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Bacterium consortium drives compost stability and degradation of organic contaminants in *in-vessel* composting process of the mechanically separated organic fraction of municipal solid waste (MS-OFMSW)

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ABSTRACT

Commercial composting of the mechanically separated organic fraction of municipal solid waste (MS-OFMSW) is employed to stabilize municipal organic waste. Its feasibility is linked to process efficacy and compost stability. Threshold values for stability are imposed by regulatory frameworks. Limited reuse options exist for this material often due to the presence of organic pollutants. The optimisation of the composting process is required to reach stability in a viable timeframe. We evaluated the effects on compost stability and the degradation of organic contaminates by using wood shavings as a bulking agent and increasing the turning frequency in a pilot scale process. The use of wood shavings decreased the time required for compost stability while turning frequency had no impact. The addition of wood shavings to the initial feedstock stimulated microbial activity that in turn decreased the time to compost stability and enhanced the degradation of detected PAHs and short-chain phthalates.

1. Introduction

Commercial composting is a process of controlled degradation of organic matter by microorganisms. Traditionally, composting has been used for agricultural wastes to reduce volume and water content, destroy pathogens and produce an odour free, nutrient and humus rich product for use as a natural enrichment of soils. Composting has been widely recognised as an eco-friendly management approach to the treatment of the mechanically separated organic fraction of municipal solid waste (MS-OFMSW). Commonly MSW is disposed of by incineration or landfill, however composting has become a well-established management approach to stabilize the organic matter contained in the MSW (Brändli et al., 2007). The amount of MSW being composted around Europe is rising due to strict limits set by the Landfill Directive (Council Directive 1999/31/EC) and the Waste Framework Directive (Directive 2008/104/EC). This restricts the amount of biodegradable waste that can be disposed of by landfilling and establishes a compulsory bio-stabilization of MSW. After household collection, MSW is often subject to mechanical screening, where the larger fraction is recycled and/or recovered (e.g co-incineration). The smaller fraction (<80 mm), designated MS-OFMSW, can comprise >50% of biodegradable organic material (López-Gómez et al., 2019), and in most cases is subject to a composting process prior to landfilling. The MS-OFMSW derived compost is also known as biostabilised residual waste (BSRW) or municipal compost. Within EU countries, *in-vessel* systems are a legal requirement (Langdon et al., 2019) for composting wastes containing food and animal by-products (e.g., MSW-derived waste).

Stability criteria for the disposal/reuse of BSRW are specified by each EU member state. Among others, the Oxygen Uptake Rate (OUR) is utilized as an indicator of compost stability that provides reliable values of microbial activity in a compost sample (Gea et al., 2004). Feedstock parameters such as pH and C:N ratio, are important for an efficient composting process (Hargreaves et al., 2008). At a commercial scale, the composting process of MS-OFMSW is often limited to time constraints

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and an understanding of how compost conditions relate to its final stability can make a major difference in the success and efficiency of a commercial composting plant. Due to the low CN ratio of the MS-OFMSW (Li et al., 2016) and lack of structural material (Oujana and Zwolinski, 2018), some commercial plants often need to blend MS-OFMSW with carbon rich bulking agents, such as wood shavings, sawdust or green waste (Goyal et al., 2005; Tognetti et al., 2007). To keep the costs low, the oversize fraction from the final compost is often reused in the process to improve the feedstock conditioning and to increase efficiency of the composting process. Despite this, full understanding of the link between the chemical and biological characteristics in the commercial process and the production of stable compost is still elusive. The use of bulking agents is therefore often seen as commercially onerous, in particular in countries where the end use of the BSRW is of low value. As a result, using a bulking agent originated from waste wood could provide a cheaper alternative.

Frequent turning of the material avoids the formation of anaerobic conditions in the process and ensures appropriate air flow. Higher turning frequencies of the feedstock have been shown to affect the evolution of temperature, allowing for a higher degree of pasteurization of the feedstock and shortest maturation times in windrow composting (Khalil et al., 2011). However, it has also been demonstrated in a small scale that lower turning frequency leads to better crop growth, despite delaying the composting process (Getahun et al., 2012). Little is known of the impact of turning frequency in an *in-vessel* composting, where the aeration and oxygen rates are controlled by software systems.

The content of pollutants in compost derived from MSW waste often limits the reuse of the BSRW leading to its disposal in landfill or incineration (Langdon et al., 2019). However, microorganisms are known degraders of a wide range of organic pollutants (Haritash and Kaushik, 2009), raising the potential of the composting process to be used for biodegradation of persistent organic pollutants (POPs) (Brändli et al., 2007) and enhancing the possibility for its reuse as a soil organic amendment.

In line with the European Circular Economy Strategy, municipal compost has the potential to be used as a soil improver. Within the EU, the criteria for its use on land is inconsistent. While it is required to meet established criteria in certain EU countries like Italy and Austria, in countries such as France and Portugal it can be marketed as compost if it meets designated national standards and regulatory frameworks (Decreto-Lei No. 103/, 2015; Langdon et al., 2019). The MS-OFMSW is known to contain a plethora of POPs including pesticides, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), as well as phthalates (e.g plasticisers) (Rigby et al., 2015; Langdon et al., 2019). Previous studies have shown that composting can be effective in reducing the PAH content of sewage sludge (Hafidi et al., 2008; Guo et al., 2020). Composting has also been shown as effective in degrading the di-(2-ethylhexyl) phthalate (DEHP) (Cheng et al., 2008) and could be used as a detoxification process for the degradation of POPs. Most investigations of the influence of composting on pollutant degradation focus on open composting processes (e.g windrows or static piles) and/or laboratory scale enclosed systems due to the difficulty in sampling commercial *in-vessel* systems and the heterogeneous nature of the MSW. Therefore, little is known about the degradation of POPs and phthalates during the MS-OFMSW *in-vessel* composting process.

In this work, a pilot scale aerobic, thermophilic *in-vessel* compost system was designed and constructed to allow sampling of the material without interference with the composting process. The nature of the system allowed for a continuous monitoring of physical, chemical and microbiological parameters under varying conditions and how these influenced the success, or otherwise, of the composting cycle. Furthermore, this study allowed the monitoring of organic contaminants in MS-OFMSW and whether composting accelerates their removal. We hypothesised that a) the bacterial consortium in the material during composting will respond to changes in the starting feedstock and process parameters affecting the final compost stability and b) the rate of removal of potential organic contaminants will be linked to improved efficiency of the composting process. The objectives of this work were to 1) relate the importance of composting parameters such as CN ratio and material turning frequency towards achieving compost stability, 2) understand the succession of the bacterial consortium during induced changes in the composting process parameters, and 3) screen the feedstock for PAHs and phthalates and monitor their degradation during composting.

