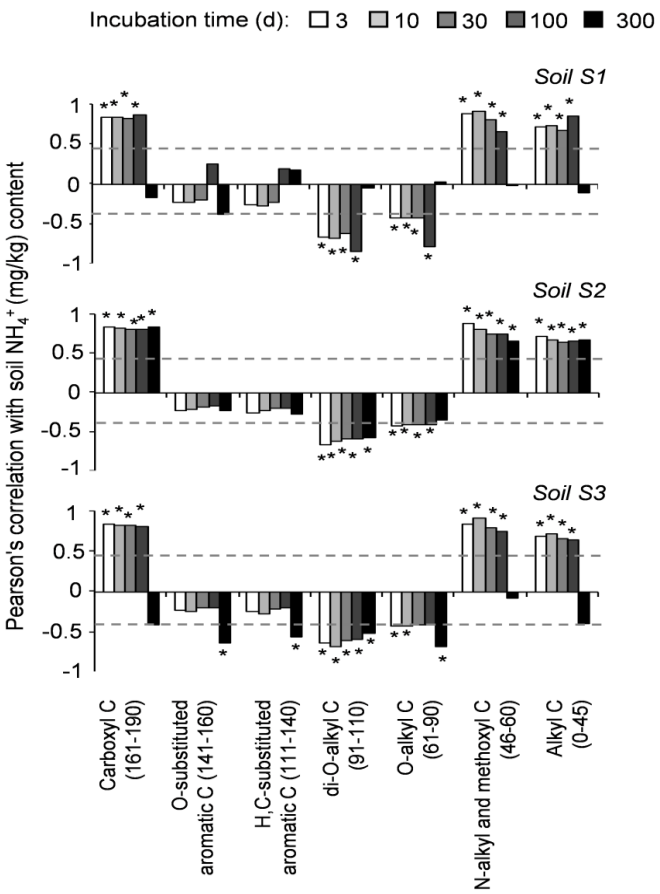

Figure 2.9. Relationships between NH_4^+ release observed in three different soils amended with nine different organic materials at five different incubation times, and molecular composition of organic materials. Data for each bar refer to correlation (Pearson's *r*) between NH_4^+ release values observed at a given incubation time in a given soil, and ^{13}C-CPMAS NMR spectral signals in a reference spectral region, corresponding to a specific C- type, detected in the organic materials used for soil amendments (N = 9 organic materials × 3 replicates). Dashed lines indicate threshold values of statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). Asterisks indicate significant *r* values.

Concerning NO_3^- release, increasing trend of positive correlation to carboxyl C (161-190 ppm) region was observed in all tested soils (Fig. 2.10). Positive correlation was statistically significant ($p < 0.01$) at different time during the incubation period (Fig. 2.10). No significant relationship was observed with O-substituted aromatic C (141–160 ppm) and H, C-substituted aromatic C (111-140 ppm) region to NO_3^- content throughout the incubation period in all tested soil (Fig. 2.10). The di-O-alkyl C (91–110 ppm) and the O-alkyl C (61–90 ppm) region, showed

a negative correlation with NO_3^- release in all three soils. Briefly, di-O-alkyl C (91–110 ppm) region showed significant negative ($p < 0.01$) correlation throughout the incubation period in S2 and S3, whereas in S1 soil significant negative ($p < 0.01$) correlation found up to 100 days. The O-alkyl C (61–90 ppm) region showed significant negative ($p < 0.01$) correlation maintaining irregular timing during the incubation period in all tested soils (Fig. 2.10). Finally, the N-alkyl and methoxyl C (46-60 ppm) and the alkyl C (0-45 ppm) region, which had an increased trend of positive correlation with NO_3^- content in all three soils (Fig. 2.10). In details, both regions had significant ($p < 0.01$) positive correlation throughout the incubation period in S2 and S3, while in S1 soil significant ($p < 0.01$) positive correlation found up to 100 days with N-alkyl and methoxyl C region and at middle stage (30 and 100 day) with alkyl C region, respectively (Fig. 2.10).

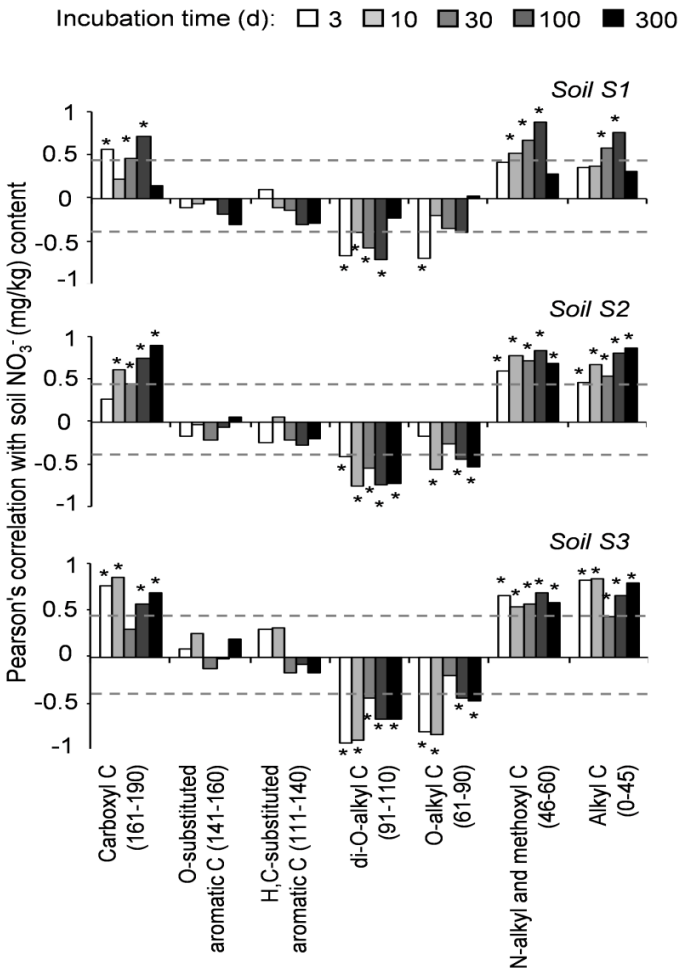

Figure 2.10. Relationships between NO_3^- release observed in three different soils amended with nine different organic materials at five different incubation times, and molecular composition of

organic materials. Data for each bar refer to the correlation (Pearson's *r*) between NO_3^- release values observed at a given incubation time in a given soil, and ^{13}C-CPMAS NMR spectral signals in a reference spectral region, corresponding to a specific C- type, detected in the organic materials used for soil amendments (N = 9 organic materials × 3 replicates). Dashed lines indicate threshold values of statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). Asterisks indicate significant *r* values.

2.4. Discussion

Our mesocosm incubation experiment, based on nine organic matters, representing a wide range of biochemical quality, three soil types showing different texture, nutrient availability and organic matter content and five incubation times, demonstrated all amending treatments combinations (i.e. soil type, organic matter type, and incubation time) largely affected mineral N dynamics. Three (e.g. fish meal, meat powder as well as alfalfa litter) out of nine OM types, mineralized massive amount of N, while others OM types (e.g. cellulose, grass litter, glucose and wood powder) immobilized N, with different intensity and duration that depends on incubation time. Finally, we found that by defining OM quality with ^{13}C-CPMAS NMR spectra is better capable to predict OM N dynamics compared to OM classical C/N ratio index. Moreover, our results contribute towards a full understanding of the relationships between OM chemistry and N dynamics.

2.4.1. Organic matters N dynamics

Mineral N release following the organic amendments on soil types have been previously investigate (Cabrera et al. 2005; Cayuela et al. 2009; Li et al. 2013; Mohanty et al. 2010, 2011). Our study demonstrates that the occurrence and magnitude of the N release were highly variable among amendment types, in combination with soil type and incubation time. We found massive initial N release with two animals remaining like meat powder, fish meal, followed by a rapid decrease (Fig. 2.4). These results are consistent with previous works dealing with the addition of animal remains to soil (Cayuela et al. 2009; Mondini et al. 2008), found strong initial N release which decreased more rapidly. A noteworthy increase in both extractable NH_4^+ and NO_3^- was observed when soil was amended with meat and bone meal (Mondini et al. 2008), and N mineralization started immediately after soil amendment showing increased extractable NH_4^+ and

NO_3^- after 2 days of incubation. Moreover, Cordovil et al. (2005) observed high N mineralization rate with OM types, those are from animal sources, in a laboratory aerobic incubation. Mondini et al. (2008) also mention that, as the incubation time elapsed the extractable NH_4^+ was readily converted into NO_3^-, which are in agreement with our findings that NH_4^+ was the predominant form of mineral N in most treatments during the initial period, after which NO_3^- dominated. Thereafter, we found mineral N release was also significant from leguminous alfalfa litter (Fig. 2.4). N mineralization following addition of leguminous residues was reported earlier by many authors (Fox et al. 1990; Johnson et al. 2007; Nakhone & Tabatabai 2008). The amount of N mineralized from legume residues treated soil increased rapidly at initial stage, followed by a slower, nearly liner decrease (Nakhone & Tabatabai 2008).

Surprisingly, we found humus release slower initial N and as the time progress N content increased at the end of the incubation (Fig. 2.4). The release of mineral N from compost product is mediated by time, and in general N mineralization from compost occurred later after 4–5 years, resulting in higher N availability and productivity (Barbarick & Ippolito 2007; Eghball 2002). In general composted matters are resistant to microbial degradation, thus are not considered to be involved in microbial metabolism (Lovley et al. 1996). Composted matters released smaller amounts of N than non-composted matters (Adegbidi & Briggs 2003) and composted fractions are known to release nutrients slower and for a longer period. Interestingly, Burgos et al. (2006) observed a short period of initial immobilization followed by a continuous mineralization with two composts (agroforest and municipal waste compost) in a sandy soil. Furthermore, Bernal et al. (1998) observed net N mineralization with mature compost and the highest concentration of inorganic N was found after 70 d, the concentration increasing with increasing incubation time.

In our experiment we found that biochar, cellulose, glucose, grass litter and wood powder, immobilized N from soil depending on incubation time (Fig. 2.5). We observed negligible amount of N immobilized by biochar application, which is really surprising when considering its high C/N ratio. Biochar additions decreased the availability of soil mineral N, probably because of immobilization by microbes (Tammeorg et al. 2012), though N content was not statistically different from the control soil (López-Cano et al. 2013). Contrast results were also reported José & Knicker (2011), observed pyrogenic organic material obtained from "*Lolium perenne*" is relatively low recalcitrance and N is slowly transferred into a plant-available form, it may

contribute to the observed improvement of soil fertility. Moreover, interesting results was observed by Ameloot et al. (2015), who found both net mineralization and immobilization by the poultry litter biochar (PL) and pine chips (P) biochar addition in two different soil for 14-week of incubations. PL biochars increased the net N mineralization, while for treatments with P biochars net N immobilization was observed in both soils. Thereafter, cellulose, grass litter and wood powder, these OM promotes very strong and long-term N immobilization. Strong N immobilization was reported from some organic matter like crops straws, woody plant materials, saw dust by number of authors (Hassan 2013; Herrmann & Witter 2008; Homyak et al. 2008; Szili-Kovács et al. 2007). Generally, incorporation of large C/N ratio substrates as wood scraps can lead to strong N immobilization due to microbial competition, with consequent plant growth inhibition (Michelsen et al. 1995). Chaves et al. (2007) found that waste straw and sawdust were able to immobilize between 54 and 68% of N in field condition. Moreover, Mohanty et al. (2010) found rapid N immobilization which reached about 40 mg N/kg by 28 days with rice straw in laboratory incubation experiment for 98 days under aerobic conditions, similar results was observed with wheat straw. Homyak et al. (2008) reported that the wood-chip application can potentially immobilize between 19 and 38 kg N/ha in the first year after harvesting.

However, the immobilization of inorganic N by labile sucrose and resistant sawdust in a laboratory incubation experiment were reported by Tilston et al. (2009), and authors observed both amendments led to net N immobilization. Authors concluded that sucrose addition effectively mobilized N from the soil organic N pool into the microbial biomass, whereas sawdust addition apparently immobilized N into a non-biomass compartment or a biomass compartment not released. Moreover, similar findings were reported earlier by Szili-Kovács et al. (2007) that sucrose and sawdust additions led to short- and long-term reductions in inorganic N concentrations, respectively. The positive association of gross N mineralization rates and several labile organic products and the negative association of recalcitrant characteristic were observed as in temperate soil ecosystems (Hart et al. 1994).

2.4.2. Linking OM chemistry with N dynamics

The C/N ratio has frequently been used to describe the OM decomposition and subsequent N release. In fact, is widely accepted that a low substrate C/N ratio implies a high mineralization rate due to N sufficiency while a C/N ratio above 30, induce soil N immobilization (Abiven et al.

2005; Bernal et al. 1998; Caricasole et al. 2011; Jalota et al. 2006). In this study, we found that this is true only for some organic matters like meat powder, fish meal, grass litter and wood powder. Instead, C/N ratio fails in predicting mineral N release form OM like glucose, humus, and especially biochar, showing contrasting, time-dependent correlations with C/N ratio. According to traditional C/N ratio indices, biochar should immobilize N very rapid and long-term considering its very high C/N ratio (149.14) but we found mild or no immobilization. On the other hands, humus should mineralize massive N as it had low C/N ratio (14.96), but we observed very little initial N release. In consistent to our results, Rowell et al. (2001) found the C/N ratio was fairly poor predictors of net N mineralization.

Here, we showed that the initial biochemical characteristics of organic matter corresponding to ^{13}C-CPMAS NMR spectral regions are suitable to explain the variability of mineral N release after organic input (Fig. 2.9 and 2.10). In particular, mineral N release is positively related with the abundance organic acids and the amide carbon (NMR spectral regions corresponding to carboxylic C). These results provide further support to the hypothesis that the high proportion of amide carbon in some OM types (i.e. meat powder, fish meal and leguminous alfalfa litter) lead to rapid initial N release onset. Besides, for such materials, the rapid N release decrease after peaking suggests that these compounds might be short–lived and rapidly subjected to chemical or microbial breakdown. Here, we found consistent positive correlations between N release and NMR regions related to proteins and peptides (methoxyl and N-alkyl C region) and the aliphatic fraction of the NMR spectra (Alkyl C region). These results can be associated to the degradation of proteins and lipids, which are major components of meat powder, fish meal and alfalfa litter. A strong correlation between mineral N release and indices of protein determined from ^{13}C NMR, suggesting that these protein indices may be useful for predicting N mineralization from organic matter (Rowell et al. 2001). In contrary, Flavel & Murphy (2006) found no relationship between the alkyl-C values and either net or gross N mineralization. Further investigation is needed to explicitly test this result.

In contrast, N release was negatively correlated with the easily decomposable carbohydrates C fractions (NMR spectral regions corresponding to di-*O*-alkyl C and *O*-alkyl C). As carbohydrates are preferential to microbial consumption, microbes consume all available nutrients and cause rapid N immobilization, as the day progress microbes dies for food deficiency and N that was the part of microbial body was available to soil, thus N level increase.

These results provide further support to our hypothesis of a rapid burst of microbial activity, sustained by the high availability of sugars and labile C compounds for soil treated with glucose leading to rapid initial short-term N immobilization. Rowell et al. (2001) reported weak relationship between rates of decomposition and net N mineralization; microorganisms decompose the more readily available compounds first, as the decomposition proceeds, more recalcitrant chemical structures tend to accumulate. Interestingly, we found no significant association between N release and aromatic C fractions (spectral regions O-substituted aromatic C and H, C-substituted aromatic C), resonating at 141–160 ppm and 111-140 ppm. Such results possibly related to biochar and humus addition, where higher aromaticity and lower carboxyl exist. The high amount of recalcitrant aromatic C has been suggested to reduce the rate of N mineralization (Vigil & Kissel 1995), as this C is resistant to most forms of microbial attack (Kögel-Knabner 2002). Contrast result was observed by Paré & Bedard-Haughn (2013), mention O-Alkyl-C to aromatic-C ratio positively influenced gross N mineralization in sub- to high Arctic. Therefore, during the decomposition the aromatic C, mostly derived from lignin structures, would accumulate as carbohydrates are utilized, but then would disappear with further decomposition to leave a residue with a high content of alkyl C (Baldock et al. 1997).

Our results indicate that classification of organic C based on the NMR C types provides better assessment of the mineralizability of N found in OM type than classifications based on the classical elemental composition. Our results supported by previous findings by Paré & Bedard-Haughn (2013) showed that gross N mineralization OM qualities and or relatively of labile C as determined using solid state ^{13}C CPMAS NMR spectra.

2.5. Conclusion

Our study provided clear-cut evidence that the use of C/N ratios as descriptors of OM quality is limited to some organic materials (i.e. meat powder, fish meal, alfalfa litter, grass litter, wood powder), with the same indices unable to predict N mineralization rate of some organic matters (i.e. glucose, biochar, humus). In contrast, an approach based on ^{13}C-CPMAS NMR analysis led to more predictive descriptions of organic matter quality throughout the whole investigated incubation process. We showed that the relationship between OM quality and its N release rate can be satisfactorily predicted by ^{13}C-CPMAS NMR spectral regions and corresponding C types better than traditional C/N ratio index form elemental chemical analyses. We are aware that our

experiment was based on a not exhaustive number of OM types, conducted under optimal conditions of temperature and water content. Consequently, further studies should investigate the consistency of the proposed index under more limiting conditions extending the analysis to materials from other ecosystems (e.g. agro-ecosystems, grasslands, boreal and tropical forests). A practical limit of our study might be related to the limited availability of solid state ^{13}C NMR spectroscopy, which is, so far, accessible to few laboratories. However, we showed that this method provides good insights on the chemical dynamics of mineralization/ immobilization processes. Further comparative studies between ^{13}C NMR spectroscopy and other analytical methods will eventually support the identification of other indicators for mineralization rate predictions.

