Environmental Technology & Innovation 30 (2023) 103104

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Effect of pre-treatment processes of organic residues on soil aggregates

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ARTICLE INFO

Article history:
Received 30 January 2023
Received in revised form 8 March 2023
Accepted 10 March 2023
Available online 13 March 2023

Keywords:
Compost
Lactic acid fermentation
Solid fraction of digestate
Aggregate formation
Microbial activity
Soil organic matter

ABSTRACT

Process technologies, such as composting, anaerobic digestion, or lactic acid fermentation, greatly influence the resulting organic amendments (OAs) characteristics even when the same raw material is used. However, it is still unclear how these process technologies indirectly modify the effect of OAs on soil microbial activity and soil aggregation. To determine the effect of OA produced using pre-treatment technologies on the soil microbial activity and soil aggregation, we ran a soil column experiment in which we applied compost, digestate and lactic acid fermentation product made of the same model bio-waste. The results indicated that OAs produced under anaerobic conditions (fermented product and digestate) increased microbial activity, biomass, and soil micro- and macro-aggregation compared to compost and control treatments. Soil microbial activity strongly correlated to C, Ca, Mg, extracellular polymeric substances (EPS), fungal biomass, and macroaggregate formation ($rs > 0.7$, $p < 0.05$). Simultaneously, soil macroaggregate formation strongly correlated to water-extractable C, EPS, cation exchange capacity, K, Mg, Na, and bacterial biomass ($rs > 0.7$, $p < 0.05$). This study demonstrated that the effect of an organic substrate on soil properties can be modified towards desired effects using different pre-treatment technologies, suggesting the possibility of "engineer" OAs.

1. Introduction

The loss of topsoil through erosion is a major issue worldwide and was classified as a key threat to sustainable soil management (FAO and ITPS, 2015; Stolte et al., 2016). Erosion rates are 100 to 1000 times higher than natural ones (FAO and ITPS, 2015). Even more so, in certain regions, the soil erodes 1.4 times faster than the natural process of soil formation (Panagos and Borrelli, 2017; Verheijen et al., 2009). In the European Union alone, 970 Tg of soil is lost yearly (Panagos

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https://doi.org/10.1016/j.eti.2023.103104

and Borrelli, 2017). The organic matter decline is one of the most important driving factors of soil erosion (FAO and ITPS, 2015). Organic matter has been identified as a critical factor in preventing soil erosion due to its fundamental connection to soil structure (FAO and ITPS, 2015).

The use of organic amendments (OAs) has been proposed as a management practice to increase soil organic matter (SOM) (FAO, 2017), but also as a measure to mitigate climate change (Amelung et al., 2020). The addition of OAs can easily increase the content of free particulate organic matter in the soil (POM) (non-stable SOM pool) (Angst et al., 2021). However, the effect of OAs produced using different technologies in the most stable SOM pools, such as mineral-associated organic matter (MAOM) and occluded-POM (inside aggregates), is still unclear (Angst et al., 2021).

SOM stabilisation is a complex process involving two main mechanisms: (1) formation of MAOM and (2) aggregate formation (Angst et al., 2021). MAOM develops from the interaction of organic matter and mineral surfaces that can result in the formation of strong and weak bonds (Angst et al., 2021; Von Lützow et al., 2006). The aggregate hierarchy model (Tisdall and Oades, 1982; Totsche et al., 2018) describes the aggregation process in the soil where MAOM can contribute to the aggregation process of the finer fraction of microaggregates (2 μm–50 μm). Inside micro- and macroaggregates, mineral particles offer physical protection to occluded-POM that can become less accessible for biological degradation (Six et al., 2004; Von Lützow et al., 2006) since occluded-POM works as a nucleus during macroaggregate formation (Bucka et al., 2019). Both processes of SOM stabilisation can co-occur, contributing to micro- and macroaggregation.

The nature of the organic compounds involved in SOM stabilisation is a highly discussed topic. On one hand, some studies suggested that the most stable SOM consists primarily of microbial compounds (Kopittke et al., 2018), where microbial necromass can account for around 50% of the stable organic C (Angst et al., 2021). On the other hand, other studies found a higher contribution of plant-derived products (Angst et al., 2017; Van der Voort et al., 2017; Whalen et al., 2022). Either microbial biomass or plant derived products, both are affected by the quantity and quality of organic input.

A high-quality organic input is sometimes referred to as substrate that can enhance microbial-substrate-use efficiency in terms of the build-up of microbial biomass (Cotrufo et al., 2013) with low C nutrient ratios and low concentrations of biochemically recalcitrant compounds (Angst et al., 2021). An increase in microbial biomass could induce higher microaggregate formation since residues of microbial cell wall envelopes have been associated with microaggregates. Even more, bacteria may operate as both a "composite building unit" and a nucleus for early aggregation when coupled to clay particles or microscopic microaggregates (Miltner et al., 2012; Totsche et al., 2018).

Pre-treatment technologies to produce OAs (i.e., composting, anaerobic digestion, and fermentation) modify the organic matter chemistry, elemental composition, and nutrient availability of organic-raw materials in different ways. Technologies that use reducing conditions (i.e., lactic acid fermentation and anaerobic digestion) can increase the availability of easily degradable C and can be more C and N rich than compost (Chavez-Rico et al., 2022), showing that under reducing conditions, characteristics that are related to high-quality OAs can be enhanced. A high-quality OA is defined here as a substrate that can simultaneously increase carbon and nutrient content and promote aggregate formation.

Considering that OAs produced under reducing conditions increase water-extractable C, we hypothesised that a raw organic residue pre-treated under anaerobic conditions will induce higher microbial biomass and soil aggregation compared to the same OA treated under aerobic conditions until stabilisation. Our experimental set-up used a model biowaste to produce different OAs (i.e., compost, digestate, and lactic acid fermented product) under controlled conditions for application in model-soil systems. Soil columns were used to represent the topsoil of an agricultural model soil with low C content and without aggregates to assess the effect of the OAs on aggregate formation and stability. After 3 months of incubation, changes in explanatory and response variables were measured. To the best of our knowledge, there is no study using standardised organic-feed material to connect the effects of pre-treatment technology to soil quality enhancement. This research aims to compare the effect of different OAs pre-treatment technologies, produced from the same standardised raw material, on soil microbial activity and aggregation to get new insights into the role of technology selection on aggregate formation.

2. Materials and methods

2.1. Preparation of OAs and characterisation

To allow an independent comparison between OAs, the same model substrate was used to produce compost, digestate and lactic acid fermented product. This initial substrate resembled biowaste composed of 20% meadow hay and 80% dog food which was used as model-domestic-food waste (Baker et al., 1999; Chavez-Rico et al., 2022; Fernandez-Bayo et al., 2018; Nakasaki et al., 1998; Schloss et al., 2000). Additionally, these OAs were produced in similar reactors under laboratory conditions. Several indicators of quality, specific for each technology, were used. All the details about the design and preparation of the model-initial substrate, the OAs production and characterisation and mass balances are described in Chavez-Rico et al. (2022). The solid-fraction of digestate was separated from the liquid one by centrifugation for 20 min at 10,000 rpm because we wanted to assess its properties as an organic soil improver which by definition should have $\geq$20% dry weight (EU, 2019). In the OAs characterisation (Table 1), water-extractable C and nutrients were measured in a cold-water extract (CWE) using the same procedure as the one used in soils described in Section 2.4 to quantify water-extractable organic C (WEOC) among other nutrients that are considered highly labile (Ghani et al., 2003).

Table 1
Characteristics of the organic amendments and the model soil (n = 3).