2. Material and methods

2.1. Description of in-vessel composting

In the *in-vessel* composting process the material is composted in an enclosed thermophilic aerobic digestion system. Temperature, humidity and oxygen levels are monitored and controlled via ventilation/aeration and percolation. The aim is to maintain temperature within the thermophilic range and moisture between 50 and 60%. The material is first required to initially reach a temperature barrier of 60–70 °C to pasteurize the material and kill off pathogens. Once this critical temperature is reached, forced aeration is used to maintain composting temperatures of 52 to 55 °C, considered the optimum for microbial activity. After a period of time the material is removed, mixed and returned to the tunnel and it must again increase to above 60 °C during this period.

After the material is composted in the *in-vessel* system, it undergoes a maturation phase, where the compost is placed on aerated floors until it complies with stability requirements for landfilling. Generally, the material undergoes thermophilic composting in the *in-vessel* system for 8 weeks and with a curing phase of 3–4 weeks.

This work focuses on evaluating the *in-vessel* process and does not take into account the curing phase of the process.

2.2. In-vessel composting pilot scale containers and feedstock description

A pilot scale of a commercial thermophilic composting process was set-up. Two bioreactors of 30 m^3 each, measuring 6 × 2.4 × 2.2 m were built as a 1:50 scale model of commercial reactors (Fig. 1a) to simulate the commercial *in-vessel* composting process. These vessels allowed precise control of composting parameters (Fig. 1b) with 16 t of capacity in each. Bioreactors were fitted with hydraulic lids, allowing the units to be opened and filled with a front loader. Bioreactors were also fitted with a back door for easy unloading in combination with a hook loader. A 20 kw fan was used to provide underfloor aeration, while an air damper and oxygen sensor ensured a minimum oxygen level of 12% was maintained within the composting mass. The system was fitted with air recirculation to remove warm air from the top of each container and then blend this with fresh air to ensure a consistent and controlled air and material temperature in each unit. The air inlet pipe was fitted with a pressure sensor indicating resistance of the material to aeration. Three temperature probes in each reactor gave an indication of biological activity. A sprinkler system was fitted to the roof of the containers and used to irrigate the compost so that optimum moisture was maintained.

The customised containers (Fig. 1) were fitted with sampling ports, allowing for non-intrusive sample collection at any moment of the process. One container acted as the control while conditions (one at a time) were varied in the test container. Two containers were important as they allowed a simultaneous control for each experimental incubation.

For the MS-OFMSW, the fine fraction (0–80 mm) generated from mechanical treatment of general household waste was used as the feedstock. In commercial composting plants, several materials can be co-composted with the MS-OFMSW in order to increase the efficiency of composting conditions. In this work two materials were used, the oversize material generated on-site from the composting process and wood shavings, generated from waste wood processing (Fig. 2). The MS-

Fig. 1. Pilot scale containers incubators with temperature, aeration and odour control ventilation in the centre (a) and the control system (b) used.

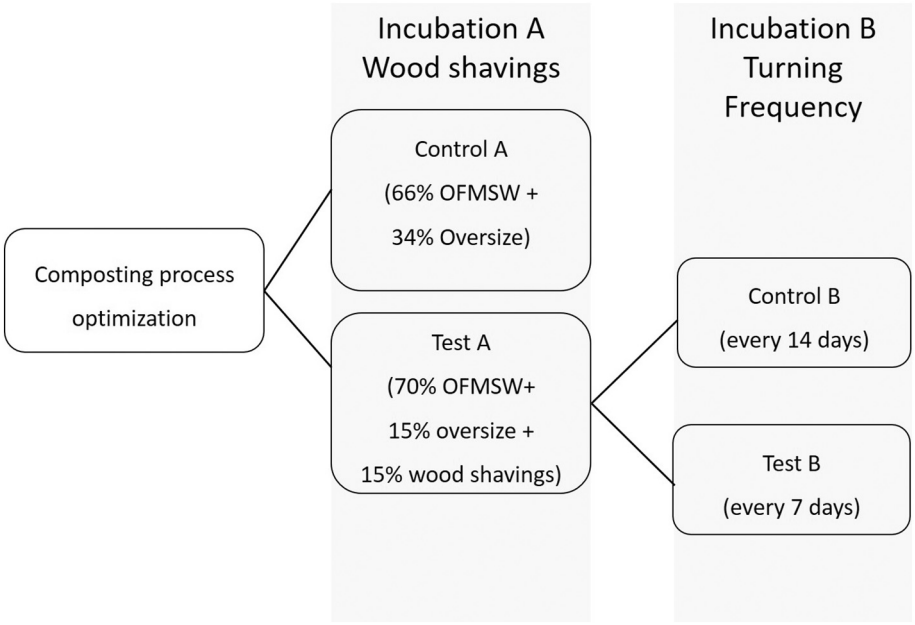


Fig. 2. Scheme of the incubation feedstock rates and tested variables in the present study.

OFMSW and the co-composting materials were mixed in open piles and loaded into the pilot vessels.

2.3. Experimental design

MS-OFMSW was collected and processed at an Irish commercial composting facility, based in Co. Meath, Ireland. The incoming material was characterised as acidic (pH of 5.5 ± 0.17) with a high moisture content (58.7 ± 0.99%) and low C:N ratio (15 ± 0.01). The oversize fraction (>40 mm) is recovered at the end of the process and reused with the incoming MS-OFMSW prior to the composting process to increase porosity and ensure the material can be easily aerated. This reuse also 'seeds' the mixture with beneficial microbiology. Initially, CN ratio optimization was assessed in Incubation A. The Control reflected current commercial approaches and the Test utilized wood shavings to increase the CN ratio (Fig. 2). The wood shavings had a C:N ratio of 65:1. Incubation A was performed over 56 days in May 2015.

In the following incubation (B), the impact of increased turning/mixing was tested. The feedstock in both Test and Control comprised of MS-OFMSW, oversize material and wood shavings (Fig. 2). The Control was physically turned every 14 days, consistent with the commercial scale process at the time in the composting plant. To evaluate if increasing turning frequency would improve the efficiency of the process, the Test was turned/mixed every 7 days. The duration of incubation B was 42 days and was performed in July 2015.

