

Universidade de Trás-os-Montes e Alto Douro

**Composting of agro-food residues:
chemical composition, greenhouse gases emissions
and compost agronomic value**

PhD thesis in Agricultural and Forestry Sciences

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Organismos não se adaptam simplesmente a condições autónomas previamente existentes; eles criam, destroem, modificam e transformam internamente aspetos do mundo exterior pelas suas próprias atividades vitais. Nem organismo, nem ambiente é um sistema fechado; cada um é aberto para o outro.

Richard C. Lewntin et al., 1984

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Abstract

Composting has been widely described and accepted as one of the most feasible and viable technologies for processing and valorising agro-industry wastes. Portugal, as member of the European Union needs accomplish the Community goals reducing the amount of waste generate and/or valorising their wastes. Beyond the recycling advantage, the application of the end product improve the soil quality acting as biologic fertilizer, enables carbon sequestration and may allow their use as a sustainable toll on management of soil-borne diseases. There exists, however an environmental risk associated to the process, such a hight warming potential (GWP) owing to the emission of greenhouse gases (GHGs), such as carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄) and other gases as ammonia (NH₃) and nitric oxide (NO).

One of the objectives of the present thesis was to assess the effect of the chemical composition (phenolic and lignocellulosic contents) of the eight diferentt raw materials used on the composting process and on the gases emmited. This evaluation was fullfilled through the physical-chemical analysis of the raw materials, samples mixtures collected along the composting process and final composts. Composting was studied in two sets on 135 L insulated reactors with forced air circulation and with a system that allow the measurement of gases emissions and temperature during the process.

In the first composting set, that use different proportions of spent coffee grounds (0 to 40%), all tratments revealed a substantial decrease in total phenolics and total tannins and an important increase in gallic acid. The GHGs emissions were very low and no significant difference between the treatments was registered. Is possible to conclude that that all spent coffee grounds treatments are suitable for composting, but the proportion of 40 % led to better conditions for composting and lower GHGs emissions.

In the second composting set, that use different agro-industry wastes (broccoli, chestnut, red grape marcs, white grape marcs, olive leaves) significant variations were observed over time and between the different wastes. The compost obtained with Chestnut wastes and olive leaves led to higher carbon dioxide, methane and nitrous oxide emissions. A positive correlation was found between phenolics and nitric oxide ($r = 0.63$; $p < 0.01$) and between lignocellulose and nitrous oxide ($r = 0.51$; $p < 0.05$). Its possible to obsserve that a higher content of hemicelluloses in the wastes seemed to entail higher N₂O emissions. These results suggest the potential use of wastes rich in lignocelluloses in composting for the purpose of reducing N losses by NH₃ and NO emissions.

After obtaining the final composts we also studied the effect of eight different composts and their chemical composition on C and N mineralization and GHGs emissions by determination of ammonium (NH_4^+) and nitrate (NO_3^-) and measurement of carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) fluxes from amended soils under laboratory conditions.

Through the results it is possible to conclude that mineralization of the C added to the soils was directly correlated to C/N, total phenolic/N, gallic acid/N and NH_4^+ and negatively correlated with total organic nitrogen (TON) and lignin. N mineralization was only correlated with C/N. All composts enhanced N_2O emissions and no significant difference in cumulative emissions were observed between treatments.

Finally a biological assay was performed with the objective of testing the suppressive potential of four composts (Chestnut, Grape marc, Coffee grounds and Olive) against the soil-borne *Botrytis cinerea* and *Fusarium oxysporum* with significant impact on lettuce cultivation. The suppressive effect was noticed in Chestnut and Olive with no significant difference between the two doses applied. These treatments also showed significantly higher values of dry matter yields of leaves and roots which denotes their bio-fungicide and bio-fertilizer potential.

The results of this thesis could be a useful instrument for planning field scale experiments giving an idea of quantification and quantification of the GHG and soil C and N pools.

Overall, the results pinpointed out that the wastes with high lignocellulose contents have additional benefits on the environment in relation to mitigating nitrogen losses. All the composts applied in pot experiments, were found to be beneficial to plant growth. Besides, composts obtained from chestnut wastes and olive leaves are promising wastes as bio-pesticides suppressing *Botrytis cinerea* and *Fusarium oxysporum* wilt diseases.

Keywords: C and N mineralisation, Composting, GHGs, Lignocellulosic compounds, Phenolics compounds, Soil-borne diseases, Suppressive effect

Resumo

A compostagem é amplamente reconhecida como uma das tecnologias mais viáveis para o tratamento e valorização de resíduos agroindustriais. Para além disso, o produto final (compostado) pode ser aplicado ao solo como fertilizante melhorando a sua qualidade, permitindo o sequestro de carbono e ainda possibilitando a gestão de doenças transmitidas pelo solo, devidas ao potencial bio-fungicida. No entanto, existe um risco ambiental associado ao processo devido à emissão de gases de efeito estufa (GEE) tais como o dióxido de carbono (CO_2), óxido nitroso (N_2O) e metano (CH_4) e outros gases como o amoníaco (NH_3) e o óxido nítrico (NO).

Um dos objetivos da presente tese foi avaliar o efeito da composição química (compostos fenólicos e lenhocelulósicos) de oito resíduos agro-industriais no processo de compostagem e nos gases gerados. Esta avaliação foi obtida através da análise físico-química dos resíduos iniciais, das amostras recolhidas ao longo do processo de compostagem e dos compostados finais. O processo de compostagem foi avaliado em dois ensaios experimentais usando reatores de 135 L com arejamento forçado. No primeiro ensaio de compostagem, foram usadas diferentes proporções de borras de café (0 %, 20 % e 40 %) e verificou-se uma diminuição substancial nos fenólicos totais e taninos totais e um aumento no ácido gálico em todos os tratamentos ao longo do processo. Relativamente às emissões dos GEEs, verificou-se que estas foram reduzidas e não se obtiveram diferenças significativas entre os tratamentos. Foi possível concluir que a proporção de 40 % de borras de café garantiu boas condições para a progressão do processo de compostagem e foi o tratamento que apresentou as emissões de GEE mais reduzidas.

No segundo ensaio de compostagem, utilizaram-se diferentes resíduos provenientes da agroindústria (brócolo, castanha, canganho de uvas tintas, canganho de uvas brancas, folhas de oliveira). Os compostados obtidos de resíduos de castanha e folhas de oliveira promoveram maiores emissões de dióxido de carbono, metano e óxido nitroso. Verificou-se a existência de uma correlação positiva entre fenólicos e óxido nítrico ($r = 0,63$; $p < 0,01$) e entre a lenhocelulose e óxido nitroso ($r = 0,51$; $p < 0,05$). Observou-se também que um maior teor de hemicelulose nos resíduos implicou maiores emissões de N_2O . Estes resultados sugerem que o uso de resíduos ricos em lenhocelulose poderão ser usados na compostagem com a finalidade de reduzir as perdas de N por emissões de NH_3 e NO .

Após a obtenção dos compostados finais provenientes dos dois ensaios experimentais, foram estudados os efeitos das suas composições químicas nas emissões de GHGs e nas

mineralizações C e N. Esta análise foi realizada num ensaio de incubação em condições laboratoriais através da determinação do amónio (NH_4^+) e nitrato (NO_3^-) e medição dos fluxos de dióxido de carbono (CO_2), óxido nitroso (N_2O) e metano (CH_4). Através dos resultados foi possível concluir que a mineralização do C adicionado ao solo se relacionou diretamente com as razões C/N, fenólicos totais/N, ácido gálico/N e NH_4^+ e evidenciou uma relação negativa com azoto orgânico total (TON) e a lenhina. A mineralização de N apenas se relacionou com C/N. Todos os tratamentos aumentaram as emissões de N_2O não se observando nenhuma diferença significativa nas emissões acumuladas entre os tratamentos.

Finalmente, realizou-se um ensaio de vasos em alface, com o objetivo de testar o potencial supressor de quatro compostados (Castanha, Canganho de uva, Borrás de Café e Oliveira) nos fungos patogénicos *Botrytis cinerea* e *Fusarium oxysporum*. Foi observado o efeito supressor nos tratamentos de Castanha e folhas de Oliveira sem diferenças significativas entre as duas doses aplicadas. Esses tratamentos também mostraram valores significativamente maiores de rendimentos de matéria seca de folhas e raízes que denotam seu potencial uso como bio-fungicida e bio-fertilizante.

Em geral, os resultados indicaram os resíduos com altos teores de lenhocelulose poderão ter benefícios adicionais para o meio ambiente devido à mitigação de perdas de azoto. Além disso, os compostados obtidos a partir de resíduos de castanha e folhas de oliveira para além de aumentarem a produção de alface demonstraram possuir um efeito supressor em *Botrytis cinerea* e *Fusarium oxysporum*.

Palavras-chave: Compostagem, Compostos fenólicos, Compostos lenhocelulósicos, Efeito supressivo, Fitopatogéneos, GEEs, Mineralização de C e N.

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List of publications

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Cátia Santos, João Fonseca, João Coutinho, Henrique Trindade, Lars Stoumann Jensen. Greenhouse gas emissions and C and N mineralization of amended soils with different composted agro-industrial-wastes. (Soil Biology and Biochemistry).

Cátia Santos, Joana Monte, João Fonseca, Natália Vilaça, Henrique Trindade, Isabel Cortez. Bio-pesticide potential of composted lignocellulosic agro-industrial wastes to suppress phytopathogenic fungus: *in vivo* and *in vitro* studies (Industrial Crops and Products).

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Cátia Santos, Andreia Santos, João Fonseca, Alfredo Aires, João Coutinho, Henrique Trindade. 2016. Composting of phytochemical-rich plant materials: Greenhouse gases emissions and evolution of total phenolic, tannins and glucosinolates compounds. XXII Encontro Luso-galego de Química, Bragança, Portugal.

Cátia Santos, João Fonseca, Joana Monte, Natália Vilaça, Henrique Trindade, Isabel Cortez. 2016. Efeito supressivo de diferentes compostados no controlo de *Botrytis* sp e *Fusarium* sp. em alfaces. 2^{as} Jornadas de Engenharia Agronómica, Vila Real, Portugal.

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List of abbreviations and nomenclature

ADF- Acid detergent fibre

ADL- Acid detergent lignin

ANOVA- Analysis of variance

BCAs – Biological control agents

C- Carbon

Ca - Calcium

CH₄- Methane

CO₂- Carbon dioxide

Ct - Total organic carbon

Cu- Copper

d - days

DM – Dry matter

DNA- Deoxyribonucleic acid

EC- Electrical conductivity

EU- European Union

FDA- Fluorescein diacetate

Fe- Iron

GAE – galic acid equivalent

GEE – Gases de efeito estufa

GHG- Greenhouse gas

GWP – Global warming potential

h – hours

H₂O – Water

H₂S- Hydrogen sulfide

IPCC – International panel on climate change

K- Potassium

kg - Kilogram

KCl – Potassium chloride

L- Litres

Mg - Magnesium

Mn- Manganese
N- Nitrogen
n.d - Not detected
N₂- Dinitrogen
N₂O- Nitrous oxide
NDF- Neutral detergent fibre
NH₃- Ammonia
NH₄⁺- Ammonium
NIR – Near infrared
NIRD – Near infrared detector
NO- Nitric oxide
NO₂- Nitrite
NO₃⁻ - Nitrate
Nt - Total organic nitrogen
O₂ – Oxygen
OC - Total organic carbon
P- Phosphorus
PC – Principal components
PCA – Principal component analysis
ppb – Parts per billion
ppm – Parts per million
S- Sulphur
SCG – Spent coffee grounds
SD- Standard deviation
SEM – Standard error mean
TFA – Trichloroacetic acid
TN – Total nitrogen
TOC – Total organic carbon
TON- Total organic nitrogen
UV – Ultraviolet
VOCs- Volatile organic compounds
Zn- Zinc

Chapter 1. Introduction

1.1. Composting background and compost potential application

At the present time, with the human population rise as well the population shift from rural areas to urban areas, large quantity of wastes are generated every day. These waste flows leads to several environmental, social and economic challenges (Awasthi et al., 2014; Sukholthaman and Sharp, 2016) that need to overcome through the adoption of effective management strategies.

There are great differences in the supply of separate collection and treatment capacity for biowaste across Europe. In contrast to countries that have count on upon separate biowaste collection and treatment systems for over 15 years (eg. Austria, Switzerland, Germany, the Netherlands, Flanders (Belgium), Sweden and Norway), Portugal present limited collection of biowastes (Figure 1) showing great potential for growth (European Compost Network, 2019).

Moreover, the implementation of composting plants in Portugal, which recycles wastes and valorise them, frames on the concept of the circular economy and could help to achieve the objectives established under the Europe 2020 strategy for a eco-sustainable growth.

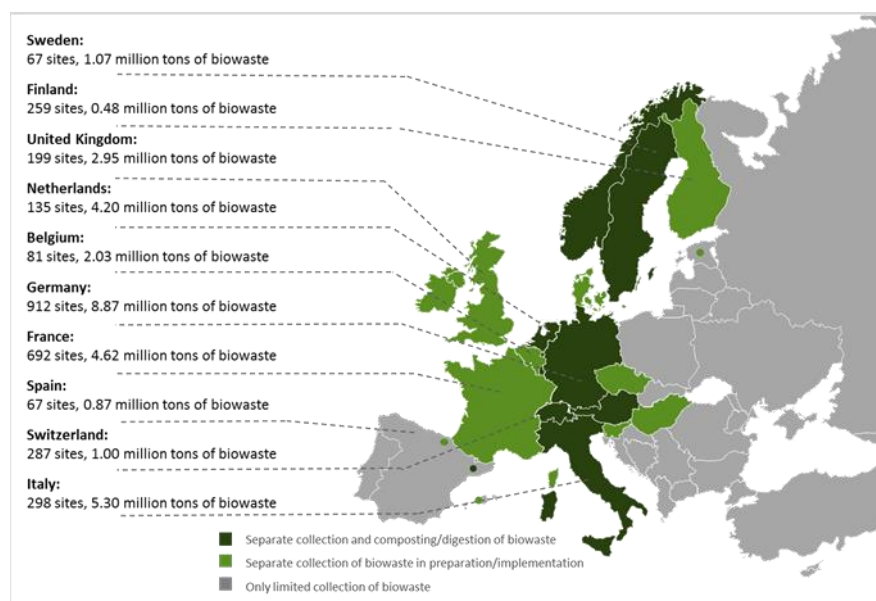


Figure 1: Status on separate collection of biowaste in Europe (European Compost Network, 2017; <https://www.compostnetwork.info/policy/biowaste-in-europe/separate-collection>).

Across the European Union, approximately 130 million tonnes of biowaste arise annually of which around 30 million tonnes of collected biowaste is effectively composted or digested annually in around 3,500 treatment plants. Composting prevails over anaerobic digestion for

the biowaste with over 90% of food and green waste being treated into compost (European Compost Network, 2017).

In southern Europe and Mediterranean countries, such as Portugal, grape and olive are broadly cultivated being the wineries and olive oil mills the dominant agro-industrial activities and the major contributors of agro-industry wastes (Salgado et al., 2014).

In recent years, use of biological treatment methods such as composting has become a reliable and promising solution in many developed countries due to increasing public concern with the environmental and health and the scarce and expensive landfill space. In fact, composting process may reduce the waste volume and weight of about 50% (Senesi, 1989) with a low operating costs and low technology requirements (Lim et al., 2016).

There are mostly two methods of waste treatment: aerobic composting and anaerobic digestion. In aerobic composting, microorganisms oxidize organic compounds to CO₂, nitrate, and nitrite in the presence of oxygen. The carbon is utilized as a source of energy, whereas nitrogen is recycled from the organic compounds. The thermophilic bacteria are mainly responsible for the breakdown of complex biodegradable organic materials and proteins through the process of oxidation (Hou et al., 2017). Anaerobic digestion takes place when bacteria (no fungi) degrade biomass under anaerobic conditions (Hermann et al., 2011). In general, the organic waste is firstly digested in anaerobic thermophilic (at 50-55°C) fermenters and after the residue is dewatered and the solid residues are post-treated by composting (Smet et al., 1999).

The process selection depends essentially on the amount and nature of organic waste as well as environmental and economic conditions (Cekmecelioglu et al., 2005).

Composts derived from agricultural and agroindustrial wastes and byproducts are generally stable, non-toxic, absent of plant and animal pathogens and free of excessive heavy metal concentrations (Ros et al., 2006). Their application to the soil and potting media is notably important for agriculture and horticulture, as they may substitute livestock manures and nonrenewable peat media (Garcia-Gomez et al., 2002; Tejada and Gonzalez 2003; Brunetti et al., 2005; Luongo et al., 2012). Chowdhury et al., (2015) have proved that composts produced from organic wastes have better quality than commercial inorganic fertilizers, since they can substitute soil conditioners in maintenance of humus generation, which is a profit that can not be attained artificially (Hermann et al., 2011; Chowdhury et al., 2015).

Besides, compost use contributes to environmental preservation: they are usually used as a natural organic amendment that is crucial for maintaining soil quality (Termorshuizen et al., 2006; Mohammad et al., 2012) and as bio-pesticide due to the suppressive properties that many

composts present, reducing significant crop losses (Hoitink and Fahy, 1986; Noble et al., 2011). Traversa et al. (2010) have found that humic-like fraction of composts ameliorates plant growth and health and also display a biocontrol effect on different soil-borne phytopathogens. The temperature reached during the composting process provides the elimination or the nonviability of pathogenic organisms present in wastes as the case of the *Parascaris equorum* eggs. This pathogenic organisms were known to have pathological consequences on animals such as horses when ingested (Gould et al., 2013).

Composts also be use as a bioremediation alternative since it can play the role of stabilizer (immobilizes metal in the soil) and as washing agent since it is a great source of humic substances that could be applied in heavy metals contaminated soils (Kulikowska et al., 2015).

Compost utilization will generally be considered as environmentally safe. However, we must always be conscious that it may lead to the introduction of pathogenic organisms or heavy metals into the soil (Innerebner et al., 2006).