Parameter	Units	Compost		Solid-fraction digestate		Fermented product		Model Soil	
		Value	SE	Value	SE	Value	SE	Value	SE
pH		8.89 ± 0.09 a		8.18 ± 0.01 b		3.81 ± 0.05 c		7.6 ± 0.01 d	
Electrical conductivity	mS cm^{-1}	19.19 ± 0.80 a		4.45 ± 0.01 ab		12.00 ± 0.00 ab		0.1 ± 0.00 b	
Dry weight	(%)	69.69 ± 0.41 a		20.51 ± 0.05 b		33.17 ± 0.58 c		99.9 ± 0.00 d	
Volatile solids/SOM*	(%)	27.34 ± 0.32 a		80.73 ± 2.08 b		94.22 ± 0.51 c		0.3 ± 0.04 d	
Cation exchange capacity	mmol kg^{-1}	353.9 ± 27.2 a		1054.8 ± 92.2 b		415.1 ± 33.6 a		42.1 ± 2.8 c	
Field capacity (pF 2)	θ %							20 ± 0.00	
Sand (> 50 μm)	%							79.3 ± 0.25	
Silt (2 μm − 50 μm)	%							16.5 ± 1.32	
Clay (< 2 μm)	%							3.3 ± 1.11	
Texture class (USDA)								Loamy sand	
Elemental composition									
C	g kg dry^{-1}	145.12 ± 10.1 a		484.68 ± 3.38 b		507.90 ± 8.28 b		1.9 ± 0.14 c	
N	g kg dry^{-1}	16.50 ± 0.88 a		49.91 ± 1.31 b		36.00 ± 0.69 c		0.2 ± 0.01 d	
P	g kg dry^{-1}	4.08 ± 0.05 a		7.26 ± 0.21 b		4.88 ± 0.12 c		2.1 ± 0.20 d	
S	g kg dry^{-1}	3.06 ± 0.03 a		8.39 ± 0.34 b		4.74 ± 0.25 c		0.5 ± 0.05 d	
Ca	g kg dry^{-1}	19.45 ± 0.25 ab		39.96 ± 1.68 a		7.11 ± 0.23 bc		3.2 ± 0.21 c	
K	g kg dry^{-1}	7.58 ± 0.05 ab		19.62 ± 0.54 c		8.40 ± 0.10 ac		3.3 ± 0.32 b	
Mg	g kg dry^{-1}	2.97 ± 0.03 a		2.24 ± 0.11 b		1.10 ± 0.02 c		2.1 ± 0.17 b	
Na	g kg dry^{-1}	1.49 ± 0.04 a		3.65 ± 0.08 b		2.00 ± 0.02 c		0.9 ± 0.03 d	
C/N		8.8		9.7		14.1		8.9	
C:N:P:S		47:5:1:1		58:6:1:1		107:8:1:1		4:0:4:1	
Immediately available C and nutrients (cold-water extract)									
Soluble total C	g kg dry^{-1}	5.43 ± 0.69 a		85.59 ± 0.79 b		83.75 ± 1.62 b		0.02 ± 0.00 c	
Organic C	g kg dry^{-1}	4.79 ± 0.63 a		83.38 ± 1.26 b		83.61 ± 1.65 b		0.02 ± 0.00 a	
C - Organic acids	g kg dry^{-1}	0		51.16		45.33		0	
Soluble total N	g kg dry^{-1}	1.85 ± 0.1 a		25.67 ± 0.58 b		6.84 ± 0.58 c		0.10 ± 0.02 a	
NO_2^-	mg/kg dry^{-1}	< 0.98		< 84.6		< 29.3		< 0.49	
NO_3^-	g kg dry^{-1}	0.01 ± 0.00 a		0.12 ± 0.00 b		0.04 ± 0.01 c		0.02 ± 0.00 a	
NH_4^+	g kg dry^{-1}	0.93 ± 0.01 ab		22.90 ± 3.76 c		1.03 ± 0.07 ac		0.00 ± 0.00 b	
Soluble total P	g kg dry^{-1}	0.38 ± 0.04 a		1.30 ± 0.10 b		3.77 ± 0.24 c		0.31 ± 0.04 a	
PO_4^{3-}	g kg dry^{-1}	0.17 ± 0.07 a		1.11 ± 0.30 b		11.77 ± 0.11 c		0.04 ± 0.00 a	
SO_4^{2-}	g kg dry^{-1}	1.28 ± 0.03 a		0.55 ± 0.11 b		1.49 ± 0.07 a		0.37 ± 0.01 b	
Ca	g kg dry^{-1}	0.04 ± 0.01 a		0.47 ± 0.03 bc		5.15 ± 0.30 b		0.10 ± 0.00 ac	
Na	g kg dry^{-1}	0.67 ± 0.04 a		3.17 ± 0.11 b		1.75 ± 0.09 c		0.02 ± 0.00 d	
K	g kg dry^{-1}	5.84 ± 0.05 ab		15.20 ± 1.62 c		7.47 ± 0.42 ac		0.09 ± 0.00 b	
Mg	g kg dry^{-1}	0.01 ± 0.00 a		0.42 ± 0.05 b		0.99 ± 0.06 c		0.02 ± 0.00 a	
Organic acid composition									
Succinic acid	g kg dry^{-1}	BD		BD		6.1 ± 3.5		BD	
Lactic acid	g kg dry^{-1}	BD		BD		43.3 ± 1.7		BD	
Acetic acid	g kg dry^{-1}	BD		42.4 ± 0.9		16.8 ± 3.7		BD	
Propionic acid	g kg dry^{-1}	BD		61.7 ± 0.2		38.5 ± 2.6		BD	
Butyric acid	g kg dry^{-1}	BD		7.14 ± 0.5		BD		BD	
Organic matter characterisation (Pyrolysis GC–MS)									
Aromatics	%rel. area	6.8		2.2		1.1		12	
Lignins	%rel. area	27.1		12.4		4.2		2.3	
Lipids	%rel. area	5.6		20.9		26.3		58.9	
Proteins	%rel. area	21.2		8.5		5.8		13.9	
Polysaccharides	%rel. area	24.6		50.4		59.1		8.3	
Unspecific origin	%rel. area	14.6		5.6		3.5		3.7	

SE means standard error.
Different letters along each line symbolise statistically different means within a parameter ($p < 0.05$).
BD: Below detection limit.
*SOM of model soil

2.2. Preparation of model soil

The model soil consisted of 90% mineral-fraction of agricultural soil and 10% of soil inoculum. This soil (Loamy sand, Fluvisol) was collected from an agricultural field in Kollumerzwaag, Friesland (Netherlands) (coordinates 53°16'01.5 "N 6°05'21.6 "E). To lower indigenous organic matter and get the mineral fraction, the soil was placed in a furnace at 550 °C for 4 h. As a source of naturally occurring microorganisms (inoculum), the fresh soil collected in Kollumerzwaag was used. After the inoculum addition, water was added to reach 60% of water holding capacity (WHC). A solution of NH_4NO_3

(8.3 mol l^{-1}) was added to restore the C/N ratio of the original agricultural soil (17.7). To stabilise the microbial community, the model soil was incubated for 14 days. After this step, the characterisation of the model soil was done (Table 1). However, C/N ratio after this period of incubation changed to the value reported (Table 1). Since C was reduced after the addition of N due to a possible priming effect, no further N was added.

2.3. Experimental set-up

Soil columns consisted of closed-cylindrical mesocosms made of transparent PVC to allow visual inspection (height: 30 cm, radius: 5 cm) with a removable base and lid. When closed, the columns were airtight with one air-sampling port on the lid. Each column contained 1.5 kg of model soil and OAs (15 cm–20 cm height in the soil column, representing the topsoil layer). The OAs were added after 3 weeks of their production and mixed with the model soil by hand to get a homogeneous distribution. The amount of OAs per experimental unit corresponded to 1.23% of the total weight of the model soil (Supplementary Table 1).