2.4. Sampling and storage

An initial composite sample (T0) was taken by employing a grab technique (minimum 30 grabs) after the feedstock had been mixed and before loading into the containers, according to I.S. EN 12579:2013. During the thermophilic composting process, samples were taken from the containers using a combination auger. Five grab samples (0.5–1.5 m depth) were taken from each port from the Test and Control vessels. The 30 grab samples were combined to form a composite sample. For Incubation A,. Incubation A was run for 56 days and the containers were sampled at days 7 (T7), 14 (T14), 28 (T28) and 56 days (T56). Test A achieved stability in 42 days, therefore, given that both incubations had the same initial feedstock (Fig. 2), incubation B was left to run for 42 days, and samples were taken at 14 (T14), 28 (T28), 35 (T35) and 42 (T42) days. The BSRW was sampled after unloading the material from the containers and mixing according to the EN 12579:2014 protocol.

From each composite sample three subsamples were prepared (1 kg each) after homogenising and transporting to the lab at 4 °C. Samples were sieved (10 mm) and blended in a stainless-steel Waring blender. A subsample was taken from the blended sample, placed in sterile

containers and stored at −20 °C for further analysis. The remaining fresh sample was divided. A subsample was kept fresh at 4 °C for determination of pH, moisture and oxygen uptake rate (OUR) and 10 g were freeze-dried for further analysis of C:N ratio and organic matter content. The remaining fresh sample was storage at −20 °C.

2.5. Chemical analysis

pH was determined using a 1:5 sample-to-water ratio (EN 13037:2009) and the moisture content was measured according to EN 13040:2007. The organic matter (% OM) was determined as loss-on-ignition of c.a 5 g of sample at 550 °C (EN 15935:2012). Total carbon (TC) and total nitrogen (TN) were determined by dry combustion using a Fisons NCS 1500 NA elemental analyser and 0.2 g of freeze- dried sample. The OUR was conducted on the samples using the WTW Oxitop OC 110 controller according to the EN 16087-1:2011 standard. Briefly, two grams of organic matter of each compost sample was mixed with 180 mL of distilled water, 10 mL of a nutrient solution, 10 mL of buffer solution and 2.5 mL of a nitrification inhibitor (allylthiourea) in 1 L Duran bottles. Each sample was performed in triplicate. The bottles were placed on an orbital shaking incubator (IKA KS 400i control) at 30 ± 3 °C for four hours to allow for temperature and pH to adjust within the samples. After the pH was measured to ensure it was between 6.5 and 7.5. Soda lime pellets, were placed in the headspace of the controller and the controller was attached to the bottle. These were used to remove carbon dioxide (CO2) by absorption, instigating a pressure change within the vessel. The pressure change is then recorded by the pressure sensing data recording heads.

2.6. Determination of organic pollutants

The degradation of polycyclic aromatic hydrocarbons (PAHs) and phthalates were investigated in this study. Due to time and budget constraints, and taking into account the fact that Incubation A showed improved composting efficiency, organic pollutants investigation was only focused in Incubation A.

Organic pollutants were extracted from air dried compost samples using solvent extraction. Triplicate 5 g (air-dried) samples were extracted in 48 mL Teflon centrifuge tubes using 25 mL 1:1 Hexane: Acetone. The samples were placed on a reciprocal shaker for 20 min at 121 spm. The samples were then sonicated for 20 min. Samples were then centrifuged for 10 min at 6000 rpm. The supernatant was decanted, and gravity filtered. The extract process was repeated with 25 mL dichloromethane. Both extracts were combined in a round bottom flask and rotovapped to near dryness. The extract was then diluted to a volume of 10 mL with hexane for storage prior to analysis by gas chromatography mass spectroscopy (GCMS). Internal standard calibration curves for contaminant quantification. Cholestane was used as the internal standard. The specific compound standards used were Bis (2-ethylhexyl)phthalate, Naphthalene, Acenaphtene, Phenanthrene, Chrysene and Perylene. Samples and standards were analysed using a GC (Agilent Model 6890 N) mass spectrometer (Agilent Model 5975C Quadropole MS Engine) system equipped with an automatic sampler. Injector settings were: 1 μL injection with a 2:1 split ratio, split flow of 2 mL/min and heater temperature set at 280 °C. The column was a fused silica capillary column (30 m × 0.25mmi.d.) with a film thickness of 0.25 μm (HP-5MS, Agilent) and flow reate of helium carrier gas was set at 1 mL/min. The oven was set to hold at 60 °C for 1.5 min and ramp at 6 °C/min to 300 °C and hold temperature for a further 20 min. This gave a total runtime of 61.5 min. The quadropole detector source was set at 230 °C and the quad was set at 150 °C, a solvent delay of 11 min was set to preserve the source. The data was processed using Chemstation Software. Qualitative analysis was confirmed through a combination of spectral libraries (NIST, Wiley), spectra interpretation, selective ion extracted chromatograms, retention times and consulting related literature. The final concentration of organic pollutants was calculated as μg compound/g of organic matter in a fresh basis, considering the that occur changes in the organic matter and moisture content during the composting process.

2.7. DNA extraction and 16S rRNA amplification

DNA was extracted from the frozen sample from each of the composite samples taken from Test and Control containers (0.25 g) using the POWERSOIL DNA isolation kit (MO BIO, Carlsbad, US). The purified DNA was used as a template for amplification with 16S rRNA primers targeting the variable region V6 – V9. DNA amplification was carried out according to Berry et al., (2011). PCR reaction of 35 cycles consisted of 1 x buffer with 2.5 mM $MgSO_4$ (Fermentas), 0.2 mM dNTPs (Thermo Fisher Scientific, Waltham, MA) 0.3 μM of reverse primer (1492R: 5′-NTACCTTGTTACGACT-3′), 0.5 μM of forward primer (909F:5′-ACTCAAAKGAATWGACGG-3), 1.25 U Pfu DNA polymerase and 10 ng of extracted DNA in a final reaction volume of 25 μL.