Although composting is known to have lesser environmental impact compared to other waste treatments (Saer et al., 2013) the process could lead to secondary pollution involving unpleasant odours and undesirable gases such as nitric oxide (NO), ammonia (NH₃) and greenhouse gases (GHG) emissions (Font et al., 2011; Ermolaev et al., 2015). CH₄ and N₂O are considered greenhouse gases (GHGs), whereas NH₃ is recognized as an indirect GHG and an odour component. The greatest odour pollutant originated in food wastes composting is ammonia (NH₃) and its release is stimulated by low C/N ratios (Zang et al., 2016). The emission of NH₃ is strongly dependent on the pH and temperature values of the composting and is well-favoured by alkaline conditions and elevated temperatures (Pagans et al., 2006)

1.2. Main aspects of composting and factors affecting the process

Composting is a biochemical process (Figure 2) where organic materials are transformed by microorganisms present in the organic material into a final product called compost. The process involves the mineralization of organic matter and incomplete humification resulting in a stabilized final product. A hygienisation effect was also achieved along the process with a reduction of the toxicity and pathogenic organisms in the final compost (Das et al., 2011).

Different microbial communities have been observed along the composting process (Mehta et al., 2014). These involved microbial association change depending to the

predominant physicochemical status of the different composting stages (Bhatia et al., 2013; Bialobrzewski et al., 2015).

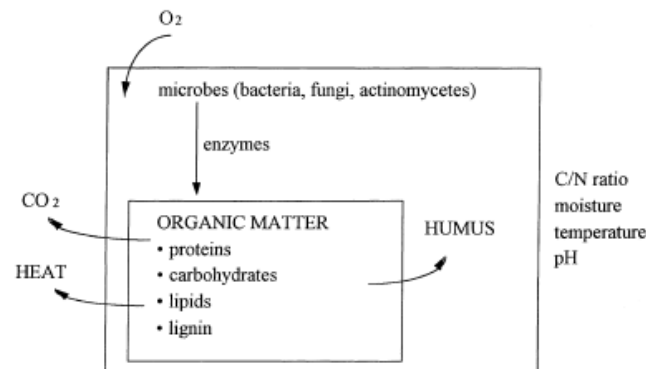


Figure 2: The composting process representation (Itävaara et al., 1995)

The three different stages of composting (Figure 3) have been extensively described by several authors (Epstein, 1997; Bhatia et al., 2013; Mehta et al., 2014).

The mesophilic stage is characterised by increasing temperatures involving a high rate of microbiological activity. Mesophilic organisms are the predominant organisms and readily degrade simple carbohydrates and proteins proceeded by thermotolerant and thermophilic microorganisms as the temperature increases over 45°C.

In the second stage (thermophilic), the biodegradation of the wastes is performed by thermophilic microorganisms that can reach the temperatures up to 70 °C. This high-temperature enables thermophiles to decompose polysaccharides, proteins and fats and is also essential for the destruction of weed seeds and pathogenic microorganisms (Bhatia et al., 2013; Pepe et al., 2013).

The final stage of the composting process is the cooling, when the compost gets the stability and maturity for their safe use and the mesophiles become again predominant at this final stage. This curing stage is characterized by lower temperatures and is essential obtain a stable compost through the biodegradation of the intermediate compounds (Epstein, 1997).

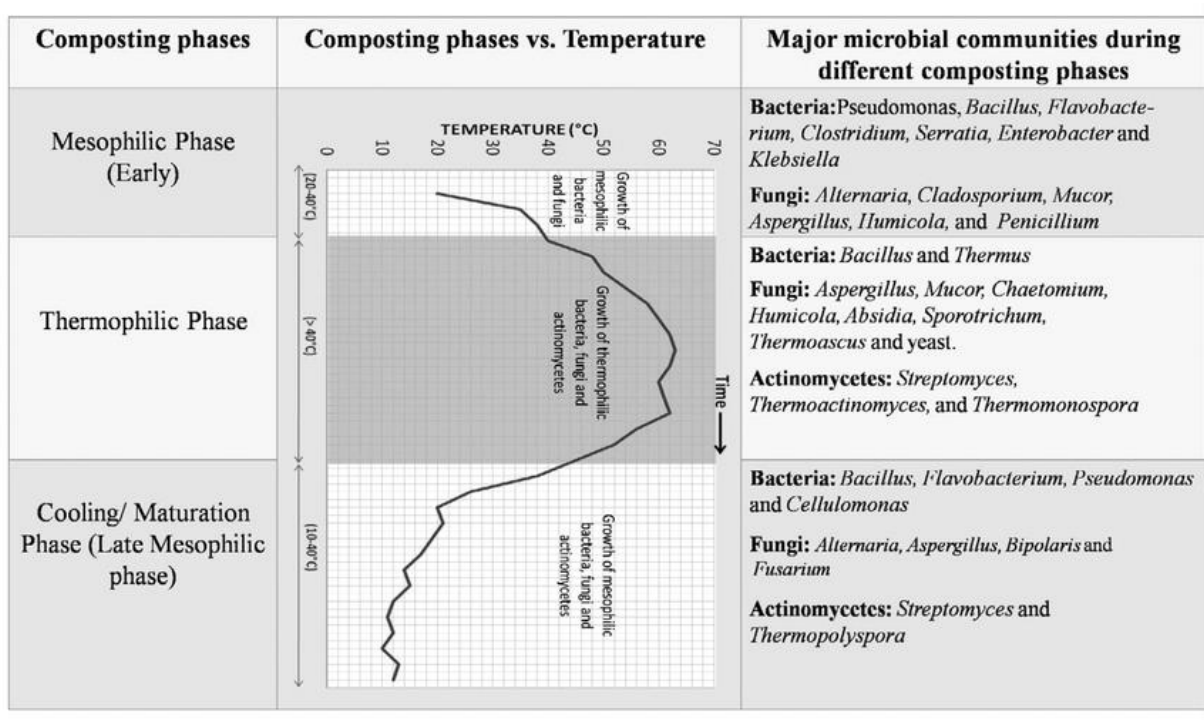


Figure 3: Schematic representation of the different stages of the composting process and the microbial communities involved (Epstein, 1997).

The presence of some microorganisms can be applied as an indicator of the stage and quality of the maturing phase (Bhatia et al., 2013; Pepe et al., 2013). The predominance of bacillales or actinobacteria is an example which can be used as an indicator of a good composting process (Sundberg et al., 2013).

Since composting depends on microorganisms, some conditions for the microorganism's survival should be maintained. Oxygen availability, C/N ratio and moisture are the most important parameters for composting process (de Guardia et al., 2008; Maeda et al., 2010). The changes in different parameters that occur with time during the composting process are interdependent and correlated to each other (Lin et al., 2016).

Oxygen is required for the composting process because the microorganisms and bacteria consume oxygen to degrade the organic material. The products are CO₂, H₂O and heat (Xu et al., 2013; Maeda et al., 2010). High temperatures in the windrow kill worm eggs, pathogens and bacteria that may be health hazards to people or animals. If oxygen is limited during the active phase of composting, the aerobic microbial activity may be inhibited and the temperature did not raise enough or gradually decreases (Fernandes et al., 1997).

The potential of microorganisms also relies on the particle size of the materials. The lower the particle size; the larger is the surface area available for microorganisms to degrade the materials (Zhao et al., 2017).

The optimum moisture contents for composting should range from 35 to 60%. However, the moisture levels from 50% and 60% have also been referred by many authors as an optimum moisture (Poincelot, 1974; Fernandes et al., 2007; Jindo et al., 2016). Should be considered that higher moisture contents lead to higher GHG emissions on account of it creates anaerobic conditions (Tamura and Osada, 2006).

There are various aeration methods applied on composting to provide oxygen for microbial activity, namely: forced aeration, natural convection and physical turning of feedstocks at regular intervals (Latifah et al., 2015). The main purpose of aeration is also to release the heat accumulation and take away extra moisture and CO₂ (Mohee and Mudhoo, 2005). During aerobic respiration, the microorganisms lead to the release of water. The aeration and the temperature increasing during the thermophilic stage, are factors that guarantee the feedstocks dry (Makan et al., 2014).

Temperature is considered a key factor which affects the composting process, since only a small variation in temperature directly influence the microbial activities (Ermolaev et al., 2015). Temperature is also considered the major parameter determining the inactivation of plant pathogens during composting under aerobic conditions and thermophilic anaerobic digestion, while toxic agents are considered to be responsible for pathogen inactivation during decomposition under mesophilic anaerobic conditions (Bollen and Volker, 1996; Ryckeboer, 2003). For phytosanitary reasons, composting conditions are required to reach a temperature of > 60°C for at least 3 days (Noble and Roberts, 2004). However there is another sources that indicate other values; for example, according to the California Compost Quality Council, for example, the reported temperature is 55 ° C for 15 days. During the thermophilic phase of composting, which may last several days, temporary and local anaerobic conditions widely occur (Fernandes and Sartaj, 1997; Veeken et al., 2002; Beck-Friis et al., 2003), leading to decomposition products that may be toxic to multiple organisms. Thus, in a compost heap, combined effects of temperature and anaerobic microsites may become responsible for pathogen eradication.

Carbon and N are the critical elements required for microbial decomposition. Carbon is used as an energy source even as the basic structure of the microbial biomass. Analogously, N is a constitutive part of various molecular forms such as the amino acids, peptides, proteins, enzymes, and DNA needed for cell function and growth (Wang and Zeng, 2017).

C/N ratios below 30:1 are recommended for rapid decomposition and rapid microbial growth, however may generate undesirable odors due to the loss of N in the form of NH₃ (de Guardia et al., 2008). Jindo et al. (2016) reported that a C/N ratio ranging from 25 to 38 promote

a satisfactory composting process. Higher C/N ratio limits microorganism activities, whereas a low C/N ratio leads to greater NH₃ emissions (He et al., 2000; Pagans et al., 2006; de Guardia et al., 2008; Möller and Stinner, 2009).

The type of waste used in composting and its own constitution play a fundamental role in the way the process is carried out. The main constituents of organic waste are cellulose, hemicellulose, and lignin. In addition, from these main polymers, organic waste comprises other structural polymers such as starch, proteins, waxes among others. These polymers are hydrolyzed by microbial extracellular enzymes into more simple molecules that are further metabolized (Vargas-García et al., 2010). Lignocellulose is a complex substrate and the biodegradation of each constituent could be different; the degradation capacity is not only dependent on environmental conditions alone, but also the degradative capacity of the microbial population (Waldrop et al., 2000).

Cellulose is the most abundant component of plants, found in almost every type of organic waste. Hemicellulose is a heteropolysaccharide composed of different hexoses, pentoses, and glucuronic acid (Riddech et al., 2002). Serramiá et al. (2010) observed a more pronounced degradation of hemicellulose comparing with cellulose, indicating that hemicellulose was the first cellulosic fraction used by microorganisms as C and energy source. Hemicellulose is a smaller branched carbohydrate compared to cellulose, which is made of very long unbranched fibrils held together by hydrogen bonding that prevents easy access by microorganisms and enzymes (Komiliis and Han, 2003). Komillis and Ham (2003) found that cellulose and hemicellulose are responsible for more than 50% of the total dry mass loss for most of the substrates used in their study. Lignin is a major structural component of plants and is the one that is degraded the slowest. Lignin acts as a protective factor for the cellulosic and hemicellulosic fractions and the biodegradation of lignin usually occurs tardily and at a very low rate (Malherbe and Cloete, 2002). Francou et al. (2008) have proposed the lignin/holocellulose ratio as an index to monitor the biodegradability of the organic material. This ratio can be useful to understand a compost resistance to degradation after soil application.

Lignocellulosic residues are rarely composted alone justly due to their high C/N ratios. However, they are commonly used as structuring agents along the composting of other wastes, particularly when these can exhibit aeration problems (Paradelo et al., 2013). Furthermore, Sanchez-Monedero et al. (2001), demonstrated that the inclusion in the mixtures of wastes with a high lignocellulosic content reduced N losses during the composting process.

Besides lignocellulosic compounds, there are another compounds, (also are characterized by a low degradation rate) that also highly influence the composting process and the degradation

of organic matter. There is the case of polyphenols, known mainly by their beneficial properties including their potential use when present in organic amendments (Spatafora, 2012).

The compost stability and maturity represent relevant requirements for safely compost use for agricultural purposes.

Carbon stability is related to the lack of a labile-C pool inducing fast organic matter decomposition, whereas maturity is associated with low phytotoxicity and high plant growth potential, usually obtained by a curing process following compost stabilization (Bernal et al., 1998; Wu et al., 2000; Benito et al., 2003; Cooperband et al., 2003).

Several strategies have been proposed to assess biological stability of organic matter, like as chemical and physical methods (López et al., 2010; Fernández et al., 2012), biological and enzymatic methods (Komilis et al., 2011; Barrena et al., 2014).

The biological tests are reported as the more suitable to realistically assess the achievement of the biological stability after a composting process. Respiration techniques, in particular, provide the more detailed compost stability characterization (Barrena et al., 2014).

There are different procedures used to describe compost maturity degree (Bernal et al., 2009), however in the most part of the cases it is usually evaluated by phytotoxicity tests. Tests used in both research studies and quality standards, using plants, can be divided into four broad categories: germination tests, growth tests, combinations of germination and growth and other biological methods.

1.3. Sources of GHGs emissions in composting

Generally, composting not only emits CO₂ and aqueous vapour but also trace amounts of other gases such as CO, NH₃, CH₄, N₂O, H₂S, NO_x and volatile organic compounds (VOCs), that are related to air quality (Lou and Nair, 2009; Peigne and Girardin, 2004; Adhikari et al., 2013; Nasini et al., 2016).

Jiang et al. (2015), reported that some by-products such as CH₄, N₂O and NH₃ are prejudicial to the environment and can cause secondary environmental pollution. Besides, according to Schlegelmilch et al. (2005) the emission of these gases could be linked with prejudicial health problems. Odorous Emissions constitute a relevant topic of the published studies in the field of gaseous emissions. Goldstein (2002), uncover alcohols, fatty acids, aldehydes, terpenes, ammonia and a scope of sulphur compounds as the main responsible of odour emissions at composting plants.

The optimization of composting conditions has been suggested as a method of avoiding a large portion of odourous emissions (Schlegelmilch et al., 2005), since studies reported that the major sources of odourous emissions usually occur when the biomass are delivered, preprocessed, along the composting process itself and during the turning over (Schlegelmilch et al., 2005; Szanto et al., 2007, Delgado-Rodríguez et al., 2012). Enclosing the composting operations and the use of biofilters are among the main mitigation strategies for both odours and GHG (Sánchez et al., 2005). The optimal initial mixture preparation and the maintenance of high levels of porosity to assure aerobic conditions in the pile are important managing practices also reported (Ruggieri et al., 2009).

The significance of GHG emissions formed during the wastes composting has been also exposed by several authors.

Ammonia is not considered a GHG, however its a composting gas product commonly studied due to its relation to the acid rains formation and due the nitrogen conservation at the end product. The pH value, C/N ratio, $\text{NH}_4^+/\text{NH}_3$ ratio, dry matter content, quantity and mineralisation intensity of N-compounds and temperature are the most relevant factors that influence NH_3 emissions (Martins and Dewes, 1992; Hellebrand and Kalk, 2001). During the composting process the increase in temperature and pH rises NH_3 emissions. One way to reduce NH_3 emission is to use biofilters equipments that has proved particularly useful for the in enclosed waste treatment plants (Hong and Park, 2004; Pagans et al., 2006).

In the composting process, the main GHG that can contribute to global warming are N_2O and CH_4 . CO_2 emissions coming from biological process are not considered to contribute to global warming since this carbon has a biogenic origin, i.e. this carbon has been previously fixed biologically (Sánchez et al., 2015). Emissions of N_2O and CH_4 depend on the management of the composting process and related to a lack of oxygen along the process (Cayuela et al., 2012; Colón et al., 2012). Even though they are generated in small amounts, these gases have a great contribution to global warming since they have higher warming potentials when compared with CO_2 (28 for CH_4 and 265 for N_2O) (IPCC, 2013).

Greenhouse gases emissions from composting processes are greatly related on the waste type and composition (Haug, 1993). Wastes with a high-water content and low C/N ratio have a wide potential for generating GHG emissions. Wastes lacking nutrients, porosity and structure, or low biodegradability, can inhibit the standard progress of the process led to a GHG emission increasing (Sánchez et al., 2015).

The aeration rate appears as the main factor affecting gaseous emissions during composting (Delgado-Rodríguez et al., 2011; Blazy et al., 2014). Coker (2012) and Gao et al.

(2010) explained this importance through an observation of wide range of functions during composting process such as: replenishing O₂, CO₂ and volatile compounds, removing water, temperature control and moisture variations.

The addition of mineral additives in agricultural waste composting is another alternative studied and effective for reduction of gaseous emission (Hao et al., 2005; Luo et al., 2014).

Is important to remember the role of compost as an end product in the role played by composting in GHG emissions. Even though the composting process could be a GHG source, the use of compost as an organic amendment can be used to mitigate GHG. Their application as an organic amendment, has a positive effect in GHG emissions since it induces that carbon stays bound to soil, although the typically lower content of other nutrients (N, P, etc.). Their application can also minimize the use of chemical fertilisers and pesticides (reduction in GHG emissions associated with their production and application).

The beneficial effects of compost application must be evaluated jointly with a real estimate of the amount of GHG emitted during the composting process. On the other hand, GHG emissions data are essential to improve knowledge about the contribution to composting in GHG emissions

Carbon dioxide (CO₂)

There are two main sources of CO₂ emissions from composting: non-biogenic and biogenic CO₂. Non-biogenic CO₂ comprises the emissions linked with energy and fuel consumption in the composting facility (Boldrin et al., 2009). The biogenic CO₂ emissions acquire from the biological degradation of the organic matter, mostly because of aerobic decomposition or from anaerobic processes or the oxidation of CH₄ by aerobic methanotrophic bacteria. Carbon dioxide emission accounts for the highest amount of gas produced during the process, since between 40 and 70 % of the original organic matter is oxidized (Haug, 1993). However, as referred above, the global warming potential of these emissions is not considered in the environmental impact of composting operations since this biological CO₂ (short C cycle) is considered to be carbon neutral (IPCC, 2007). Nowadays this gas is mainly studied for the stability evaluation of the end product (Barrena et al., 2006), index of the overall microbial activity (Hobson et al., 2005; Sánchez-Monedero et al., 2010) and mass balances establishment of composting operations (Boldrin et al., 2009).