This value was selected to ensure that the measurements of the remaining carbon pools at the end of the incubation will not fall under the detection limit of the methods. It represents ∼5 times the addition rate typically used in agricultural practice for residue addition (20 ton · ha^{-1}) (Diacono and Montemurro, 2010). The amount of OA used was normalised based on dry weight because the differences of C, nutrients and particle size among OAs were assumed as intrinsic to the effect of each technology on the same initial substrate and thus not modified. Further grinding and sieving would affect the physicochemical characteristics of the OAs, and it is not a common practice in the use of these OAs.

For three months, the experimental units were incubated in the dark using a daily temperature regime of 22±0.5 °C (16 h) and 14±0.5 °C (8 h), simulating summer temperatures in the Netherlands. The water content was kept around 60 ± 1% of WHC as an approximation of the watering in agricultural land and to reduce the effects on soil structure of swelling and shrinking particle movement (Bucka et al., 2019). Initial water content was corrected in each treatment to ensure that all columns started with the same amount of water (Supplementary Table 1). Water loss, measured by weight difference, was compensated whenever the column was opened after gas sampling (headspace air refreshing). After 3 months, changes in soil characteristics were evaluated.

2.4. Analytical methods

Gas sampling: Ten millilitres of the gaseous sample were taken from the sampling ports using gas-tight syringes and stored in exetainer (5.9 ml) vials (Labco Limited, Lampeter, UK). Concentrations in volume % of CO_2, O_2, N_2, CH_4, H_2 and H_2S were measured on a gas chromatograph (Varian CP4900 Micro GC, TCD detector and two separate column models Mol Sieve 5 ÅPLOT (MS5) to detect CO_2, O_2, N_2 and Pora PLOT U (PPU) to detect CH_4, H_2 and H_2S). The C-mineralised was calculated as it is described in Supplementary Table 3.

Soil C pools and nutrient assessment: Total C and N were determined on an Elemental Analyser (Thermo Scientific™ FlashSmart™, Courtaboeuf, France) while other elements (P, Na, K, Ca, and Mg) were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 5300 DV, Perkin Elmer) (Chavez-Rico et al., 2022). A cold-water and hot-water soil extraction (at 20 °C and 80 °C, correspondingly) were used to assess immediately- and short-term available C, N, and P correspondingly following Ghani et al. (2003). Cold water extracts (CWE) are considered to have highly labile C since this fraction includes the dissolved organic matter (DOM) and other suspended organics more susceptible to microbial degradation than other settleable organics. DOM is considered the most labile and mobile form of soil organic carbon (SOC) (Kalbitz and Kaiser, 2008), and it has been reported to be the organic fraction more efficiently used by soil microbes (Cotrufo et al., 2013). Hot-water extract (HWE) is frequently used to measure potentially available SOM since it measures the resulting OM from hydrolysis (Von Lützow et al., 2007). HWE is associated with microbial biomass, N-containing compounds, amides, and carbohydrates, including those related to extracellular polymeric substances (EPS) (Ghani et al., 2003; Von Lützow et al., 2007).

Total C and organic C were determined in CWE and HWE using a Shimadzu TOC Analyser. Total N and P content were quantified using the Hach Lange kits LCK 338 and LCK 348. Concentrations of organic acids, anions, and cations were determined in the cold-water extract. Dissolved anions and cations were measured using Ion chromatography, and the organic acids were measured in an HPLC Dionex ultimate 3000RS (Thermo Scientific™) (Chavez-Rico et al., 2022). All the results were expressed on a dry weight basis. Methods described in Van Ranst et al. (1999) were followed to determine: Dry weight (DW), SOM (LOI method), pH (water and $CaCl_2$), electrical conductivity (EC), cation exchange capacity (CEC) and plant-available nutrients (Extraction with $CaCl_2$ 0.01M).

Bacterial C (BC) was calculated from Q-PCR (Details in Supplementary Note 1). Fungal C (FC) was calculated from soil–ergosterol content. The extraction and purification of ergosterol were done following Drost et al. (2020). A conversion factor of 5.4 mg ergosterol.g biomass C^{-1} (Klamer and Bååth, 2004) was used to convert the result into FC. Soil extracellular polymeric substances (EPS) were extracted and quantified (Details in Supplementary Note 2). The organic matter characterisation was done using Pyrolysis GC – MS (Details in Supplementary Note 3).

Soil aggregates size distribution and stability: Air-dried soil was sieved through a 2 mm mesh sieve. Total C and N were measured in both fractions (> 2 mm and < 2 mm). The further determination of the size distribution of aggregates < 2 mm was performed using a laser-diffraction analyser (Malvern Mastersizer 3000 MU) (Details in Supplementary Note 4).

Aggregate stability was assessed by following the change of the relative proportion of particle size classes when suspended in water under continuous agitation (Details in Supplementary Note 5). All the measurements were done in triplicate. The aggregates morphology was observed in sieved air-dried samples (250 μm) using a scanning electron microscope (SEM) JEOL JSM-6480 LV.

2.5. Statistical analysis

A completely randomised experimental design was used ($n = 3$) where each soil column was an experimental unit. Differences among treatments (OA types and the control) were assessed on biological replicates by using one-way ANOVA. The homogeneity of variance and normality were verified using Levene's Test, a visual inspection of the QQ-plots, followed by Shapiro Wilks normality test. Data transformation was performed when the variance was heterogeneous and/or the normality could not be confirmed. Post-hoc analysis to identify significant differences between the means was done using Tukey's range test. When data did not meet the homoscedasticity assumption Welch's ANOVA test was used. If the data did not meet the normality assumption after transformation, the Kruskal–Wallis test was used, followed by Dunn test for post-hoc analysis. Pearson correlation analysis to examine relationships between OA characteristics and effects in soils was used; when normality was not confirmed, Spearman's rank correlation was used. Principal component analysis (PCA) was done to explore relationships among the measured parameters in soil. The significance was evaluated at $p < 0.05$. The software used was R (R Core Team, 2021) with RStudio version 2021.09.1+372 "Ghost Orchid" for the statistical analysis and graphs.

3. Results

3.1. Effect of organic amendments in pH, carbon, and nutrient content in soils

After 3 months of incubation, the soils were removed from the columns. The analysis showed that the pH of the soils treated with digestate, and compost were high (8.1 and 8.9, respectively) but showed no significant differences compared to the control (Table 1 and Fig. 1). In contrast, soil pH decreased significantly from 6.0 to 5.4 following fermented product addition ($p < 0.05$). Other parameters such as CEC, CEC saturation, and EC were not different among treatments ($p > 0.05$) (Fig. 1).

The final total C was the highest in the soil treated with digestate, followed by the soil treated with the fermented product (Fig. 1), although digestate and fermented product had similar initial C contents at the beginning of the incubation and showed high mineralisation of C–CO_2 during the incubation period (Table 1). Total C content in the soil treated with compost was the lowest among treatments, which also had the lowest CO_2 production. However, total C was still higher than the control ($p < 0.01$). The final short-term available C in the soils treated with the fermented product and digestate was similar ($p < 0.05$) but higher by one order of magnitude than the soils treated with compost and the control, which were not significantly different from one another ($p > 0.05$) (Fig. 1). The final immediately available C was 13% higher in the soil treated with the fermented product than in soil treated with digestate ($p < 0.01$) (Fig. 1). This difference was seen regardless digestate and fermented product had similar initial immediately available C, one order of magnitude higher than compost (Table 1). Soil treated with compost showed the lowest immediately available C, but it was still 50% higher than the control ($p < 0.01$).