Supplementary Table

Supplementary Table 2.S1. 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**
Sand, %	60.38	45.60	23.48
Silt, %	21.22	46.42	40.14
Clay, %	18.40	7.98	36.38
Bulk density, g cm^{-3}	1.37	1.19	1.46
Electrical conductivity, dS m^{-1}	0.14	0.61	0.29
pH	6.25	7.72	8.07
Organic carbon, g kg^{-1}	9.68	13.14	10.2
Total nitrogen, g kg^{-1}	4.45	1.91	3.10
C/N ratio	2.17	7.38	3.27

Chapter – 3

Linking organic matter chemistry with soil aggregate stability: insight from ^{13}C NMR spectroscopy

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In collaboration with Riccardo Spaccini, Alessandro Piccolo, Giuliano Bonanomi, Stefano Mazzoleni

Abstract

Soil aggregation is an important ecosystem process, and considered as a crucial aspect of agro-system sustainability due to its involvement in soil physical, chemical and biological processes. In this context, we test the hypothesis that the initial chemical characteristics of organic matter (OM) are suitable to explain the variability of aggregation dynamic after incorporation. OM was characterized by ^{13}C-CPMAS NMR and elemental chemical parameters to investigate the effects of amended chemistry on soil aggregate stability. Incubation experiment was carried out in laboratory condition, using three soil types (Capasso, Castel volturno and Torino), ten organic substrates (alfalfa litter, biochar, cellulose, glucose, green compost, maize litter, manure compost, meat powder, solid digestate, and wood powder), replicated 3 times for each of 4 incubation times, for a total of 396 microcosms. We found that alfalfa litter, glucose, meat meal often induces a rapid initial increase of aggregation index (AI), likely acting as a C source for microbes, while biochar barely affects AI when incorporated into the soil. Considering ^{13}C-CPMAS NMR spectral regions, the di-*O*-alkyl C and *O*-alkyl C (carbohydrate fraction) had significant positive relation with AI, while the H, C- substitute aromatic C and O- substitute aromatic C (aromatic fraction) had a significant negative correlation with AI. This study suggests that the chemical quality of OM is a major controlling factor of soil aggregation. OM quality defined by ^{13}C-CPMAS NMR spectroscopy explains the aggregation process better than classical elemental parameter. However, our results provide a significant novel contribution towards a full understanding of the relationships between OM chemistry and soil aggregation.

3.1. Introduction

Soil aggregation is an important ecosystem process resulting in the formation and stabilization of soil structure, consisting of soil aggregates and the resulting matrix of pore spaces (Rillig et al. 2015). Soil aggregation can be form by rearrangement, flocculation and cementation of mineral and organic particles (Bronick & Lal 2005), and is considered a crucial aspect of soil quality and then a keystone for the sustainability of agro-ecosystems. In fact, soil structure facilitates diffusion of gases and water movement in soils, and so promote root penetration and growth, reduce the susceptibility to erosion (Annabi et al. 2007; Six et al. 2004).

The ecological factors that affects aggregation processes have been well documented and reviewed (Amezketa 1999; Bronick & Lal 2005; Diacono & Montemurro 2010; Six et al. 2004). The formation and stability of soil aggregates depends on several processes, affected by chemical, physical and biological factors. Soil texture, clay mineralogy, cation content, and organic matter (OM) are considered the main abiotic determinants. OM can act directly as a binding agent (Karami et al. 2012), or indirectly by promoting soil microbial activity and then the formation and maintenance of aggregate stability (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 organic matter decomposition (Schmidt et al. 2011).

OM is an especially important factor controlling aggregate stability because its amount and properties can be modified trough agronomic management. A large variety of organic matter types including crop residues, composts, peats, organic wastes from agro-industries, and biochar are widely used as soil amendments. Most of the published studies assessed the immediate effects of organic amendments on soil aggregate stability reporting a general positive effect. However, inconsistencies (Busscher et al. 2010; Zhang et al. 2015) as well successfully (Abiven et al. 2007; Annabi et al. 2007, 2011; Six et al. 2004) applications of organic amendments to improve soil structure has been reported. Then, a better understanding of the impact of different OM types on soil aggregate structure is required. The first step in this direction was made more than 50 year ago by Monnier (1965), who proposed a conceptual model that link soil aggregate stability with organic amendment quality across time scales, varying from weeks to months till years after their incorporation. Specifically, easily decomposable OM such as green manure had an intense, but short term (week to month) effect on aggregate stability, other materials as wheat straw have a maximum effect at a monthly scale, while more recalcitrant products such as

decomposed manure had a lower initial effect that develop over time. Later, the meta-analysis of Abiven et al. (2009), based on 48 empirical studies, largely validated the conceptual model proposed by Monnier. Abiven et al. (2009), however, pointed out that to properly translate the Monnier's conceptual model in effective agronomic practice a better link between OM quality and aggregate stability is required. In fact, in the Monnier's model the quality of the organic amendment is largely assumed by simple naming the organic input, i.e. green manure *vs* straw *vs* recalcitrant substrate, which is very simple but not enough to characterize their effect on aggregate stability. In this context, some studies used the well know C/N ratio as predictor of organic matter quality, but reported inconsistent relationships with aggregate stability (Martens & Frankenberger 1992; Sonnleitner et al. 2003).

The limited effort have been made in the search of chemical indicators that consistently describe OM chemistry and predict aggregate stability, also considering the recent advance in chemical analytic techniques. In this perspective, several chemical throughput methods are currently available and have been applied to collect direct information on the characteristics of OM, including pyrolysis-gas chromatography/mass spectrometry (Huang et al. 1998), near infrared reflectance spectroscopy (Gillon et al. 1999) and ^{13}C-cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Keiluweit et al. 2015; Kögel-Knabner 2002). In detail, ^{13}C-CPMAS NMR has been proven useful to provide a description of the total organic 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 inhibition (Mazzoleni et al. 2015).

In this study, we combined a detailed OM characterization by ^{13}C-CPMAS NMR in solid state (Kögel-Knabner 2002), with a medium-term microcosms incubation experiment to investigate the link between OM chemistry and soil aggregation stability. In detail, we evaluated the capability of 10 organic amendment types, spanning a wide range of chemical quality, to induce soil aggregation in three soil type with contrasting texture. Specific aims of the study were to:

(1) assess the aggregation capability of different organic amendment types and describe their effects in terms of time scale;
(2) explore the relationships between soil aggregation and OM initial chemical quality, as defined by ^{13}C-CPMAS NMR spectroscopy and standard chemical parameters; and

(3) identify the ^{13}C-CPMAS NMR spectral regions that better predict the effects of OM on soil aggregate stability.

3.2. Materials and methods

3.2.1. Organic amendment collection and chemical characterization

Ten organic amendment types (Fig. 3.1A) representing a wide range of OM chemistry were selected, i) alfalfa litter; ii) biochar; iii) cellulose; iv) glucose; v) green compost; vi) maize litter vii) manure compost; viii) meat powder; ix) solid digestate; and x) wood powder.

Organic amendments were characterized for total C and N content by flash combustion of micro samples (5 mg of sample) in an Elemental Analyser NA 1500 (Fison 1108 Elemental Analyzer, Thermo Fisher Scientific). All organic amendments were characterized by ^{13}C-CPMAS NMR in solid state under the same condition, thus allowing a quantitative comparison among NMR spectra. A spectrometer (Bruker AV-300, Bruker Instrumental Inc, Billerica, MA, USA) equipped with a magic angle spinning (MAS) probe with wide-bore of 4 mm was used, set up with MAS of 13,000 Hz of rotor spin, a recycle time of 1s, a contact time of 1 ms, an acquisition time of 20 ms, and 2000scans (for details see Bonanomi et al. 2013). Selection of spectral regions and identification of corresponding classes of C types were performed according to previous studies (Bonanomi et al. 2015; Kögel-Knabner 2002; Li et al. 2015; Mathers et al. 2007; Pane et al. 2011). The following seven regions and 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.

3.2.2. Aggregation experiment

Three soils showing contrasting texture, nutrient availability and organic matter content were selected to represent a range of soil types (Capasso, Castel volturno and Torino, see supplementary Table 3. S1). Soils were collected from the top layer (first 20 cm), sieved at 2 mm and were oven air-dried at 25-30°C.

The aggregation experiment was carried out in microcosms, in laboratory condition (Fig. 3.1). Plastic jars were filled with 200 g of dry soil and were incorporated homogeneously with 4 g (2% w/w) of each dry organic matter type. Microcosms were kept in a growth chamber under

controlled temperature (18±2°C night and 24±2°C day) and watered every seven days to field capacity with distilled water. The soil with added organic material (amended treatment, AT) or without (control treatment, CT) were incubated for 10, 30, 100, and 300 days. The full experimental design entailed three soil types, ten organic substrates, replicated 3 times for each of 4 incubation times, for a total of 396 experimental units (Fig. 3.1). At each harvesting time, the soil was collected, air dried and submitted to the assessment of soil aggregation stability.

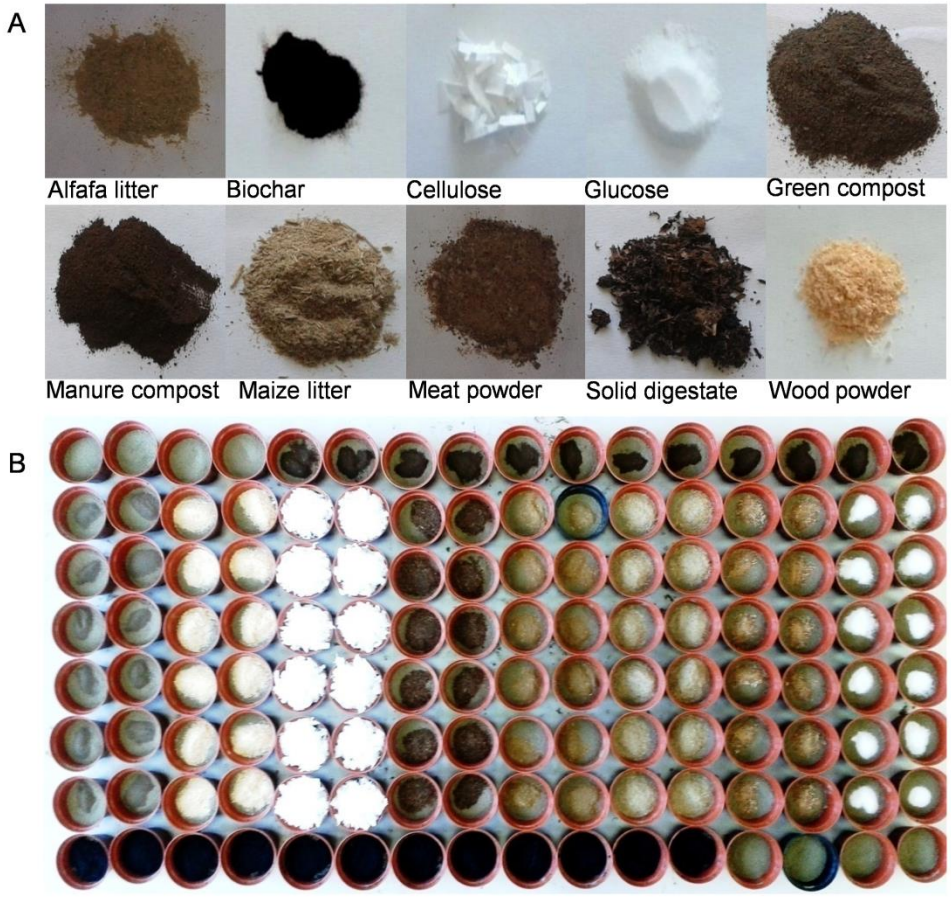

Figure 3.1. Aggregation incubation experiment using three soil types (Capasso, Castel volturno, and Torino soil) and ten organic matter types. (A) showing ten different organic materials (alfalfa litter, biochar, cellulose, glucose, green compost, maize litter, manure compost, meat powder, solid digestate, and wood powder) used for soil amendment; and (B) showing experiment set up mentioning treatments combinations used. Treatment as follows- control (without OM), soil + alfalfa litter, soil + biochar, soil + cellulose, soil + glucose, soil + green compost, soil + manure compost, soil + maize litter, soil + meat powder, soil + soil digestate, and soil + wood powder.

3.2.3. Assessment of soil aggregates stabilities

Water stability of soil aggregates (WSA) were assessed according to the method of Kemper & Rosenau (1986). Twenty grams of air dried soil were sieved through 4.75 mm mesh 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 its contents were oscillated vertically in water 20 times using a 4 cm amplitude at the rate of one oscillation per sec. After wet-sieving, 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 weighed. Aggregates stability are expressed as aggregation index (AI) value, which is the sum of the mass fraction of soil remaining on each sieve after sieving, multiplied by the mean diameter of the adjacent meshes (Spaccini et al. 2004). The percentage ratio of the aggregates in each sieve represents the water-stable aggregates of size classes: 1.500 mm, 0.750 mm, 0.375 mm and <0.125 mm. Aggregate stability was measured as the AI of water-stable aggregates as by equation 3.1:

$$AI = \sum_{i=1}^{n} XiWi \qquad (3.1)$$

where, Xi is the mean diameter of the i^{th} sieve size and Wi is the 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.

3.2.4. Data analysis

Data from the microcosms experiment were analyzed by a Generalized Linear Model (GLM), considering main and interactive effects of soil type (S, three levels), organic matter type used for soil amendment (OM, ten levels) and incubation time (treated as a continuous covariate) on soil aggregation index (AI). Pairwise differences were tested using Tukey's HSD post-hoc test.

To address the relationships between with OM chemistry and AI recorded at different incubation time, different approaches were considered. First, simple linear correlation analysis was separately tested between AI of soil and each OM chemical parameter, including both elemental chemical parameters (i.e. N content, labile C, C/N ratio) and regions of the ^{13}C-CPMAS NMR spectra selected from reference literature (Kögel-Knabner 2002; Mathers et al. 2007). In a more detailed analysis, correlation was extensively tested between AI of the tested soil amended with the 10 amendment types and ^{13}C-NMR data recorded for the same OM at each

resonance signal (n = 190), providing a fine-resolution profile of the variation in C types in the tested organic material associated with the effect on AI. This analysis allows identifying restricted ^{13}C-CPMAS NMR spectral regions showing significant correlation with AI. The correlation was tested for statistical significance controlling for multiple comparisons, according to Bonferroni's correction at $p < 0.01$. Dendrogram of used OM obtained by complete linkage and Pearson's correlation coefficient applied to a data matrix of signals recorded in the ^{13}C-CPMAS NMR spectra. Finally, a Principal Component Analysis (PCA) was carried out on a data matrix reporting, the reference spectral regions in the organic materials. Data refer to loading vectors of the spectral regions and factorial scores of the organic materials, following Legendre & Legendre (1998).

3.3. Results

3.3.1. Organic matter initial biochemistry

Ten organic amendment types representing a wide range of OM chemistry in term of C, N content and C/N ratio, characterized by elemental analyzer presented in Table 3.1. ^{13}C-CPMAS NMR spectra showed remarkable differences in organic C components among OM types in respect to spectral regions (Fig. 3.2A). The alkyl-C (0-45 ppm) region, characteristic of lipid such as waxes and cutins, and the methoxyl and N-alkyl C (46-60 ppm) region are present in a greater intensity in meat powder followed by green compost and alfalfa litter (Fig. 3.2A), while being almost absent in biochar, glucose and cellulose. These two regions are less pronounced in manure compost, solid digestate, wood powder and maize litter. The O-alkyl-C (61-90 ppm) region, mainly associated with sugars and polysaccharides, and the di-O-alkyl-C (91-110 ppm) region are abundant in glucose and maize litter followed by others, while in biochar both regions are almost absent (Fig. 3.2A). The most pronounced difference exists in the H- C-substituted aromatic C (111-140 ppm) region, which is highly abundant in biochar, while this region is not pronounced in all other OM. Finally, the O-substituted aromatic C (141-160 ppm) region is not abundant in most OM types; with the carboxyl C (161-190 ppm) region that is abundant in meat powder and alfalfa litter (Fig. 3.2A).

Table 3.1. Initial C, N content and the 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	500
Glucose	0.00	43.0	-
Green compost	1.52	31.0	20.39
Maize litter	0.49	40.38	82.40
Manure compost	2.0	34.3	17.15
Meat powder	8.26	43.88	5.31
Solid digestate	1.91	43.8	22.93
Wood powder	0.11	49.88	453.45

Dendrogram (Fig. 3.2B) provides a comparison among the used OM, in term of signals recorded in the ^{13}C-CPMAS NMR spectra. Biochar and meat powder are clearly different from other OM types, as well as one another (Fig. 3.2B). Glucose showed dissimilarity from all other OM, while similarities were observed between alfalfa litter and green compost, so does manure compost and solid digestate (Fig. 3.2B). Principal component analysis (PCA) provided a satisfactory ordination of the ^{13}C-CPMAS NMR data across OM types (Fig 3.2C), with the first two eigenvalues accounting for 91.6% (54.4, 37.2 %) of the total variance. The PCA reported the loading vectors of amendment quality parameters (i.e. relative abundance of each ^{13}C-NMR region measured in each sample and how they relate to the PC axes), and the factorial scores of the ten OM on the bi-dimensional space. The PCA showed that biochar is characterized by aromatic C, meat powder by alkyl C, N-alkyl C and carboxyl C, while cellulose, maize litter by the O-alkyl C and di-o-alkyl C. Others OM did not show any pronounced differences in term of signals recorded in the ^{13}C-CPMAS NMR spectra (Fig. 3.2C).