Methane (CH₄)

When the diffusion of O₂ is insufficient or in anaerobic degradation systems the formation of CH₄ occurs (Font et al., 2011). Methane is oxidised by microorganisms in the upper layer of the compost raw material (Hao, 2001; Szanto et al., 2007; Andersen et al., 2010). The optimum conditions for methanogenic bacteria development are: an absence of oxygen (strict anaerobic microorganisms), neutral pH, redox potentials below -200 mV and the presence of nutrients and substrates rich in organic matter (Kebreab et al., 2006). Usually, these requirements can be temporarily found in the early stages of the composting process, where available sources of organic compounds and large amounts of nutrients promote microbial growth. Thus, most CH₄ emissions have been recorded during the first weeks of the process at the beginning of the thermophilic phase (Beck-Friis et al., 2000; Sánchez-Monedero et al., 2010). The high temperatures reduce the solubility of oxygen (Pel et al., 1997), facilitating the creation of anaerobic microsites inside the pile. In addition, high NH₄ concentrations can inhibit CH₄ oxidation. High ammonia concentrations or the presence of electron acceptors such as sulphates are other variables which inhibited the production of CH₄ by competition with sulphate-reducing bacteria (Hao et al., 2005; Kebreab et al., 2006; Sánchez-Monedero et al., 2010).

Methanotrophs, aerobic microorganisms colonising the surroundings of anaerobic zones, can play a considerable role in the production and consumption of other relevant GHG emitted during composting, such as N₂O and CO (Topp and Hanson, 1991).

Some researchers highlighted the importance of the composting feedstock, the control of moisture content, the height and shape of the pile, and turning frequency as the principal elements controlling CH₄ emissions during the process, since these variables will affect both the oxygen availability and gas diffusion in the composting pile (Kebreab et al., 2006, Brown et al., 2008). Sánchez-Monedero et al. (2010) reported a CH₄ emissions reduction associated with turning frequencies and watering. Humer et al. (2011), found that water holding capacity, electrical conductivity, bulk density, total organic carbon, organic matter and nutrient content, influence methane oxidation rate.

Nitrous oxide (NO₂)

Despite the fairly negligible amounts of N₂O emitted during composting, there is an increasing awareness about their emission from composting owing to the high global warming potential of this gas and its impact on the ozone layer (Smith et al., 2010).

There are distinct microbial pathways associated to the N_2O generation (Figure 4) namely nitrification, nitrifier denitrification and denitrification, among others, which may simultaneously occur at different locations (Czepiel et al., 1996; Kebreab et al., 2006; Maeda et al., 2011). Due to this complexity, the recognition of N_2O sources as well as the microorganisms involved in these processes still remains an important research subject (Maeda et al., 2011).

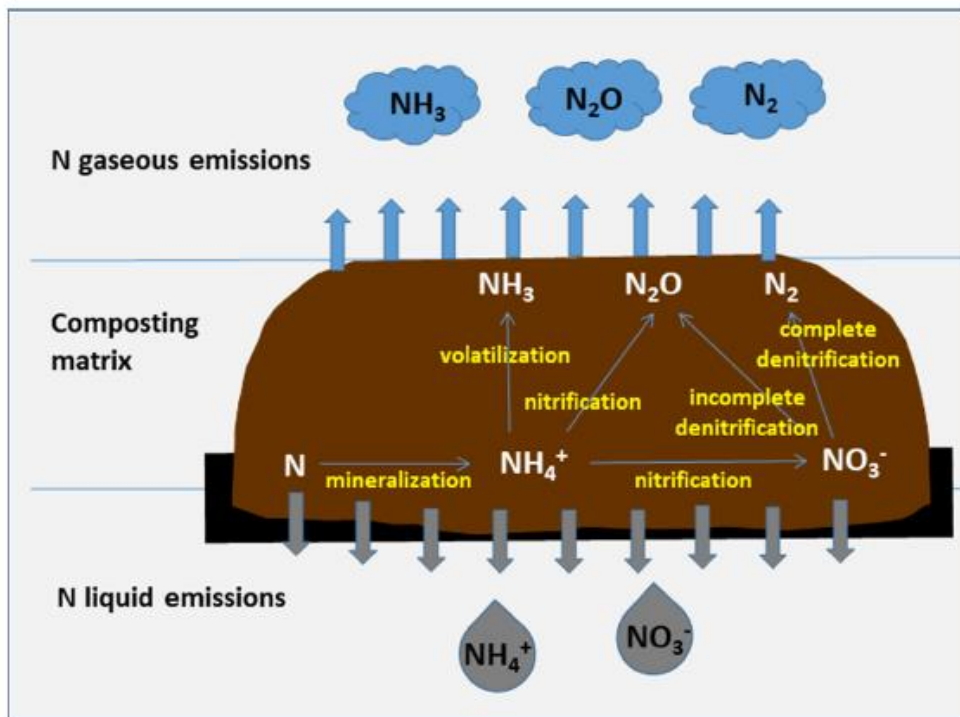


Figure 4: Nitrogen cycle within composting (Cáceres et al., 2018)

Nitrification is one of the main microbial processes leading to the emission of N_2O during composting. Aerobic nitrification involves the initial conversion of ammonia to nitrite by different genera of ammonia-oxidising bacteria, such as *Nitrososporas* and *Nitrosomonas*, and the oxidation of nitrite to nitrate by nitrite-oxidising bacteria, such as *Nitrobacter* (Kowalchuk et al., 1999; Maeda et al., 2010). The main precursor of nitrification is NH_4^+ , produced at early stages of the process, by ammonification of organic matter. The alkaline pHs found in composting matrices favour the transformation of soluble NH_4^+ into NH_3 , which is then firstly oxidised by ammonia-oxidising bacteria into NO_2^- and then converted to NO_3^- by nitrite-oxidising bacteria (Sánchez-Monedero et al., 2001).

The denitrification process (anoxic) is attained by heterotrophic microorganisms, the denitrifiers, which can use NO_3^- as the electron acceptor, inducing the reduction of NO_3^- to N_2 .

The nitrificants microorganisms require aerobic conditions, mesophilic temperatures (below 40°C), pH values above 5 and the presence of NH_4^+ . On the other hand, the denitrifiers

require anaerobic conditions or low O₂ concentration, the presence of available C sources and the presence of electron acceptors (NO₃⁻, NO₂⁻, or NO) (Kebreab et al., 2006).

Either aerobic or anaerobic conditions, nitrous oxide (N₂O) could be emitted. However, in absence of O₂, NO₃⁻ is reduced to N₂ without significant N₂O production (Czepiel et al., 1996). In this case, nitrifier denitrification (incomplete nitrification at low O₂ concentrations) can be responsible for the generation of N₂O during the initial step of ammonia oxidation and also as a consequence of NO₂ reduction. Under the O₂ absence and when available C was exhausted, nitrifier denitrification would be the major mechanisms leading to N₂O emissions, as stated by He et al. (2000), who observed an increase in the N₂O emission when the ratio between water-soluble C and dissolved N was lower than 5.

Hwang and Hanaki (2000), referred that the denitrification was the main source of N₂O at oxygen concentrations around 10 % and moisture levels between 40 and 60 %, and, in opposition, nitrification became more prevalent at higher oxygen concentrations.

Nitrous oxide emissions are influenced by aeration rate, temperature and nitrate content (Hellebrand, 1998). Amlinger et al. (2008) proposed that high aeration and effective stripping of NH₃ during the early stages of composting can reduce N₂O formation.

For the correct evaluation of the benefit of compost application we must have in mind the real knowledge about the amount of GHG emitted along the composting process like as the relation of GHG with some operational conditions and the technology applied. Studies on GHG emissions during the composting process are important to improve the knowing about their contribution to GHG emissions. In addition, we must also consider the role of the compost as final product. The compost application as an organic amendment can benefit to mitigate the GHG due to the reduction requirement in the use of chemical fertilizers and pesticides (Favoino and Hogg, 2008).

1.4. Potential use of composts as bio-fungicide

Soil-borne fungal plant pathogens represent one of the major causes for the decreasing on productivity of agro-ecosystems (McDonald and Linde, 2002). These pathogens could induce other problems like as vascular wilt, corm rot, yellows, root rot, and damping-off that could compromise the quality of foods and related products (Haouala et al., 2008).

During the last decade of the 20th century, the most common method to control soil-borne fungal diseases was the use of synthetic fungicides and the use of fumigants like methyl bromide (Martin, 2003). However, their constant and indiscriminate use lead to the

development of fungicide resistance in pathogens, atmospheric pollution, reduces the crop quality and increases the labour and costs to control the pathogen (Rice, 1995; Christopher et al., 2010).

In this context, the traditional strategies, such as the use of synthetic fungicides and resistant cultivars, are not completely adequate to control soil-borne pathogens (Bonanomi et al., 2010) and the search alternatives with less negative environmental effects, low cost and effectiveness is much needed for a eco-sustainable agriculture (Bonanomi, 2007; Mehta et al., 2014).

Different methods include biofumigation (Kirkegaard et al., 2000), biological soil disinfestations (Blok et al., 2000), solarisation (Katan, 1996) and application of biocontrol agents (Hoitink and Boehm, 1999; Ryckeboer, 2001) or organic amendments such as composts (Paulitz and Belanger, 2001; Bailey and Lazarovits, 2003). Indeed, greater attention is towards the use of composts to control plant disease due to their low cost investment and biological suppressive abilities that allow the reduction of chemical fungicide use (Mehta et al., 2014).

The use of compost in disease suppression was first proposed by Hoitink et al. (1975) that proposed their use as peat substitute. Afterwards, Lumsden et al. (1983), suggested the inclusion of compost in the growing media of different crops in order to suppress some soil-borne plant pathogens like *Fusarium oxysporum f. sp. cucumerinum*, *Rhizoctonia solani*, and *Sclerotinia sclerotium*.

Hoitink and Fahy (1986) demonstrated the potential of several types of agricultural and forestry wastes for compost preparation and their potential suppression of the *Rhizoctonia*, *Pythium*, *Fusarium* and *Phytophthora* soil-borne plant pathogens.

Since these primordial studies the disease-suppressive effect of composts has been broadly reported for several soil-borne pathogens and different composts (Table 1).

Some studies have shown that composts can be very effective in controlling diseases caused by pathogens like as *Pythium spp.* (Erhart et al., 1999; Alfano et al., 2011; Pane et al., 2011; Borrero et al., 2013; Vestberg et al., 2014), *Fusarium spp.* (Saadi et al., 2010; Huang et al., 2012; Suárez-Estrella et al., 2012, 2013; Blaya et al., 2013; Morales et al., 2016), *Rhizoctonia solani* (Pane et al., 2011; Xu et al., 2012; Borrero et al., 2013; Mengesha et al., 2017), *Phytophthora spp.* (Kyung-Sang et al., 2010; Alfano et al., 2011; Blaya et al., 2015), *Sclerotinia spp.* (Alfano et al., 2011; Pane et al., 2011), *Sclerotium spp.* (Coventry et al., 2005), *Botrytis spp.* (Yogev et al., 2010; Pane et al., 2011; Borrero et al., 2013) and *Verticillium dahliae* (Alfano et al., 2011). Several authors revealed in their studies, a suppression ability after compost application between 20 % and 90 % on suppression of *Fusarium* wilt (Serra-Wittling

et al., 1996; Cotxarrera et al., 2002; Reuveni et al., 2002; Postma et al., 2003). The authors attributed the main reason of this activity to the microbial activity.

Noble (2011), in their recent review, explore the positive and negative impacts of soil amendment with composts relatively to plant pathogens and verified that the risk of introduced diseases for the compost application was negligible. Meanwhile, when the effect of field application is compared with experiments as potting media, the compost effects became smaller and more inconstant.

Termorshuizen et al. (2006) tested 18 commercial composts in 7 plant pathogens i.e. *F. oxysporum* on flax (*Linum usitatissimum*), *Cylindrocladium spathiphylli* on spathiphyllum (*Spathiphyllum wallisii* Hort. cv. Ceres), *R. solani* on pine (*Pinus nigra* var. *austriaca*), *V. dahliae* on eggplant (*Solanum melongena*), *Phytophthora nicotianae* on tomato (*Lycopersicon esculantum* Mill.), *R. solani* on cauliflower (*Brassica oleracea* var. *botrytis*) and *P. cinnamomi* on lupin (*Lupinus spp.*). The results showed that disease suppressiveness vary from 14 to 61% per compost. After the application of 20 % of the chosen composts into potting sand or soil only 3 % revealed significant increasing of the disease, 43 % did not revealed significant differences and 54 % reduced the disease symptoms when compared to the control (without compost).

Bonanomi et al. (2007) analysed 2423 experimental case studies comprised in a total of 250 articles that testing several types of organic materials amendments including composts. The application of OM amendments significantly increased the disease incidence in 20 % of the cases and was found to be pathogen suppressive in 45% of the cases. The authors concluded that composts were the more effective material on disease suppression, with more than 50% of suppressive effect. Through the results of this study the authors pinpointed the need of more investigations for allow the application of these amendments more predictable and avoid the actual inconsistencies in their application.

Table 1: Summary of the composts studied as inducer of plant disease suppression, soil pathogens, main effects and mechanisms associated. The data referred to studies made since 2010, once Noble et al., made a similar review in 2011.

Compost materials	Disease suppression	Effects and mechanisms associated	References
Commercial compost and vineyard compost	<i>Rhizoctonia solani</i> AG3 (R299 and R422); <i>Alternaria solani</i> ,	Hyperparasitism and/or competition for <i>Alternaria</i>	Mengesha et al., 2017
Different residues and by-products from the fruit and vegetable processing industry	<i>Fusarium oxysporum f.sp. melonis</i>	The suppressive effect was probably related to high pH and pepper wastes and high content of pruning waste as initial raw materials	Morales et al., 2016
Different agro-industrial wastes; artichoke sludge; chopped vineyard pruning waste and various	<i>Fusarium oxysporum f.sp. melonis</i> ; <i>Phytophthora capsici</i>	Biological and microbiological parameters such as specific enzymatic activities (protease, NAGase, chitinase) in the case of <i>Fusarium</i> wilt caused by FOM and general enzymatic activities (dehydrogenase activity) in the case of <i>Phytophthora</i> root rot caused by <i>P. capsici</i> , could be used as potential indicators of compost suppressiveness.	Blaya et al., 2014
Biowaste composts, Poultry manure (PM) compost; sewage sludge compost;	<i>Pythium sp.</i>		Vesteberg et al., 2014
Different compost teas	<i>P. capsici</i> ; <i>P. parasitica</i>	Non-aerated compost teas were more beneficial than aerated compost teas.	Marin et al., 2014
<i>Bacillus</i> strain with mature composts	<i>Fusarium oxysporum f.sp. cubense</i>	All the results confirmed that application of bio-organic fertilizer could more effectively control fusarium wilt disease in field conditions by improving soil microbial communities.	Shen et al., 2013
Vineyard pruning wastes compost inoculated with <i>Trichoderma</i>	<i>Fusarium sp.</i>	The higher degree of compost suppressiveness achieved after the addition of <i>T. harzianum</i> may be due not only to its biocontrol ability, but also to changes promoted in both abiotic and biotic characteristics of the growing media	Blaya et al., 2013
Cork compost; grape mark; olive marc; cotton gin trash; mushroom	<i>Fusarium oxysporum f.sp. lycopersici</i> ; <i>Fusarium oxysporum f.sp. dianth</i> <i>Pythium aphanidermatum</i> , <i>Rhizoctonia solani</i> and <i>Botrytis cinerea</i>		Borrero et al., 2013
Agro-industrial waste-based compost	<i>Xanthomonas campestris</i> and <i>Fusarium oxysporum f.sp. melonis</i>	The suppression could be caused by induced resistance mechanisms	Suaréz-Estrela et al., 2013
Pig manure compost; cattle manure compost; medicine residue compost	<i>Fusarium</i> wilt disease	Suppressiveness effect in field conditions occurred by improving soil microbial communities.	Shen et al., 2013

Table 1 (Cont.)