The final total N was the highest in soils treated with digestate and fermented product, which were not different from each other (Fig. 1). Despite that initial-N content in OAs was significantly higher in digestate than in the fermented product ($p < 0.05$) (Table 1). The final available pool of short-term N was ~80% higher in the soils treated with fermented product and digestate compared to the control (Fig. 1). Finally, the immediately available N was the highest in the soils treated with digestate (114% higher than the control) followed by the soils treated with the fermented product (87% higher than the control). The soils treated with compost did not show significant differences from the control ($p = 0.57$). There were initial differences in the OAs organic matter chemistry (composition and concentration) (Table 1 and Supplementary Fig. S3). Nevertheless, at the end of the 3 months of incubation, the final SOM chemistry (composition) became similar in all the treatments (Fig. 1B and Supplementary Table S7–S10). In all OAs, polysaccharide- and lignin-derived fractions were reduced while protein- and lipid-derived fractions increased. The most important fraction in the control was the lipidic (40%), while in the soil treated with compost, the dominant fraction was the protein (28%), closely followed by the lipid fraction (27%). In the soils treated with fermented product and digestate, polysaccharide-derived products were more important, with 28% and 26% of the relative abundance, respectively.

All samples yielded a predominance of smaller polysaccharide-derived pyrolysis products compared to the relatively intact ones (e.g., levoglucosan, maltol, and 2-hydroxy-3-methyl-2-cyclopenten-1-one). The control and soil treated with fermented product reported the largest proportions of more intact polysaccharide-derived compounds (44% of the total polysaccharides derived). In contrast, in the soils with compost and digestate, these compounds accounted for 22%–26% (Supplementary Tables S7 to S10).

The same aromatic-derived products were reported in all treatments (benzene, ethylbenzene, 1, 3- and 1, 4-dimethylbenzene) representing 9%–16% of the relative areas for all the treatments. Aliphatic, and lignin represented less than 5% of the relative abundance in all the treatments. Among the lignin-derived compounds, 2-methoxy-4-vinylphenol was dominant in all the treatments (22%–54% of the relative peaks of lignin).

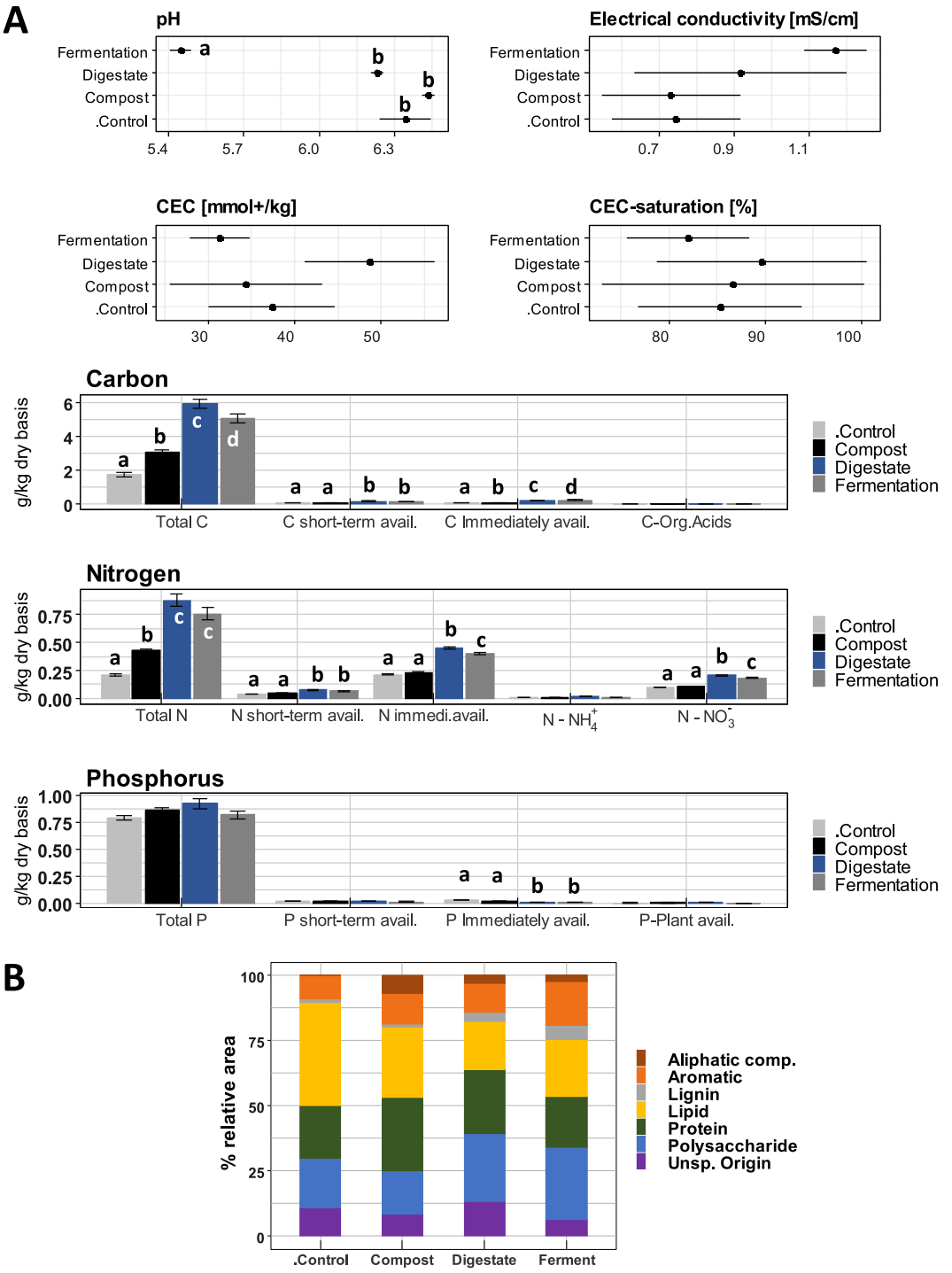

Fig. 1. Characterisation of the soils at the end of the experiment (3 months of incubation) (n = 3). (A) Physicochemical properties in the soil. Different letters indicate statistically different means between treatments for each parameter ($p < 0.05$). The absence of letters means no significant differences. Error bars represent ± one standard error. (B) SOM chemistry determined using pyrolysis GC–MS after 3 months expressed as relative peak area.