2.8. Pyrosequencing 454 of 16S rRNA and bioinformatic analysis

The amplicon from 16S rRNA was diluted (1:25) was subject to a PCR reaction another 5 cycles with barcode primers (Hamady et al., 2008). The fusion primers were designed according to manufacturer's instruction (454 Life Sciences). PCR product is purified with Roche High pure PCR product purification kit as per the protocol (www.roche-applied-science.com). DNA was quantified using Promega Quantus Fluorometre until a final concentration of 10 ng/μl of DNA is obtained. Pyrosequencing with 454 genome sequencer FLX platform was performed at the DNA sequencing facility Cambridge University.

16S rDNA sequences were analysed using the Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) as described by Caporaso et al. (2010). Sequences with quality scores lower than 25 were eliminated and sequences with 200–600 bp were assigned to samples with 8 bp barcodes. Following removal of chimeras, sequences were clustered at 97% similarity into relevant OTUs using Usearch (Edgar, 2010). Representative OTU sequences were picked, and aligned using the PyNast algorithm (Caporaso et al., 2010; DeSantis et al., 2006) prior to taxonomy assignment using the GreenGenes (core 13_8_2013) classifier at 97% identity (Wang et al., 2007).

2.9. Statistical analysis

Analysis of variance between the Control and Test for pH, %Moisture, % OM, C:N ratio, compost stability (OUR) and organic contaminants were determined using one-way ANOVA (R software; normality and equal variance assumptions were met). Bacterial diversity was estimated using the Shannon diversity index (Gardener, 2014) using the Biodiversity package (R software). Spearman correlations (two-sided, pairwise, $p < 0.05$) were conducted for compost parameters (OUR, CN ratio, pH, moisture), Shannon diversity and the relative abundance of the bacterial phyla and family taxa using the Hmisc package (R software).

3. Results & discussion

3.1. The effect of the addition of wood shavings on the microbial profile and activity during the composting process

The pH, C:N ratio and the moisture content of the composting process are presented in Fig. 3. All the parameters showed similar variation throughout the composting incubations. The Control A feedstock presented an acidic pH (Fig. 3a), with its bacterial profile (Fig. 5a) dominated by *Lactobacillales* (phylum Firmicutes) at the order level. The acidic nature of MS-OFMSW feedstock has been extensively reported (López-Gómez et al., 2019) and reflects the production of lactic acid by the bacteria *Lactobacillus*. In a large-scale composting plant, the low pH

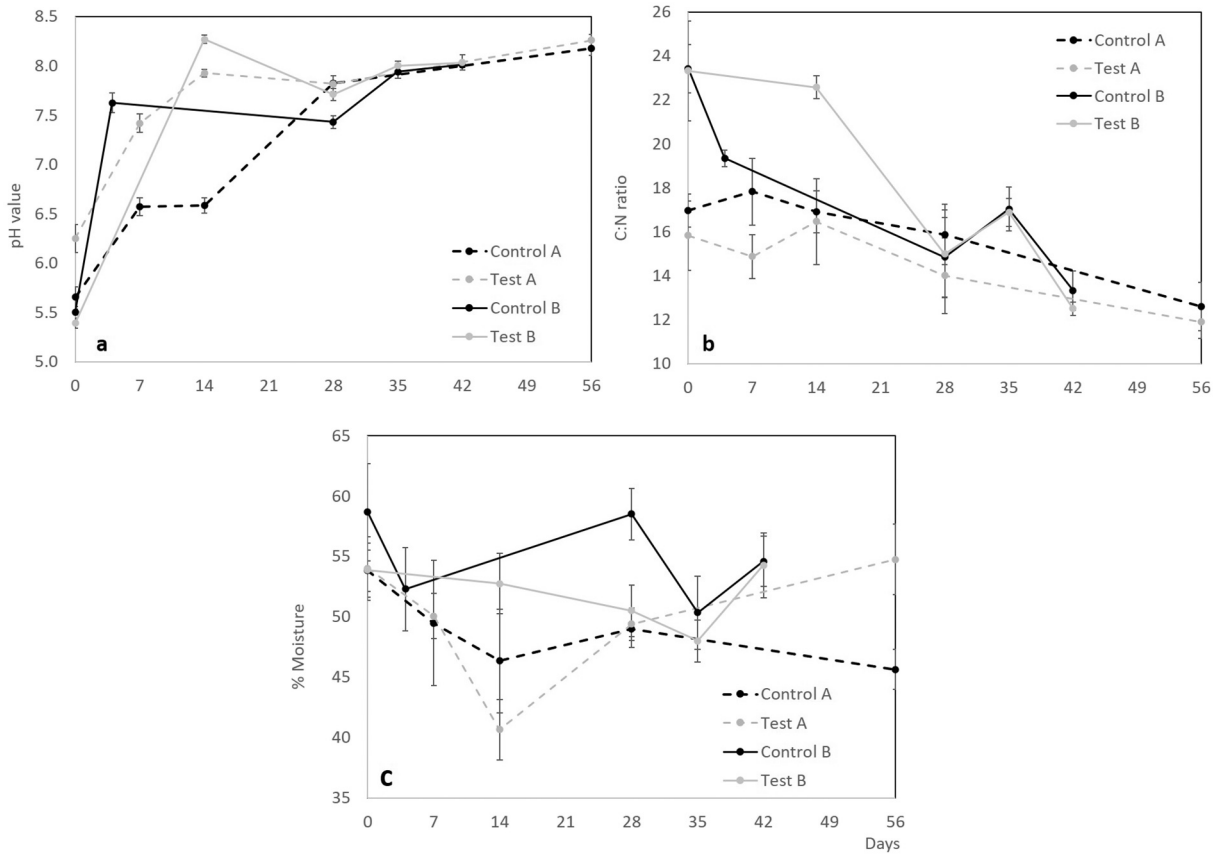


Fig. 3. Variation of the pH (a), C:N ratio (b) and % moisture (c) throughout the composting process in the incubations A and B.