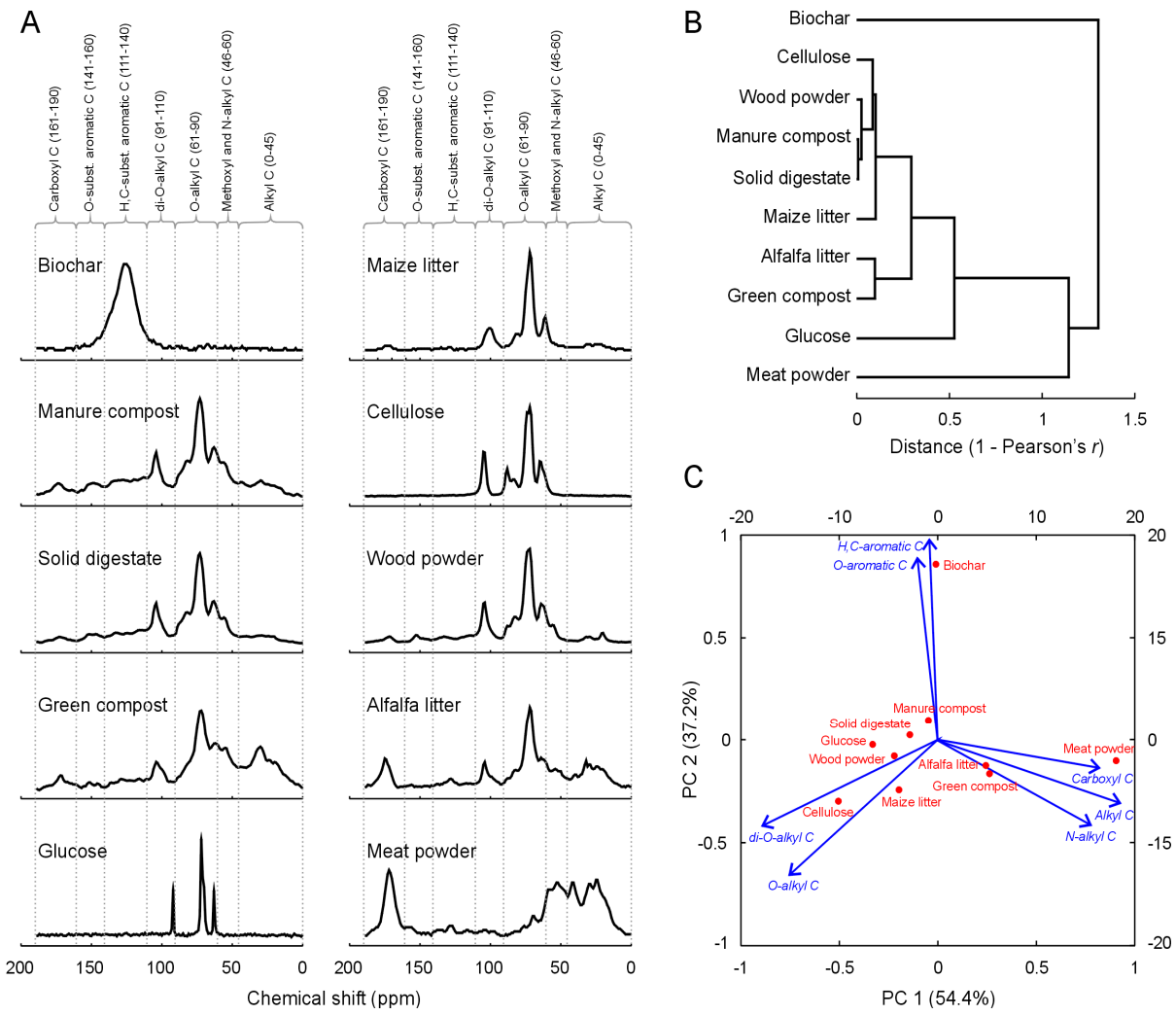

Figure 3.2. Chemical differences among organic materials used for soil amendment. (A) ^{13}C-CPMAS NMR spectra of the materials. Reference spectral regions and corresponding C types are reported on top of the panels, with chemical shift ranges indicated in brackets and by vertical dotted lines. (B) Dendrogram of organic materials, obtained by complete linkage and Pearson's correlation coefficient applied to a data matrix of on 1-ppm wide signals recorded in the ^{13}C-CPMAS NMR spectra. (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* axis) and factorial scores of the organic materials (red circles, top *x* and right *y* axis), obtained following Legendre & Legendre (1998).

3.3.2. Aggregation dynamics of soil amended with organic matter

In the mesocosm experiment, all experimental factors (i.e. amended type, soil type and incubation time) significantly affected AI, with either main or interactive significant effects (Table 3.2 and supplementary Tables 3.S2 - 3.S4). In general, the application of OM enhanced soil aggregation; however, occurrence and magnitude of the AI were highly variable among the tested conditions (Fig. 3.3). In particular, the AI increased steep outbreaks for meat powder, alfalfa litter and glucose, as well as with maize litter (Fig. 3.3). We found that cellulose and wood powder had intermediate effect on AI, while lower levels when amended with manure compost, solid digestate and green compost (Fig. 3.3) in all tested soil. In contrast, the addition of biochar barely affected AI, with the exception in soil Castel volturno, where a slight increase of AI was observed compared to the control (Fig. 3.3).

Table 3.2. Summary of the general linear model (GLM) testing for main and interactive effects of soil type (S, three levels: Capasso, Castel volturno, Torino), organic matter type used for soil amendment (OM, ten levels: alfalfa litter, biochar, cellulose, glucose, green compost, maize litter, manure compost, meat powder, solid digestate, wood powder) and incubation time (treated as a continuous covariate) on soil aggregation index (AI). Results of post-hoc tests for pairwise AI differences between treatment combinations are in Supplementary Tables 3.S2 - 3.S4.

	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		

Incubation time of amended soil samples greatly affected AI, with both main and interactive effects in combination with amendment type, and with soil type (Fig. 3.3 and

supplementary Table 3.S2 – 3.S4). In other words, over the incubation period (300 days), soil treated with OM showed variable response dynamics according to the OM types, with differences in times of AI onset, peak and overall magnitude (Fig. 3.3). Initially, all amended types (except biochar) caused an increase in AI, particularly meat powder, glucose and alfalfa litter produced a rapid initial increased, mostly peaking within the initial 10 days from OM application, followed by a relatively rapid decrease (Fig. 3.3). Cellulose rich OM such as maize litter, cellulose had also a strong initial positive effect on AI, that persists up to 30 days of incubation then decrease slowly throughout the incubation period. In case of wood powder there was an initial increase of AI followed by decreased and increased again up to 100 days. Manure compost, solid digestate and green compost showed a slower initial increased of AI up to 100 days, an effect that was maintained throughout the incubation period with the exception of soil Torino. Finally, application of biochar had negligible effects on AI throughout the incubation period in all tested soils (Fig. 3.3).

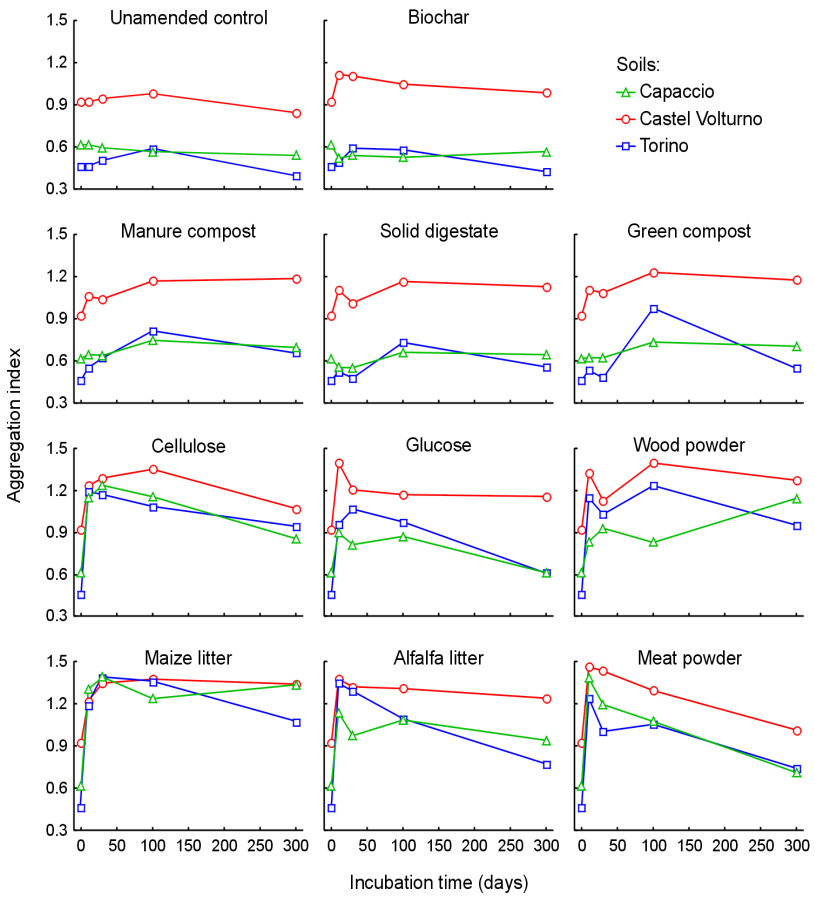

Figure 3.3. Aggregation dynamics in three soils with different properties (Table 3.1) amended with 10 different organic matters. Data refer to mean of three replicates for each treatment combination. Deviation bars are omitted to improve readability, statistical analysis are in Table 3.2 and Supplementary Tables 3.S2 – 3.S4.

2.3.3. Relationships between AI and organic matter chemistry

The correlation between AI and OM chemical quality parameters greatly depend on incubation time (Fig. 3.4A, B). Concerning N content and C/N ratio, such parameters showed a generally positive, but weak correlation with AI (Fig. 3.4A). Specifically, the initial N content of OM was positively correlated during the early and intermediate period (up to 100 days) of incubation. The correlation was significant ($p < 0.01$) at initial 10 days in soil Capasso, whereas in case of soil Castel volturno significant positive correlation showed at initial 10 to 30 days of incubation. There was no significant correlation between OM initial N content and AI in Torino soil over the incubation period (Fig. 3.4A). Considering the C/N ratio of OM, correlations were positive and statistically significant ($p < 0.01$) at the intermediate (100 days), and latter stage (300 days) of incubation for Castel volturno and Torino soil, respectively (Fig. 3.4A).

Considering OM chemical quality defined by ^{13}C-CPMAS NMR reference regions we found a general trend of positive correlations of carboxylic C (161-190 ppm) region with AI in the early period of incubation (up to 30 days). The positive correlation was statistically significant ($p < 0.01$) at the initial 10 days for Capasso, and 10 and 30 days for Castel volturno soil, but no significant correlations were observed in case of Torino soil (Fig. 3.4B). A negative correlation was observed with AI and O-substituted aromatic C and H, C-substituted aromatic C regions throughout the incubation period in all tested soils (Fig. 3.4B). In detail, significant ($p < 0.01$) negative correlation between AI and spectral data of the OM signals resonating at O-substituted aromatic C (141–160 ppm) region and H, C-substituted aromatic C (111-140 ppm) region was observed in Capasso and Torino soil over the incubation time, while in Castel volturno soil significant negative correlation was observed at intermediate to latter stages (100-300 days) (Fig. 3.4B). The di-O-alkyl C (91–110 ppm) and O-alkyl C (61–90 ppm) regions showed a trend of positive correlations with AI in all tested soils (Fig. 3.4B). However, the di-O-alkyl C (91–110 ppm) showed significant ($p < 0.01$) positive correlations at the end of the incubation (300 days) in Castel volturno and Torino soils. The O-alkyl C (61–90 ppm) region

showed significant ($p < 0.01$) positive correlations in Capasso and Castel volturno soils at 100 and 300 days of incubation, while for Torino soil, significant ($p < 0.01$) positive correlations were observed from 30 to 300 days of incubation (Fig. 3.4B). No significant correlation was observed between AI and the N-alkyl and methoxyl C (46-69 ppm) region throughout the incubation period in all soils (Fig. 3.4B). Finally, the alkyl C (0-45 ppm) region showed significant positive correlations with AI at 10 for Capasso and 30 days for Castel volturno soil (Fig. 3.4B).

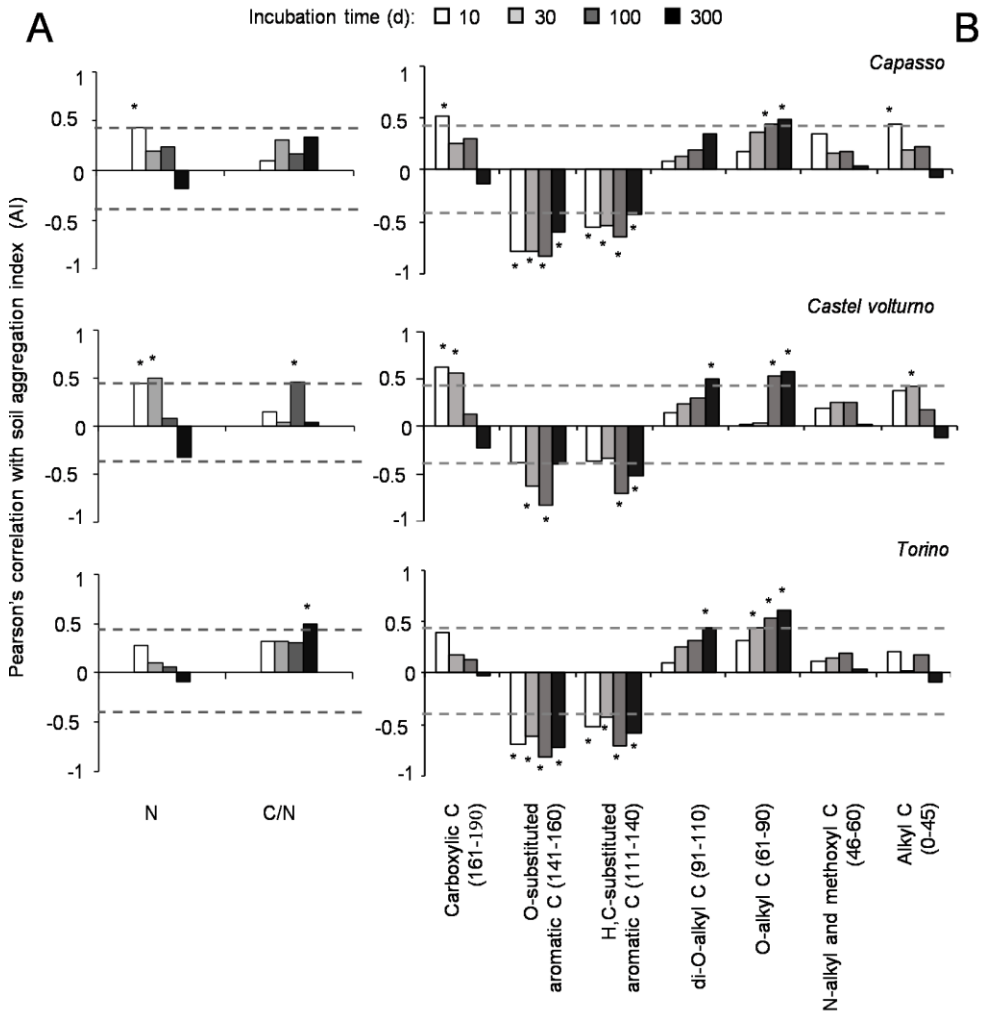

Figure 3.4. Relationships between aggregations observed in three different soils amended with ten different organic materials at four different incubation times, and molecular composition of the organic materials. Data for each bar refer to correlation (Pearson's *r*) between aggregation index (AI) values observed in a given soil at a given incubation time. The correlation (Pearson's

r) between aggregation index and elemental N content, C/N ratio (A), and correlation (Pearson's *r*) between aggregation index and ^{13}C-CPMAS NMR spectral signals in a reference spectral region, corresponding to a specific C-type (B), detected in the organic materials used for soil amendments (N = 10 organic materials × 3 replicates). Dashed lines indicate threshold values of statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). Asterisks indicate significant *r* values.

3.4. Discussion

Our experiment, based on ten organic matters representing a broad range of biochemical quality, three soil types showing contrasting texture, demonstrated all amending treatments combinations (i.e. soil type, organic matter type, and incubation time) largely affected soil AI. OM with high decomposability induces large AI, while stable OM less capable to increase AI when incorporated into the soil. Finally, by defining OM quality with ^{13}C-CPMAS NMR, our results provide a significant novel contribution towards a full understanding of the relationships between OM biochemistry and AI.

3.4.1. Organic amendment and soil aggregation dynamics

Improvements in aggregate stability following organic amendments on soil types have been previously reported (Abiven et al. 2007; Annabi et al. 2007, 2011; Spaccini et al. 2004). Our study demonstrates that occurrence and magnitude of the aggregation dynamics were highly variable among amendment types, in combination with incubation time (Fig 3.3). We found intense initial effect on aggregation with meat powder, glucose and alfalfa litter followed by rapid decrease. These results is consistent with previous findings by Abiven et al. (2007), strong initial increase in aggregate stability by the labile cauliflower residue was observed, which decreased more rapidly. Initially, for these less mature organic residues, bacteria dominate microbial activity due to the high concentration of soluble C, low C/N ratio (Eiland et al. 2001; Hu et al. 1999). The resultant bacterial by-products (extra-cellular polysaccharides) have been shown to aid the formation of soil aggregates (Alami et al. 2000; Amellal et al. 1999). This support the hypothesis that the rapid microbially induced improvement in aggregate stability that follows fresh organic residue additions involves labile polysaccharides (Abiven et al. 2007). Moreover, Tisdall & Oades (1982) observed a significant but transient increase in aggregate

stability when glucose was added to soil, because the glues are decomposed readily. Degens & Sparling (1996) observed that, glucose amendments did not affect the MWD of the > 2 mm, rather < 0.25 mm aggregate size class. They observed only the high rate treatments generally increased the MWD of the 0.5-2 mm aggregate size classes.

In our experiment; maize litter, cellulose and wood powder had an intermediate effect on aggregation (Fig. 3.3). In general, these organic products were richer than the other OM in hemicellulose- and cellulose-like fractions but poorer in soluble fractions, thus more recalcitrant to microorganism and persist for longer time. Earlier, Clark et al. (2007) observed sawdust resulted in less intense microbial activity and residue breakdown, and therefore produced less bacterial by-products, resulting in less aggregate formation. Tisdall & Oades (1982) found weaker but more persistent effect when the soil was enriched with cellulose. Furthermore, we found that the manure compost, green compost and solid digestate; these three composted matter showed smaller and slower increased in AI (Fig. 3.3), but long lasting effect on aggregation. Our results are consistent with previous studies (Annabi et al. 2007, 2011; Tejada et al. 2006, 2008, 2009), reported long-term positive effect of on aggregation by the compost application on soil. Generally the composts are slowly decomposed in the soil, and the continuous release of nutrients can sustain the microbial biomass population for longer periods of time (Murphy et al. 2007). Tejada et al. (2009) observed late positive effect of compost application on aggregates stability in a long-term (4 years) experiment. More specifically, the soil structural stability was highest at the end of the experimental period with the non-leguminous plant compost treatment (28.3%), these result was due to greater amounts of humic acids provided to the soil 63.6 kg^{-1} which was then directly involved in clay–organic complex formation. Moreover, Annabi et al. (2011) reported that, compost maturity has significant effect on aggregate stability, the addition of immature compost had an intense but transient effect while mature compost had slow but persistent effects on aggregation. Transient and temporary effects of OM on aggregate stability were due to the turnover of microbial products and cells while the persistent effects were due to humified compounds (Monnier 1965; Tisdall & Oades 1982).