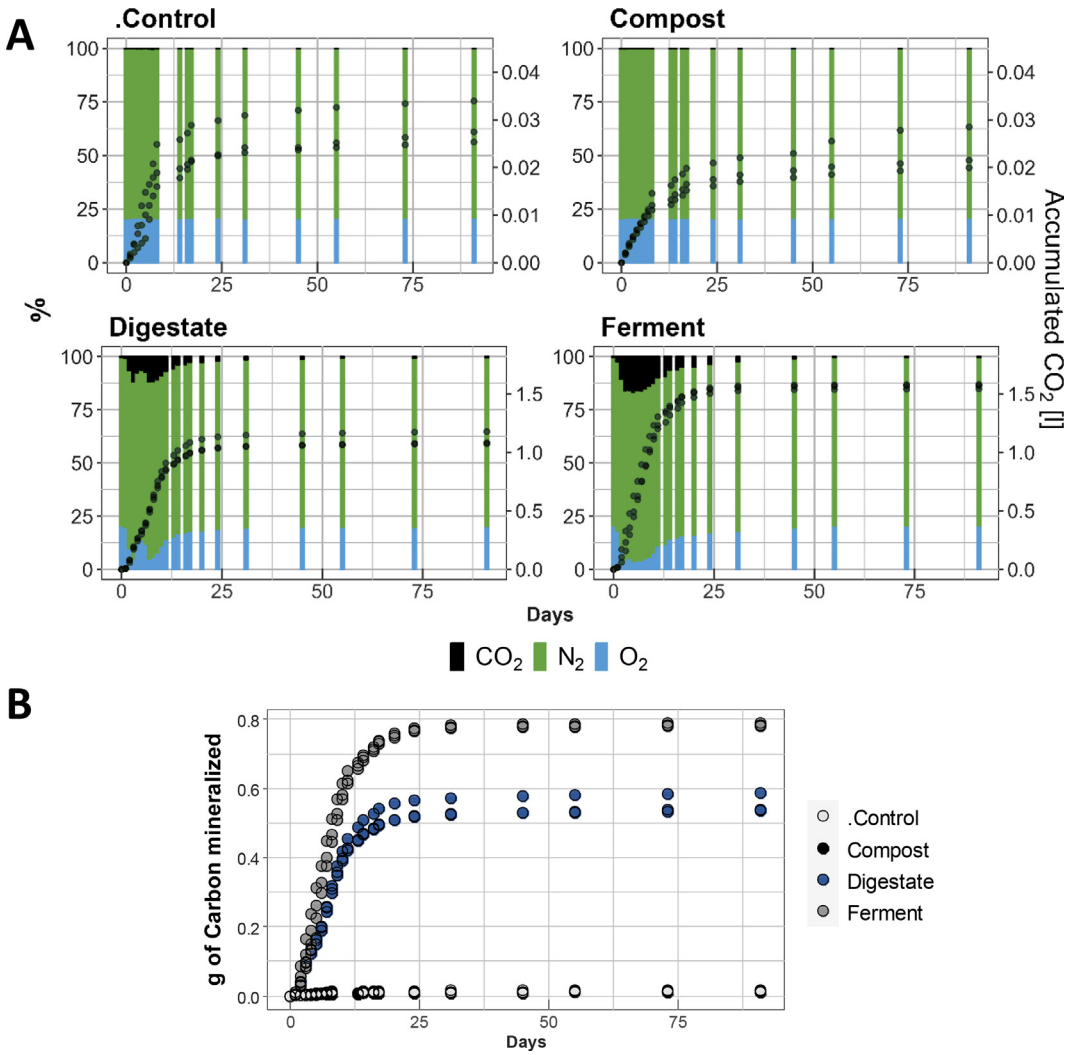

Fig. 2. Gas composition and carbon mineralisation inside the soil column headspace (A) Gas composition and accumulated CO_2 production (n = 3). (B) Accumulated grams of C mineralised in all treatments (n = 3).

3.2. Effect of organic amendments on microbial activity and biomass

After adding OAs, microbial activity was evident by a logarithmic increase in CO_2 efflux. Soils treated with fermented product and digestate showed a steep increase in CO_2 during the first 15 days (Fig. 2B). After 25 days, the production of CO_2 decreased to values comparable to the control. The total C–CO_2 accumulated in the soils treated with digestate was significantly higher than in those treated with the fermented product ($p < 0.01$). Both treatments showed values two orders of magnitude higher than the soil with compost and the control. The total C–CO_2 accumulated in soils treated with compost and the control did not differ ($p = 0.9$), and after 25 days, CO_2 values were closer to atmospheric values. After the incubation, soils treated with fermented product and digestate showed 4% higher water content than the control ($p < 0.05$).

Total C mineralised in each soil correlated better with total C present in OAs (Spearman, $r_s = 0.82$, $p < 0.01$) and polysaccharide pyrolysis-derived products (Spearman, $r_s = 0.85$, $p < 0.01$) than with water-extractable total C (Spearman, $r_s = 0.72$, $p = 0.01$). After five days, the soils treated with fermented product and digestate presented apparent cotton-like microorganisms spread on the surface and along the soil column. In the same treatments, a hydrophobic layer covering the soil surface was evident during watering that disappeared after two weeks (Supplementary Fig. S1). A mass balance over C shows that despite a higher C mineralisation in the soil treated with fermented product and digestate, the final soil C was still higher than in the soil treated with compost (Supplementary Table S3). The N mass balance reported the same trend.

The highest bacterial and fungal biomass (BC and FC, respectively) were observed in the soil treated with the fermented product (Fig. 4A). It was followed by the soil treated with digestate, which had a higher BC contribution than FB.

Soil treated with compost contained more BC in comparison to the control ($p < 0.01$), while the FC was not significantly different to the control ($p = 0.88$). Similar trends were observed in C-EPS content, where the soils treated with fermented product and digestate had the highest amounts of EPS, one order of magnitude higher than the soil treated with compost and the control (Fig. 4B). Control and the soil treated with compost were not significantly different for C- and N- EPS ($p = 0.99$ and $p = 0.57$).

3.3. Effect of organic amendments on aggregate formation

Aggregate formation occurs as a "flow production process" where smaller particles aggregate continuously with the potential of being part of bigger structures. The smallest measured group was the non-aggregated building units $< 2\ \mu m$ (NABU). Control and the soils treated with compost showed the biggest proportion of these particles, while the soils treated with digestate were the lowest (Fig. 3A). In the category SMI ($2\ \mu m$ to $20\ \mu m$ — small microaggregates and similar size particles), the same trend was observed with the control and soil treated with compost showing the biggest proportion of these particles ($p = 0.7$) with 24%–26% more particles than the soils treated with fermented products and digestate. The same pattern was observed in the following size category, $20\ \mu m - 50\ \mu m$ of microaggregates and similar-size particles (MI) (Fig. 3A). In the size group of $50\ \mu m - 250\ \mu m$ of large microaggregates and fine sand (LMI), only the soil treated with digestate had significantly lower material. SEM imaging was used to explore the soil visually and distinguish sand particles from microaggregates (Fig. 3C). In this size fraction ($\sim 250\ \mu m$) there was a higher number of microaggregates in the soils treated with fermented product and digestate. The control and the soil treated with compost mainly showed fine sand and a smaller proportion of aggregated structures.

There were no significant changes in any treatment in the range of $250\ \mu m$ to $2000\ \mu m$ of small macroaggregates (SMA) and medium sand ($p = 0.91$). The formation of > 2 mm large macroaggregates (LMA) was used as the leading indicator of aggregate formation since the model soil lacked this fraction before incubation.

Soils treated with fermented product and digestate had the highest proportion of LMA, one order of magnitude higher than soils treated with compost and the control ($p < 0.02$), which did not show significant differences between each other ($p = 0.99$) (Fig. 3A). In all soils, C was present primarily in the fraction < 2 mm. However, there was more C per Kg of soil in LMA than in fractions smaller than < 2 mm (Fig. 3B). Soils treated with digestate had the highest proportion of C in this fraction, followed by the soils treated with fermented product. The C in the LMA in the soils treated with compost was not significantly different to the control ($p = 0.99$). Similarly, N physical distribution between these fractions reported corresponding trends.

In all the treatments, aggregates < 2 mm showed a similar ratio of weak and strong aggregates, and similar disintegration coefficient of the weak and stable aggregates population (Supplementary Fig. 4). The presence of persistent stable aggregates (i.e., aggregates still present after ninety minutes of water suspension and circulation) did not show significant differences for most size classes (Fig. 3D). These indicators suggested similar aggregate stability in soil despite the type of OA used. However, the soils treated with the fermented product had more persistent stable SMA than the other soils ($p = 0.04$).

The PCA biplot showed further relationships between soil properties and treatments (Fig. 4C). The first two principal components accounted for 78.6% of the total variance. In the first principal component, the soil parameters that had a high impact on the clustering were: C (Total and water extractable), N (Total and water extractable), water-extractable P, BC, EPS (C and N), Ca, Mg, Na, and LMI. The most important parameters in the second principal component were C-LMA and the persistent stable SMA.

4. Discussion

This study assessed the use of pre-treated OAs as a soil quality improver emphasising aggregate formation. In short, our hypothesis was that a raw organic residue pre-treated under anaerobic conditions would have induced higher microbial biomass and soil aggregation upon application to soil than the same raw material treated under aerobic conditions until stabilisation. This would ultimately lead to stable carbon due to associations with mineral particle or protection in soil aggregates. This was tested with compost, digestate, and lactic-acid ferment produced from the same substrate. To assess the effect of the OAs on aggregate formation in relation to microbial biomass build-up, a model soil with low C content and no aggregates was selected. A part of the analysis of direct response variables (aggregate formation, microbial activity, and biomass) a broad range of possible explanatory variables (i.e., C, nutrient content and availability, microbial activity, CEC, pH, and biomass formation) linked to aggregate formation was utilised.