of the MS-OFMSW can inhibit other bacterial activity and severely delay a successful composting process (Sundberg et al., 2004). These effects are suggested in our analysis by the strong negative correlation of the *Lactobacillales* order with feedstock pH and bacterial diversity (Table 1). Wood shavings, originating from waste wood, were chosen to balance the poor CN ratio presented by the MS-OFMSW. Positive impacts of high CN ratio bulking agents in compost stability have been previously reported in open windrow systems for MS-OFMSW (Tognetti et al., 2007) and agricultural wastes (Goyal et al., 2005). Despite some of the carbon in the wood shavings could be recalcitrant carbon, when blended with high nitrogen materials, such as MS-OFMSW, it showed to boost microbial activity. Despite, in our study the use of wood shavings in Test A did not change the C:N ratio (Fig. 3b) result when tested as intended. Instead a significant increase of 0.6 units in the pH value (Fig. 3a) was observed. Despite this pH change, the feedstock in Test A presented a similar abundance of the *Lactobacillales* than Control A (Fig. 5a). The temperature profile (Fig. 4) shows that the Control A incubation was slower to reach pasteurizing temperatures than Test A. When microbial activity increases, the temperature increases earlier in the process to levels that *Lactobacillales* cannot function at due to the thermophilic temperature. This substantial shift from *Lactobacillales* to *Bacillales* dominated the bacterial community (Fig. 5) in both Control and Test A and happened after a week of the composting process. This shift occurred in the bacterial profile indicating a successful transition from the initial mesophilic phase to the thermophilic phase (Fig. 4) of the process (Partanen et al., 2010). This shift is accompanied by a substantial increase in bacterial diversity (Fig. 6b). Although the bacterial profile is similar between Control and Test A, the microbial activity (OUR) in the Test A reaches its maximum fourteen days earlier than in the Control A (Fig. 6a), followed by a steady decrease throughout the remaining *in-vessel* composting process. During the last four weeks of the process, a more diverse bacterial community was observed (Fig. 6b) in the material where the wood shavings were added (Test A). Moreover, in the same period, the Control A incubation was subjected to more periods of higher temperatures (Fig. 4). Composting temperature is regulated by microbial activity. The higher composting temperature observed in the Control A is due to the higher bacterial activity (Fig. 6a). These results are concomitant with the observed lower bacterial diversity (Fig. 6b), since fewer bacterial groups have the ability to survive in a pasteurization temperature. It is likely that the composting process in the Control A incubation was slower due to an influence of high temperatures. The addition of wood shavings initially favoured a diverse bacterial profile in the tested material by improving the pH of the feedstock and

Table 1
Spearman correlation ($p < 0.01$) between bacterial phyla and order, composting parameters and stability and bacterial diversity (Shannon index).

	pH	CN ratio	OUR	Bacterial diversity
OUR	−0.60		1	
Bacterial diversity	0.77		−0.71	1
Bacterial Phyla				
Acidobacteria	0.68		−0.75	0.80
Actinobacteria			−0.62	0.79
Bacteroidetes				0.73
Firmicutes	−0.65		0.68	−0.87
Proteobacteria	0.83		−0.68	0.92
Bacterial order				
Acidobacteria_iii1.15	0.63		−0.83	0.72
Actinomycetales			−0.58	0.75
Lactobacillales	−0.77	0.58		−0.60
Rhizobiales	0.61		−0.61	0.78
Burkholderiales	0.77			0.89
Myxococcales	0.72		−0.86	0.86
Alteromonadales			−0.66	0.87
Xanthomonadales	0.76		−0.65	0.90

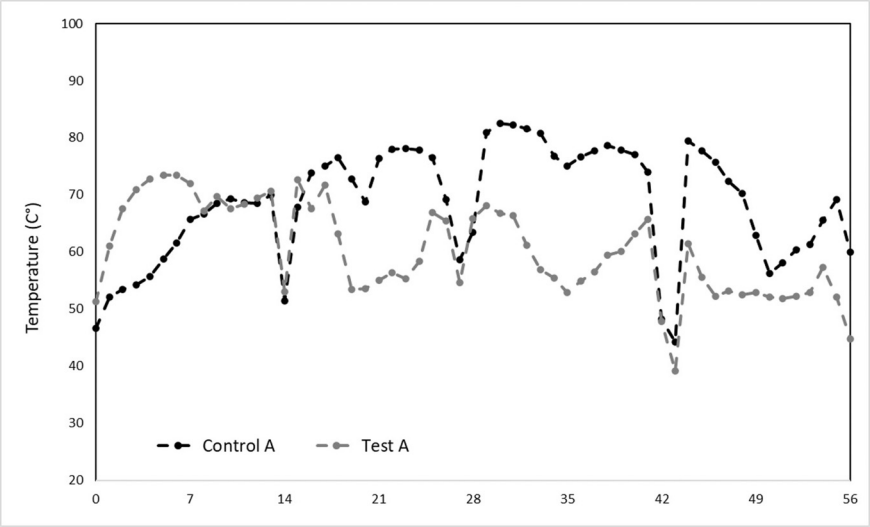


Fig. 4. Temperature (°C) profile during the composting process in the incubation A.

overcoming the initial acidic pH. The results suggest that the higher temperatures in Control A incubation (Fig. 4) may have affected the evolution of the bacterial activity and diversity. The optimum composting temperature in Test A caused greater microbial activity and lead to the achievement of the compost stability requirements in less time (Fig. 6a). By T56, Control A reached an OUR value of 22.32 mmol O_2/kg organic solids/h and Test A had reached a value of 13.34 mmol O_2/kg organic solids/h. Our results suggest that the use of wood shavings improved the feedstock composition this positively affected the bacterial succession in the composting process leading to a more efficient composting process and earlier stabilization of the BSRW.

3.2. Degradation of polycyclic aromatic hydrocarbons (PAHs) during the composting process

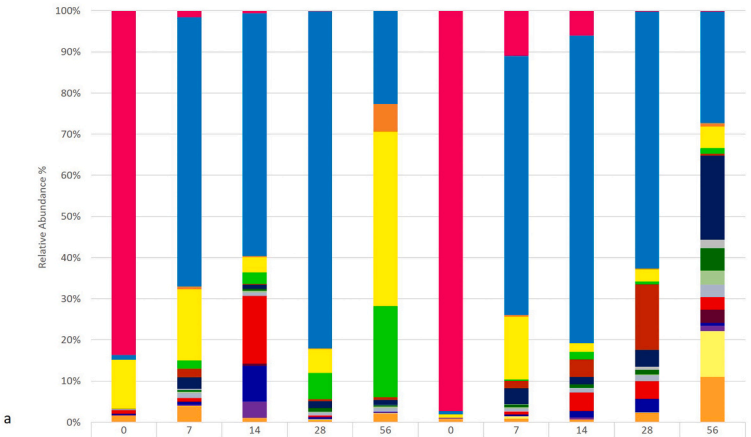
Degradation of PAHs during the composting process of MS-OFMSW was studied in Incubation A. From the 16 US EPA PAHs listed as priority pollutants only phenanthrene and fluoranthene were detected in the material. The concentrations of phenanthrene and fluoranthene ranged between 0.091 and 0.732 μg/g OM (fresh basis) and <0.0015–0.732 μg/g OM (fresh basis), respectively, and were below the PAH threshold limit in the recent regulations for source segregated compost (Regulation EU 2019/1009).