Compost materials	Disease supression	Effects and mechanisms associated	References
Aerated fermentation extracts of compost (AFEC) and non-aerated fermentation extracts of compost (NAFEC)	<i>Rhizoctonia solani</i> AG4	The microbial population in compost extracts was considered to be the most Significant factor of inhibition pathogenic fungal growth	Xu et al., 2012
Pig manure and sewage sludge composts	<i>Fusarium oxysporum</i>	PCR-DGGE results indicated that the fungal community had a significant correlation to the disease severity	Huang et al., 2012
Aerated compost teas (CTs) from five types of compost	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> and <i>Pyrenochaeta lycopersici</i> .	Antibiotic-like antagonism effect due to active microorganisms in suspension	Pane et al., 2012
Agroindustrial subproduct-based composts that	<i>Fusarium oxysporum</i> f. sp. melonis (FOM),	The causal agent of Fusarium wilt of melon, was due to a combination of biotic and abiotic factors	Suaréz-Estrela et al., 2012
Olive waste composts	<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i> , <i>Pythium ultimum</i> , <i>Phytophthora infestans</i> , <i>Sclerotinia sclerotiorum</i> and <i>Verticillium dahliae</i>	The disease suppressive effect of OW composts seems to be due to the combined effects of suppression phenomena caused by the presence of microorganisms competing for both nutrients and space as well as by the activity of specific antagonistic microorganisms	Alfano et al., 2011
Composted residues obtained from a viticulture and enological factory; composted organic fraction of differentiated municipal bio-waste; composted organic fraction of undifferentiated municipal bio-waste; composted cow manure	<i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> and <i>Sclerotinia minor</i>	The most useful parameters to predict disease suppression were different for each pathogen: extractable carbon, O-aryl C and C/N ratio for <i>P. ultimum</i> , alkyl/O-alkyl ratio, N-acetyl-glucosaminidase and chitobiosidase enzymatic activities for <i>R. solani</i> and EC for <i>S. minor</i> .	Pane et al., 2011
Water extracts of composts from commercial compost facilities	<i>Phytophthora capsici</i>	The suppressions effect might result from direct inhibition of development and population of <i>P. capsici</i> for root infection, as well as indirect inhibition of foliar infection through ISR with broad-spectrum protection.	Kyung-Sang et al., 2010
Compost prepared from tomato plants and separated cow manure	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>	It was demonstrated that compost suppressiveness against fusarium wilt of melon can be maintained for at least one year under a wide range of storage conditions, without any loss of suppressive capacity	Saadi et al., 2010
Compost made of tomato-plant residues mixed with the coarse fraction of separated cattle manure	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> ; <i>Botrytis cinerea</i>	It is suggested that induced resistance could be an additional mechanism involved in Fusarium disease suppression by composted manure and tomato residues.	Yogev et al., 2010

Vestberg et al. (2011) within the framework of an Indo-Finnish project tested twenty-one commercially composts on their ability to suppress the pathogens *Pythium spp* and *Phytophthora cactorum* and concluded that there aboutt one third of the composts manifest suppressiveness capacity against the tested pathogens.

Despite all these studies, we must also keep in mind that composts are living substrates and their suppressive ability prediction is hard due to the high variability of their characteristics. These variations happen due to the composting processs conditions (eg. temperature and moisture), the raw materials chosen, and the proportions used (Raviv, 2013). In fact, the production of reproducible and stable composts during two or more successive years is very difficult and laborious (Vestberg et al., 2014). Some authors referred that the most important factors for predicting composting suppressivity were feedstock origin (Termorshuizen et al., 2007), compost maturity (Tuitert et al., 1998), and application rate (Serra-Whittling et al., 1996; Tilston et al., 2002). Besides, the suppressive ability is directly related on the activities of extant antagonistic microorganisms, plant host and pathogen strains (Morales et al., 2016). Study and understanding all these interactions at ecological, biological and molecular level will make it possible to overcome the gaps that still exist in this field.

Two approaches can be carried out to rise the level of compost suppressiveness. The first one should be the development of consistent and reproducible composting conditions for guarantee the production of composts with predictable biological and physicochemical characteristics. Second, composts can be inoculated with biological control agents (BCAs) in order to increase their suppressive capacity. This possibility has been investigated and there was observed a significant increase in the suppressive effect (Noble and Coventry, 2005). In many studies, *Trichoderma* BCAs have been used for rising the suppressive effect (Hoitink, 1990, Trillas et al., 2006, Pugliese et al., 2011, Bernal-Vicente et al., 2012). Scheuerell et al. (2005) concluded that currently available composts could potentially provide commercially acceptable control of *Pythium spp.*, but it is necessary to fortify composts with BCAs for the control of *Rhizoctonia solani* (Suarez-Estrela, 2007).

Suppression mechanisms in composts

The disease suppressiveness phenomenon has been divided in “specific” and “general” suppressiveness; the specific suppressiveness is related to a specific activity, e.g. antagonism,

of defined organisms whereas general suppressiveness is conferred by non-specific activities of a numberless of organisms (Van Bruggen and Semenov, 2000).

The disease suppressiveness phenomenon comprise an intricate series of mechanisms (Figure 5) that have been suggested to explain the suppressive ability of composts imputed to their abiotic and biotic characteristics (Hadar and Papadopoulos, 2012; Vestberg, 2014).

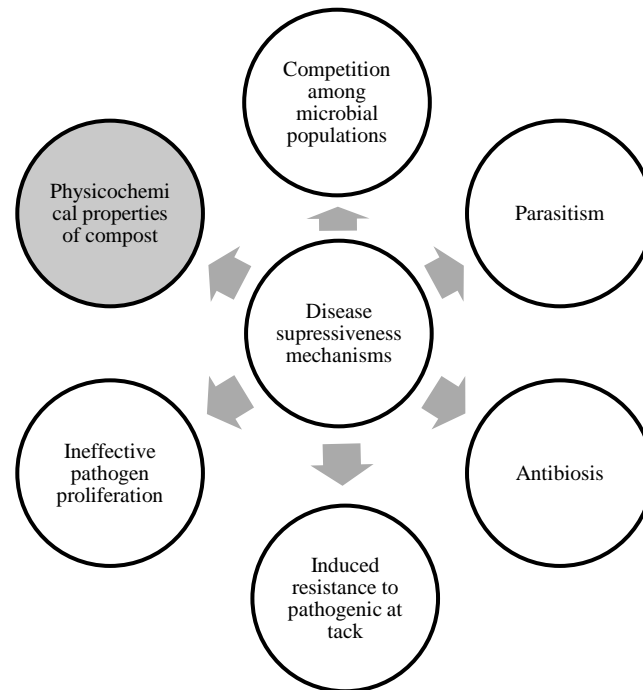


Figure 5: Scheme of the reported mechanisms that explain the suppressive phenomenon.

These include physical and chemical mechanisms, such as competition for carbon and nutrients, effects of humic and fulvic acids, release of toxic compounds during organic matter decomposition (Smolinska, 2000; Tenuta and Lazarovits, 2007) or biological mechanisms, like antagonism through antibiosis (Hoitink and Boehm, 1999), parasitism, incremented competition against pathogens for resources that cause fungistasis (Lockwood, 1990) antibiosis and systemic induced resistance in the host plants (Zhang et al., 1998; Pharand et al., 2002). This last mechanism has become a promising option for controlling plant pathogens through composts (Blaya et al., 2013). Composts that are suppressive to some pathogens and conducive to others cannot be extensively applied unless their effects on diseases can be accurately predicted (Bonanomi et al., 2010). The suppression capacity of composts is just in a few cases because to eradication of pathogens. Systemic resistance (Zhang et al., 1996; Pharand et al., 2002) and induction of fungistasis (Serra-Whittling et al., 1996) constitute the more accepted explanations, although not exclusive. Suppressive effects against soil-borne pathogens also

have been associated to carbohydrate availability (Chen et al., 1988; Wu et al., 1993; Borrero et al., 2006) and to the microbial activity of composts, in particular, the esterase enzyme activity measured by fluorescein diacetate (FDA) hydrolysis (Chen et al., 1988; Inbar et al., 1991; Craft and Nelson, 1996; Hoitink and Boehm, 1999) and β -glucosidase activity (Borrero et al., 2004).

Ntougias et al. (2008) exposed that the capacity of composts to suppress *F. oxysporum* could be imputed to the transition of microbial communities to actinomycetes, which could be included in the mechanisms of disease suppression due to the ability to produce chitinases and antibiotics (Patel et al., 2010).

Borrero et al. (2004) clarified some of the underlying mechanisms; however, little is known about the relationships between the microbiological and chemical characteristics of a compost and their disease suppression ability. Bonanomi et al. (2010) has made disease suppressive correlations with microbiological and physicochemical to predict the suppressive abilities of composts. They found that the most useful parameters are FDA activity, microbial biomass, total culturable bacteria, substrate respiration, *Trichoderma* population and fluorescent *Pseudomonas*. Regardless there are very few studies that identify the structure microbial community. The diversity of compost microbial communities may be the key to the capacity of composts to suppress soil-borne plant diseases, since the various mechanisms involved in compost-based disease suppression suggest an important role of the microbial communities present in composts and their relationship present in composts (Blaya et al., 2013, Metha et al., 2014). Each mechanism of suppression might be independently responsible for suppression of a specific pathosystem, but several mechanisms may function simultaneously in suppression of another disease (Hadar, 2012). Indeed, a diversity of microorganisms present in compost amended substrate are able of inducing systemic resistance in plants (Wei et al., 1991; Liu et al., 1995).

Some studies also suggest that the physicochemical properties of composts, such as phenolic or bioactive compounds, nutrients and organic molecules such as humic acids (Hoitink et al., 1997; Siddiqui et al., 2008; Spatafora and Tringali, 2012), may shield plants against pathogens by improving nutritional state, direct toxicity or induced systemic resistance (Metha et al., 2014)

Suppressive compost supplies a medium in which plant disease growth is reduced. Notwithstanding the great number of studies that demonstrate their bioactive effect, its practicable use is still limited. This fact is related to the absence of consistent and reliable prediction of the suppression effect and the compost behaviour variation (Hadar and Papadopolou, 2012). So, the individual evaluation of compost products for specific uses and

the development of standardized compost production and storage protocols is an actual and crucial need (Noble, 2011).

1.5. Aims of the study and thesis structure

The overall aim of this thesis is to analyse how the chemical composition of agroindustrial residues influence the composting process, the gas emissions involved and soil C and N mineralisation. Simultaneously was evaluated how the composting process itself influences the degradation of the different residues. Disease suppressive potential was also evaluated using four of the produced composts.

To achieve the aims above, the thesis was divided into five Chapters with the following specific objectives:

Chapter 1 – presents a general introduction about the main topics covered in this thesis;

Chapter 2 – the specific goal was to study the composting process of phytochemical rich materials to evaluate if their composition affects the composting process. Throught this task the production of bioactive composts were achieved. Determination and quantification of phytochemicals in the initial agroindustrial residues, intermediate mixtures and final composts were also performed. The global warming potential of the direct greenhouse gas emissions from composting were assessed.

Chapter 3 – the specific purpose was to determine the effects of amending composts on the mineralization of organic C and N added to the soil and on the emissions of the C and N gaseous compounds with environmental significance.

Chapter 4 – the target was determining disease suppressiveness potential of four selected composts against *Botrytis cinerea* and *Fusarium oxysporum* and simultaneously evaluate the effects on lettuce yield.

Chapter 5 – presents the general discussions and conclusions of the results of this thesis. Future perspectives were proposed based on the knowledge originated by this project.

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Chapter 2. Study of the composting process of different rich-phytochemical wastes materials

2.1. Effect of different rates of spent coffee grounds (SCG) on composting process, gaseous emissions and quality of end-product

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Abstract

The use of spent coffee grounds (SCG) in composting for organic farming is a viable way of valorising these agro-industrial residues. In the present study, four treatments with different amounts of spent coffee grounds (SCG) were established, namely, C₀ (Control), C₁₀, C₂₀ and C₄₀, containing 0, 10, 20 and 40% of SCG (DM), respectively; and their effects on the composting process and the end-product quality characteristics were evaluated. The mixtures were completed with *Acacia dealbata* L. shoots and wheat straw. At different time intervals during composting, carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) emissions were measured and selected physicochemical characteristics of the composts were evaluated. During the composting process, all treatments showed a substantial decrease in total phenolics and total tannins, and an important increase in gallic acid. Emissions of greenhouse gases were very low and no significant difference between the treatments was registered. The results indicated that SCG may be successfully composted in all proportions. Treatment C₄₀, was the treatment which combined better conditions of composting, lower GHG emissions and better quality of end product.

Keywords: *Acacia dealbata*, Coffee grounds, Compost, Greenhouse gases, Phytochemicals.

Introduction

Coffee is one of the most popular beverages in the world and the second largest traded commodity after petroleum (Daglia et al., 2000), as attested by the over 8.5 million tonnes produced in 2014 (www.ico.org).

During the production of espresso or hydrosoluble coffee, large amounts of solid residues (550 to 670 g/kg coffee beans) known as spent coffee grounds (SCG) are generated. Generally, these SCG do not have any commercial value or substantial application (Panusa et al., 2013). However, recently, different authors have reported some potential interesting uses of SCG, such as additives for biodiesel (Kondamudi et al., 2008) and as a source of natural phenolic antioxidants and bioactive compounds with great interest for pharmaceutical and cosmetic industries (Acevedo et al., 2013, Panusa et al., 2013). Furthermore, SCG have shown potential as fertilizers for agriculture and even as pellets for burning (Kondamudi et al., 2008, Limousy et al., 2013). However, burning of SGC is very controversial due to greenhouse gases emissions and their building up in the atmosphere (Limousy et al., 2013) which results in increased air pollution. The growing concern about the negative impacts of SGC on the environment and the increased social pressure to reduce pollution have forced researchers to look for new and effective recycling alternatives. Moreover, the findings about the high content of organic matter; the presence of bioactive compounds such as polyphenols; and other types of phytochemicals in SGC have led to new approaches. In this direction the bio-conversion of SCG into high added-value products seems to be a valid and promissory solution (Panusa et al., 2013).

Another alternative under investigation is the incorporation of SGC in soil as amendments. Soil amendment with organic residues is a common practice improving physical and nutritional soil properties. However, due to their chemical composition, SCG can be very toxic to many life processes. The incorporation of organic wastes in soil without complete degradation can immobilize plant nutrients causing phytotoxicity (Butler et al., 2001, Ros et al., 2006). To ensure that a compost respects the previous assumptions, compost's maturity can be assessed by different methods, based on physical, chemical and biological characteristics (Lasaridi and Stentiford, 1998). The most well-worn biological test used standard plants and microorganisms as models. However, organisms from other trophic levels are increasingly used in order to integrate all information and to access to the real environmental hazard. Pivato et al. (2016), tested solid and leaching composts to assess toxic effects on different organisms: Plant *Lepidium sativum*, earthworm *Eisenia foetida*, aquatic crustacean *Daphnia magna* and bacteria

Vibrio fischeri. Thus, prior to soil incorporation, SCG needs to be properly treated in order to eliminate or reduce any compounds that may have toxicity in the soil, and in that regard, composting has been considered effective. Composting has been a common practice for recycling biowastes, and it is considered a simple and efficient way of transforming agro-industrial wastes into a stable, non-toxic, pathogen free and plant nutrient-rich product, suitable for use as soil conditioner and plant fertilizer (Ros et al., 2006). Although composting is usually considered environment friendly, its effects can be seen as beneficial or harmful, depending on the associated gases' emissions.

Composting consumes oxygen (O_2) and releases into the atmosphere carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ammonia (NH_3) and other biogenic volatile compounds. Methane (CH_4) and nitrous oxide (N_2O) are of the highest importance since they are considered strong greenhouse gases (GHG) highly associated with global warming potential (GWP), thereby contributing to climate change (IPCC, 2013). The Fifth Assessment Report of the International Panel on Climate Change (IPCC) states that the GWP of CH_4 and N_2O , on a 100-year time frame excluding climate-carbon feedbacks, is 28 and 265 times higher than that of CO_2 , respectively. Ammonia is not considered to be a direct GHG but contributes to global warming because once deposited in the soil, it may be converted into N_2O after nitrification and denitrification reactions. Due to its high GWP, N_2O can contribute strongly to the carbon footprint, thus it is important to understand its formation during the composting process. The role of CO_2 emissions from composting activities emerges as less relevant since these emissions are considered as part of the C short cycle and therefore usually accounted as neutral (i.e. the GWP of CO_2 is considered zero) (Christensen et al., 2009). The quantification of GHG emissions from composting facilities is thus a very important step towards the improvement of composting emission models, and the development of environmental friendly technologies for GHG mitigation. In addition, available quantitative data on country-level GHG emissions from composting facilities can increase consistency and precision of data reported by different national and international databases (Andersen et al., 2010).

Composting of lignocellulosic wastes such as wheat straw and *A. dealbata* may be used together for supply extra carbon for microbial activity, balance the moisture and C/N ratio and avoid compaction of the composting materials (Kulcu and Yaldiz, 2007). Furthermore, Sanchez- Monedero et al., 2001, demonstrated that the inclusion in the mixtures of wastes with a high lignocellulosic content reduced N losses during the composting process.

Acacia dealbata represents one of the most critical invaders in some European countries such as Portugal, Italy, France and Spain (Sheppard et al., 2006). The plant affects the

sustainability and biodiversity of agro and natural ecosystems through modifications of some soil properties, nutrient cycles, as well as physical-chemical properties beneath plant canopy and the control of its spread is a key-issue in above mentioned countries. (Fried et al., 2014). Apart from *A. dealbata*, wheat straw is a great feedstock for composting considering its characteristics and since it is the second largest biomass in the world (Talebnia et al., 2010) with a low commercial value.

Therefore, the main objectives of this study were: (1) to study, during the composting process, the changes of physical and chemical characteristics of mixtures of SCG at different rates with *A. dealbata* and wheat straw (2) to measure the emissions of methane, carbon dioxide and nitrous oxide during composting, and; (3) to evaluate the end-product quality characteristics and the efficiency of the entire process.

Material and methods

Treatments and composting procedure

The study was conducted at University of Trás-os-Montes e Alto Douro (UTAD) campus, N 41°17'7.28"; W 7°44'36.83". The compost was produced in 135 L insulated reactors with continuous forced air circulation; the composting system was developed to allow the measurement of gases emissions during the process (Figure 6).