However, in our study we found that biochar addition had no positive effects on aggregation throughout the incubation period compare to control (Fig. 3.3). This finding is in line with previous study by Zhang et al. (2015), observed that neither soil aggregation nor aggregate stability was significantly affected by biochar amendments. The principle of

underpinning this phenomenon is that microorganisms prefer organic C forms that require less activation energy for their metabolism. However, biochar is both chemically unusual and energetically less adventitious to mineralize than most other organic C forms in the soil ecosystem (all other factors being equal) (Lehmann et al. 2015). Contrast to our findings, it was also reported that biochar might interact with mineral soils, including inter-relation within clay minerals and surface hydrophobic–hydrophilic interactions (Joseph et al. 2010), and thus positively improved the formation of soil macroaggregates (Herath et al. 2013).

3.4.2. Linking organic matter chemistry with soil aggregation

In our microcosms experiment, we found that soil aggregation was dynamically variable during the 300 days of incubation due to many biological, chemical, and physical processes involve in the aggregation process. Results from our present study suggest that soil aggregation cannot be satisfactorily explained using information limited to organic matter N content and C/N ratio, which were often used to describe as the OM chemical characteristics but gave no particular relationships to soil aggregate stability or soil aggregation (Sonnleitner et al. 2003). Instead, we found that OM quality defining by ^{13}C-CPMAS NMR spectra better explain AI, showing statistically significant ($p < 0.01$) positive correlation with the aliphatic C, carbohydrate fraction (corresponding C type di-*O*-alkyl C and *O*-alkyl C) and carboxylic C, while negatively associated with aromatic C (corresponding C type O-substituted aromatic C and H, C-substituted aromatic C) (Fig. 3.4B). These results is consistent with previous finding by Kavdır et al. (2005), found that carbohydrate content of OM was directly related (r^2=0.92) to the stability of soil aggregates but not to the total amount of organic matter. Moreover, Tisdall & Oades (1982) mentioned generally aggregate stability is strongly associated with SOM content but is sometimes even more strongly correlated with labile pools of organic matter such as microbial biomass C or extractable carbohydrates (Haynes & Beare 1996).

In addition, we also found short term significant positive correlations between AI and NMR regions related to the aliphatic (Alkyl C, 0-45 ppm) and carboxylic fraction (carboxyl C, 161-190 ppm) (Fig. 3.4B), which are rich in some OM like meat powder, alfalfa litter. For such materials, the rapid decrease of AI after peaking suggests not only those microbes may produce strongly transient polysaccharide compounds, but also that these compounds might be short–lived and rapidly subjected to chemical or microbial breakdown. Our results provide further

support to our hypothesis of a rapid burst of microbial activity, sustained by the high availability of sugars and labile C compounds in soil treated with labile organic products leading to the rapid initial increase onset. The dominance of easily degradable carbohydrates and proteinaceous compounds in some OM (such as glucose, meat powder, and alfalfa litter) confirms the findings of previous studies that these OM are relatively young (Leifeld & Kögel-Knabner 2005) and easy to microbial growth. Besides, Chevallier et al. (2010) reported structural retention could occur by preferential microbial consumption of carbohydrates or alkyl-rich compounds. Moreover, a general positive correlation was observed between AI and NMR regions related to lignin and proteins and peptides (methoxyl and N-alkyl C region, 46-60 ppm) though correlation was not significant (Fig. 3.4B). This result is in contrast with previous findings by Martens (2000) found a strong relationship ($r = 0.89$) between aggregate stability and the initial protein content of crop residues. The possible explanation of our result may be the low signal intensity of this region was attributable not only to mobile methoxyl groups in lignin moieties, but also to N-alkyl carbons of protein residues (Baldock et al. 1990; Knicker 2000).

Finally, we found significant negative correlations between AI and NMR spectral regions related to aromatic C (corresponding to H, C - and O-substitute aromatic C) (Fig. 3.4B). These results are related to the effect of aromatic C rich, labile C poor biochar. Compared to carbohydrates, aromatic C forms characteristics of biochar actually generate much greater energy yields when reacted with O_2 creating a strong energetic intensive for decomposer to use aromatic rings as an energy source (Lehmann et al. 2015). The best explanation for such effect of biochar on aggregation may be the change of the O-Alkyl C to aromatic C during pyrolysis observed by Czimczik et al. (2002). Besides, the abundance of aromatics groups indicates a greater degree of humification (Zech et al. 1997), thus is unsuitable to sustain microbial growth, and can even inhibit microbes by the presence of recalcitrant and/or fungi toxic compounds (Incerti et al. 2013). Kramer et al. (2012) reported most persistent mineral-bound carbon is comprised of aromatic compounds with strong chemical resemblance to dissolve. Hence, our hypothesis is that aromatic C rich organic product is unable to induce AI because do not support a substantial microbial growth.

3.5. Conclusion

Proper management of organic matter 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 organic matter additions to achieve 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 C source for microbes. An opposite response was found for biochar, barely affects AI when incorporated into the soil. We also found a strong effect on aggregation with some cellulose rich organic matters such as maize litter, cellulose, and wood powder. Moreover, some composted organic matter showed lower and smaller initial but persistent effect on soil aggregation. The use of ^{13}C-CPMAS NMR provides an improved definition of organic matter biochemical quality, helping to explain the variable effects of organic matter on aggregation dynamics. In detail, ^{13}C-CPMAS NMR revealed that the four restricted spectral regions such as di-*O*-alkyl C and *O*-alkyl C (roughly corresponding to carbohydrate fraction), and H, C - substitute aromatic C and O-substitute aromatic C (roughly corresponding to aromatic fraction) are crucial to understand amendment effects on aggregate stability better than classical elemental chemical parameter. However, as a major novel contribution, our study is the first attempt to linking litter biochemistry with dynamics of soil aggregation.

Supplementary Tables

Supplementary Table 3. S1. Physical and chemical properties (mean values of three replicates) of Capasso, Castel volturno and Torino soil types at the beginning of the experimental activity.

Parameter	Capasso	Castel volturno	Torino
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

Supplementary Table 3.S2. Statistically significant results of post-hoc tests for pair-wise differences of soil aggregation index (AI) between treatment combinations (i.e. soil type, organic matter used for soil amendment, and incubation time in days), limited to combination pairs including Castel volturno soil type. For each treatment combination, data refer to treatment levels, AI (mean values ± standard deviation of three replicates), pair-wise AI difference (mean and 95% confidence interval) and associated p-value according to Bonferroni's test. Pair-wise comparisons resulting in non-significant p-values are not shown.

Treatment combination 1				Treatment combination 2				Post-hoc test	
Soil type	Organic matter	Time	AI	Soil type	Organic matter	Time	AI	AI difference	p
Castel volturno	Alfalfa litter	10	1.37±0.03	Castel volturno	Manure compost	10	1.06±0.05	0.32 (0.01÷0.63)	$3.4 \cdot 10^{-2}$
Castel volturno	Alfalfa litter	30	1.32±0.01	Capasso	Alfalfa litter	30	0.97±0.02	0.35 (0.04÷0.66)	$3.9 \cdot 10^{-3}$
Castel volturno	Alfalfa litter	300	1.24±0.06	Torino	Alfalfa litter	300	0.77±0.08	0.47 (0.16÷0.78)	$2.7 \cdot 10^{-7}$
Castel volturno	Biochar	10	1.12±0.02	Capasso	Biochar	10	0.52±0.02	0.6 (0.29÷0.91)	$1.6 \cdot 10^{-12}$
Castel volturno	Biochar	10	1.12±0.02	Castel volturno	Meat powder	10	1.46±0.01	-0.35 (0.04÷0.66)	$4.1 \cdot 10^{-3}$
Castel volturno	Biochar	10	1.12±0.02	Torino	Biochar	10	0.49±0.05	0.63 (0.32÷0.94)	$< 10^{-14}$
Castel volturno	Biochar	30	1.1±0.02	Capasso	Biochar	30	0.54±0.03	0.57 (0.26÷0.88)	$2.9 \cdot 10^{-11}$
Castel volturno	Biochar	30	1.1±0.02	Castel volturno	Meat powder	30	1.43±0.02	-0.33 (0.02÷0.64)	$1.6 \cdot 10^{-2}$
Castel volturno	Biochar	30	1.1±0.02	Torino	Biochar	30	0.59±0.03	0.51 (0.2÷0.82)	$4.0 \cdot 10^{-9}$
Castel volturno	Biochar	100	1.05±0.04	Capasso	Biochar	100	0.53±0.01	0.52 (0.21÷0.83)	$2.2 \cdot 10^{-9}$
Castel volturno	Biochar	100	1.05±0.04	Castel volturno	Maize litter	100	1.37±0.03	-0.33 (0.02÷0.64)	$1.5 \cdot 10^{-2}$
Castel volturno	Biochar	100	1.05±0.04	Castel volturno	Wood powder	100	1.4±0.02	-0.35 (0.04÷0.66)	$3.3 \cdot 10^{-3}$
Castel volturno	Biochar	100	1.05±0.04	Torino	Biochar	100	0.58±0.04	0.47 (0.16÷0.78)	$2.8 \cdot 10^{-7}$
Castel volturno	Biochar	300	0.99±0.09	Capasso	Biochar	300	0.57±0.05	0.42 (0.11÷0.73)	$1.6 \cdot 10^{-5}$
Castel volturno	Biochar	300	0.99±0.09	Castel volturno	Maize litter	300	1.34±0.04	-0.35 (0.04÷0.66)	$2.4 \cdot 10^{-3}$
Castel volturno	Biochar	300	0.99±0.09	Torino	Biochar	300	0.42±0.03	0.56 (0.25÷0.87)	$4.4 \cdot 10^{-11}$
Castel volturno	Glucose	10	1.4±0.04	Capasso	Glucose	10	0.9±0.24	0.5 (0.19÷0.81)	$1.3 \cdot 10^{-8}$
Castel volturno	Glucose	10	1.4±0.04	Castel volturno	Manure compost	10	1.06±0.05	0.34 (0.03÷0.65)	$6.1 \cdot 10^{-3}$
Castel volturno	Glucose	10	1.4±0.04	Torino	Glucose	10	0.96±0.08	0.44 (0.13÷0.75)	$2.8 \cdot 10^{-6}$
Castel volturno	Glucose	30	1.21±0.02	Capasso	Glucose	30	0.82±0.04	0.39 (0.08÷0.7)	$1.6 \cdot 10^{-4}$
Castel volturno	Glucose	300	1.16±0.06	Capasso	Glucose	300	0.62±0.08	0.54 (0.23÷0.85)	$2.5 \cdot 10^{-10}$
Castel volturno	Glucose	300	1.16±0.06	Torino	Glucose	300	0.61±0.11	0.55 (0.24÷0.86)	$1.9 \cdot 10^{-10}$
Castel volturno	Green compost	10	1.11±0.03	Capasso	Green compost	10	0.62±0.01	0.48 (0.17÷0.79)	$5.8 \cdot 10^{-8}$
Castel volturno	Green compost	10	1.11±0.03	Castel volturno	Meat powder	10	1.46±0.01	-0.36 (0.05÷0.67)	$2.0 \cdot 10^{-3}$
Castel volturno	Green compost	10	1.11±0.03	Torino	Green compost	10	0.53±0.04	0.57 (0.26÷0.88)	$1.2 \cdot 10^{-11}$
Castel volturno	Green compost	30	1.09±0.06	Capasso	Green compost	30	0.62±0.04	0.46 (0.15÷0.77)	$4.1 \cdot 10^{-7}$
Castel volturno	Green compost	30	1.09±0.06	Castel volturno	Meat powder	30	1.43±0.02	-0.35 (0.04÷0.66)	$4.0 \cdot 10^{-3}$
Castel volturno	Green compost	30	1.09±0.06	Torino	Green compost	30	0.48±0.03	0.61 (0.3÷0.91)	$7.9 \cdot 10^{-13}$
Castel volturno	Green compost	100	1.23±0.04	Capasso	Green compost	100	0.73±0.11	0.5 (0.19÷0.81)	$2.1 \cdot 10^{-8}$
Castel volturno	Green compost	300	1.18±0.01	Capasso	Green compost	300	0.7±0.05	0.47 (0.16÷0.78)	$2.0 \cdot 10^{-7}$
Castel volturno	Green compost	300	1.18±0.01	Torino	Green compost	300	0.55±0.04	0.63 (0.32÷0.94)	$< 10^{-14}$
Castel volturno	Maize litter	30	1.35±0.02	Castel volturno	Solid digestate	30	1.01±0.07	0.33 (0.02÷0.64)	$1.0 \cdot 10^{-2}$
Castel volturno	Maize litter	300	1.34±0.04	Castel volturno	Meat powder	300	1.01±0.04	0.33 (0.02÷0.64)	$1.6 \cdot 10^{-2}$
Castel volturno	Manure compost	10	1.06±0.05	Capasso	Manure compost	10	0.65±0.03	0.41 (0.1÷0.72)	$2.6 \cdot 10^{-5}$
Castel volturno	Manure compost	10	1.06±0.05	Castel volturno	Meat powder	10	1.46±0.01	-0.4 (0.09÷0.71)	$5.4 \cdot 10^{-5}$
Castel volturno	Manure compost	10	1.06±0.05	Torino	Manure compost	10	0.55±0.06	0.51 (0.2÷0.82)	$7.2 \cdot 10^{-9}$
Castel volturno	Manure compost	30	1.04±0.04	Capasso	Manure compost	30	0.64±0.09	0.4 (0.09÷0.71)	$6.1 \cdot 10^{-5}$
Castel volturno	Manure compost	30	1.04±0.04	Castel volturno	Meat powder	30	1.43±0.02	-0.39 (0.08÷0.7)	$1.5 \cdot 10^{-4}$
Castel volturno	Manure compost	30	1.04±0.04	Torino	Manure compost	30	0.62±0.04	0.42 (0.11÷0.73)	$1.4 \cdot 10^{-5}$
Castel volturno	Manure compost	100	1.17±0.02	Capasso	Manure compost	100	0.75±0.08	0.42 (0.11÷0.73)	$1.2 \cdot 10^{-5}$
Castel volturno	Manure compost	100	1.17±0.02	Torino	Manure compost	100	0.81±0.11	0.35 (0.05÷0.66)	$2.2 \cdot 10^{-3}$
Castel volturno	Manure compost	300	1.19±0.07	Capasso	Manure compost	300	0.7±0.09	0.49 (0.18÷0.8)	$3.5 \cdot 10^{-8}$
Castel volturno	Manure compost	300	1.19±0.07	Torino	Manure compost	300	0.66±0.06	0.53 (0.22÷0.84)	$8.8 \cdot 10^{-10}$
Castel volturno	Meat powder	10	1.46±0.01	Castel volturno	Meat powder	300	1.01±0.04	0.45 (0.14÷0.76)	$1.2 \cdot 10^{-6}$
Castel volturno	Meat powder	10	1.46±0.01	Castel volturno	Solid digestate	10	1.1±0.04	0.36 (0.05÷0.67)	$1.5 \cdot 10^{-3}$
Castel volturno	Meat powder	30	1.43±0.02	Castel volturno	Meat powder	300	1.01±0.04	0.42 (0.11÷0.73)	$1.6 \cdot 10^{-5}$
Castel volturno	Meat powder	30	1.43±0.02	Castel volturno	Solid digestate	30	1.01±0.07	0.42 (0.11÷0.73)	$1.6 \cdot 10^{-5}$

Castel volturno	Meat powder	30	1.43±0.02	Torino	Meat powder	30	1±0.3	0.43 (0.12÷0.74)	$6.8 \cdot 10^{-6}$
Castel volturno	Solid digestate	10	1.1±0.04	Capasso	Solid digestate	10	0.55±0.01	0.55 (0.24÷0.86)	$1.5 \cdot 10^{-10}$
Castel volturno	Solid digestate	10	1.1±0.04	Torino	Solid digestate	10	0.52±0.01	0.58 (0.27÷0.89)	$7.1 \cdot 10^{-12}$
Castel volturno	Solid digestate	30	1.01±0.07	Capasso	Solid digestate	30	0.55±0.06	0.46 (0.15÷0.77)	$4.4 \cdot 10^{-7}$
Castel volturno	Solid digestate	30	1.01±0.07	Torino	Solid digestate	30	0.48±0.04	0.53 (0.22÷0.84)	$5.8 \cdot 10^{-10}$
Castel volturno	Solid digestate	100	1.17±0.05	Capasso	Solid digestate	100	0.66±0.04	0.5 (0.2÷0.81)	$9.2 \cdot 10^{-9}$
Castel volturno	Solid digestate	100	1.17±0.05	Torino	Solid digestate	100	0.73±0.01	0.43 (0.12÷0.74)	$4.5 \cdot 10^{-6}$
Castel volturno	Solid digestate	300	1.13±0.1	Capasso	Solid digestate	300	0.64±0.06	0.48 (0.17÷0.79)	$6.1 \cdot 10^{-8}$
Castel volturno	Solid digestate	300	1.13±0.1	Torino	Solid digestate	300	0.56±0.04	0.57 (0.26÷0.88)	$1.7 \cdot 10^{-11}$
Castel volturno	Wood powder	10	1.33±0.03	Capasso	Wood powder	10	0.84±0	0.49 (0.18÷0.8)	$4.0 \cdot 10^{-8}$
Castel volturno	Wood powder	100	1.4±0.02	Capasso	Wood powder	100	0.83±0.1	0.57 (0.26÷0.88)	$2.9 \cdot 10^{-11}$
Castel volturno	Wood powder	300	1.27±0.05	Torino	Wood powder	300	0.95±0.03	0.32 (0.01÷0.63)	$2.5 \cdot 10^{-2}$

Supplementary Table 3.S3. Statistically significant results of post-hoc tests for pair-wise differences of soil aggregation index (AI) between treatment combinations (i.e. soil type, organic matter used for soil amendment, and incubation time in days), limited to combination pairs including Capasso soil type. See caption of Supplementary Table 3.S2 for further details.