The concentration of phenanthrene was similar in the feedstock material in Control A and Test A before the composting process ($p = 0.358$). However, the concentration of fluoranthene in the feedstock material of Test A was higher ($p < 0.05$) than in the Control A. The changes in the initial concentrations of fluoranthene could be linked to the different proportions in the mixing material used (Fig. 2) or the use of wood shavings from waste wood. However, the high heterogeneity of these material could also contribute to the variability of PAHs in the feedstock (Oujana and Zwolinski, 2018).

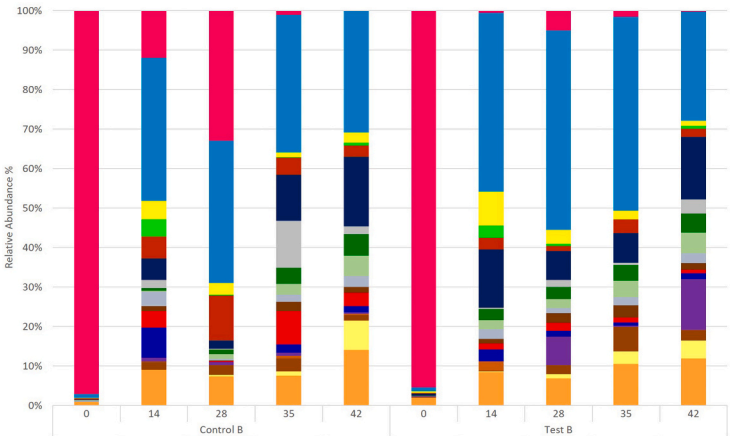
Concentrations of the detected PAHs did not change from T0 to T56 in Control A whereas, in Test A, the concentration of phenanthrene decreased 3.3-fold while the fluoranthene concentration was below the detection limit (<0.0015 μg/g OM) at T56 (Table 2). Since the concentration of PAHs already takes into account changes in the %OM and moisture content, our results strongly suggest that the changes in the microbial profile in Test A lead to the removal of PAHs during a more efficient compost process of 8 weeks. Recent studies have also reported a significant degradation of PAHs during composting/co-composting of sewage sludge (Guo et al., 2020) and biomass fly ash (Košnář et al., 2019). Our study also shows no evidence of thermal degradation of PAHs. The temperature profile of Incubation A (Fig. 4) reveals that microbial activity was affected by high temperatures (>70 °C), suggesting that optimum thermophilic conditions in Test A enhanced microbial degradation of PAHs during composting. This finding was also reported by Mehetre et al. (2019) and Rabodonirina et al. (2019) while studying the biodegradation of PAHs using *Bacillus* and *Pseudomonas* strains. The role of PAHs-degrading microbes indicates that composting MS-OFMSW would guaranty the degradation of PAHs in the organic municipal waste, ensuring its potential safety for further reuse.

3.3. Degradation of phthalates during the composting process

The degradation of the four phthalates; diethyl phthalate (DEP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP) and the Bis(2-ethylhexyl) phthalate (DEHP) was assessed during Incubation A. The degradation of phthalates during composting is not yet fully understood as well as the extent of the contribution of microbial degradation over other chemical and physical process (Liang et al., 2008). The concentrations of the phthalates DEP, DIBP and DBP were similar in the feedstocks used in the Control and Test incubations. However, DEHP showed higher initial concentrations ($p < 0.05$) in the Control A feedstock (19.45 μg/g OM fresh basis) than in the Test A (14.13 g/g OM fresh basis), indicating that only the concentration of DEHP was impacted by the change in the feedstock composition, due to the use of wood shavings (Fig. 2). The phthalate concentrations found in the MS-OFMSW and in the final compost are in general similar to previously reported results in UK biosolids and municipal compost (Rigby et al., 2015). It has been suggested that the degradation of phthalates is linked to their chemical properties, such as molecular weight as is the case with many PAHs (Net et al., 2015). In our study, the phthalate's molecular weight increases in the order DEP < DIBP = DBP < DEHP. DEP has been categorized as a short chain phthalate whereas DIBP, DBP and DEHP are classified as medium chains (Net et al., 2015). The phthalates DEP, DIBP and DBP showed a significant ($p < 0.05$) decrease in concentrations at day 56 in both the Control and Test in the incubation A (Table 2). Degradation rates of these phthalates ranged between 70.8 and 97.4% and no difference was observed between the degradation rate of DEP and DBP. However, DIBP degradation rate was higher ($p < 0.05$) in the Test A incubation (94.3%) that in the Control A (70.8%). Our results indicate that the removal of short and medium chain phthalates occurs during the MS-OFMSW composting process. While the degradation or/and removal of short chain phthalates occurred independent of microbial activity, the higher degradation of DIBP in Test A suggests that microbial