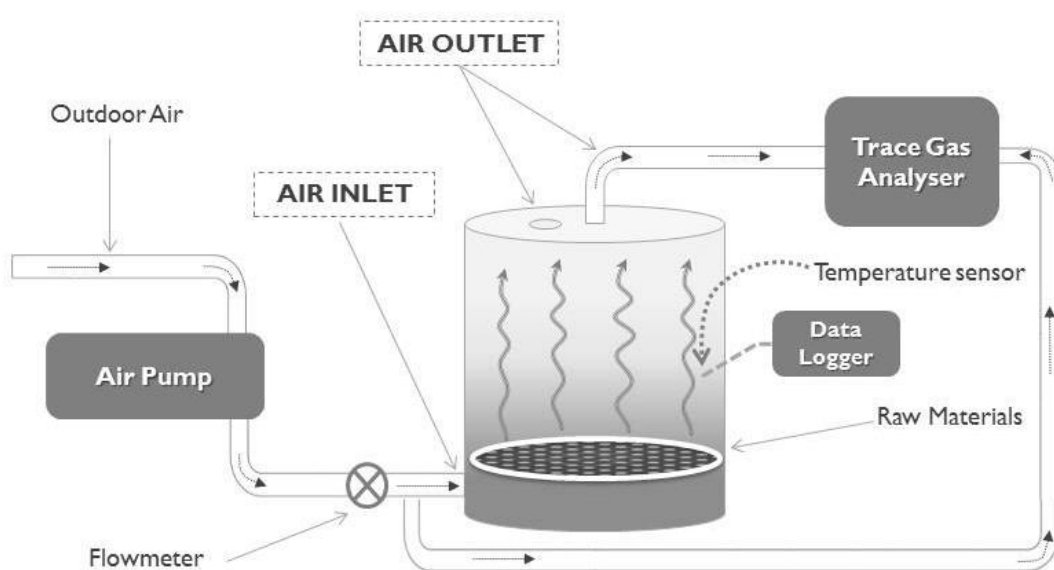


Figure 6: Schematic representation of the composting system and gas measurements.

Aeration was achieved by blowing air using an air pump (KNF, model N010. KN.18) connected to a punctured plastic plate located at the bottom of the reactor. A flowmeter equipped with a needle valve (Aalborg, model P, 5L min⁻¹) was used to control the aeration rate at 20 L h⁻¹ kg⁻¹ DM (dry matter) of the composting mixture. This rate was chosen to create an optimum aeration level that allows simultaneously reducing the CH₄, N₂O and NH₃ losses. Jiang et al. (2011), demonstrated that values of lower aeration rates exhibited longer emission periods and higher emission rates. In the opposite, higher aeration rates reduced the CH₄ emission but increase the N₂O and NH₃ emissions.

The structural materials used in the current study were *A. dealbata* and wheat straw. *A. dealbata* shoots were collected in the UTAD campus and were grinded using a shredder (Viking model GB 370 S); the wheat straw was grinded using a knife mill (Yike, 9FQ-360 straw hammer mill). Four different treatments were then established, and they consisted of four different rates of SCG co-composted with *A. dealbata* and wheat straw. The four rates of SCG considered were 0, 10, 20 and 40% of total DM, and are referred in all the study as treatments C₀, C₁₀, C₂₀ and C₄₀, respectively. In all treatments, the proportion (dry weight basis) of *A. dealbata* and straw in the portion of structural material was kept at 35:65, respectively. Each treatment was done in triplicate and the treatment C₀ was prepared with only *A. dealbata* and straw and was considered as control.

The coffee grounds were supplied by a private company and dried in a laboratory oven (Memmert, UL 60) at 60 °C to remove all the humidity and inhibit microbial processes. After, these materials were mixed and used in composting according to their properties (Table 2) to achieve a mixture with a C/N ratio of ca. 20, the the idea being to obtain a compost rich in nitrogen for plant fertilization.

Table 2: Main characteristics of the raw materials.

	TOC (g C kg ⁻¹ of DM)	TN (g N kg ⁻¹ of DM)	C/N
Wheat straw	467.6±5.59	5.06±0.20	92.5±2.51
<i>A. dealbata</i>	500.7±3.10	33.5±4.33	15.1±1.86
Coffee grounds	512.1±0.99	25.4±0.06	20.2±0.09

About 10 kg DM of each mixture was composted over 156 days. The moisture was maintained in the range 45-60% of water content by adding water when necessary. The mixture was homogenized manually revolving each mixture every week during the most active phase of compositing, and then every 15 days throughout the maturation period. The temperature in

the center of each reactor was measured daily during the 10 first days, and thereafter at increasing intervals until day 105 of the composting period, using a digital thermometer (model Prima Long).

Samples were taken on days 0, 3, 9, 22, 41, 64, 105 and 156, mixing subsamples from different areas of each reactor. Each sample was divided into 3 parts: one part was frozen and stored for determination of physical and chemical properties; the second part was used to determine DM; and the third was dried at 40°C for phytochemicals determination. Samples from the period with highest gases emissions i.e., first six weeks (day 0, 3, 9, 22 and 41), were analysed for mineral N.

Physical and chemical analysis

For physical and chemical properties analyses, the samples were ground with a coffee mill and sieved (particle size < 1 mm). The following analysis were carried out in fresh samples: electrical conductivity (EC), pH and Kjeldahl N were determined by standard procedures (CEN, 1999); Soluble Organic Carbon (Soluble OC) and Soluble Organic Nitrogen (Soluble ON) were determined by an elemental analyser (Formac, Skalar) by NIRD and chemiluminescence detection, respectively, after extraction with CaCl_2 0.01 M (1:10 w/v); ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) were extracted by shaking 3 g of sample with 30 ml KCl 1M and concentrations were determined in filtered extracts by molecular absorption spectrophotometry (Houba et al., 1995). The concentration of the elements Ca, Mg and K were measured in dry samples by atomic absorption spectroscopy after nitric-perchloric acid digestion and total P was measured by flame photometry (CEN, 1999); total organic carbon and total organic nitrogen were determined also in dry samples by NIRD (near infrared detector) and thermic conductivity, respectively. Then, the C/N ratio was calculated.

The determination of polyphenols included the extraction and quantification of total phenolics, simple phenolics, total tannins, and individual phenolics. The total and simple phenolics content were determined by spectrophotometric methods prior to extraction with the Folin-Ciocalteu reagent, according to the method of Javanmardi et al. (2003). The total tannin content was calculated by the difference between the average content of total and simple phenolics. Quantification was done using a calibration curve of gallic acid (Sigma-Aldrich, Taufkirchen, Germany) as commercial standard, and all the data were expressed as mg gallic acid equivalent (GAE) g^{-1} DM.

The individual polyphenol profile of each sample was evaluated by a HPLC-DAD system, with a mobile phase of water with 1% of trichloroacetic acid (TFA) (solvent A) and acetonitrile with 1% TFA (solvent B), at a flow rate of 1 mL min⁻¹. The separation was done using a C18 column (250 × 46 mm, 5 µm). The gradient used started with 100 % of water, injection volume of 10 µL, and chromatograms were recorded at 280, 320, 370 and 520 nm. Polyphenols were identified using peak retention times, UV spectra and UV maximum absorbance bands, compared with those found in literature and with external commercial standards. The quantification was performed using internal (naringin, Sigma-Aldrich, Germany) and external standards. The external standards gallic acid, (+)(-) catechin, (+) epicatechin, ellagic acid, chlorogenic acid, protocatechuic acid, caffeic acid, ferulic acid, and luteolin-7-*O*-glucoside were purchased from Extrasynthese (Cedex, France). All standards were freshly prepared in 70% methanol (methanol:water) at 1.0 mg mL⁻¹ and ran in HPLC-DAD before the samples. Methanol and acetonitrile were purchased from Panreac chemistry (Lisbon, Portugal) and Sigma-Aldrich (Taufkirchen, Germany), respectively. The aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore). All data were expressed as µg g⁻¹ DM.

Gas measurement

The measurements of CO₂, N₂O and CH₄ emissions were performed using a photo-acoustic analyzer (1412 Photoacoustic Field Gas Monitor, Innova AirTech Instruments, Ballerup, Denmark) with internal filters for small particles and water vapor, and optical filters for CO₂ (filter UA0982), N₂O (filter UA0985) and CH₄ (filter UA0969). The photo-acoustic analyzer was connected to Teflon tubes placed on air inlet and outlet entries of each composter (Figure 6). The CO₂, N₂O and CH₄ detection limits of the analyzer were 1.5, 0.03 and 0.4 ppm, respectively. Gas measurements were carried out daily during the first 10 days of composting and thereafter on days 12, 14, 16, 19, 22, 26, 29, 36, 41, 43, 49, 57, 64, 70 and 93. The flux of CO₂, N₂O and CH₄ in each sampling period was calculated using the gas concentration in the outlet air of each composter and discounting the concentration of each gas in the inlet air. The accumulated gas emissions between sampling periods were calculated as the average of the flux between successive measurements multiplied by the time interval between sampling periods.

Statistical analysis

All the experiments were conducted in triplicate, and the data are presented as mean values \pm standard error mean (SEM). A Two-way repeated measures ANOVA analysis was performed and the differences between the means were separated by the Tukey's test at the probability level of 0.05. Posteriorly, the mean separation was performed using a software routine that allow analyzing simple main effects of one factor (treatment) limited to a particular level of another factor (day). Pearson's correlation tests were computed for the variables to assess their linear association. Principal Components Analysis (PCA) was conducted as a mean to identify the best composts and variables that best described the separations between treatments. All these analyses were done using the software Statistix 10.0 (Analytical Software, Tallahassee, USA).

Results and discussion

Temperature

It is largely accepted that each stage of fermentation is characterized by a different temperature and different types of microorganism activities, and hence the names, i.e., mesophilic, thermophilic, and cure stages. Therefore, each stage is characterized by a specific group of chemical and physical properties. Temperature is considered one of the most important tools for monitoring the quality of the composting process, since it can help to define the actual stage, the type of microbial activities going on, and consequently, the final quality of the compost (Tiquia et al., 1996; Kulcu and Yadiz, 2014).

According to our temperature results presented in Figure 7, a similar trend of variation in all treatments occurred. We noted, a quick sharp increment of temperature, reaching a peak of 57.6°C in the third day of composting, followed by a fall to near 54°C on day 4 (Figure 7). The decrease in temperatures after day 4 was more accentuated for the treatments C₀ and C₁₀ for which the thermophilic phase (over 40°C) ended by day 6. For the treatments C₂₀ and C₄₀, the duration of the thermophilic phase was longer (8 days).

The sharp rise in temperatures in the first days of fermentation can be explained by the increment of microbial metabolic activities (Cunha-Queda et al., 2007), mainly resulting from the regular aeration of the medium, but also from the intrinsic nature of the raw material, particularly its level of moisture, organic matter and C/N ratio. In fact, at the beginning of the composting process, there is higher amount of readily degradable compounds such as sugars,

fatty acids, pectins, amino acids, among others, boosting the microbial metabolic activity and thus, sharply incrementing the temperatures.

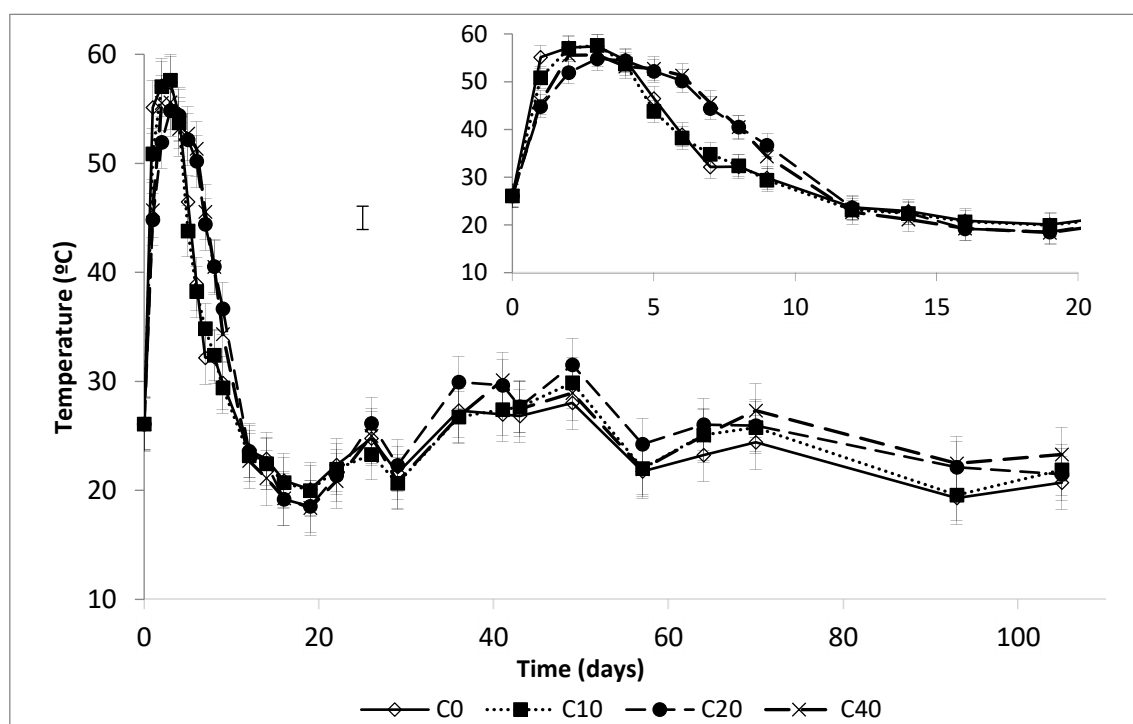


Figure 7: Temperature profile during the composting period. The inner figure represents the temperature profile in the first 20 days.

On the opposite side, the temperature decrease during the curing period is due to the decay of microbiological activities caused by depletion of easily degradable organic materials (Caceres et al., 2006). Although some authors have reported that aeration can delay the fermentation process through release of heat from fermentation piles (Himanen and Hanninen, 2011), our results from small-scale reactors seems to contradict this, as aeration, at least at the beginning of the process, seems to have helped incrementing microbial activities through increasing the oxygen flow, and causing a rise in temperatures. Since there was a very short period with high temperatures to guarantee *Acacia* seed destruction, it is important that this feedstock do not include viable seeds. Simple management approaches, as the harvest of *Acacia* shoots before flowering, must be considered to avoid collection of seeds.

After 14 days of experiment, the average temperature returned gradually to a mesophilic phase of around 22°C in all treatments. The return of temperatures to values near the ambient conditions at this stage is a good indicator that the most active fermentation process was almost complete, and the thermophilic phase and respective microbial activities have slowed down.

Physic-chemical characteristics of the compost

The maturity, stability and quality of any compost can be assessed by determining changes in chemical and physical properties. The changes in physic-chemical characteristics of the mixtures during the composting process are presented in Table 3.

The interaction between treatment x composting time period showed significant effects on all the parameters with the exception of C/N ratio and TOC.

Moisture loss during the first phases of composting can be used as an index of decomposition rate (Epstein, 1997) because the heat generation from microbial activities drove water evaporation from soluble solids.

Despite this initial loss, the moisture content remained in the optimal range for the composting process to proceed (Richard et al, 2002), and since water content was adjusted frequently, no significant differences ($P>0.05$) were observed in moisture levels between treatments.

The electrical conductivity (EC) measures the salinity of the compost and it is also a good indicator of its maturity. The values between 0.75 and 1.99 dS m⁻¹ are considered the most acceptable for the germination of seeds and for seedling establishment (Epstein, 1997). In this study, the EC values in final composts ranged from 0.78 to 1.62 which indicates an efficient composting process and attests to the usefulness of the compost produced as crop amendment.

Altogether, a slightly decrease in EC was seen in the final stage of composting and this could be related with the precipitation of minerals or the volatilization of ammonia (Epstein, 1997). The variations in the EC values can also be explained, in part, by the variations in water moisture, given that a negative correlation (data not shown) was observed between the moisture content and the EC ($r = -0.46$; $p < 0.01$). Thus, the lower value of EC measured at the end of composting for the treatment C₄₀ (0.78 ± 0.04 dS m⁻¹) may be related with the highest value of moisture ($74.1\% \pm 1.0$) observed at that specific time period (Table 3).

Table 3: Evolution of physic-chemical parameters during composting.