Treatment combination 1				Treatment combination 2				Post-hoc test	
Soil type	**Organic matter**	**Time**	**AI**	**Soil type**	**Organic matter**	**Time**	**AI**	**AI difference**	**p**
Capasso	Alfalfa litter	10	1.14±0.13	Capasso	Biochar	10	0.52±0.02	0.62 (0.31÷0.93)	$< 10^{-14}$
Capasso	Alfalfa litter	10	1.14±0.13	Capasso	Green compost	10	0.62±0.01	0.51 (0.2÷0.82)	$4.2 \cdot 10^{-9}$
Capasso	Alfalfa litter	10	1.14±0.13	Capasso	Manure compost	10	0.65±0.03	0.49 (0.18÷0.8)	$3.7 \cdot 10^{-8}$
Capasso	Alfalfa litter	10	1.14±0.13	Capasso	Solid digestate	10	0.55±0.01	0.58 (0.27÷0.89)	$5.5 \cdot 10^{-12}$
Capasso	Alfalfa litter	30	0.97±0.02	Capasso	Biochar	30	0.54±0.03	0.43 (0.12÷0.74)	$4.6 \cdot 10^{-6}$
Capasso	Alfalfa litter	30	0.97±0.02	Capasso	Green compost	30	0.62±0.04	0.35 (0.04÷0.66)	$3.1 \cdot 10^{-3}$
Capasso	Alfalfa litter	30	0.97±0.02	Capasso	Maize litter	30	1.39±0.01	-0.42 (0.11÷0.73)	$2.0 \cdot 10^{-5}$
Capasso	Alfalfa litter	30	0.97±0.02	Capasso	Manure compost	30	0.64±0.09	0.33 (0.02÷0.64)	$9.5 \cdot 10^{-3}$
Capasso	Alfalfa litter	30	0.97±0.02	Capasso	Solid digestate	30	0.55±0.06	0.42 (0.11÷0.73)	$1.3 \cdot 10^{-5}$
Capasso	Alfalfa litter	30	0.97±0.02	Castel volturno	Alfalfa litter	30	1.32±0.01	-0.35 (0.04÷0.66)	$3.9 \cdot 10^{-3}$
Capasso	Alfalfa litter	30	0.97±0.02	Torino	Alfalfa litter	30	1.29±0.13	-0.32 (0.01÷0.63)	$3.3 \cdot 10^{-2}$
Capasso	Alfalfa litter	100	1.08±0.1	Capasso	Biochar	100	0.53±0.01	0.56 (0.25÷0.87)	$6.6 \cdot 10^{-11}$
Capasso	Alfalfa litter	100	1.08±0.1	Capasso	Biochar	300	0.57±0.05	0.37 (0.06÷0.68)	$5.9 \cdot 10^{-4}$
Capasso	Alfalfa litter	100	1.08±0.1	Capasso	Glucose	300	0.62±0.08	0.32 (0.01÷0.63)	$1.9 \cdot 10^{-2}$
Capasso	Alfalfa litter	100	1.08±0.1	Capasso	Green compost	100	0.73±0.11	0.35 (0.04÷0.66)	$3.2 \cdot 10^{-3}$
Capasso	Alfalfa litter	100	1.08±0.1	Capasso	Manure compost	100	0.75±0.08	0.34 (0.03÷0.65)	$7.8 \cdot 10^{-3}$
Capasso	Alfalfa litter	100	1.08±0.1	Capasso	Solid digestate	100	0.66±0.04	0.42 (0.11÷0.73)	$1.2 \cdot 10^{-5}$
Capasso	Alfalfa litter	300	0.94±0.1	Capasso	Maize litter	300	1.33±0.04	-0.4 (0.09÷0.7)	$1.1 \cdot 10^{-4}$
Capasso	Biochar	10	0.52±0.02	Capasso	Cellulose	10	1.15±0.25	-0.63 (0.32÷0.94)	$< 10^{-14}$
Capasso	Biochar	10	0.52±0.02	Capasso	Glucose	10	0.9±0.24	-0.38 (0.07÷0.69)	$3.8 \cdot 10^{-4}$
Capasso	Biochar	10	0.52±0.02	Capasso	Maize litter	10	1.3±0.01	-0.78 (0.47÷1.09)	$< 10^{-14}$
Capasso	Biochar	10	0.52±0.02	Capasso	Meat powder	10	1.38±0.05	-0.86 (0.55÷1.17)	$< 10^{-14}$
Capasso	Biochar	10	0.52±0.02	Capasso	Wood powder	10	0.84±0	-0.32 (0.01÷0.63)	$2.9 \cdot 10^{-2}$
Capasso	Biochar	10	0.52±0.02	Castel volturno	Biochar	10	1.12±0.02	-0.6 (0.29÷0.91)	$1.6 \cdot 10^{-12}$
Capasso	Biochar	30	0.54±0.03	Capasso	Cellulose	30	1.24±0.05	-0.7 (0.39÷1.01)	$< 10^{-14}$
Capasso	Biochar	30	0.54±0.03	Capasso	Maize litter	30	1.39±0.01	-0.85 (0.54÷1.16)	$< 10^{-14}$
Capasso	Biochar	30	0.54±0.03	Capasso	Meat powder	30	1.19±0.04	-0.65 (0.35÷0.96)	$< 10^{-14}$
Capasso	Biochar	30	0.54±0.03	Capasso	Wood powder	30	0.93±0.14	-0.39 (0.08÷0.7)	$1.2 \cdot 10^{-4}$
Capasso	Biochar	30	0.54±0.03	Castel volturno	Biochar	30	1.1±0.02	-0.57 (0.26÷0.88)	$2.9 \cdot 10^{-11}$
Capasso	Biochar	100	0.53±0.01	Capasso	Cellulose	100	1.16±0.22	-0.63 (0.32÷0.94)	$< 10^{-14}$
Capasso	Biochar	100	0.53±0.01	Capasso	Glucose	100	0.87±0.09	-0.35 (0.04÷0.66)	$3.8 \cdot 10^{-3}$
Capasso	Biochar	100	0.53±0.01	Capasso	Maize litter	100	1.24±0.01	-0.71 (0.4÷1.02)	$< 10^{-14}$
Capasso	Biochar	100	0.53±0.01	Capasso	Meat powder	100	1.07±0.04	-0.55 (0.24÷0.86)	$1.7 \cdot 10^{-10}$
Capasso	Biochar	100	0.53±0.01	Castel volturno	Biochar	100	1.05±0.04	-0.52 (0.21÷0.83)	$2.2 \cdot 10^{-9}$
Capasso	Biochar	300	0.57±0.05	Capasso	Maize litter	300	1.33±0.04	-0.77 (0.46÷1.08)	$< 10^{-14}$
Capasso	Biochar	300	0.57±0.05	Capasso	Wood powder	300	1.15±0.11	-0.58 (0.27÷0.89)	$8.7 \cdot 10^{-12}$
Capasso	Biochar	300	0.57±0.05	Castel volturno	Biochar	300	0.99±0.09	-0.42 (0.11÷0.73)	$1.6 \cdot 10^{-5}$
Capasso	Cellulose	10	1.15±0.25	Capasso	Green compost	10	0.62±0.01	0.53 (0.22÷0.84)	$1.3 \cdot 10^{-9}$
Capasso	Cellulose	10	1.15±0.25	Capasso	Manure compost	10	0.65±0.03	0.5 (0.19÷0.81)	$1.2 \cdot 10^{-8}$
Capasso	Cellulose	10	1.15±0.25	Capasso	Solid digestate	10	0.55±0.01	0.59 (0.29÷0.9)	$1.6 \cdot 10^{-12}$
Capasso	Cellulose	10	1.15±0.25	Capasso	Wood powder	10	0.84±0	0.31 (0÷0.62)	$4.5 \cdot 10^{-2}$
Capasso	Cellulose	30	1.24±0.05	Capasso	Cellulose	300	0.86±0.12	0.38 (0.07÷0.69)	$3.6 \cdot 10^{-4}$
Capasso	Cellulose	30	1.24±0.05	Capasso	Glucose	30	0.82±0.04	0.42 (0.11÷0.73)	$1.2 \cdot 10^{-5}$
Capasso	Cellulose	30	1.24±0.05	Capasso	Green compost	30	0.62±0.04	0.61 (0.31÷0.92)	$< 10^{-14}$
Capasso	Cellulose	30	1.24±0.05	Capasso	Manure compost	30	0.64±0.09	0.6 (0.29÷0.91)	$7.9 \cdot 10^{-13}$
Capasso	Cellulose	30	1.24±0.05	Capasso	Solid digestate	30	0.55±0.06	0.69 (0.38÷1)	$< 10^{-14}$
Capasso	Cellulose	100	1.16±0.22	Capasso	Green compost	100	0.73±0.11	0.42 (0.11÷0.73)	$1.2 \cdot 10^{-5}$
Capasso	Cellulose	100	1.16±0.22	Capasso	Manure compost	100	0.75±0.08	0.41 (0.1÷0.72)	$3.3 \cdot 10^{-5}$
Capasso	Cellulose	100	1.16±0.22	Capasso	Solid digestate	100	0.66±0.04	0.5 (0.19÷0.8)	$2.2 \cdot 10^{-8}$
Capasso	Cellulose	100	1.16±0.22	Capasso	Wood powder	100	0.83±0.1	0.33 (0.02÷0.64)	$1.7 \cdot 10^{-2}$
Capasso	Cellulose	300	0.86±0.12	Capasso	Maize litter	300	1.33±0.04	-0.48 (0.17÷0.79)	$1.2 \cdot 10^{-7}$
Capasso	Glucose	10	0.9±0.24	Capasso	Solid digestate	10	0.55±0.01	0.34 (0.04÷0.65)	$4.6 \cdot 10^{-3}$
Capasso	Glucose	10	0.9±0.24	Castel volturno	Glucose	10	1.4±0.04	-0.5 (0.19÷0.81)	$1.3 \cdot 10^{-8}$
Capasso	Glucose	30	0.82±0.04	Castel volturno	Glucose	30	1.21±0.02	-0.39 (0.08÷0.7)	$1.6 \cdot 10^{-4}$
Capasso	Glucose	300	0.62±0.08	Capasso	Wood powder	300	1.15±0.11	-0.53 (0.22÷0.84)	$8.9 \cdot 10^{-10}$

Capasso	Glucose	300	0.62±0.08	Castel volturno	Glucose	300	1.16±0.06	-0.54 (0.23÷0.85)	$2.5 \cdot 10^{-10}$
Capasso	Green compost	10	0.62±0.01	Capasso	Maize litter	10	1.3±0.01	-0.68 (0.37÷0.99)	$< 10^{-14}$
Capasso	Green compost	10	0.62±0.01	Capasso	Meat powder	10	1.38±0.05	-0.76 (0.45÷1.07)	$< 10^{-14}$
Capasso	Green compost	10	0.62±0.01	Castel volturno	Green compost	10	1.11±0.03	-0.48 (0.17÷0.79)	$5.8 \cdot 10^{-8}$
Capasso	Green compost	30	0.62±0.04	Capasso	Maize litter	30	1.39±0.01	-0.77 (0.46÷1.08)	$< 10^{-14}$
Capasso	Green compost	30	0.62±0.04	Capasso	Meat powder	30	1.19±0.04	-0.57 (0.26÷0.88)	$1.7 \cdot 10^{-11}$
Capasso	Green compost	30	0.62±0.04	Capasso	Wood powder	30	0.93±0.14	-0.31 (0÷0.62)	$5.0 \cdot 10^{-2}$
Capasso	Green compost	30	0.62±0.04	Castel volturno	Green compost	30	1.09±0.06	-0.46 (0.15÷0.77)	$4.1 \cdot 10^{-7}$
Capasso	Green compost	100	0.73±0.11	Capasso	Maize litter	100	1.24±0.01	-0.5 (0.19÷0.81)	$1.2 \cdot 10^{-8}$
Capasso	Green compost	100	0.73±0.11	Capasso	Meat powder	100	1.07±0.04	-0.34 (0.03÷0.65)	$6.5 \cdot 10^{-3}$
Capasso	Green compost	100	0.73±0.11	Castel volturno	Green compost	100	1.23±0.04	-0.5 (0.19÷0.81)	$2.1 \cdot 10^{-8}$
Capasso	Green compost	300	0.7±0.05	Capasso	Maize litter	300	1.33±0.04	-0.63 (0.32÷0.94)	$< 10^{-14}$
Capasso	Green compost	300	0.7±0.05	Capasso	Wood powder	300	1.15±0.11	-0.44 (0.13÷0.75)	$2.6 \cdot 10^{-6}$
Capasso	Green compost	300	0.7±0.05	Castel volturno	Green compost	300	1.18±0.01	-0.47 (0.16÷0.78)	$2.0 \cdot 10^{-7}$
Capasso	Maize litter	10	1.3±0.01	Capasso	Glucose	10	0.9±0.24	0.41 (0.1÷0.71)	$4.8 \cdot 10^{-5}$
Capasso	Maize litter	10	1.3±0.01	Capasso	Manure compost	10	0.65±0.03	0.66 (0.35÷0.97)	$< 10^{-14}$
Capasso	Maize litter	10	1.3±0.01	Capasso	Solid digestate	10	0.55±0.01	0.75 (0.44÷1.06)	$< 10^{-14}$
Capasso	Maize litter	10	1.3±0.01	Capasso	Wood powder	10	0.84±0	0.47 (0.16÷0.78)	$3.0 \cdot 10^{-7}$
Capasso	Maize litter	30	1.39±0.01	Capasso	Glucose	30	0.82±0.04	0.57 (0.26÷0.88)	$1.3 \cdot 10^{-11}$
Capasso	Maize litter	30	1.39±0.01	Capasso	Manure compost	30	0.64±0.09	0.75 (0.44÷1.06)	$< 10^{-14}$
Capasso	Maize litter	30	1.39±0.01	Capasso	Solid digestate	30	0.55±0.06	0.84 (0.53÷1.15)	$< 10^{-14}$
Capasso	Maize litter	30	1.39±0.01	Capasso	Wood powder	30	0.93±0.14	0.46 (0.15÷0.77)	$6.9 \cdot 10^{-7}$
Capasso	Maize litter	100	1.24±0.01	Capasso	Glucose	100	0.87±0.09	0.36 (0.05÷0.67)	$1.3 \cdot 10^{-3}$
Capasso	Maize litter	100	1.24±0.01	Capasso	Manure compost	100	0.75±0.08	0.49 (0.18÷0.8)	$3.7 \cdot 10^{-8}$
Capasso	Maize litter	100	1.24±0.01	Capasso	Solid digestate	100	0.66±0.04	0.57 (0.27÷0.88)	$1.2 \cdot 10^{-11}$
Capasso	Maize litter	100	1.24±0.01	Capasso	Wood powder	100	0.83±0.1	0.41 (0.1÷0.71)	$4.8 \cdot 10^{-5}$
Capasso	Maize litter	300	1.33±0.04	Capasso	Glucose	300	0.62±0.08	0.72 (0.41÷1.03)	$< 10^{-14}$
Capasso	Maize litter	300	1.33±0.04	Capasso	Manure compost	300	0.7±0.09	0.64 (0.33÷0.95)	$< 10^{-14}$
Capasso	Maize litter	300	1.33±0.04	Capasso	Meat powder	300	0.71±0.11	0.62 (0.31÷0.93)	$< 10^{-14}$
Capasso	Maize litter	300	1.33±0.04	Capasso	Solid digestate	300	0.64±0.06	0.69 (0.38÷1)	$< 10^{-14}$
Capasso	Manure compost	10	0.65±0.03	Capasso	Meat powder	10	1.38±0.05	-0.73 (0.42÷1.04)	$< 10^{-14}$
Capasso	Manure compost	10	0.65±0.03	Castel volturno	Manure compost	10	1.06±0.05	-0.41 (0.1÷0.72)	$2.6 \cdot 10^{-5}$
Capasso	Manure compost	30	0.64±0.09	Capasso	Meat powder	30	1.19±0.04	-0.55 (0.25÷0.86)	$8.2 \cdot 10^{-11}$
Capasso	Manure compost	30	0.64±0.09	Castel volturno	Manure compost	30	1.04±0.04	-0.4 (0.09÷0.71)	$6.1 \cdot 10^{-5}$
Capasso	Manure compost	100	0.75±0.08	Capasso	Meat powder	100	1.07±0.04	-0.33 (0.02÷0.64)	$1.5 \cdot 10^{-2}$
Capasso	Manure compost	100	0.75±0.08	Castel volturno	Manure compost	100	1.17±0.02	-0.42 (0.11÷0.73)	$1.2 \cdot 10^{-5}$
Capasso	Manure compost	300	0.7±0.09	Capasso	Wood powder	300	1.15±0.11	-0.45 (0.14÷0.76)	$1.3 \cdot 10^{-6}$
Capasso	Manure compost	300	0.7±0.09	Castel volturno	Manure compost	300	1.19±0.07	-0.49 (0.18÷0.8)	$3.5 \cdot 10^{-8}$
Capasso	Meat powder	10	1.38±0.05	Capasso	Glucose	10	0.9±0.24	0.48 (0.17÷0.79)	$7.0 \cdot 10^{-8}$
Capasso	Meat powder	10	1.38±0.05	Capasso	Meat powder	300	0.71±0.11	0.67 (0.36÷0.98)	$< 10^{-14}$
Capasso	Meat powder	10	1.38±0.05	Capasso	Solid digestate	10	0.55±0.01	0.83 (0.52÷1.14)	$< 10^{-14}$
Capasso	Meat powder	10	1.38±0.05	Capasso	Wood powder	10	0.84±0	0.54 (0.23÷0.85)	$2.5 \cdot 10^{-10}$
Capasso	Meat powder	30	1.19±0.04	Capasso	Glucose	30	0.82±0.04	0.38 (0.07÷0.69)	$3.9 \cdot 10^{-4}$
Capasso	Meat powder	30	1.19±0.04	Capasso	Meat powder	300	0.71±0.11	0.48 (0.17÷0.79)	$6.2 \cdot 10^{-8}$
Capasso	Meat powder	30	1.19±0.04	Capasso	Solid digestate	30	0.55±0.06	0.64 (0.33÷0.95)	$< 10^{-14}$
Capasso	Meat powder	100	1.07±0.04	Capasso	Meat powder	300	0.71±0.11	0.36 (0.05÷0.67)	$1.1 \cdot 10^{-3}$
Capasso	Meat powder	100	1.07±0.04	Capasso	Solid digestate	100	0.66±0.04	0.41 (0.1÷0.72)	$2.6 \cdot 10^{-5}$
Capasso	Meat powder	300	0.71±0.11	Capasso	Wood powder	300	1.15±0.11	-0.44 (0.13÷0.74)	$4.2 \cdot 10^{-6}$
Capasso	Solid digestate	10	0.55±0.01	Castel volturno	Solid digestate	10	1.1±0.04	-0.55 (0.24÷0.86)	$1.5 \cdot 10^{-10}$
Capasso	Solid digestate	30	0.55±0.06	Capasso	Wood powder	30	0.93±0.14	-0.38 (0.07÷0.69)	$3.3 \cdot 10^{-4}$
Capasso	Solid digestate	30	0.55±0.06	Castel volturno	Solid digestate	30	1.01±0.07	-0.46 (0.15÷0.77)	$4.4 \cdot 10^{-7}$
Capasso	Solid digestate	100	0.66±0.04	Castel volturno	Solid digestate	100	1.17±0.05	-0.5 (0.2÷0.81)	$9.2 \cdot 10^{-9}$
Capasso	Solid digestate	300	0.64±0.06	Capasso	Wood powder	300	1.15±0.11	-0.5 (0.19÷0.81)	$1.4 \cdot 10^{-8}$
Capasso	Solid digestate	300	0.64±0.06	Castel volturno	Solid digestate	300	1.13±0.1	-0.48 (0.17÷0.79)	$6.1 \cdot 10^{-8}$
Capasso	Wood powder	10	0.84±0	Castel volturno	Wood powder	10	1.33±0.03	-0.49 (0.18÷0.8)	$4.0 \cdot 10^{-8}$
Capasso	Wood powder	10	0.84±0	Torino	Wood powder	10	1.15±0.03	-0.31 (0÷0.62)	$4.7 \cdot 10^{-2}$
Capasso	Wood powder	100	0.83±0.1	Castel volturno	Wood powder	100	1.4±0.02	-0.57 (0.26÷0.88)	$2.9 \cdot 10^{-11}$
Capasso	Wood powder	100	0.83±0.1	Torino	Wood powder	100	1.24±0.07	-0.4 (0.1÷0.71)	$5.0 \cdot 10^{-5}$
Capasso	Wood powder	300	1.15±0.11	Capasso	Wood powder	100	0.83±0.1	0.31 (0÷0.62)	$3.7 \cdot 10^{-2}$

Supplementary Table 3.S4. Statistically significant results of post-hoc tests for pair-wise differences of soil aggregation index (AI) between treatment combinations (i.e. soil type, organic matter used for soil amendment, and incubation time in days), limited to combination pairs including Torino soil type. See caption of Supplementary Table 3.S2 for further details.