This study provided insights into fundamental questions related to how the selection of pre-treatment technologies can indirectly modify the effect of OAs on soil properties related to aggregate formation through their influence in C-turnover, microbial activity, microbial biomass, EPS, and content and availability of nutrient pools. In agreement with the hypothesis, significantly better outcomes in soil aggregation were observed after the addition of fermented product and digestate (produced under anaerobic conditions) than after the use of compost. Compost addition did not increase microbial activity, aggregate formation, EPS content and fungal biomass in comparison to the control, despite the increase

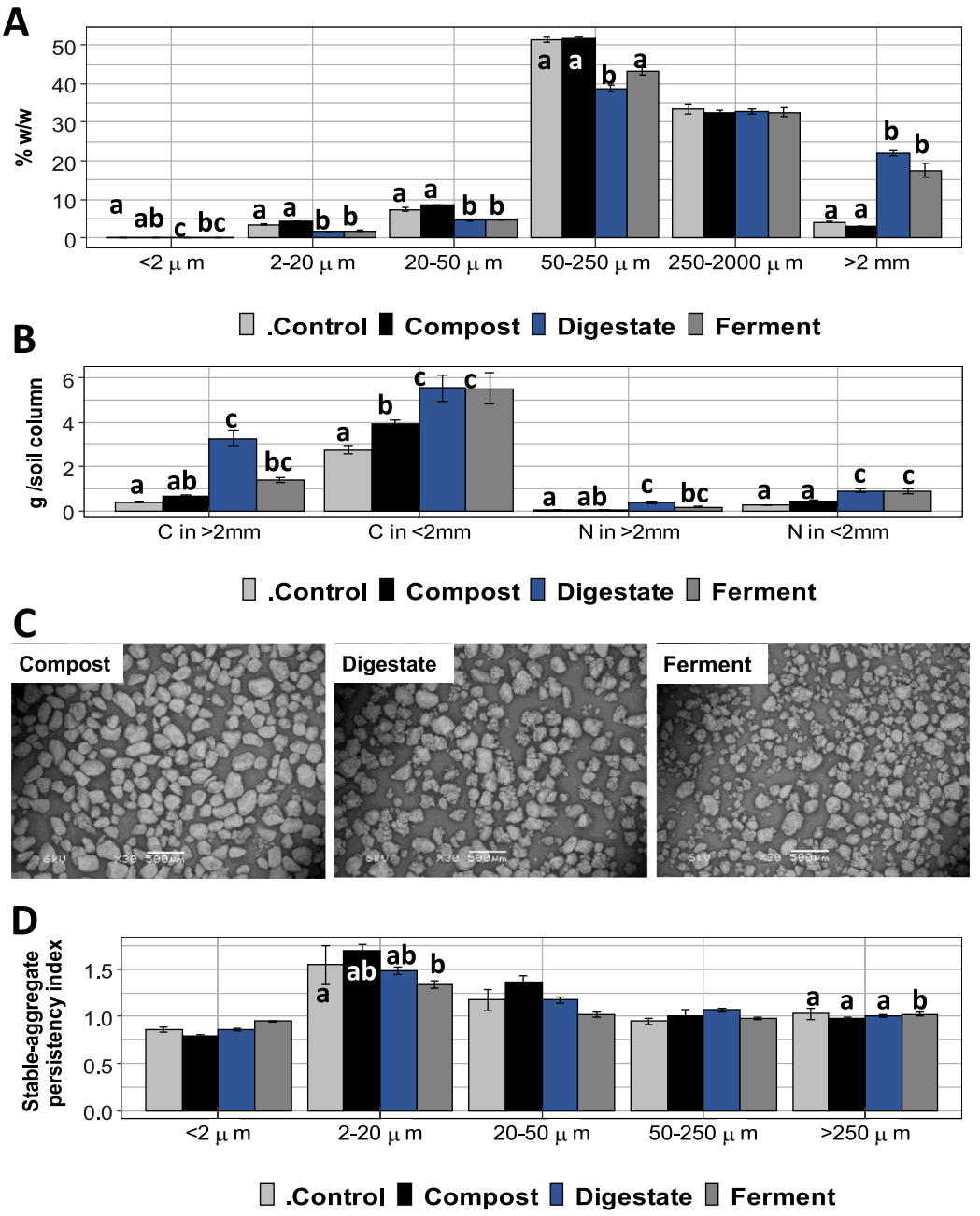

Fig. 3. Effects of the addition of OAs on aggregation, aggregate stability, and physical carbon distribution after incubation. (A) Aggregate size distribution. (B) Physical distribution of C in two different size groups. (C) SEM images of the fraction 50 μm – 250 μm in the treated soils. (D) Index of stable aggregates persistency (High index value indicates high persistence of stable aggregate). Different letters indicate statistically different means (n = 3) between treatments for each parameter ($p < 0.05$). Absence of letters mean no significant differences. Error bars represent ± one standard error.

in soil total C (80% more). Moreover, a better impact on C, plant available nutrient content, and microbial biomass in soils was observed after adding fermented product and digestate. OAs that increased soil microbial biomass might have created conditions for the increase of MAOM since microaggregates are stabilised by organic material from microbial or faunal origin which has been found onto clay particles (Oades and Waters, 1991; Totsche et al., 2018).

4.1. Microbial development as affected by treatment technology

The pre-treatment technologies modified the physicochemical characteristics of the initial substrate (Table 1). The most important changes that correlated to microbial activity and biomass were the quantity of total C, N, polysaccharide-derived