	Moisture (%)	EC (dS m ⁻¹) (water 1:5)	pH	Soluble OC (g C kg ⁻¹ of DM)	TOC (g C kg ⁻¹ of DM)	Soluble ON (mg N kg ⁻¹ of DM)	TN (g N kg ⁻¹ of DM)	NH ₄ ⁺ -N (mg N kg ⁻¹ of DM)	NO ₃ ⁻ -N (mg N kg ⁻¹ of DM)	C/N
Day 0										
C₀	60.5±0.3 ^a	1.40±0.04 ^a	5.7±0.0 ^a	11.56±0.00 ^{bc}	535.9±12.3	3.79±0.09 ^b	24.2±0.4 ^a	878.9±25.3 ^a	2.1±0.2 ^b	22.2±0.9
C₁₀	61.3±0.4 ^a	1.38±0.02 ^a	5.8±0.1 ^a	13.12±0.10 ^a	536.7±9.9	4.82±0.11 ^a	25.2±0.6 ^a	674.6±16.4 ^{ab}	1.9±0.1 ^b	21.5±1.9
C₂₀	60.1±0.4 ^a	1.26±0.02 ^a	5.8±0.0 ^a	12.27±0.11 ^{ab}	493.1±4.0	4.65±0.07 ^{ab}	25.4±0.6 ^a	558.6±16.8 ^b	7.0±0.1 ^a	19.4±0.3
C₄₀	52.0±0.1 ^a	1.24±0.01 ^a	5.9±0.1 ^a	10.11±0.00 ^c	496.0±7.5	4.12±0.10 ^{ab}	26.5±0.8 ^a	622.5±14.9 ^{ab}	8.7±0.2 ^a	18.7±0.7
Day 3										
C₀	68.6±1.1 ^a	0.91±0.13 ^a	7.2±0.1 ^a	6.60±0.07 ^c	486.7±2.3	2.42±0.18 ^c	30.1±1.3 ^a	444.1±92.4 ^b	2.7±1.0 ^b	16.4±1.5
C₁₀	64.2±2.2 ^a	1.05±0.16 ^a	6.5±0.3 ^b	6.43±0.48 ^c	487.4±9.8	2.53±0.25 ^{bc}	28.3±0.8 ^a	533.4±54.2 ^{ab}	2.5±0.5 ^b	17.4±1.5
C₂₀	66.5±0.3 ^a	1.11±0.08 ^a	5.5±0.2 ^c	8.71±0.53 ^b	509.8±8.0	3.17±0.27 ^b	28.1±0.4 ^a	579.4±38.1 ^{ab}	3.8±0.3 ^{ab}	18.2±0.7
C₄₀	56.2±0.3 ^a	1.31±0.08 ^a	5.3±0.1 ^c	10.51±0.18 ^a	521.3±2.7	4.25±0.06 ^a	25.7±0.9 ^a	744.6±23.4 ^a	5.2±0.1 ^a	20.6±1.8
Day 9										
C₀	64.4±2.0 ^{ab}	2.11±0.25 ^a	7.1±0.1 ^a	23.69±2.55 ^a	519.3±9.2	3.53±0.49 ^a	30.8±1.6 ^a	353.1±56.0 ^a	n.d.	17.2±1.7
C₁₀	67.6±0.2 ^a	1.63±0.04 ^a	7.1±0.0 ^a	15.04±0.66 ^a	521.6±2.8	2.59±0.11 ^a	24.2±2.8 ^a	394.9±61.2 ^a	n.d.	25.0±3.7
C₂₀	69.0±1.6 ^a	1.79±0.24 ^a	7.1±0.1 ^a	18.53±0.82 ^a	539.9±32.8	3.96±0.19 ^a	30.6±2.9 ^a	426.4±36.1 ^a	n.d.	19.5±1.3
C₄₀	52.3±2.2 ^b	1.80±0.16 ^a	6.5±0.1 ^b	22.36±2.35 ^a	459.8±47.6	5.27±0.62 ^a	23.4±1.4 ^a	520.2±69.7 ^a	n.d.	19.3±0.3
Day 22										
C₀	56.3±2.4 ^a	2.59±0.19 ^a	6.5±0.2 ^a	24.95±0.40 ^a	544.7±2.2	4.68±0.07 ^a	29.9±1.2 ^a	262.0±36.6 ^a	n.d.	18.4±1.5
C₁₀	52.7±1.3 ^a	2.53±0.08 ^{ab}	6.7±0.1 ^a	27.22±1.47 ^a	551.6±1.8	4.74±0.22 ^a	26.3±0.4 ^a	256.5±16.0 ^a	n.d.	21.0±0.7
C₂₀	46.0±1.5 ^a	2.64±0.13 ^a	6.6±0.0 ^a	24.20±0.88 ^a	542.8±9.1	4.45±0.18 ^a	25.8±1.0 ^a	273.5±21.5 ^a	n.d.	21.2±1.7
C₄₀	55.3±3.0 ^a	1.96±0.23 ^b	6.3±0.0 ^a	25.39±1.69 ^a	541.1±3.8	4.80±0.37 ^a	30.7±0.6 ^a	295.8±42.9 ^a	n.d.	17.7±0.7
Day 41										
C₀	64.7±1.2 ^a	2.20±0.07 ^a	7.3±0.1 ^a	22.39±1.13 ^a	514.4±7.9	5.42±0.29 ^a	27.3±0.5 ^a	459.9±32.7 ^a	n.d.	18.9±0.5
C₁₀	59.6±1.2 ^a	2.14±0.04 ^a	6.9±0.1 ^a	22.59±1.11 ^a	486.5±3.3	5.26±0.19 ^a	20.7±2.3 ^a	428.4±46.2 ^a	n.d.	26.1±3.1
C₂₀	65.8±1.9 ^a	1.78±0.13 ^{ab}	7.1±0.1 ^a	20.32±0.31 ^a	491.7±3.6	5.12±0.04 ^a	20.1±0.5 ^a	318.8±20.2 ^a	n.d.	24.5±0.4
C₄₀	71.8±0.5 ^a	1.44±0.08 ^b	7.0±0.0 ^a	24.68±0.77 ^a	507.6±13.2	6.61±0.22 ^a	23.2±1.5 ^a	281.6±18.9 ^a	n.d.	22.6±1.6

Day 64										
C₀	62.8±0.8 ^a	2.35±0.11 ^{ab}	6.9±0.0 ^a	18.64±0.25 ^a	560.7±4.4	4.75±0.03 ^a	34.1±1.6 ^a	n/a	n/a	16.7±0.8
C₁₀	59.0±1.2 ^a	2.45±0.09 ^{ab}	6.6±0.1 ^{ab}	20.74±0.12 ^a	552.0±17.0	5.21±0.11 ^a	31.9±1.4 ^a	n/a	n/a	18.9±1.0
C₂₀	60.8±1.7 ^a	2.53±0.03 ^a	6.5±0.1 ^b	24.10±0.31 ^a	550.2±5.3	5.87±0.15 ^a	32.7±0.6 ^a	n/a	n/a	16.9±0.8
C₄₀	67.0±0.7 ^a	1.94±0.01 ^b	6.5±0.0 ^b	25.41±0.43 ^a	548.9±6.0	5.67±0.04 ^a	30.6±0.4 ^a	n/a	n/a	18.0±0.8
Day 105										
C₀	62.3±1.5 ^{ab}	2.65±0.01 ^a	6.8±0.1 ^a	18.65±1.50 ^a	555.8±20.0	4.17±0.28 ^a	35.9±1.9 ^{ab}	n/a	n/a	16.6±0.7
C₁₀	49.1±2.2 ^b	2.52±0.16 ^{ab}	6.6±0.1 ^a	17.71±1.16 ^a	432.3±33.1	4.39±0.31 ^a	30.3±2.1 ^b	n/a	n/a	14.2±0.4
C₂₀	55.6±4.9 ^{ab}	2.05±0.05 ^{bc}	6.8±0.0 ^a	21.61±2.88 ^a	492.9±63.0	4.83±0.62 ^a	37.9±3.6 ^{ab}	n/a	n/a	12.7±0.8
C₄₀	67.6±2.5 ^a	1.79±0.09 ^c	6.6±0.1 ^a	30.13±3.63 ^a	497.7±39.5	6.98±0.96 ^a	44.5±2.6 ^a	n/a	n/a	11.1±0.5
Day 156 (Final composts)										
C₀	61.0±1.1 ^{ab}	1.33±0.17 ^{ab}	7.0±0.1 ^a	3.22±0.09 ^b	509.5±1.4	1.33±0.07 ^b	42.4±0.7 ^b	n/a	n/a	12.1±0.5
C₁₀	55.5±3.1 ^b	1.53±0.35 ^a	6.8±0.1 ^a	3.72±0.23 ^b	510.2±10.8	1.84±0.07 ^b	41.8±2.1 ^b	n/a	n/a	12.5±0.8
C₂₀	62.7±2.9 ^{ab}	1.62±0.40 ^a	7.0±0.2 ^a	3.98±0.12 ^b	484.4±16.9	2.03±0.07 ^b	46.0±0.6 ^b	n/a	n/a	10.6±0.5
C₄₀	74.1±1.0 ^a	0.78±0.07 ^b	7.0±0.1 ^a	5.48±0.24 ^a	507.6±6.3	2.84±0.25 ^a	59.7±1.4 ^a	n/a	n/a	8.6±0.6
ANOVA										
Treatment	0.457	0.000	0.000	0.003	0.812	0.002	0.232	0.843	0.012	0.474
Day	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.000	0.000	0.000
Treatment x Day	0.000	0.005	0.000	0.000	0.689	0.000	0.024	0.001	0.000	0.364

¹ Values are expressed as mean ± SEM (standard error of the mean) of three replicates.

² n.d. - not detected.

³ n/a - not analyzed.

Values within a column, for the same day, with the same letter are not significantly different. The columns without any letter did not shown significant differences.

The pH analysis of composted samples from the different treatments showed a gradual increment, from slightly acidic to neutral, probably due (i) to the mineralization of proteins, peptides and amino acids into ammonia, (ii) to the degradation of acidic compounds, such as phenolic acids, organic acids, and carboxylic groups during the thermophilic phase (Hachicha et al., 2009), and/or (iii) to the production of humic substances which can act as buffers (Zenjari et al., 2006) and attenuate extra pH changes due to their neutralizing capacity.

The final pH, near to 7.0 in all treatments, indicates a mature compost suitable for most cultivated crops (Lasaridi and Stentiford, 1998). In addition, this pH reflects a classical evolution of the compost properties in aerobic conditions, suggesting the formation of humic substances (Hachicha et al., 2009).

The evolution of total organic carbon (TOC) during composting is another parameter that can be used as an index of compost stability and maturity, allowing the monitoring of the decomposition throughout the process. No significant differences were observed in TOC along the composting process, although a tendency for a decreasing trend was noticed. This is related with the mineralization of labile organic compounds. Throughout the composting process, the organic compounds are being lost, increasing the proportion of the conservative compounds. As a consequence, TOC decrease slowly during the process.

We observed a significant ($p < 0.001$) difference between treatments for soluble organic carbon (soluble OC), particularly at the beginning and end of the composting process. At the beginning of the experiment, the treatment C₁₀ showed the highest content of soluble OC ($13.12 \pm 0.1 \text{ g C kg}^{-1} \text{ DM}$) and the treatment C₄₀ presented the lowest soluble OC content ($10.11 \pm 0.0 \text{ g C kg}^{-1} \text{ DM}$); but as the experiment progressed, this tendency changed. For example, on day 3, the C₁₀ treatment presented soluble OC values significantly lower than those of the C₂₀ and C₄₀ treatments. These results are probably due to the fact that the former treatment contained lower levels of coffee grounds and so, the carbon sources were rapidly depleted. At the end of the maturation period, the C₄₀ treatment showed the highest values of soluble OC ($5.48 \pm 0.24 \text{ g C kg}^{-1} \text{ DM}$) while the C₀ treatment instead showed the lowest values ($3.22 \pm 0.09 \text{ g C kg}^{-1} \text{ DM}$). A similar tendency was observed for the soluble organic nitrogen (soluble ON) fraction. We noted significant differences ($p < 0.01$) among treatments in the initial and final periods of the process. On day 3, the soluble ON in treatment C₄₀ ($4.25 \pm 0.06 \text{ g N kg}^{-1} \text{ DM}$) was significantly higher ($p < 0.05$) than the values found for C₀, C₁₀ and C₂₀ treatments. After this day, all treatments presented similar values of soluble ON throughout the experiment until day 156 when the C₄₀ treatment showed the highest value of ON ($2.84 \pm 0.25 \text{ g N kg}^{-1} \text{ DM}$) and the C₀ treatment showed the lowest value ($1.33 \pm 0.07 \text{ g N kg}^{-1} \text{ DM}$).

Total nitrogen (TN) increased during composting for all treatments. At the end of the experiment (day 156), the C₄₀ treatment ($59.7 \pm 1.4 \text{ g N kg}^{-1} \text{ DM}$) has a significantly higher value of TN ($p < 0.05$) than the other treatments. From these results, we can state that the addition of 40% of SCG in the C₄₀ treatment resulted in a compost with up to 40% more TN than that of the C₀ treatment.

The dynamics of mineral N (NH_4^+ and NO_3^-) in the first six weeks are presented in Table 3. The NH_4^+ content in all treatments decreased throughout the 41 days; however, the decrease was without significant differences among treatments, except for treatment C₄₀ on day 41, which showed a lower value ($281.6 \pm 9.5 \text{ mg NH}_4^+ \text{-N kg}^{-1} \text{ DM}$) compared to the other treatments (318.8 to 459.9 $\text{mg NH}_4^+ \text{-N kg}^{-1} \text{ DM}$). The highest NO_3^- concentration was observed on day 1 in C₂₀ ($7.0 \pm 0.0 \text{ mg NO}_3^- \text{-N kg}^{-1} \text{ DM}$) and C₄₀ ($8.7 \pm 0.1 \text{ mg N kg}^{-1} \text{ DM}$) treatments. From day 9 to day 41, we observed a total absence of NO_3^- (values below detection limit), probably due to the oxidation of NH_4^+ .

The C/N ratio is usually used as a parameter for the determination of the compost maturity. A compost with a C/N ratio below 20 is considered acceptable and labelled as “mature”. Nonetheless, it’s preferable to achieve a C/N ratio of 15 or lower (Morais and Queda, 2003). Although, in the present work, the initial C/N ratios of the mixtures were already low, this parameter decreased from the initial values of 18.7 – 22.2 to the final values of 8.6-12.1 (Table 3); suggesting a greater decomposition of organic matter (Liu and Price, 2011), and thus, adopting the criteria set by Morais and Queda (2003), we can state that at the end of the experiment our compost was mature. The decrease of the C/N ratio can be explained by higher losses of carbon compared to nitrogen, resulting from microbial activities during the fermentation process. Consequently, as discussed before, TN concentration increase in the final composts.

Regarding the mineral composition (Table 4), at the end of the experiment no significant differences ($p > 0.05$) were found between treatments for P and Ca.

Regarding K and Mg, C₄₀ presented the lower values. However, an increase in all concentrations was observed in the final compost, as a result of absolute carbon losses of the materials during the process, as have been previously reported in the literature (Kulku and Yaldiz, 2014, Liu and Price, 2011).

Table 4: Chemical analysis of macronutrients at selected composting times

Treatments	P (g kg ⁻¹ DM)	K (g kg ⁻¹ DM)	Ca (g kg ⁻¹ DM)	Mg (g kg ⁻¹ DM)
Day 0				
C ₀	0.95±0.03	7.75±0.04 ^a	0.56±0.05 ^a	8.82±0.33 ^a
C ₁₀	1.13±0.05	5.43±0.40 ^{ab}	0.74±0.07 ^a	9.98±0.42 ^a
C ₂₀	1.09±0.02	4.51±0.02 ^b	0.54±0.01 ^a	9.14±0.06 ^a
C ₄₀	1.02±0.03	3.62±0.03 ^b	0.51±0.05 ^a	10.08±0.45 ^a
Day 3				
C ₀	0.94±0.07	5.31±0.49 ^a	0.97±0.12 ^a	9.66±1.67 ^a
C ₁₀	1.22±0.08	6.33±0.56 ^a	1.51±0.29 ^a	9.45±0.21 ^a
C ₂₀	1.11±0.07	4.91±0.12 ^a	1.22±0.08 ^a	9.17±0.07 ^a
C ₄₀	1.24±0.05	5.47±0.92 ^a	1.19±0.02 ^a	8.26±0.14 ^a
Day 22				
C ₀	1.39±0.28	7.85±1.23 ^a	1.46±0.22 ^a	12.88±0.81 ^a
C ₁₀	1.17±0.04	6.61±0.20 ^a	1.43±0.03 ^a	10.57±0.28 ^{ab}
C ₂₀	1.46±0.13	7.14±0.66 ^a	1.67±0.43 ^a	10.64±0.60 ^{ab}
C ₄₀	1.46±0.11	5.46±0.13 ^a	0.79±0.02 ^b	9.52±0.31 ^b
Day 156 (Final composts)				
C ₀	2.17±0.06	19.88±0.71 ^a	2.38±0.86 ^a	10.96±0.17 ^a
C ₁₀	2.11±0.13	19.53±0.85 ^{ab}	2.55±0.51 ^a	11.14±0.10 ^a
C ₂₀	1.77±0.40	16.8±1.64 ^{bc}	2.45±0.53 ^a	9.73±0.09 ^{ab}
C ₄₀	2.33±0.04	16.17±1.11 ^c	2.38±0.39 ^a	8.23±0.09 ^b
ANOVA				
Treatment	0,543	0,006	0,012	0,045
Day	0,000	0,000	0,000	0,001
Treatment x Day	0,188	0,111	0,232	0,023

Values are expressed as mean ± SEM (standard error of the mean) of three replicates.

Values within a column, for the same day, with the same letter are not significantly different. The columns without any letter did not shown significant differences.

Phenolic compounds

The results for the quantitative determination and qualitative composition of polyphenols, at the beginning and at the end of the experiment are presented in Table 5.

Different types of polyphenols were found in the material used to produce the composting matrix. The coffee grains presented the highest diversity of phenolic compounds (6 compounds), whilst *A. dealbata* showed lesser diversity, but higher contents, particularly of total tannins.

Table 5: Average content of polyphenols in raw materials and at the beginning and at the end of the composting process in each treatment

Treatments	Total phenolics (mg g ⁻¹ DM)	Simple phenolics (mg g ⁻¹ DM)	Total tannins (mg g ⁻¹ DM)	Tannins (µg·g ⁻¹ DM)	Phenolic acids (µg g ⁻¹ DM)				Flavonoids (µg g ⁻¹ DM)
				Gallic acid	Chlorogenic acid	Protocatechuic acid	Caffeic acid	Ferulic acid	Luteolin-7- <i>O</i> - glucoside
Initial material									
Straw	8.73 ± 0.27 ^b	0.01 ± 0.00	8.72 ± 0.27 ^b	23.99 ± 1.79 ^a	20.75 ± 6.86 ^a	n.d.	n.d.	n.d.	n.d.
<i>A. dealbata</i>	63.65 ± 2.41 ^a	0.05 ± 0.00	63.60 ± 2.41 ^a	24.16 ± 1.18 ^a	n.d.	n.d.	n.d.	n.d.	83.74 ± 11.87 ^a
SCG	12.02 ± 0.10 ^b	0.01 ± 0.00	12.01 ±0.09 ^b	23.83 ± 6.09 ^a	31.83 ± 2.36 ^a	172.41 ± 3.82 ^a	14.52 ± 0.95 ^a	12.18 ± 0.82 ^a	n.d.
Initial composts									
C ₀	44.43 ± 1.66 ^a	0.03 ± 0.00	44.40 ± 1.66 ^a	24.10 ± 1.19 ^a	7.26 ± 2.40 ^b	n.d.	n.d.	n.d.	54.43 ± 7.71 ^a
C ₁₀	41.46 ±1.50 ^{ab}	0.03 ± 0.00	41.43±1.50 ^{ab}	24.07 ± 0.58 ^a	9.61 ± 1.89 ^b	17.24 ± 0.38 ^c	1.45 ± 0.09 ^c	1.22 ± 0.08 ^c	49.41 ± 7.00 ^{ab}
C ₂₀	38.50 ± 1.34 ^b	0.03 ± 0.00	38.47 ±1.34 ^b	24.04 ± 0.54 ^a	11.97 ± 1.38 ^{ab}	34.48 ± 0.76 ^b	2.90 ± 0.19 ^b	2.44 ± 0.16 ^b	44.38 ± 6.29 ^{ab}
C ₄₀	32.57 ± 1.02 ^c	0.03 ± 0.00	32.54 ± 1.02 ^c	23.99 ± 1.81 ^a	16.68 ± 0.37 ^a	68.96 ± 1.53 ^a	5.81 ± 0.38 ^a	4.87 ± 0.33 ^a	34.33 ± 4.87 ^b
Final composts									
C ₀	1.18 ± 0.03 ^a	0.00 ± 0.00	1.17 ± 0.03 ^a	18.43 ± 0.91 ^c	n.d.	n.d.	n.d.	n.d.	n.d.
C ₁₀	1.47 ± 0.20 ^a	0.01 ± 0.00	1.46 ± 0.20 ^a	24.14 ± 2.64 ^{bc}	n.d.	n.d.	n.d.	n.d.	n.d.
C ₂₀	0.61 ± 0.04 ^a	0.01 ± 0.00	0.60 ± 0.04 ^a	44.15 ± 3.04 ^a	n.d.	n.d.	n.d.	n.d.	n.d.
C ₄₀	0.89 ± 0.04 ^a	0.00 ± 0.00	0.89 ± 0.04 ^a	28.02 ± 1.06 ^b	n.d.	n.d.	n.d.	n.d.	n.d.