	Control A					Test A				
	0	7	14	28	56	0	7	14	28	56
Lactobacillales (Firmicutes)	83.6836	1.6129	0.6303	0.1032	0.0000	97.3658	11.0043	6.0606	0.2066	0.2227
Bacillales (Firmicutes)	1.1125	65.3763	59.0336	81.9401	22.7027	0.7092	62.9274	74.7126	62.5000	27.0601
Clostridia i.s. (Firmicutes)	0.0000	0.7527	0.2101	0.2064	6.7027	0.0000	0.4274	0.0000	0.1033	0.8909
Clostridiales (Firmicutes)	11.9901	17.2043	3.7815	5.7792	42.3784	0.8105	15.2778	2.0899	2.9959	5.2339
Rhodocyclales (Proteobacteria)	0.0000	2.0430	2.7311	6.3983	22.1622	0.0000	0.3205	1.8809	0.6198	1.3363
Pseudomonadales (Proteobacteria)	0.0000	2.0430	0.2101	0.4128	0.6486	0.1013	1.8162	4.2842	16.0124	0.4454
Xanthomonadales (Proteobacteria)	0.1236	2.9032	1.0504	1.7544	1.1892	0.0000	3.8462	1.7764	4.0289	20.4900
Alteromonadales (Proteobacteria)	0.1236	0.3226	0.0000	0.0000	0.1081	0.0000	0.1068	0.0000	0.8264	2.0045
Gammaproteobacteria Other (Proteobacteria)	0.0000	0.3226	0.4202	0.8256	0.3243	0.0000	0.6410	0.8359	1.1364	5.4566
Myxococcales (Proteobacteria)	0.0000	0.3226	0.4202	0.2064	0.3243	0.0000	0.3205	0.1045	0.2066	3.4521
Burkholderiales (Proteobacteria)	0.0000	1.1828	0.8403	0.7224	0.8649	0.1013	0.7479	1.0449	1.3430	3.0067
Flavobacteriales (Bacteroidetes)	0.8653	0.8602	16.3866	0.4128	0.0000	0.0000	0.6410	4.4932	4.3388	3.0067
Saprospirales (Bacteroidetes)	0.1236	0.1075	0.6303	0.0000	0.0000	0.0000	0.1068	0.1045	0.1033	3.2294
Sphingobacteriales (Bacteroidetes)	0.2472	0.6452	8.6134	0.4128	0.1081	0.1013	0.3205	1.4629	3.2025	0.6682
Bacteroidales (Bacteroidetes)	0.1236	0.3226	3.9916	0.2064	0.3243	0.0000	0.1068	0.5225	0.0000	1.3363
Group iii1-15 (Acidobacteria)	0.0000	0.1075	0.0000	0.1032	0.1081	0.0000	0.5342	0.0000	0.1033	11.1359
Other Orders with less than 2%	1.6069	3.8710	1.0504	0.5160	2.0541	0.8105	0.8547	0.6270	2.2727	11.0245



	Control B					Test B				
	0	14	28	35	42	0	14	28	35	42
Lactobacillales (Firmicutes)	96.7	11.7	33	1	0	94.9	0.5	4.9	1.5	0.2
Bacillales (Firmicutes)	1	35.4	36	33.6	30.1	1	44.7	49.5	48.3	26.8
Clostridiales (Firmicutes)	0.1	4.6	3	1.1	2.5	0.4	8.5	3.5	2.2	1.2
Rhodocyclales (Proteobacteria)	0	4.2	0.2	0.1	0.7	0	3	0.5	0	0.7
Pseudomonadales (Proteobacteria)	0.3	5.5	11.4	4.2	2.8	0.2	3	1.2	3.4	2
Xanthomonadales (Proteobacteria)	0.1	5.3	2.1	11.3	17.2	0.5	14.5	7.2	7.4	15.3
Alteromonadales (Proteobacteria)	0	2	0.2	11.4	1.9	0	0.3	1.8	0.6	3.5
Gammaproteobacteria Other (Proteobacteria)	0	0.7	1.2	3.9	5.4	0.1	2.9	3	3.9	4.6
Myxococcales (Proteobacteria)	0.2	0.2	1.2	2.7	5	0	2.2	2.2	4.1	5.1
Burkholderiales (Proteobacteria)	0.1	3.6	0.4	1.7	2.7	0.1	2.5	1.3	2	2.4
Rhizobiales (Proteobacteria)	0	1.2	0.2	2.3	1.4	0	1.1	2.4	3	1.6
Flavobacteriales (Bacteroidetes)	0	4.1	0.2	8.1	3.3	0.1	1.5	1.9	1.3	0.9
Sphingobacteriales (Bacteroidetes)	0.1	7.5	0	2	1.6	0.1	2.9	1.5	0.8	1.4
Bacteroidales (Bacteroidetes)	0	0.9	0.8	0.8	0.4	0	0.1	7.1	0.3	12.5
Group VC2_1_Bac22 (Bacteroidetes)	0	0.1	0	0.6	0.2	0	2.3	0	0	0.1
Actinomycetales (Actinobacteria)	0	2	2.4	3.2	1.4	0.1	0.2	2.2	6.1	2.5
Group iii1-15 (Acidobacteria)	0.1	0	0.4	1	7.2	0	0.2	1.1	3.1	4.4
Other Orders with less than 2%	0.9	8.8	7.4	7.3	13.8	2	8.3	6.7	10.4	11.5

Fig. 5. Succession of bacterial order taxa and relative abundance (cut-off 2%) during composting of the mechanically separated organic fraction of municipal solid waste in the a) incubation A and b) incubation B.

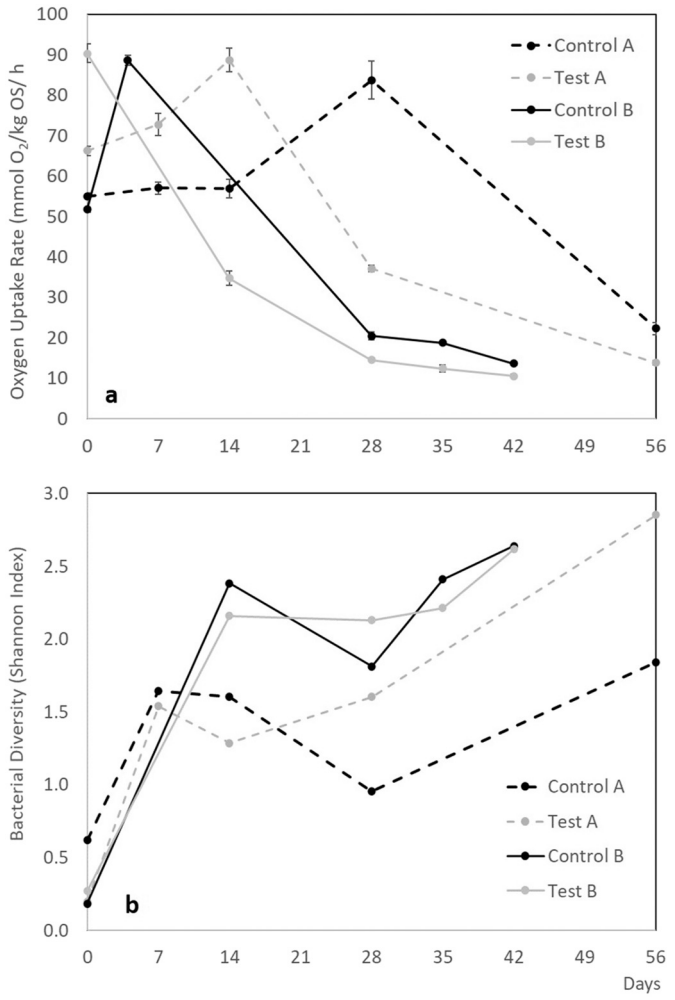


Fig. 6. Oxygen uptake rate (mmolO_2/kg OS/h) (a) and diversity (Shannon Index) of bacterial community (b) throughout the composting process in the incubations A and B.