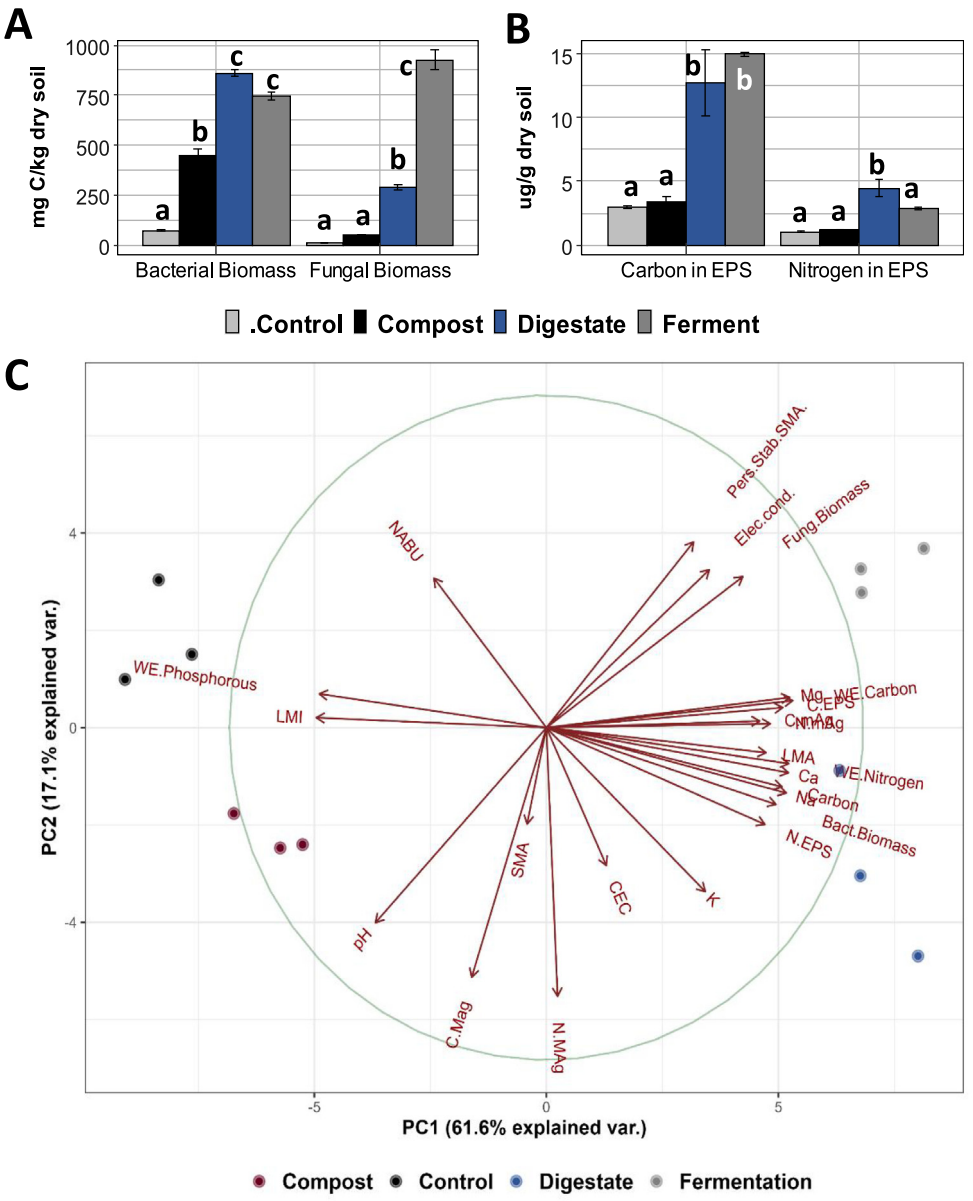

Fig. 4. (A) Bacterial and fungal biomass. (B) C and N content on EPS among different treatments. Different letters indicate statistically different means (n = 3) between treatments for each parameter ($p < 0.05$). Error bars represent ± one standard error. (C) PCA showing: > 2 mm large macroaggregates (LMA), 2000 μm – 250 μm small macroaggregates (SMA), 250 μm – 53 μm large microaggregates (LMI), < 2 μm non-aggregated building units (NABU), C in macroaggregates > 2 mm (C-MAg), C in microaggregates < 2 mm (C-mAg), N in macroaggregates > 2 mm (N-MAg), N in microaggregates < 2 mm (N-mAg), Water-extractable C (WE. Carbon), water-extractable N (WE. Nitrogen), Water-extractable P (WE. Phosphorus), cation exchange capacity (CEC), Persistent Stable SMA after 90 min of agitation in water (Pers.Stab.SMA.), Electrical conductivity (Elec.cond), C in Extracellular polymeric substances C-EPS, N in Extracellular polymeric substances N-EPS and cations in solution.

compounds and water-extractable C, N and P. The OA pH seemed to have influenced the bacterial:fungal ratio, given the positive correlation of fungal biomass with acidic pH. Finally, Ca, Na, and Mg in solution also reported a high correlation to microbial biomass and, in the case of Mg, high correlation to EPS content (Fig. 4B).

Technologies that use anaerobic conditions are more preserving of the C and N of the initial substrate than technologies that use aerobic conditions facilitating more availability of C and other nutrients in the water-extractable phase (Chavez-Rico et al., 2022). This preservation of C and N was seen as a higher content of C and N per kg of dry OA in digestate and fermented product (Table 1). Additionally, the organic fraction in these OAs showed more polysaccharide-derived content from the initial substrate than compost. At the same time, compost showed more compounds indicating biodegradation after the pre-treatment (Supplementary Fig. S4). The increased soil microbial activity and microbial biomass correlated

slightly better to the total C and polysaccharide-derived compounds in OAs than to the water-extractable C ($r_s = 0.82$, $r_s = 0.85$ and $r_s = 0.72$ correspondingly). It is possible that as microbial breakdown advances, more C becomes available, making total C a better predictor of the C sources for microbial activity, despite the fact that water soluble compounds are more easily available and can increase the proportion of active soil microorganisms (Macias-Benitez et al., 2020; Odlare et al., 2008). However, the effect of readily available C sources was evident in the soil treated with digestate, where the CO_2 production seemed to indicate a diauxic growth pattern (Fig. 2A) which is an indicator of microorganism adaptation moving from the uptake of easily degradable compounds to more recalcitrant ones (Kim and Kim, 2017).

The application of fermented products stimulated bacterial (BC) and fungal biomass (FC) equally while digestate and compost mainly stimulated bacteria over fungi. Since the pH significantly correlated only to fungal biomass (r_s= −0.91, $p < 0.01$), it is possible that the decrease in soil pH after fermented product addition (Fig. 1A and Fig. 4A) induced a selective advantage for fungi development since fungi tolerate better lower pH than bacteria (Rousk et al., 2009). EPS was high only in soils treated with ferment and digestate. Since EPS turnover times vary greatly among different EPS-producing species (Costa et al., 2018), it is not possible to estimate how much of the EPS was formed during soil incubation and how much was formed inside the reactors during OA production. According to the PCA, EPS (C and N) correlated better to BC than FC (Fig. 4B). Soils treated with fermented product and digestate reported a similar amount of BC and EPS content even though soil treated with fermented product showed ∼50% additional biomass from fungi (Fig. 4A and B), which supports that EPS was linked mainly to bacterial development.

Despite the high C-mineralisation reported in the soils treated with digestate and fermented product (Fig. 2), these treatments still showed the highest final total C in soils (Fig. 1A). Moreover, the resulting increase in biomass seen in the soils treated with digestate and the fermented product was reflected as well in the change in hot water-extractable C (HWC) (short-term available C), which is considered an integrated measure of soil quality (Ghani et al., 2003). Additionally, the remaining immediately available C seen after 3 months in the soils treated with digestate and fermented product (Fig. 1A) can be used to promote more biomass formation. Finally, the resulting SOM chemistry, which initially showed differences, changed towards a similar pattern displayed by the control indicating degradation of all organic fractions (Fig. 1 and Supplementary Note 7). This observation is usually referred to as the "decomposer funnel" and is used to describe the conversion of highly varied plant material into more uniform leftover organic matter when the conditions governing decomposition are the same (Hoffland et al., 2020; Swift et al., 1979) attributed to the microbiological signature on decaying OM (Fierer et al., 2009; Hoffland et al., 2020). Biological and environmental factors dominated even the degradation of organic compounds produced using OA pre-treatment technologies, evenmore than molecular structure alone (Schmidt et al., 2011).

4.2. Aggregate formation as controlled by treatment technology

The addition of OAs produced under anaerobic conditions (digestate and fermented product) improved aggregate formation in a wide range of aggregate sizes, especially large macroaggregates (LMA) (Fig. 3A), while the effect of compost was almost negligible. We observed that LMA formation can be achieved after the addition of OAs, despite the absence of plant root hairs for macroaggregate stabilisation process. However, the OAs effect contributes mainly to the aggregation of the fine-mineral fractions < 250 μm.

Effect on microaggregate formation: Assessing the effect on microaggregate formation is not as straightforward as LMA since mineral particles can have similar dimensions to microaggregates, making it difficult to differentiate both groups. Additionally, microaggregates can bind together, forming macroaggregates (Totsche et al., 2018). In this sense, an increase in LMA can be seen as an implicit increase in microaggregation. In this set-up, an effective microaggregation was seen as a decrease in the smallest fractions of loose particles < 2 μm (NABU — predominantly clays among other building units). The soils treated with digestate and fermented product reduced NABU better than those treated with compost and the control (Fig. 3A).