¹ Values are expressed as mean ± SEM (standard error of the mean) of three replicates.

² n.d. - not detected.

Values within a column, for the same day, with the same letter are not significantly different. The columns without any letter did not shown significant differences.

Our results are in agreement with Anjos et al. (2014), who recently reported high contents of the flavonoid luteolin and gallotannins such as gallic acid and its respective isomers in different *A. dealbata* accessions. The wheat straw exhibited the lowest contents in polyphenols.

The compounds catechins, epicatechins and its respective isomers were not found in the current study; this is in agreement with findings from several other authors, which showed low contents of such compounds in coffee beans, probably resulting from degradation during the course of industrial treatments such as roasting, composting or fermentation (Verzelloni et al., 2011). After the mixing of initial materials with different proportions of SCG, the polyphenol content varied in all treatments due to the differing contribution of each material, and in general the differences were statistically significant ($p < 0.05$) (Table 5). After composting, except for gallic acid, the contents of all polyphenols decreased sharply. These decreases could be associated with microbial activities during the composting process. Ghaly et al. (2012), reported a reduction of 73.9% of phenolics during the composting process. However, Volf et al. (2014) contradicted some of these findings and stated that any degradation of phenolic compounds will be strongly dependent on the occurrence of three conditions: temperature, type of phenolics and thermal stability of phenolics. In addition, according to the same authors, gallotannins like gallic acid, ellagic acid and vanillic acid, have strong thermal stability and are only metabolized when temperature reaches values above 60°C, which was not the case in the current study. The maximum temperature reached during the composting process was 57°C, lower than 60°C, and thus, this seems to explain the similar contents of gallic acid in the beginning and at the end of the experiment.

Gas emissions

Carbon dioxide (CO₂) and methane (CH₄) emissions

The measurement of CO₂ emissions during the composting process is important because of the correlation between CO₂ levels and microbial activities, which can be used as a direct method for assessing the stability of the final compost (Kalamdhad et al., 2008). Moreover, CO₂ emissions measurements are surrogates for mineralization and full degradation of organic matter measurements; and composts can be considered “very stable” when the CO₂ fluxes are lower than 2 mg CO₂-C g⁻¹ day⁻¹. The results of CO₂-C emissions (mg h⁻¹ kg⁻¹ initial DM) during the composting process are shown in Figure 8.

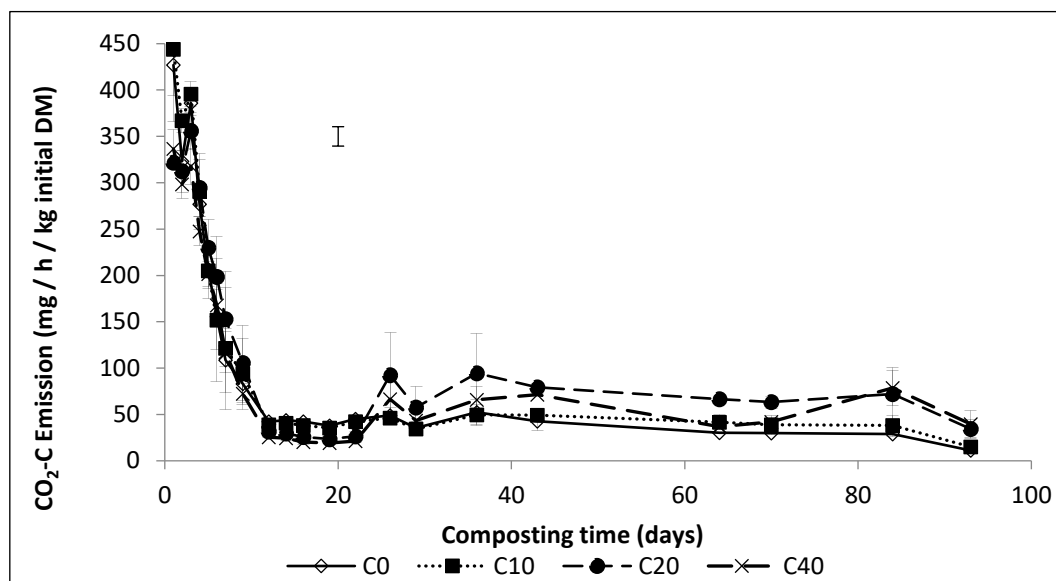


Figure 8: Emission patterns of CO₂-C during the composting period. The vertical bar represents the maximum standard error observed.

Adopting the criteria of Epstein (1997), we can state that the final composts obtained were very stable since the average values detected were always below the limit of 2 mg CO₂-C g⁻¹ day⁻¹. In addition, there were no significant differences ($p > 0.05$) between treatments over time. The highest emission value was observed during the first day (444.16 ± 50.34 mg CO₂-C h⁻¹ kg⁻¹ initial DM) of the experiment (C₁₀ treatment, Figure 3); and then, a slow decrease was observed during the first 4 days, followed by a sharp decrease from day 4 until day 9 (Figure 3). After day 9 and until the end of the experiment, the values were maintained below 100 mg CO₂-C h⁻¹ kg⁻¹ initial DM.

An increase in CO₂ emissions is associated with high microbial activities during the bio-oxidation of organic residues, and this can be used to state a thermophilic phase since the more labile organic matter is degraded, leading to high temperatures. On the other hand, a decrease in CO₂ levels indicates a lower microbial activity, a more stable composted organic matter, a lower temperature, and thus a mesophilic phase (Ros et al., 2006; Liu and Price, 2011). According to our data (Figures 7 and 8), the temperature and CO₂ emissions followed the same trends of change, which is in agreement with the previous sentence. Thus, we can state that the evolution of temperatures and CO₂ emissions during the composting process of our samples are highly correlated with each other ($r = 0.92$; $p < 0.001$). The mesophilic phase was extremely short and corresponded to the first day of composting. Afterwards, the highest CO₂ emissions were reached, corresponding to the thermophilic phase of the composting process with temperatures over 40°C, which lasted approximately until day 6. Later, the analysis of our data suggested two main periods, the first one characterized by a still active composting phase that

occurs until day 12 with high emissions of CO₂, and the second one with slightly constant temperatures corresponding to the phase of maturation with reduced CO₂ emissions.

Methane is formed by the deoxidization of CO₂/H and acetic acid by methanogens under anaerobic conditions (Epstein, 1997). The results from CH₄-C emissions (mg h⁻¹ kg⁻¹ initial DM) measured in different treatments during the composting process are shown in Fig. 4. Statistical analysis showed that CH₄ emissions were only affected by the sampling date ($p < 0.001$). Although the average values of CH₄ emissions were different between treatments, the trend of change of CH₄ emissions was very similar in all treatments (Figure 9). In the first 3 days of composting, we observed the highest emissions of CH₄ and then a sharp decrease to negative or near zero values until the end of the experiment. On day 1, CH₄ emissions were significantly different between treatments, with lower emissions for the treatment C₄₀ (-0.32 ± 0.09 CH₄-C h⁻¹ kg⁻¹ initial DM) compared to C₀ (0.61 ± 0.21 CH₄-C h⁻¹ kg⁻¹ initial DM) and C₂₀ treatments (0.85 ± 0.18 CH₄-C h⁻¹ kg⁻¹ initial DM). The highest CH₄ emission was recorded on day 3 for the C₁₀ treatment (1.48 ± 0.29 mg CH₄-C h⁻¹ kg⁻¹ initial DM).

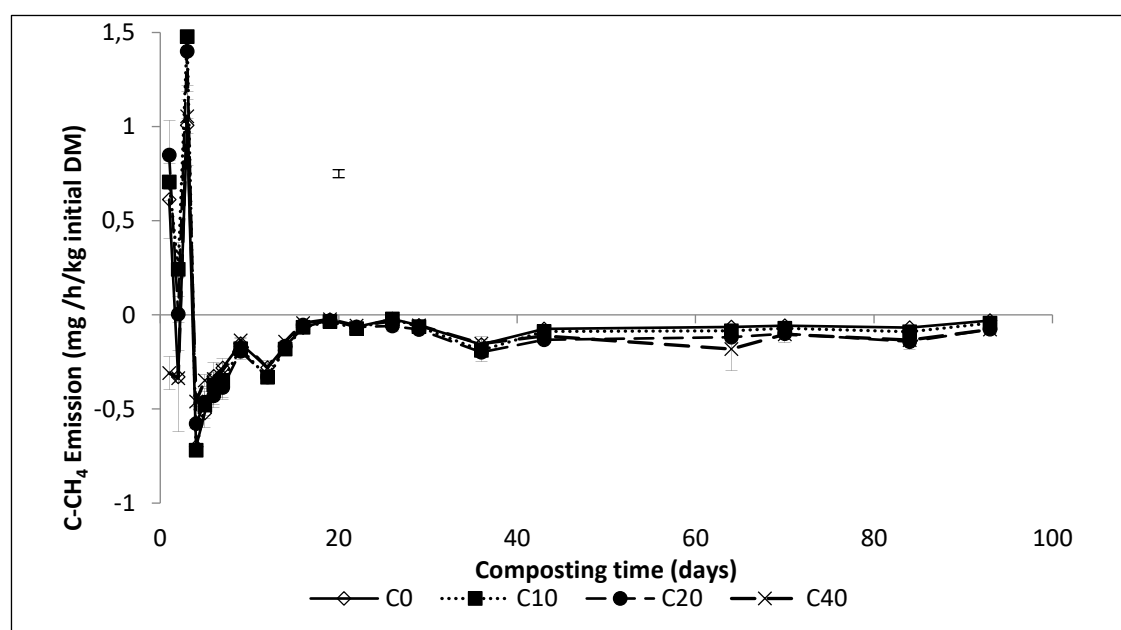


Figure 9: Emission patterns of CH₄-C during the composting period. The vertical bar represents the maximum standard error observed.

Methane emissions from the treatments in our study occurred mainly in the thermophilic phase, which is in agreement with the observations made by Li et al. (2013) using different organic materials and food wastes for composting. The largest emission of CH₄ in the first three days occurred probably due to the large amounts of nutrients and easily degradable organic

compounds that stimulated microbial activities, thereby reducing available oxygen and promoting optimum conditions for methanogenic bacteria.

From the third day till the end of composting, there was a stable trend in the emissions in all the treatments and during that period, the composts acted as CH₄ sinks. Furthermore, the negative fluxes of CH₄ seem to indicate an absence of CH₄ emissions, probably due to their oxidation as a consequence of the constant aeration performed during the composting process. In addition, we believe that the pronounced decrease of CH₄ emissions can also be related with the quick exhaustion of carbon compounds by microbes in the composting materials (Table 2).

Nitrous oxide (N₂O) emissions

The formation of N₂O occurs during the incomplete nitrification/denitrification processes that normally convert NH₄⁺ into N₂, a non-polluting gas. During denitrification, N₂O can be synthesized where there is a lack of O₂ and/or nitrate (or nitrite) accumulation (Philippe et al., 2012). During nitrification, N₂O is produced in the presence of O₂ and/or low availability of degradable carbohydrates (Epstein, 1997). Therefore, N₂O can be produced under both aerobic and anaerobic conditions. The results for nitrogen emissions in the form of nitrous oxide (mg N₂O-N h⁻¹ kg⁻¹ initial DM) are shown in Figure 10. Emissions of N₂O were only affected by the sampling date ($p>0.05$), with the results being very similar in all treatments except in the first two days of composting. The highest value of N₂O was recorded on day 2 in C₁₀ treatment (0.65 ± 0.11 mg NO₂-N h⁻¹ kg⁻¹ initial DM), followed by C₀ treatment (0.64 ± 0.12 mg NO₂-N h⁻¹ kg⁻¹ initial DM), and C₄₀ treatment (0.41 ± 0.04 mg NO₂-N h⁻¹ kg⁻¹ initial DM). The same trend was observed on day 1 where the emissions were significantly higher for the treatments C₀ (0.59 ± 0.12 mg NO₂-N h⁻¹ kg⁻¹ initial DM) and C₁₀ (0.49 ± 0.04 mg NO₂-N h⁻¹ kg⁻¹ initial DM) compared to C₂₀ (0.26 ± 0.08 mg NO₂-N h⁻¹ kg⁻¹ initial DM) and C₄₀ treatments (0.26 ± 0.11 mg NO₂-N h⁻¹ kg⁻¹ initial DM). After day 9 and until the end of the experiment, N₂O emissions were similar and the values remained close to zero.

These results indicate that N₂O production is greatly dependent of the composting materials; the specific characteristics of SCG and *A. dealbata* have determined N transformations, thereby promoting the activity of soil microbes, increasing nitrification and denitrification rates and subsequently N₂O emissions.

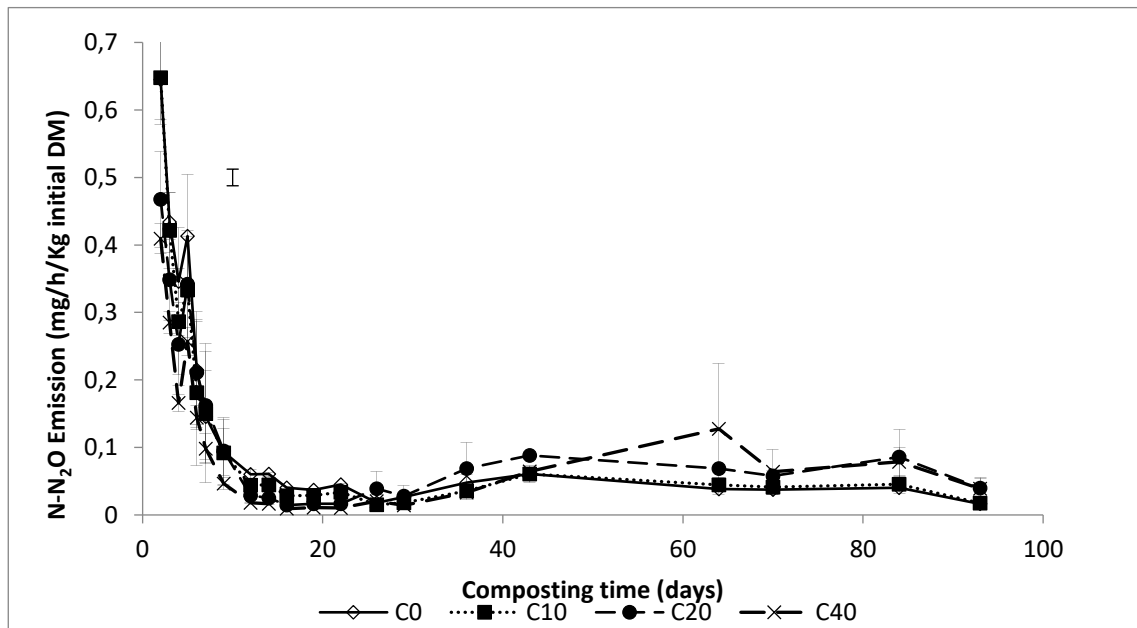


Figure 10: Emission patterns of N_2O -N during the composting period. The vertical bar represents the maximum standard error observed.

Treatments C_0 and C_{10} with lower rates of SCG, showed higher N_2O emissions; these emissions occurred with ammonia oxidation and depletion of nitrates as may be concluded by the evolution of mineral N content in the treatments (Table 3) simultaneously with an high composting activity, suggestion that denitrification may have been the main source of N_2O .

As with CH_4 emissions, we also noted that N_2O in all treatments were mostly emitted during the thermophilic phase; these results are similar to the emissions patterns previously reported by other authors (Sømmer and Møller, 2000), but contrary to the assumption of Thompson et al. (2004) that the activity of nitrifiers would be inhibited by high temperatures ($>40^\circ C$) and N_2O would be unable to be produced in the thermophilic phase. Additionally, emissions of N_2O during aerobic conditions may be caused by the transportation (denitrification) of NO_2^- - NO_3^- from aerobic portions to low O_2 concentration microsites in the middle of composting mixtures. Thus, high emissions of N_2O at the beginning of the composting process seem to be related with the composition of the materials, the temperature, and the intensive aeration and its effects, as described above.

Effect of the different treatments on composting process, gaseous emissions and quality of end products

Finally, an overview of changes observed during composting was displayed using PCA. Plotting (figure 11) the first two PC and calculating the distances between C₀, C₁₀, C₂₀, and C₄₀ on the PC1 axis clearly showed that there was an influence of the treatments on the combined measured parameters, which was stronger with increasing spent-coffee ground concentrations.

C₀ composts tended to cluster together with C₁₀ composts; this was mainly reflected in the CO₂ and N₂O emissions which remained similar between the two treatments, and decreased with increasing SCG concentrations, indicating that C₀ and C₁₀ were the least desirable treatments in terms of gaseous emissions.