Table 2
Polycyclic aromatic hydrocarbons (PAHs) and phthalates content, calculated on a fresh weight basis, in the starting material (day 0) and at the end (day 56) in the Incubation A.

	PAHs (μg/g OM)		Phthalates (μg/g OM)			
	Phenanthrene	Fluoranthene	DEP	DIBP	DBP	DEHP
Control A						
Day 0	0.56	0.30	0.44a	1.02a	5.47a	19.45
Day 56	0.58	0.41	0.09b	0.37b	0.26b	18.89
Test A						
Day 0	0.46 A	0.69 A	0.70A	1.51A	3.77A	14.13
Day 56	0.14 B	<0.0015 B	0.02B	0.13B	0.24B	13.29

Diethyl phthalate – DEP; Diisobutyl phthalate – DIBP; Dibutyl phthalate – DBP; Di2(ethylhexyl) phthalate – DEHP;
Different letters indicate significant differences ($p < 0.05$) between organic compounds concentrations measured at T0 and T56, in the Control (lowercase letters) and in the Test (capital letters).

activity could enhance the rates of degradation of some phthalates. The non-removal of DEHP in our study was surprising taking into account previous studies reporting removals of 80–90% in sewage sludge composting (Moeller and Reeh, 2003; Cheng et al., 2008). Although phthalates degradation has been studied in several environments such as soils, water and air (Gao and Wen, 2016) little is known about the specific pathways of phthalates removal during the composting process. Aerobic microbial degradation is thought to be the main mineralization process for phthalates (Gao and Wen, 2016). In our study, only the low molecular weight phthalates showed a decrease in concentration during composting, across both incubations, suggesting that biological degradation together with other physical and chemical process have a role in the removal of phthalates. Solubilization of phthalates and consequent leaching into wastewater during the composting process may also be possible. Although phthalates have low water solubility (Net et al., 2015), the high temperatures during the thermophilic composting phase and the changes in pH over the process, could lead to changes in the surface charges of the phthalates, increasing their leaching potential. Our study suggests that the higher temperatures observed in the Control A incubation (Fig. 4) do not indicate a higher degradation rate of phthalates. The work conducted by Fudala-Ksiazek et al. (2017) support the idea that physical treatment in the MSW sorting facilities and the biological treatment of MS-OFMSW could alter the properties of phthalates and potentially increase their solubility rate. Due to their ubiquitous occurrence in the environment and detrimental health effects, future work should focus on the flow of phthalates in composting systems to understand a) if the composting process could be used as a solution to degrade organic pollutants from organic waste and b) understand the role of temperature, leaching and/or volatilization of organic pollutants during the composting process.

3.4. The effect of turning frequency on the microbial profile and activity during the composting process

The effect of turning frequency of the material in the pilot scale tunnels was evaluated in Incubation B. Studies performed in windrow systems/static piles showed a strong link between optimized turning frequency and the improvement of compost stability in composting of agriculture waste (Parkinson et al., 2004; Jiang-ming, 2017) and sewage sludge (Khalil et al., 2011). The turning frequency of the material has been understudied in *in-vessel* systems, but there is great potential due to the control of compost parameters, such as temperature and oxygen rate. In *in-vessel* composting, the material is still turned to allow for temperature homogenization and pathogen reduction (Getahun et al., 2012). In this study, Control B was turned every 14 days whereas the material in Test B was turned every 7 days. The composting process in the pilot scale vessel lasted 42 days. No changes in pH, CN ratio and moisture were observed in the starting material in incubation B (Fig. 3). These parameters also presented similar evolution curves throughout the composting process. The OUR (Fig. 6a) was 1.6-fold higher in Test B ($F = 268.47$, $p < 0.001$) than Control B in the starting material, leading to a different evolution of microbial activity in the composing process in the two incubations. Changes in pH and CN ratio between Control B and Test B were detected only at day 14, with Test B presenting higher pH and CN ratios. Due to substantially different microbial activity (OUR) at the beginning of incubation B (Fig. 6a), it is not clear if the changes in pH and CN ratio at day 14 were due to the turning frequency or microbial activity. However, they could be linked to the slower decrease in *Lactobacillales* relative abundance in the Control B (Fig. 5b). Test B achieved an OUR value of 12.42 mmol O_2/kg OS/h by T35, while Control B reached a OUR of 12.34 mmol O_2/kg OS/h by T42. Our data suggests that increasing the turning frequency did not significantly impact the final compost stability (Fig. 6a) nor the bacterial profile (Fig. 5b) and diversity (Fig. 6b) of the BSRW. However, the increased turning frequency of Test B does may be responsible for the material reaching stability earlier than Control B. It is important to note that the OUR in the incubation B (Control and Test) decreases sharply at 28 days of composting and does not change significantly throughout the remaining composting time. Similarly, Boyle et al. (2015) reported no effect of turning frequency in food waste compost quality using the *in-vessel* system Earth Tubs™. Nevertheless, both Control B and Test B reached stability values lower than 13 mmolO_2/kg OS/h, meeting the Irish stability criteria for quality compost (Prasad and Foster, 2009).

4. Conclusion

Compost stability defines the efficacy of the composting process. The respose of bacterial profiles and compost stability to adding wood shavings as bulking agent and increasing the material turning frequency was evaluated. The wood shavings affected the bacterial succession, leading to a faster compost stability, whereas turning frequency had less impact. Degradation of PAHs was observed when wood shavings were used, whereas phthalates removal was observed regardless of the feedstock composition. Our work indicates that biological activity is responsible for the degradation of organic pollutants during the composting of MS-OFMSW.

CRediT authorship contribution statement

Brian Kelleher, Christopher Allen and Tim Duggan: Funding acquisition.

Brian Kelleher, Christopher Allen, Brian Murphy[a,b], Jessica Graca[a], and Tim Duggan: Conceptualisation, supervision, review and editing.

Jessica Graca[a], Brian Murphy[a,b], Prasanna Pentlavalli[c]: Methodology, validation, formal analysis, writing, visualisation, data curation, investigation.

Eoin Bird[b], Michael Gaffney[d]: Methodology, resources, validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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