Effect on macroaggregate formation: Soil treated with fermented product and digestate, which had the largest LMA, showed an overall decrease in particles (< 250 μm) with respect to the control. This observation shows how single microaggregates (< 250 μm) are bound to form macroaggregates. However, not all mineral fractions (clay, silt, and sand) are involved in the same way in the macroaggregation process. When looking at macroaggregates in SEM images, we see that medium and coarse sands (> 250 μm) were less frequently seen in macroaggregates. This observation agrees with the results of the aggregate size distribution (Fig. 3A). In this graph, medium to coarse sands (from 250 μm to 2000 μm) did not change significantly among treatments and were present in the same initial proportion as in the non-incubated model soil. These observations could indicate that OAs mainly affects microaggregates and mineral particles < 250 μm (fine sand, silt, and clay).

The development of filamentous-saprophytic fungi has an important role in macroaggregation (> 250 mm) (Lehmann et al., 2017). In this study, ergosterol was measured to estimate saprotrophic fungal biomass (FC) (Joergensen and Wichern, 2008). However, macroaggregation was not higher in the soil treated with the fermented product, which reported more FC than in the soil treated with digestate (Fig. 4A). The explanation for this observation could be related to the specific fungal traits (i.e., phylogeny, mycelium density, and negative activity of leucine aminopeptidase) that were found to be important for the macroaggregation process (Lehmann et al., 2020). Fungi without these traits could have been stimulated in the soils treated with fermented products.

The PCA (Fig. 4C) showed a positive correlation between LMA and bacterial biomass (BC), Ca, Na, Mg, EPS (C and N) and a negative correlation to small microaggregates (SMI). These correlations might indicate the main factors contributing to LMA formation, BC, Ca, Na, Mg, EPS (C and N), that could contribute to SMI formation and later aggregation towards LMA formation. The positive relationship between EPS and divalent cations has been reported (Somerton et al., 2013; Song and Leff, 2006) for several species in soil (Simoni et al., 2000).

The PCA showed no correlation between C and N present in the LMA fraction (C.Mag and N.Mag) and microbial carbon (BC and FC)(Fig. 4C). The C and N present in macroaggregates (C.Mag and N.Mag) could be linked to occluded POM, which is reported to have a more important role in this size scale (Bucka et al., 2019).

Effect on aggregate stability: Binding mechanisms among microaggregates can include roots, hyphae and extracellular polymeric substances (EPS), among others (Totsche et al., 2018). Further than BC and FC, water repellency has been found to be positively correlated to higher aggregate stability (Chenu et al., 2000; Mao et al., 2019). During incubation, soils treated with digestate and the fermented product formed a hydrophobic layer covering the soil surface. This process has been reported previously for digestate (Voelkner et al., 2019), and it has been associated with high fatty acid content (Bayer and Schaumann, 2007). The production of water-repellent substances can be induced by the addition of nutrients (Hallett and Young, 1999) that exacerbate the exudates production by soil microorganisms (Navarro and Navarro, 2003).

Although soil treated with digestate and the fermented product showed this hydrophobic layer, the fermented product was the only treatment that reported a significantly higher persistence of large stable aggregates 250 μm $-$ 2000 μm (Fig. 3D). The production of water-repellent substances in the soil is frequently attributed to fungal (Mao et al., 2019) hydrophobin production (Rillig, 2005), explaining why soil treated with fermented product reported higher aggregate stability given its relationship with FC stimulation.

A wide range of significant effects on soil properties were observed, some of them not previously described, like the limits of the effect of OAs in the aggregation of sand particles > 250 μm that was less successful in soil treated with compost than in the soil treated with fermented product and digestate. The numerous measured parameters provided an integral view of the multifactorial processes related to aggregate formation at different size scales and a mechanistic explanation of several effects in the soil.

The effect of the initial substrate must be further explored. In this study, a high correlation was observed between polysaccharide-derived products and microbial activity, biomass, and EPS production. This could have been related to the MOR composition, which comprised 80% dog food (readily available substrate) and 20% hay (more biochemically recalcitrant). Increasing the amount of biochemically recalcitrant compounds in the organic residue could have led to different results. The long-term effect of these pre-treatment technologies on OAs should also be studied since macroaggregates are less stable over time than microaggregates (Finn et al., 2017). Ultimately, the comparison among technologies should be repeated in field trials to assess the effects in a complex system and the interactions with biotic and abiotic factors.

The application of this knowledge in the field could be improved by extending this study to the properties of different stable and unstable OAs. Legislation and regulations (EU, 2019) restrict the use of unstable OAs in agricultural fields. However, given all the new insights on how the soil mechanisms benefit from the addition of unstable OA, it seems paradoxical that all the benefits that can come with the addition of un-stable OA have been passed over, which is probably justified only in the adverse effects on crop seedling development and the competition with microbes for N sources in soils. In the transition of this knowledge to the field, the particularities of each type of pre-treated OA should be considered recognising their potentialities and limitations in an integral management framework.

5. Conclusion

This research demonstrates the significant influence of pre-treatment technology on the characteristics of the resulting organic amendment (OAs) and its subsequent effect on soil. The results support our hypothesis that raw organic matter treated anaerobically during OA production will likely generate higher microbial biomass and greater soil aggregation than the same raw substrate treated aerobically until stabilisation. OAs pre-treated under reducing conditions were found to be richer in carbon and nitrogen than compost, as well as having more water-extractable C and nutrients. These characteristics are correlated with increased microbial activity, biomass, and aggregate formation. Though compost may increase total C in soil, its effect on the aforementioned parameters is not significantly different from the control. Despite the similarities in the effects of digestate and the fermented product, they have different impact on the soil. Finally, the SOM chemistry of all treatments showed chemical convergence during biodegradation, demonstrating that there are no lasting effects on SOM characteristics despite the distinct natures of the OAs.

Our results can provide the foundation for developing specific OAs that are designed to enhance distinct soil properties. This method can also provide invaluable information that can be used as a decision-making tool in waste management, since not all technologies produce the same benefits or are equally successful in preserving C and N in raw substrates. The selection of technologies that can generate C-rich OAs may be essential to maximising stable SOM, minimising GHG emissions, and improving the productivity of utilising high-quality organic streams in a growing circular economy.

CRediT authorship contribution statement

Vania Scarlet Chavez-Rico: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Stijn van den Bergh:** Methodology, Data curation, Formal analysis. **Paul L.E. Bodelier:** Conceptualization, Supervision, Resources, Writing – review & editing. **Miriam van Eekert:** Conceptualization, Supervision, Writing – review & editing. **Yujia Luo:** Methodology, Data curation, Formal analysis. **Klaas G.J. Nierop:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Valentina Sechi:** Conceptualization, Supervision, Writing – review & editing. **Adrie Veeken:** Conceptualization, Resources, Supervision, Writing – review & editing. **Cees Buisman:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Adrie Veeken reports a relationship with Attero that includes: employment.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the Wetsus' technical and analytical teams for contributing to the experimental work. Maria Hundscheid and Ciska Raaijmakers from Netherlands Institute of Ecology (NIOO-KNAW) for the extract and analysis of ergosterol. This work has been done within the cooperation framework of Wetsus, European Centre of Excellence for Sustainable Water Technology and Wageningen University and Research. Wetsus is co-funded by the Dutch Ministry of Economic Affairs and Climate Policy, the European Union Regional Development Fund, the City of Leeuwarden, the Province of Fryslân, and the Northern Netherlands Provinces. Wetsus also coordinates WaterSEED project that received funding from European Union's Horizon 2020 research and innovation program under Marie Skłodowska-Curie grant agreement No. 665874. Recognitions to the members of the research soil theme (Agriton, Mulder Agro, Vereniging Afvalbedrijven, Koninklijke Oosterhof Holman, Waterketen Onderzoek Noord (WON), and Waterschap Zuiderzeeland) for the fruitful discussions and financial support.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2023.103104.

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