Treatment combination 1				Treatment combination 2				Post-hoc test	
Soil type	Organic matter	Time	AI	Soil type	Organic matter	Time	AI	AI difference	P
Torino	Alfalfa litter	10	1.35±0.14	Torino	Alfalfa litter	300	0.77±0.08	0.57 (0.26÷0.88)	$1.3 \cdot 10^{-11}$
Torino	Alfalfa litter	10	1.35±0.14	Torino	Biochar	10	0.49±0.05	0.86 (0.55÷1.17)	$< 10^{-14}$
Torino	Alfalfa litter	10	1.35±0.14	Torino	Glucose	10	0.96±0.08	0.39 (0.08÷0.7)	$2.2 \cdot 10^{-4}$
Torino	Alfalfa litter	10	1.35±0.14	Torino	Green compost	10	0.53±0.04	0.81 (0.5÷1.12)	$< 10^{-14}$
Torino	Alfalfa litter	10	1.35±0.14	Torino	Manure compost	10	0.55±0.06	0.79 (0.48÷1.1)	$< 10^{-14}$
Torino	Alfalfa litter	10	1.35±0.14	Torino	Solid digestate	10	0.52±0.01	0.82 (0.51÷1.13)	$< 10^{-14}$
Torino	Alfalfa litter	30	1.29±0.13	Capasso	Alfalfa litter	30	0.97±0.02	0.32 (0.01÷0.63)	$3.3 \cdot 10^{-2}$
Torino	Alfalfa litter	30	1.29±0.13	Torino	Alfalfa litter	300	0.77±0.08	0.52 (0.21÷0.83)	$3.0 \cdot 10^{-9}$
Torino	Alfalfa litter	30	1.29±0.13	Torino	Biochar	30	0.59±0.03	0.7 (0.39÷1.01)	$< 10^{-14}$
Torino	Alfalfa litter	30	1.29±0.13	Torino	Green compost	30	0.48±0.03	0.81 (0.5÷1.12)	$< 10^{-14}$
Torino	Alfalfa litter	30	1.29±0.13	Torino	Manure compost	30	0.62±0.04	0.67 (0.36÷0.98)	$< 10^{-14}$
Torino	Alfalfa litter	30	1.29±0.13	Torino	Solid digestate	30	0.48±0.04	0.81 (0.5÷1.12)	$< 10^{-14}$
Torino	Alfalfa litter	100	1.09±0.13	Torino	Alfalfa litter	300	0.77±0.08	0.32 (0.01÷0.63)	$2.3 \cdot 10^{-2}$
Torino	Alfalfa litter	100	1.09±0.13	Torino	Biochar	100	0.58±0.04	0.51 (0.2÷0.82)	$4.3 \cdot 10^{-9}$
Torino	Alfalfa litter	100	1.09±0.13	Torino	Solid digestate	100	0.73±0.01	0.36 (0.05÷0.67)	$1.3 \cdot 10^{-3}$
Torino	Alfalfa litter	300	0.77±0.08	Castel volturno	Alfalfa litter	300	1.24±0.06	-0.47 (0.16÷0.78)	$2.7 \cdot 10^{-7}$
Torino	Alfalfa litter	300	0.77±0.08	Torino	Biochar	300	0.42±0.03	0.35 (0.04÷0.66)	$3.7 \cdot 10^{-3}$
Torino	Biochar	10	0.49±0.05	Castel volturno	Biochar	10	1.12±0.02	-0.63 (0.32÷0.94)	$< 10^{-14}$
Torino	Biochar	10	0.49±0.05	Torino	Cellulose	10	1.19±0.04	-0.7 (0.4÷1.01)	$< 10^{-14}$
Torino	Biochar	10	0.49±0.05	Torino	Glucose	10	0.96±0.08	-0.47 (0.16÷0.78)	$1.8 \cdot 10^{-7}$
Torino	Biochar	10	0.49±0.05	Torino	Maize litter	10	1.19±0.13	-0.7 (0.39÷1.01)	$< 10^{-14}$
Torino	Biochar	10	0.49±0.05	Torino	Meat powder	10	1.24±0.2	-0.75 (0.44÷1.06)	$< 10^{-14}$
Torino	Biochar	10	0.49±0.05	Torino	Wood powder	10	1.15±0.03	-0.66 (0.35÷0.97)	$< 10^{-14}$
Torino	Biochar	30	0.59±0.03	Castel volturno	Biochar	30	1.1±0.02	-0.51 (0.2÷0.82)	$4.0 \cdot 10^{-9}$
Torino	Biochar	30	0.59±0.03	Torino	Cellulose	30	1.17±0.16	-0.58 (0.27÷0.89)	$6.3 \cdot 10^{-12}$
Torino	Biochar	30	0.59±0.03	Torino	Glucose	30	1.07±0.09	-0.48 (0.17÷0.79)	$1.1 \cdot 10^{-7}$
Torino	Biochar	30	0.59±0.03	Torino	Maize litter	30	1.38±0	-0.79 (0.48÷1.1)	$< 10^{-14}$
Torino	Biochar	30	0.59±0.03	Torino	Meat powder	30	1±0.3	-0.41 (0.1÷0.72)	$2.8 \cdot 10^{-5}$
Torino	Biochar	30	0.59±0.03	Torino	Wood powder	30	1.03±0.07	-0.44 (0.13÷0.75)	$2.8 \cdot 10^{-6}$
Torino	Biochar	100	0.58±0.04	Castel volturno	Biochar	100	1.05±0.04	-0.47 (0.16÷0.78)	$2.8 \cdot 10^{-7}$
Torino	Biochar	100	0.58±0.04	Torino	Cellulose	100	1.09±0.17	-0.51 (0.2÷0.82)	$7.8 \cdot 10^{-9}$
Torino	Biochar	100	0.58±0.04	Torino	Glucose	30	1.07±0.09	-0.4 (0.09÷0.71)	$9.1 \cdot 10^{-5}$
Torino	Biochar	100	0.58±0.04	Torino	Green compost	100	0.98±0.06	-0.39 (0.09÷0.7)	$1.1 \cdot 10^{-4}$
Torino	Biochar	100	0.58±0.04	Torino	Maize litter	100	1.35±0.03	-0.77 (0.46÷1.08)	$< 10^{-14}$
Torino	Biochar	100	0.58±0.04	Torino	Meat powder	100	1.05±0.14	-0.47 (0.16÷0.78)	$1.6 \cdot 10^{-7}$
Torino	Biochar	100	0.58±0.04	Torino	Wood powder	100	1.24±0.07	-0.66 (0.35÷0.96)	$< 10^{-14}$
Torino	Biochar	300	0.42±0.03	Castel volturno	Biochar	300	0.99±0.09	-0.56 (0.25÷0.87)	$4.4 \cdot 10^{-11}$
Torino	Biochar	300	0.42±0.03	Torino	Cellulose	300	0.95±0.1	-0.52 (0.21÷0.83)	$1.9 \cdot 10^{-9}$
Torino	Biochar	300	0.42±0.03	Torino	Maize litter	300	1.07±0.04	-0.64 (0.34÷0.95)	$< 10^{-14}$
Torino	Biochar	300	0.42±0.03	Torino	Meat powder	300	0.74±0.06	-0.32 (0.01÷0.63)	$3.1 \cdot 10^{-2}$
Torino	Biochar	300	0.42±0.03	Torino	Wood powder	300	0.95±0.03	-0.53 (0.22÷0.84)	$9.3 \cdot 10^{-10}$
Torino	Cellulose	10	1.19±0.04	Torino	Green compost	10	0.53±0.04	0.66 (0.35÷0.97)	$< 10^{-14}$
Torino	Cellulose	10	1.19±0.04	Torino	Manure compost	10	0.55±0.06	0.64 (0.33÷0.95)	$< 10^{-14}$
Torino	Cellulose	10	1.19±0.04	Torino	Solid digestate	10	0.52±0.01	0.67 (0.36÷0.98)	$< 10^{-14}$
Torino	Cellulose	30	1.17±0.16	Torino	Green compost	30	0.48±0.03	0.69 (0.38÷1)	$< 10^{-14}$
Torino	Cellulose	30	1.17±0.16	Torino	Manure compost	30	0.62±0.04	0.55 (0.24÷0.86)	$1.2 \cdot 10^{-10}$
Torino	Cellulose	30	1.17±0.16	Torino	Solid digestate	30	0.48±0.04	0.69 (0.38÷1)	$< 10^{-14}$
Torino	Cellulose	100	1.09±0.17	Torino	Solid digestate	100	0.73±0.01	0.36 (0.05÷0.66)	$2.1 \cdot 10^{-3}$
Torino	Cellulose	300	0.95±0.1	Torino	Glucose	300	0.61±0.11	0.33 (0.02÷0.64)	$1.0 \cdot 10^{-2}$
Torino	Cellulose	300	0.95±0.1	Torino	Green compost	300	0.55±0.04	0.4 (0.09÷0.71)	$7.9 \cdot 10^{-5}$
Torino	Cellulose	300	0.95±0.1	Torino	Solid digestate	300	0.56±0.04	0.39 (0.08÷0.7)	$1.7 \cdot 10^{-4}$
Torino	Glucose	10	0.96±0.08	Castel volturno	Glucose	10	1.4±0.04	-0.44 (0.13÷0.75)	$2.8 \cdot 10^{-6}$
Torino	Glucose	10	0.96±0.08	Torino	Glucose	300	0.61±0.11	0.35 (0.04÷0.66)	$3.8 \cdot 10^{-3}$
Torino	Glucose	10	0.96±0.08	Torino	Green compost	10	0.53±0.04	0.43 (0.12÷0.74)	$7.7 \cdot 10^{-6}$
Torino	Glucose	10	0.96±0.08	Torino	Manure compost	10	0.55±0.06	0.41 (0.1÷0.72)	$3.7 \cdot 10^{-5}$
Torino	Glucose	10	0.96±0.08	Torino	Solid digestate	10	0.52±0.01	0.44 (0.13÷0.75)	$3.4 \cdot 10^{-6}$

Torino	Glucose	30	1.07±0.09	Torino	Glucose	300	0.61±0.11	0.46 (0.15÷0.77)	$7.1 \cdot 10^{-7}$
Torino	Glucose	30	1.07±0.09	Torino	Green compost	30	0.48±0.03	0.59 (0.28÷0.9)	$3.2 \cdot 10^{-12}$
Torino	Glucose	30	1.07±0.09	Torino	Manure compost	30	0.62±0.04	0.45 (0.14÷0.76)	$1.4 \cdot 10^{-6}$
Torino	Glucose	30	1.07±0.09	Torino	Solid digestate	30	0.48±0.04	0.59 (0.28÷0.9)	$2.4 \cdot 10^{-12}$
Torino	Glucose	100	0.98±0.07	Torino	Glucose	300	0.61±0.11	0.36 (0.06÷0.67)	$1.1 \cdot 10^{-3}$
Torino	Glucose	300	0.61±0.11	Castel volturno	Glucose	300	1.16±0.06	-0.55 (0.24÷0.86)	$1.9 \cdot 10^{-10}$
Torino	Glucose	300	0.61±0.11	Torino	Wood powder	300	0.95±0.03	-0.34 (0.03÷0.65)	$5.8 \cdot 10^{-3}$
Torino	Green compost	10	0.53±0.04	Castel volturno	Green compost	10	1.11±0.03	-0.57 (0.26÷0.88)	$1.2 \cdot 10^{-11}$
Torino	Green compost	10	0.53±0.04	Torino	Maize litter	10	1.19±0.13	-0.65 (0.34÷0.96)	$< 10^{-14}$
Torino	Green compost	10	0.53±0.04	Torino	Meat powder	10	1.24±0.2	-0.71 (0.4÷1.02)	$< 10^{-14}$
Torino	Green compost	10	0.53±0.04	Torino	Wood powder	10	1.15±0.03	-0.62 (0.31÷0.93)	$< 10^{-14}$
Torino	Green compost	30	0.48±0.03	Castel volturno	Green compost	30	1.09±0.06	-0.61 (0.3÷0.91)	$7.9 \cdot 10^{-13}$
Torino	Green compost	30	0.48±0.03	Torino	Maize litter	30	1.38±0	-0.9 (0.59÷1.21)	$< 10^{-14}$
Torino	Green compost	30	0.48±0.03	Torino	Meat powder	30	1±0.3	-0.52 (0.21÷0.83)	$1.7 \cdot 10^{-9}$
Torino	Green compost	30	0.48±0.03	Torino	Wood powder	30	1.03±0.07	-0.55 (0.24÷0.86)	$1.2 \cdot 10^{-10}$
Torino	Green compost	100	0.98±0.06	Torino	Green compost	10	0.53±0.04	0.44 (0.13÷0.75)	$2.1 \cdot 10^{-6}$
Torino	Green compost	100	0.98±0.06	Torino	Green compost	30	0.48±0.03	0.5 (0.19÷0.8)	$2.2 \cdot 10^{-8}$
Torino	Green compost	100	0.98±0.06	Torino	Green compost	300	0.55±0.04	0.43 (0.12÷0.74)	$7.5 \cdot 10^{-6}$
Torino	Green compost	100	0.98±0.06	Torino	Maize litter	100	1.35±0.03	-0.38 (0.07÷0.69)	$3.9 \cdot 10^{-4}$
Torino	Green compost	300	0.55±0.04	Castel volturno	Green compost	300	1.18±0.01	-0.63 (0.32÷0.94)	$< 10^{-14}$
Torino	Green compost	300	0.55±0.04	Torino	Maize litter	300	1.07±0.04	-0.52 (0.21÷0.83)	$1.9 \cdot 10^{-9}$
Torino	Green compost	300	0.55±0.04	Torino	Wood powder	300	0.95±0.03	-0.41 (0.1÷0.72)	$4.3 \cdot 10^{-5}$
Torino	Maize litter	10	1.19±0.13	Torino	Manure compost	10	0.55±0.06	0.64 (0.33÷0.94)	$< 10^{-14}$
Torino	Maize litter	10	1.19±0.13	Torino	Solid digestate	10	0.52±0.01	0.66 (0.35÷0.97)	$< 10^{-14}$
Torino	Maize litter	30	1.38±0	Torino	Glucose	30	1.07±0.09	0.31 (0÷0.62)	$3.7 \cdot 10^{-2}$
Torino	Maize litter	30	1.38±0.01	Torino	Maize litter	300	1.07±0.04	0.31 (0÷0.62)	$3.8 \cdot 10^{-2}$
Torino	Maize litter	30	1.38±0	Torino	Manure compost	30	0.62±0.04	0.76 (0.45÷1.07)	$< 10^{-14}$
Torino	Maize litter	30	1.38±0	Torino	Meat powder	30	1±0.3	0.38 (0.07÷0.69)	$3.4 \cdot 10^{-4}$
Torino	Maize litter	30	1.38±0	Torino	Solid digestate	30	0.48±0.04	0.9 (0.59÷1.21)	$< 10^{-14}$
Torino	Maize litter	30	1.38±0	Torino	Wood powder	30	1.03±0.07	0.35 (0.04÷0.66)	$2.8 \cdot 10^{-3}$
Torino	Maize litter	100	1.35±0.03	Torino	Glucose	30	1.07±0.09	0.38 (0.07÷0.69)	$4.6 \cdot 10^{-4}$
Torino	Maize litter	100	1.35±0.03	Torino	Manure compost	100	0.81±0.11	0.54 (0.23÷0.85)	$3.8 \cdot 10^{-10}$
Torino	Maize litter	100	1.35±0.03	Torino	Solid digestate	100	0.73±0.01	0.62 (0.31÷0.93)	$< 10^{-14}$
Torino	Maize litter	300	1.07±0.04	Torino	Glucose	300	0.61±0.11	0.46 (0.15÷0.77)	$6.7 \cdot 10^{-7}$
Torino	Maize litter	300	1.07±0.04	Torino	Manure compost	300	0.66±0.06	0.41 (0.1÷0.72)	$2.6 \cdot 10^{-5}$
Torino	Maize litter	300	1.07±0.04	Torino	Meat powder	300	0.74±0.06	0.33 (0.02÷0.64)	$1.4 \cdot 10^{-2}$
Torino	Maize litter	300	1.07±0.04	Torino	Solid digestate	300	0.56±0.04	0.51 (0.2÷0.82)	$4.8 \cdot 10^{-9}$
Torino	Manure compost	10	0.55±0.06	Castel volturno	Manure compost	10	1.06±0.05	-0.51 (0.2÷0.82)	$7.2 \cdot 10^{-9}$
Torino	Manure compost	10	0.55±0.06	Torino	Meat powder	10	1.24±0.2	-0.69 (0.38÷1)	$< 10^{-14}$
Torino	Manure compost	10	0.55±0.06	Torino	Wood powder	10	1.15±0.03	-0.6 (0.29÷0.91)	$1.6 \cdot 10^{-12}$
Torino	Manure compost	30	0.62±0.04	Castel volturno	Manure compost	30	1.04±0.04	-0.42 (0.11÷0.73)	$1.4 \cdot 10^{-5}$
Torino	Manure compost	30	0.62±0.04	Torino	Meat powder	30	1±0.3	-0.38 (0.07÷0.69)	$3.0 \cdot 10^{-4}$
Torino	Manure compost	30	0.62±0.04	Torino	Wood powder	30	1.03±0.07	-0.41 (0.1÷0.72)	$3.2 \cdot 10^{-5}$
Torino	Manure compost	100	0.81±0.11	Castel volturno	Manure compost	100	1.17±0.02	-0.35 (0.05÷0.66)	$2.2 \cdot 10^{-3}$
Torino	Manure compost	100	0.81±0.11	Torino	Wood powder	100	1.24±0.07	-0.42 (0.11÷0.73)	$1.3 \cdot 10^{-5}$
Torino	Manure compost	300	0.66±0.06	Castel volturno	Manure compost	300	1.19±0.07	-0.53 (0.22÷0.84)	$8.8 \cdot 10^{-10}$
Torino	Meat powder	10	1.24±0.2	Torino	Meat powder	300	0.74±0.06	0.5 (0.19÷0.81)	$1.6 \cdot 10^{-8}$
Torino	Meat powder	10	1.24±0.2	Torino	Solid digestate	10	0.52±0.01	0.72 (0.41÷1.03)	$< 10^{-14}$
Torino	Meat powder	30	1±0.3	Castel volturno	Meat powder	30	1.43±0.02	-0.43 (0.12÷0.74)	$6.8 \cdot 10^{-6}$
Torino	Meat powder	30	1±0.3	Torino	Solid digestate	30	0.48±0.04	0.52 (0.21÷0.83)	$1.6 \cdot 10^{-9}$
Torino	Meat powder	100	1.05±0.14	Torino	Meat powder	300	0.74±0.06	0.31 (0÷0.62)	$4.1 \cdot 10^{-2}$
Torino	Meat powder	100	1.05±0.14	Torino	Solid digestate	100	0.73±0.01	0.32 (0.01÷0.63)	$2.2 \cdot 10^{-2}$
Torino	Solid digestate	10	0.52±0.01	Castel volturno	Solid digestate	10	1.1±0.04	-0.58 (0.27÷0.89)	$7.1 \cdot 10^{-12}$
Torino	Solid digestate	10	0.52±0.01	Torino	Wood powder	10	1.15±0.03	-0.63 (0.32÷0.93)	$< 10^{-14}$
Torino	Solid digestate	30	0.48±0.04	Castel volturno	Solid digestate	30	1.01±0.07	-0.53 (0.22÷0.84)	$5.8 \cdot 10^{-10}$
Torino	Solid digestate	30	0.48±0.04	Torino	Wood powder	30	1.03±0.07	-0.55 (0.24÷0.86)	$1.1 \cdot 10^{-10}$
Torino	Solid digestate	100	0.73±0.01	Castel volturno	Solid digestate	100	1.17±0.05	-0.43 (0.12÷0.74)	$4.5 \cdot 10^{-6}$
Torino	Solid digestate	100	0.73±0.01	Torino	Wood powder	100	1.24±0.07	-0.5 (0.19÷0.81)	$1.0 \cdot 10^{-8}$
Torino	Solid digestate	300	0.56±0.04	Castel volturno	Solid digestate	300	1.13±0.1	-0.57 (0.26÷0.88)	$1.7 \cdot 10^{-11}$
Torino	Solid digestate	300	0.56±0.04	Torino	Wood powder	300	0.95±0.03	-0.4 (0.09÷0.71)	$9.5 \cdot 10^{-5}$
Torino	Wood powder	10	1.15±0.03	Capasso	Wood powder	10	0.84±0	0.31 (0÷0.62)	$4.7 \cdot 10^{-2}$
Torino	Wood powder	100	1.24±0.07	Capasso	Wood powder	100	0.83±0.1	0.4 (0.1÷0.71)	$5.0 \cdot 10^{-5}$
Torino	Wood powder	300	0.95±0.03	Castel volturno	Wood powder	300	1.27±0.05	-0.32 (0.01÷0.63)	$2.5 \cdot 10^{-2}$

Chapter – 4

A new modeling approach to describe and predict soil aggregation dynamics

Tushar C. Sarker

In collaboration with Francesco Giannino, Giuliano Bonanomi, Stefano Mazzoleni

Abstract

Modeling soil aggregation is a fundamental to understand soil physical process. The goal of the current modeling approach is, to purposely overcome the limitations of using C/N as a single OM quality indicator, and to explore the relationship between OM quality and soil aggregation. Our SOMDY model is based on an advanced description of OM chemical quality by ^{13}C-CPMAS NMR instead of traditional C/N ratio. The approach was taken to define model compartments representing water-stable soil aggregate size (micro, meso and macro) fractions and describing the relation with OM chemical quality defined by ^{13}C-CPMAS NMR spectroscopy. Soil aggregate data from 1 year laboratory incubation study with 10 different organic matters (OM) types and 5 incubation date (0, 10, 30, 100 and 300 days). To calibrate the model, we select four OM types (biochar, meat powder, glucose and solid digestate) out of ten OM types covering a different aggregation range (null to very high aggregation). The simulation results showed the model capability, to predict aggregation behavior of organic amendments and the distribution of water-stable aggregate size fractions of biochar and solid digestate and glucose, while the models have several limitations to describe the aggregation behavior of rapid and very high aggregations inducing OM, such as meat powder. However, our model describe as well as the impact of OM chemical quality on the physical structure of soil aggregation.

4.1. Introduction

Soil aggregation is an important ecosystem process resulting in the formation and stabilization of soil structure, consisting of soil aggregates and the resulting matrix of pore spaces (Rillig et al. 2015). OM is an especially important factor controlling aggregate stability because its amount and properties can be modified trough agronomic management. Then, a better understanding of the impact of different OM types on soil aggregate structure is required. No significant effort has been made before 1965, to explain the link between OM addition and soil aggregation. However, the first step in this direction was made by Monnier (1965), who proposed a conceptual model that link soil aggregate stability with organic amendment quality across time scales, varying from weeks to months till years after their incorporation.

Defined OM quality in terms of organic chemical composition (Swift et al. 1979) is operationally complex because OM consist of different organic compounds with particular susceptibility to decomposition (e.g. cellulose, organic acids, amino acids, simple sugars, lignin, tannins, humic substances) and diverse inorganic elements (e.g. N, P, S) whose relative fractions differ with decaying stages (Rovira & Vallejo 2007). During the last decades, a significant effort has been made to find out effective indicators of OM quality, which is capable to serve reliable predictions of decay rate. The traditional approach has been based on the assessment of selected characteristics to identify parameters or indexes correlated with decay rates, and thus useful for predictive purposes (Meentemeyer 1978; Melillo et al. 1982). In the last decade, several chemical throughput methods as pyrolysis-gas chromatography/ mass spectrometry (Huang et al. 1998), near infrared reflectance spectroscopy (Gillon et al. 1999), and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy either as such (Kögel-Knabner 2002) or in combination with chemo-metry (Šmejkalová et al. 2008) have been applied to characterize organic matter at molecular level. In particular, ^{13}C-CPMAS NMR has been proven useful to provide an overview of the total organic chemical composition of complex matrices, such as soil organic matter (Bonanomi et al. 2013; Kögel-Knabner 2002). However, such novel experimental applications have not yet been exploited by current modeling implementation for an improved description of OM quality.

The current paradigm for soil aggregation stems from a progression of work proposing a hierarchical model of soil aggregation (Dexter 1988; Oades & Waters 1991; Tisdall & Oades 1982; Williams et al. 1967). The hierarchical model was supported by experiments observing

porosity exclusion (Currie 1966), aggregate strength (Hadas 1987), and aggregate breakdown with differing levels of applied energy (Oades & Waters 1991).

Several researchers (Beare et al. 1994; Golchin et al. 1994; Six et al. 2000) have proposed conceptual models of soil aggregation that dynamically link the decomposition of organic materials in soil to the formation of aggregates. Generally, the models propose that large soil aggregates form around nuclei of particulate organic matter, and that smaller aggregates are subsequently released by the dispersion of the macroaggregates as the organic matter decomposes and is no longer able to stabilize the macroaggregate. The necessity for a quantitative model of soil aggregation is significant. Aggregation plays a key role in the stabilization of OM by physically protecting it through occlusion within aggregates, but Elliott et al. (1996) reported that our current knowledge is too weak. Majority of the concurrent models of SOM turnover use an over simplified approach based on soil texture (Parton et al. 1987) or land use (Van Veen & Paul 1981) to account for the processes of physical protection and tillage. Balesdent et al. (2000) suggested that modeling of SOM dynamics should be more mechanistic and reproduce the processes of physical protection as described in the current conceptual models of soil aggregation. The first step in developing a mechanistic model of SOM dynamics that accounts for physical protection and physical disturbances such as tillage is the development of a quantitative model for soil aggregates themselves. A quantitative model of soil aggregate formation and disruption may provide data concerning the rates of occlusion or release of labile organic materials and, therefore, their availability for mineralization or stabilization.

Though several empirical models have been developed during the last few decades that point out that a better link between OM quality and aggregate stability is required, but in fact, no mathematical model has been developed describing the effect of OM quality on soil aggregation process. However, last year Incerti et al. (2017) published a model called OMDY (Organic Matter DYnamics): a new model of organic matter decomposition based on biomolecular content as assessed by ^{13}C-CPMAS-NMR spectroscopy.

In this work, a new model called SOMDY (Soil Organic Matter DYnamics) is developed, calibrated and validated. The model is based on a novel implementation of OM quality by ^{13}C-CPMAS NMR, to purposely overcome the limitations of C/N as a single OM quality indicator and to explore the relationship between OM quality and soil aggregation.

4.2. Materials and methods

4.2.1. Model concept and description

The SOMDY model presented here has been conceived to represent soil organic matter dynamics taking into account the following major issues:

- improved definition of chemical composition of SOM;
- assessment of SOM physical structure;
- chemical and physical protective effects on aggregation processes;
- microbial turnover and chemical evolution of SOM during the decomposition.

Then, the general structure and logic of SOMDY model is represented in Fig. 4.1. The model is modular and developed according to a system dynamic approach. It has been implemented by the Simile software (Muetzelfeldt & Massheder 2003). The initial requirements to run the integrated model are the definition of soil texture (% of sand, silt and clay particles), bulk density, adsorbing mineral surface area, and the initial content and chemical composition of soil organic matter. The model is an extension of OMDY model (Incerti et al. 2017), in particular here we present effect of organic matter quality on soil aggregation process.

4.2.2. SOM chemical composition and physical aggregation

In the model, soil organic matter (SOM) is a state variable represented by the sum of dissolved (DOM) and aggregated organic matter (AOM). Addition of exogenous organic matter is also taken into account with external inputs added to the DOM and partitioned according to their specific chemical composition.

Solid state ^{13}C-NMR spectroscopy has been used to assess the chemical composition of organic matter in litter decomposition studies, with different classes of organic-C compounds related to specific NMR spectral regions (Bonanomi et al. 2013; Kögel-Knabner 2002). In the frame of the this model, seven resonance regions have been considered, as reported by previous reference studies (Kögel-Knabner 2002; Li et al. 2015; Mathers et al. 2007; Pane et al. 2011): 0-45 ppm = alkyl C; 46-60 ppm = methoxyl and N-alkyl 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); 161-190 ppm = carboxyl C. For calibration purposes, within each wide reference region, a restricted sequence of signals was selected by choosing those most correlated with litter decay rate. Then, the following ranges have been used and referred to the

different model layers (Fig. 4.1) to represent SOM quality: 10-19; 53-57; 70-75; 103-106; 132-136; 149-153; 175-180 ppm.

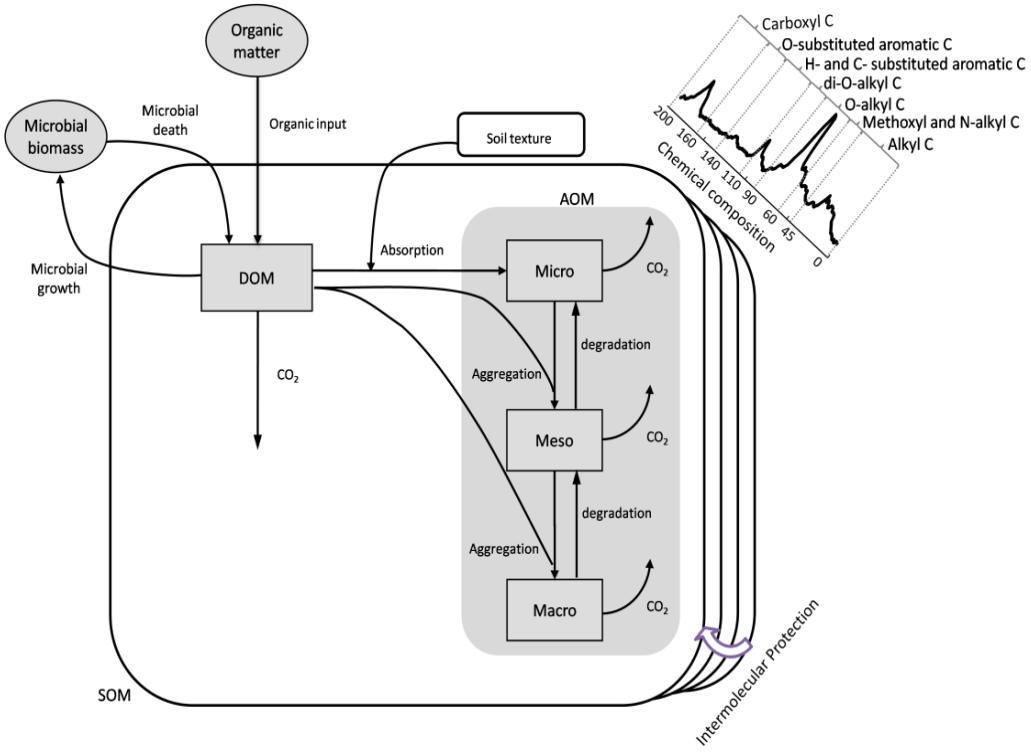

Figure 4.1. Schematic representation of the SOMDY model. Soil organic matter (SOM) is described in terms of chemical composition (different spectral regions in solid-state ^{13}C NMR spectra correspond to model layers) and physical structure of mineral/organic aggregation status (DOM- dissolved organic matter; AOM- aggregated organic matter). Soil aggregation proceeds with rates depending on intermolecular interactions. Mineralization produces CO_2 emissions with rates depending on both chemical and physical characteristics (see text for details). Microbial turnover is accounted by the change in chemical composition during mineralization processes.

The mathematical formulation of such representation of organic matter quality in the model is then the following:

$$SOM = \sum_{i=1}^{7} \left(DOM + AOM \right)_i \qquad (4.1)$$

where $i=1, \ldots 7$, is the chemical class index

The physical aggregation of organic matter is represented in the model by either dissolved organic materials (*DOM*) or three different dimensional classes of aggregates: *Micro* (micro-

aggregates, particle diameter < 0.25 μm), *Meso* (meso-aggregates, between 0.25 μm and 1 μm), and *Macro* (macro-aggregates, >1 μm) (see grey box in Fig. 4.1).

According to the physical structure of *SOM*, the equation (4.1) then becomes:

$$SOM = \sum_{i=1}^{7} \left(DOM + Micro + Meso + Macro \right)_i \qquad (4.2)$$

The following processes are considered in the *SOM* system dynamics:

- Mineral and organic adsorption
- Physical aggregation
- Mineralization
- Microbial turnover

4.2.3. Mineral and organic adsorption

The adsorbing surface on which organic compounds are aggregated is a function of both the adsorption surface area of the soil mineral fraction and the residual exposed surface area of neo-formed organic aggregates.

This is implemented in the model by calculating the available mineral adsorbing surface, $AS_{mineral}$, as the sum of surface area in each textural class (sand, silt and clay):

$$AS_{mineral} = sand \cdot AS_{sand} + silt \cdot AS_{silt} + clay \cdot AS_{clay} \qquad (4.3)$$

where AS_{sand}, AS_{silt} and AS_{clay} are the texture class adsorbing parameters.

Then, the rate of DOM adsorption is the product of an adsorption coefficient and the available free mineral surface area.

Additionally, as aggregation proceeds through adsorption of organic molecules on soil particles, the model calculates the new surface adsorption created on the newly formed soil aggregates aggregates. Differently from the mineral adsorption surface, such organic adsorption surface cannot be saturated, because the aggregation process progressively produces new available binding sites for additional *DOM*, thereby maintaining a somewhat available adsorption surface. For simplicity, organic matter surface adsorption is modelled considering a spherical geometry for organic C aggregates.

In particular the adsorption equation becomes:

$$Absorption = AS_{\mathrm{min}eral} \cdot k_{chemical} \cdot DOM_i \cdot \left(1 - \frac{\sum \mathrm{AOM}}{\sum \mathrm{AOM} + DOM} \right) \text{ with i = 1,..., 7.} \qquad (4.4)$$

Where, $AS_{mineral}$ is the soil adsorption coefficient according texture and $k_{chemical}$ is the aggregation index according the chemical composition of the organic substance.

In particular the $k_{chemical}$ is calculated as follow:

$$k_{chemical} = \frac{\sum_{i=1}^{7} a_i \cdot DOM_i}{\sum_{i=1}^{7} DOM_i} \quad (4.5)$$

Where a_i is the weighing score of aggregation of that chemical class attributed to each biomolecular class that reflects its relative contribution to intermolecular interactions.

4.2.4. Physical aggregation

The exogenous organic matter is considered in the model as an external input to *DOM* compartment, in form of alfalfa litter, biochar, cellulose, glucose, green compost, maize litter, manure compost, meat powder, solid digestate and wood powder. Aggregation experiment was carried out in laboratory condition, using three soil types, ten organic substrates, replicated 3 times for each of 4 incubation times, for a total of 396 microcosms. Aggregates fractions were separated according to the method of Kemper & Rosenau (1986). *DOM* can be mineralized with consequent CO_2 release or adsorbed by the mineral and organic soil components. Newly formed *Micro*-aggregates can then further aggregate forming larger particles (*Meso* and *Macro* aggregates). The aggregation process is reversible, i.e. the model also simulates degradation from *macro*- to *meso*-, and from *meso*- to *micro*- fractions. During aggregation a part of the DOM is consumed to make different aggregated fractions.

Here I write a simple equation for MESO compartment that can be used also for the other aggregation level:

$$\frac{dMESO}{dt} = MICRO_{agg} + DOM_{agg} - MICRO_{degrad} - MACRO_{agg} + MACRO_{degrad} - MESO_{mineral} \quad (4.6)$$

4.2.5. Mineralization

The process of mineralization in the model is represented separately for each chemical class of organic compounds and varies according to the level of physical aggregation (e.g. differences among SOM and Micro, Meso, Macro). For details about mineralization, see Incerti et al. (2017).

4.2.6. Microbial turnover

The model structure based chemical differences among layers also provide a conceptual frame for implementing a submodel on microbial turnover. During the mineralization processes a percent fraction of the organic C is converted into microbial biomass. The model does not explicitly describe the processes of microbial feeding, growth, and reproduction, but simply calculates the total microbial biomass according to a "metabolic ratio" of all mineralization flows. Then, microbial death is implicitly modelled by re-entering the microbial mass in the system through a partitioning related to a reference microbial composition (Kögel-Knabner 2002). In other words, every time the mineralization occurs, the involved microbes are re-cycled in the *DOM* compartments (model layers), in coherence with a chemical description of microbial composition, and, thus, the overall organic matter chemical composition is changed in turn. For details about microbial turnover, see Incerti et al. (2017).

4.3. Results

4.3.1. Simulation and validation

To run the simulation, we select four OM types (biochar, meat powder, glucose and solid digestate) out of ten OM types (mention earlier) covering a different aggregation range (null to very high aggregation), and compare with real data. In general terms, simulated data shows significant correlation ($p < 0.001$) with empirical data, with a very high regression coefficient (R^2) for biochar ($R^2 = 0.995$) and solid digestate ($R^2 = 0.884$) followed by glucose and meat powder (Table 4.1).

Table 4.1. The value of Pearson's correlation coefficient, p-value, linear regression equation and regression coefficient between experimental and simulated data for four organic matters

OM type	Pearson correlation	p-value	Equation	Regression coefficient (R^2)
Biochar	0.982	< 0.001	0.963x+0.012	0.965
Meat powder	0.856	0.047	1.000x-0.005	0.733
Glucose	0.910	0.003	0.889x+0.036	0.828
Solid digestate	0.940	< 0.001	0.869x+0.043	0.884
All	0.924	< 0.001	0.929x+0.022	0.854

The calibrated models fit the water-stable aggregate size distribution data well (Fig. 4.2). We found the model fitting process yielded different patterns of aggregation dynamics. Simulated data of all OM types showed well fit with experimental data observed (Fig. 4.2). Considering different aggregate fractions (micro, meso and macro), different OM types showed a different trend of fitting during the incubation time. Specifically, for biochar we found all simulated aggregate fractions showed very good fit, with experimental data throughout the incubation time (Fig. 4.2). In case of meat powder, simulated micro aggregate fraction had fit, while meso and macro aggregates differ from the experimental data, especially in the intermediate to end period (30-300 days) (Fig. 4.2). Fitting was fair for glucose, in particular micro aggregate fraction showed good fit throughout the incubation time, while meso and macro aggregates showed dissimilarity at the middle stages (30 –100 days) (Fig. 4.2). Finally, solid digestate had also better fit, in case of micro fraction throughout the incubation time, while other aggregate fractions did not show good similarity at initial and end period (Fig. 4.2).

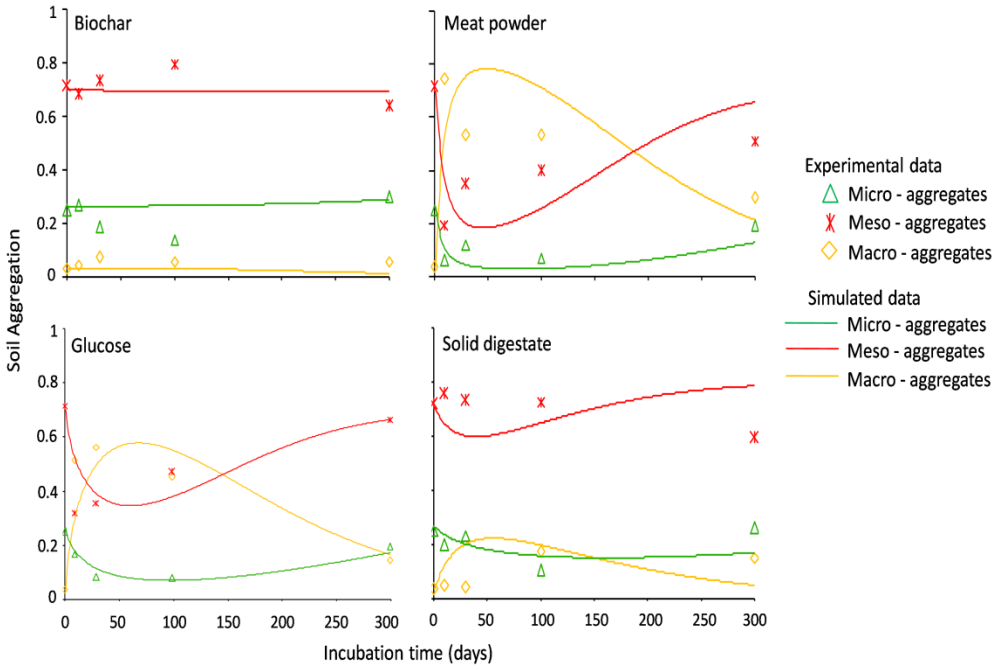

Figure 4.2. Simulation dynamics of different aggregate fractions (micro, meso and macro) of four OM types (biochar, meat powder, glucose and solid digestate), at 5 incubation time (0, 10, 30, 100 and 300 days).

Moreover, we put all aggregation data (experimental and simulated data from four OM types, 5 incubation time and 3 aggregate fractions) in a regression plot to compare experimental

and simulation data. We observed a very good fit of simulation data *vs* experimental data with regression coefficient equal to R^2= 0.854 (Fig. 4.3). Among OM types, biochar and solid digestate showed better fit with a regression line.

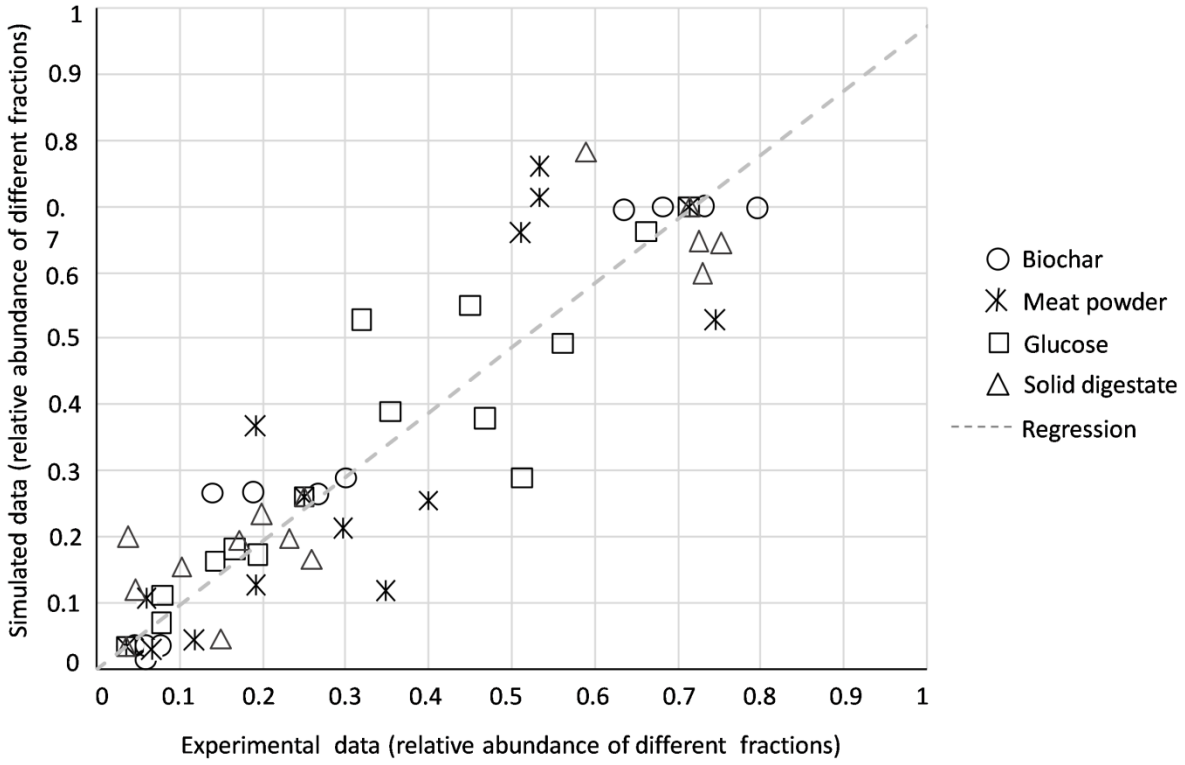

Figure 4.3. Model validation against experimental data. Scatter plot and correlation (Pearson's *r* and associated p-value) between simulated and observed value of different aggregate fractions (micro, meso and macro), at 5 incubation time (0, 10, 30, 100 and 300 days). Symbols indicate different OM types.

4.4. Discussion

The lack of universality of the models is expected and reflects the impact of differing soil properties on the dynamics of the aggregate components. Properties such as OM chemical quality and clay contents may alter the patterns and rates of aggregate dynamics by supplying varying amounts of reactive surfaces and binding agents.

To the best of our knowledge, the SOMDY approach provides for the first time a chemically based interpretation of experimental observations that depict the coexistence of distributions of different aggregate fractions. Moreover, the SOMDY model is an integrated

model to combine OM chemical quality (defined by ^{13}C NMR spectroscopy) and physical soil data to simulate the process of aggregation and mineralization. Our mathematical model well fitted with verbal model proposed by Monnier (1965), that was the first step in this direction was made more than 50 year ago to link soil aggregate stability with organic amendment quality across time scales, varying from weeks to months till years after their incorporation.

The literature (Beare et al. 1994; Six et al. 2000) and observations of the tracer Dy distribution in soil aggregate size fractions (Plante et al. 2002) suggest that macro aggregates are form first, when degradation occurred macro aggregates can form micro and meso aggregates that can be supported or refuted by our model structures (Fig. 4.1), the patterns of aggregate formation and disruption proposed by the model structures provide sufficient evidence to suggest that aggregation is not a random association of aggregate constituents and can be predicted.

In a modeling approach to quantifying soil macro-aggregate dynamics, Inconsistent behavior observed by (Plante et al. 2002), they observed model is not able to make any predictions of the dynamics of micro aggregates. As illustrated by the relatively flat lines for the 1–2 mm and 2–4 mm aggregate size fractions, a better model structure might consist of one pool for large aggregates (> 1 mm), one for intermediate macroaggregates (0.25–1 mm), and one or more pools for microaggregates (< 0.25 mm). In case of our model, we found that the model predicts the behavior of micro aggregates better than meso and macro aggregates.

4.5. Conclusion

The SOMDY model is a new soil structure model that can represent, simultaneously, within a multiscale context, SOM, particles and aggregates. We have presented a model for sequential incomplete fragmentation based on the OMDY model for soil structure (Incerti et al. 2017). While the model developed here fits the soil aggregate data well and was able to predict the aggregation behavior of biochar, glucose and solid digestate, it has some limitations, such as not sufficient to describe the initial phases of aggregation behavior due to meat powder. This will require further calibration work. Prediction accuracy emerged from the OM-specific partitioning of molecular types and from the representation of intermolecular interactions. The modeling approach adopted here suggests several directions for future efforts. Development and application of an improved model, calibration and validation based field measurements are also needed in terms of model calibration experiments with a wide range of OM types.

Chapter – 5

General conclusion

Tushar C. Sarker

General conclusions

A sustainable use of soil means its exploitation in a way and at a rate that preserves at the long-term its multitude of functions and protects or improves its quality, thereby maintaining its potential to meet the likely needs and aspirations of present and future generations. Appropriate agricultural utilization of soil needs equal attention for all essential components of soil, such as biological, chemical and physical, thus attaining a sustainable agricultural system. Organic amendments can increase SOM content and thus influence soil characteristics by the interdependent modification of biological, chemical and physical properties. This thesis demonstrates the importance of not only the organic amendment, but also the chemical quality of the OM for various soil processes including nitrogen mineralization and soil aggregation.

This thesis was motivated by the lack of scientific agreement to identify the OM chemical qualities, which are the key factor for controlling soil processes. *"Chapter 1"* provides an overview of previous research framed in support of the hypothesis that organic amendment is a major driving force of soil processes, which linked to its quality parameters. I showed that the ^{13}C-CPMAS NMR has been useful to provide a full description of the total organic chemical composition of complex matrices. Moreover, selected spectral regions and corresponding C types showed better correlation with soil processes, including N dynamics and soil aggregation. By linking OM quality as an indicator of microbial processes in both controlled incubation experiments and statistically explorative studies, this thesis aimed to provide evidence for the link of OM chemical quality in these processes and support the hypothesis that OM is an important component in agricultural soil. This was done by two separate experiments, presented as two thesis chapters.

Objective of "*Chapter 2*" was, to investigate the effects of organic input on soil N dynamics, and how this process correlates with OM quality parameters. In this context, we proposed a hypothesis that OM quality characterization by ^{13}C-CPMAS NMR spectra predicts N mineralization process better than the classical C/N ratio index. Mineralization experiment was carried out in laboratory condition, using three soil types, nine organic substrates and replicated 3 times for each of 5 incubation times. Observed results were then correlated with OM chemical quality parameters defined by solid-state NMR spectra and the classical elemental analyzer. Considering ^{13}C-CPMAS NMR spectral regions, we found the carboxyl C (161-190 ppm), N-alkyl and methoxyl C (46-60 ppm) and alkyl C (0-45 ppm) regions had significant positive

relation with N mineralization, while the di-*O*-alkyl C (61-90) and *O*-alkyl C (91-110) had a significant negative correlation, depending on soil type and incubation time. This result supports our hypothesis that OM quality characterized by ^{13}C-CPMAS NMR spectra explains N mineralization process better than the classical C/N ratio index.

The aim of the *"Chapter 3"* was, linking OM intrinsic biochemical quality with soil aggregation dynamics. In this context, we test the hypothesis that the initial biochemical characteristics of OM are suitable to explain the variability of aggregation dynamic after organic input, as related to the OM capability of sustaining microbial growth. OM was characterized by ^{13}C-CPMAS NMR spectra and elemental chemical parameters and then correlates with soil aggregation. Aggregation experiment was carried out in laboratory condition, using three soil types, ten organic substrates and replicated 3 times for each of 4 incubation times. Considering ^{13}C-CPMAS NMR spectral regions, we found the di-*O*-alkyl C and *O*-alkyl C (carbohydrate fraction) had significant positive relation with soil aggregation, while the H, C - substitute aromatic C and O-substitute aromatic C (aromatic fraction) had a significant negative correlation. Our study proved that OM specific C types, identified by ^{13}C-CPMAS NMR spectra showed better correlation than elemental chemical parameters with soil aggregation process after organic input depending on incubation time.

Finally, the aim of my "*4th Chapter"* was to develop a new empirical model SOMDY (Soil Organic Matter DYnamics), based on a novel implementation of OM quality by ^{13}C-CPMAS NMR, to purposely overcome the limitations of C/N as a single OM quality indicator and to explore the relationship between OM quality and soil aggregation.

However, I strongly encourage researchers to consider the value of organic amendment and amendment quality in the context of their research questions. The results of this thesis also contribute evidence to support the hypothesis that amendment quality characterized by the ^{13}C-CPMAS NMR spectra, explains its efficiency to regulate soil functions after incorporation, better than classical chemical parameters such as C/N and lignin/N ratio characterized elemental analyzer. However, the implications of this thesis extend beyond soil science and advance our fundamental understanding, exploring OM chemistry that linked with biological, chemical and physical processes occurring in the soil.

Chapter – 6

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List of Abbreviations

^{13}C-NMR	carbon-13 nuclear magnetic resonance
AI	aggregation index
AM	arbuscular mycorrhizal
ANOVA	analysis of variance
AOM	aggregated organic matter
BGC	bio geochemical cycles
C	carbone
C/N	carbon-to-nitrogen ratio
CEC	cation exchange capacity
CO_2	carbon dioxide
DOM	dissolve organic matter
EC	electrical conductivity
GLM	generalized linear model
HCA	hierarchical cluster analysis
K	potassium
lignin/N	lignin-to-nitrogen ratio
N	nitrogen
NH_4^+	ammonium
NO_3^-	nitrate
OC	organic carbon
OM	organic matter
OMDY	organic matter dynamics
P	phosphorus
PCA	principle component analysis
PL	poultry litter
S	sulfur
SOC	soil organic carbon
SOM	soil organic matter
SOMDY	soil organic matter dynamics
SWR	soil water repellency
WSA	water stable aggregates

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