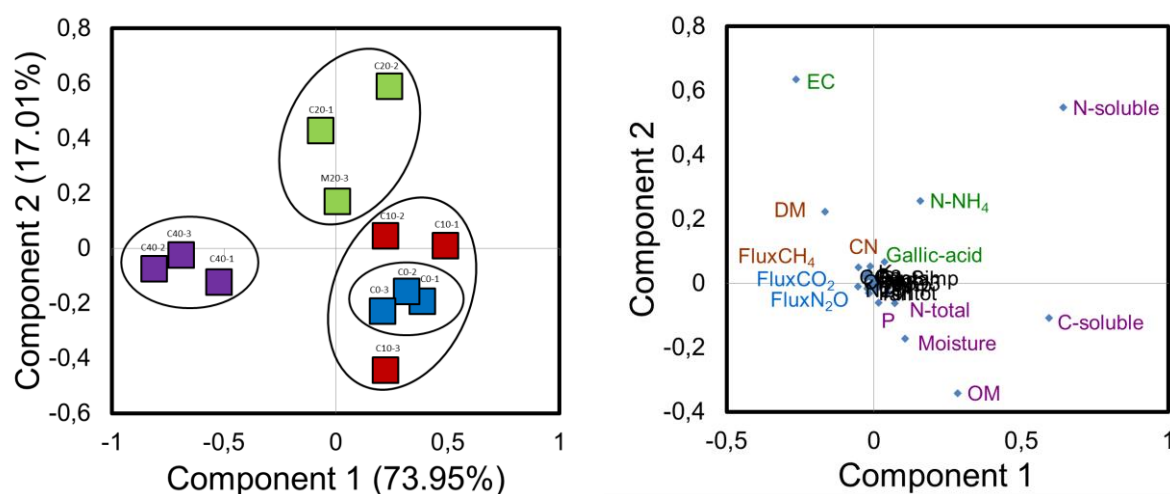


Figure 11: PCA biplots showing the effect of the different treatments on all parameters obtained: (A) separation of treatments (B) distribution of all parameters in relation to the treatments. Parameters near the centre of plot B are temperature, pH, N-NO₃, TOC, K, Ca, Mg, Total phenolics, Simple phenolics and Total tannins.

In general, however, the coefficient of variation of the PC scores was higher for C₁₀ than for C₀ (i.e., C₁₀ data sets were more dispersed on the PCA plots), which was related to higher C/N, DM and CH₄ contents in C₁₀ compared to all other treatments. Therefore, if composts with low C/N (denoting a more complete maturity state) and CH₄ emissions (denoting lower environmental impacts) are sought, C₁₀ is not the ideal choice.

The C₂₀ set of samples formed a distinct cluster near to C₁₀, but on the positive coordinates of the PC, in agreement with the less homogeneity of the responses. For example, EC increased from C₀ to C₁₀, peaked at C₂₀, and then decreased at C₄₀.

The N-NH₄⁺ content on the other hand increased at C₁₀, and then decreased at C₂₀ to peak at C₄₀. Although C₁₀ favoured higher gallic acid contents, its high EC and the observed

variability in the responses as compared with other treatments could disqualify its use in agriculture.

Changes observed were greater and more beneficial when using C₄₀, whose replicates formed a closer cluster some 1.25 distance away from C₀ on the PC1 axis. Differences were primarily caused by larger amounts of dissolved C (15%, 23%, and 70% from C₀ to C₁₀, C₂₀, and C₄₀ respectively) and N (37%, 47%, and 103% from C₀ to C₁₀, C₂₀, and C₄₀ respectively) as the concentrations of SCG increased. Minor contributors were OM, moisture, total N and P contents, which remained unchanged between C₀ and C₁₀, but increased by up to 19% with C₂₀ and up to 54% with C₄₀.

Conclusions

During the composting process of mixtures containing different proportions of spent coffee grounds (0 to 40%) an increase in pH and TN and a decrease in C/N ratio and TOC were observed. A substantial decrease in total phenolics and total tannins, and an important increase in gallic acid occurred.

Emissions of greenhouse gases, CO₂, N₂O and CH₄ were very low and no significant difference between the treatments was registered. Overall, it is possible to conclude that all SCG treatments are suitable for composting. However, C₄₀ which led to better conditions for composting (via moisture levels), quality end product (via EC, OM, dissolved C and N contents) and lower gaseous emissions, would be desirable compared with the other treatments.

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2.2. Effect of lignocellulosic and phenolic compounds on ammonia, nitric oxide and greenhouse gas emissions during composting

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Abstract

Composting is recognised a promising technology for recycling and adding value to agro-food wastes. There are, however, potential environmental risks associated with composting, such as the emission of greenhouse gases (GHGs). The aim of this study was to quantify the emissions of carbon dioxide, methane, nitrous oxide, nitric oxide and ammonia during composting of five agro-food wastes originated from the broccoli, chestnut, olive and grape industries, and to study the relationship between the emissions and the lignin, cellulose, hemicellulose and phenolic contents of the wastes. According to physicochemical indicators monitored during the process, all agro-food residues tested were suitable for composting, with chestnut in the top and broccoli in the bottom of the range. Composting of chestnut and olive led to higher carbon dioxide, methane and nitrous oxide emissions, whereas lower emissions were observed with white grape. A positive correlation was found between phenolics and nitric oxide ($r = 0.63$; $p < 0.01$), but not with the other gases. Lignocellulose showed a positive correlation with nitrous oxide ($r = 0.51$; $p < 0.05$), but not with carbon dioxide and methane. The accumulation of lignin was highest in the chestnut compost, which was associated with lower nitric oxide and ammonia

emissions relative to the other waste materials. Overall, the results show that wastes with high lignocellulose can be managed using composting with additional benefits on the environment in relation to mitigating nitrogen losses.

Keywords: Agro-food residues, Ammonia, Carbon fractions, Composts, Greenhouse gases, Nitric oxide, Organic wastes.

Introduction

Large amounts of agro-food wastes, generated as a consequence of rapid population growth and subsequent increase in food production and consumption, nowadays pose serious environmental and economic risks (Mirabella et al., 2014). The agro-food sector contributes to the loss of an abundant pool of organic valuable materials that could be used as a substitute for mineral fertilisers and pesticides, thereby promoting a more sustainable agriculture.

Crop residues represent a substantial portion of organic wastes. In southern Europe, grape, olive and chestnut crops are widely cultivated and wineries, chestnut and olive oil mills constitute the dominant agro-industrial activities in the region (Salgado et al., 2014). The winery industry for example, reportedly produces 14.5 million tonnes of grape byproducts in the region (Pinelo et al., 2006).

Direct soil application of agro-food wastes, especially those rich in bioactive compounds (e.g., polyphenols), is cautiously recommended due to toxic effects on microorganisms and plants (Medina et al., 2015). For these reasons, transformation and stabilisation of these wastes before their application to soil are imperative; composting provides a viable and secure technology option for achieving that goal. Composting can be used to effectively counter the toxicity of wastes while yielding a pathogens-free material. The composting process also reduces the waste volume and weight by about 50 % (Senesi, 1989) with a low investment cost compared with other waste treatment technologies (Lim et al., 2016); the reduction in weight ensures a stable final product which can be applied to improve the chemical and physical properties of soils (Bustamante et al., 2011), including the performance of plants (Santos et al., 2016).

Composting could lead to secondary pollution involving ammonia (NH₃), nitric oxide (NO) and greenhouse gases (GHG) emissions (Ermolaev et al., 2015), which are known drivers

of global warming and stratospheric ozone depletion. The primary GHGs in earth's atmosphere are methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) (IPCC, 2013). Although composting is considered to have a lesser environmental impact compared to other waste treatments (Saer et al., 2013), its advantages can be negated if the composition process is not optimized. Productivity gains following compost application should also be weighed against the amount of gases released during the composting process (Sanchez-Garcia et al., 2015). Therefore, quantitative studies on gaseous emissions during the composting process are needed to make such comparisons possible and to develop models; emission models would be useful for environmental assessments involving different conditions such as the waste type, chemical composition, the bulking agents and the processing unit airflow.

The last decade has seen a growing interest in GHG emissions during composting. For example, Sánchez-Monedero et al., (2001) evaluated the effect of olive mill wastes mixed with different agroindustrial by-products on GHG emissions; Cayuela et al. (2012) studied GHG emissions during composting of a lignocellulosic mixture added with different N sources. GHG emissions have also been addressed in composting studies involving varying experimental units and operation schemes: Amlinger et al. (2008) evaluated the influence of turning frequency and different aeration rates on GHG, NH_3 and N_2O emissions; Ermolaev et al. (2015) studied N_2O and CH_4 emissions at different temperatures. These studies collectively demonstrate that the rate of gas production, diffusion and emission from composting piles are highly dependent on the physical characteristics of the starting material, such as its temperature, and its microbial population. There is, however, a lack of information on the relationship between the physical properties of the wastes and GHG emissions during composting. It is reasonable to assume that chemical compounds, such as lignocelluloses and phenolics which are characterized by a low degradation rate, would highly influence the mechanisms involved in GHG emissions during composting.

The objectives of this work were to (i) assess the reuse/recycling potential of broccoli, chestnut, grape and olive wastes as composts, with consequent environmental implications regarding gaseous emissions during the composting process, and (ii) assess the relationship between the mechanisms involved in CO_2 , CH_4 , N_2O , NO and NH_3 emissions and the lignin, cellulose, hemicellulose and phenolic contents of the wastes. This information would be crucial for disposing and/or valorising agro-food wastes based on sound environmental decision criteria, and through implementation of improved management practices.

Materials and methods

Agro-food wastes and experimental design

The composting experiment was carried out at the Universidade de Trás-os-Montes e Alto Douro (N 41°17'7.28"; W 7°44'36.83"), and lasted ca. 5 months. The compost was produced in 135-L insulated reactors. The air was supplied continuously with a diaphragm pump (KNF, model N010.KN.18, Neuberger GmbH, Freiburg, Germany) from the underside of the reactor, where a perforated plate was positioned between the base and the composting mixture. The photoacoustic and chemiluminescent N oxide analysers used to measure gas concentrations were connected to the reactor with Teflon tubes positioned on the air inlets and outlets. Additional information about the composting system can be found in Santos et al. (2017). Aeration of the composting mixture was at the rate of 20 L h⁻¹ kg⁻¹ DW (dry weight) during the first 15 d, and 10 L h⁻¹ kg⁻¹ DW thereafter, using a flowmeter equipped with a needle valve (Aalborg, model P, Orangeburg, USA) operating at 5 L min⁻¹.

Five types of agro-food wastes were used in the composting experiments: (i) broccoli; (ii) chestnut; (iii) white grape marc from *Vitis vinifera* L. var. Moscatel; (iv) red grape marc from *Vitis vinifera* L. var. Alfrocheiro; and (v) olive. Broccoli wastes consisted of rejected flowering inflorescences; chestnut wastes comprised of peels and rejected fruits; grape marc were collected during the grape stripping step of the winemaking process; and olive leaves were obtained from an olive oil extraction plant. Dried wheat straw previously milled (2 – 4 cm) using a knife mill (Yike, 9FQ-360 straw hammer mill, Zhengzhou, China) was used as structural material. A control treatment was prepared with wheat straw complemented with urea to adjust the carbon-to-nitrogen (C/N) ratio to 40.

Twenty-four (24) hours before the composting experiment starts, agro-food raw materials were grinded into pieces of ca. 1 cm in length using a shredder (Viking model GB370S, Langkampfen, Austria) and stored fresh at 4 °C until use.

Four reactors were used per treatment, representing the replicates. Each reactor was filled with 10 kg dry matter (DM) of the waste-straw mixture, in a proportion allowing an initial C/N ratio of ca. 40, as calculated from the elemental contents of the raw materials (Table 6).

Treatments consisted of a mixture of the wastes with wheat straw and urea (except for broccoli) and are herein referred to as: “Control”, “Broccoli”, “Chestnut”, “WhiteGrape”, “RedGrape” and “Olive”. Moisture was maintained approximately between 45 % and 60 % by adding water when necessary. The temperature was measured using TM1 temperature probes

(Delta-T Devices, Cambridge, UK) positioned inside the reactors and in the centre of the composting mixture, where the highest temperatures were recorded. The mixture was turned manually once a week during the most biooxidative phase and then every 15 d till the end of the maturation period.

A representative sample of each compost was taken on days 0, 3, 8, 17, 30, 76 and 147, by mixing sub-samples from six different locations in the reactor and at depths between 20 and 80 cm.

Each composite sample was disassembled into 3 subsamples, one of which was ground (particle size < 1 mm), frozen and stored for chemical and physical analyses; another subsample was dried at 40 °C for phenolic and lignocellulosic determination; the last subsample was oven dried at 65 °C during 48 h to determine the DM content.

Measurements of physicochemical properties of the composts

Fresh composts were analysed for their pH, electrical conductivity (EC), and content of organic matter (OM), using standard procedures (CEN, 1999).

Soluble organic nitrogen (ONsol) and soluble organic carbon (OCsol) were determined after extraction of dry samples with 0.01 M CaCl₂ (1:10 w/v) in an elemental analyser (Formac, Skalar, Analytical B.V, Breda, Netherlands), followed by chemiluminescence and near infrared (NIR) detection. Total organic N (Nt) and total organic C (Ct) were evaluated by thermoconductivity and NIR detection in a Skalar Primacs SNC-100 and the C/N ratio calculated.

Ammonium N (NH₄⁺-N) and nitrate N (NO₃⁻-N) concentrations were determined in filtered extracts by molecular absorption spectrophotometry with a segmented flow system (SanPlus, Skalar) prior to extraction of samples with 1M KCl (1:10 v/v) (Houba et al. 1995). Iron (Fe), Zn, Ca, Cu, Mn and Mg were measured by atomic absorption spectroscopy, after nitric-perchloric acid digestion of dry samples. Total P was determined by molecular absorption spectrophotometry with the Skalar segmented flow system.

Table 6: General characterisation of the initial wastes straw mixtures used for composting.

	pH	EC (dS m ⁻¹) (water 1:5)	OCsol (g C kg ⁻¹ of DM)	OM (g C kg ⁻¹ of DM)	ONsol (g N kg ⁻¹ of DM)	Nt (g N kg ⁻¹ of DM)	NH ₄ ⁺ -N (mg N kg ⁻¹ of DM)	NO ₃ ⁻ -N (mg N kg ⁻¹ of DM)
Control	8.11 ± 0.48 a	1.79 ± 0.07 bc	8.89 ± 1.84 cd	938.0 ± 24.0 a	4.84 ± 2.00 a	13.8 ± 0.5 a	693.4 ± 167.9ab	0.18 ± 0.06 c
Broccoli	8.04 ± 0.24 a	2.18 ± 0.27 ab	6.99 ± 1.03 cd	927.1 ± 19.6 a	4.06 ± 0.28 a	19.8 ± 3.2 a	1,075.5 ± 156.4 a	7.18 ± 2.77 a
Chestnut	7.69 ± 0.55 a	1.26 ± 0.21 d	6.06 ± 1.40 d	959.0 ± 4.7 a	4.21 ± 0.92 a	15.0 ± 2.2 a	686.0 ± 295.1 ab	0.44 ± 0.10 bc
WhiteGrape	5.18 ± 0.39 c	2.55 ± 0.22 a	19.65 ± 3.67 a	946.6 ± 4.8 a	5.69 ± 1.25 a	15.0 ± 2.2 a	378.4 ± 151.7 b	4.88 ± 1.03 ab
RedGrape	4.51 ± 0.34 c	2.47 ± 0.28 a	18.51 ± 5.29 ab	943.8 ± 5.6 a	4.97 ± 0.74 a	16.1 ± 1.1 a	222.2 ± 63.3 b	5.24 ± 1.14 a
Olive	6.24 ± 0.63 b	1.61 ± 0.16 cd	12.70 ± 1.20 bc	948.2 ± 6.8 a	4.21 ± 1.02 a	16.6 ± 2.8 a	280.0 ± 127.1 b	0.20 ± 0.09 c

EC = electrical conductivity; OCsol = soluble organic carbon; OM = organic matter; ONsol = soluble organic nitrogen; Nt = total nitrogen; Values within a column, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

Phytochemical analyses consisted of the extraction and quantification of total phenolics and gallic acid. Total phenolics were extracted using the Folin-Ciocalteu reagent, and detected by spectrophotometry, as described by Javanmardi et al. (2003). A calibration curve with different gallic acid concentrations (Sigma-Aldrich, Taufkirchen, Germany) was constructed and all data quantified as mg gallic acid equivalent (GAE) g⁻¹ DM. Gallic acid was determined by high performance liquid chromatography as reported by Santos et al. (2017).

The lignocellulosic fractions were determined in the raw materials, waste-straw mixtures and final composts. Neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined by the sequential method of Van Soest et al. (1991). Hemicellulose and cellulose were calculated as the difference between NDF and ADF, and between ADF and ADL. All analyses were done in four replicates.

Measurements of gas emissions

Emissions of CO₂, CH₄ and N₂O were measured using a 1412 Photoacoustic Field Gas Monitor (Innova AirTech Instruments, Ballerup, Denmark), with internal filters for water vapour, and particulate and optical filters for CO₂ (UA0982), CH₄ (UA0969) and N₂O (UA0985). The CO₂, CH₄ and N₂O detection limits specified for the analyser were 1.5, 0.4 and 0.03 ppm, respectively. Emissions of NO and NH₃ were performed with a chemiluminescent N oxide analyser (SIR MODEL S-5012, Madrid, Spain) with a detection limit of 0.5 ppb. Emission rates at the different events were determined after discounting the concentration of each gas in the inlet air to the outlet air in INNOVA and SIR data.

All gases were sampled once a day during the first 8 d of composting, every two days for the following three weeks and then on days 31, 36, 42, 49, 59. Thereafter, NH₃ and NO were measured on days 66, 73, 80, 87, 94, 104, 110, 117, 125, 130; and CO₂, N₂O and CH₄ on days 80, 104, 117 and 147. To minimize the influence of ambient air on the gas concentrations, measurements were always done before homogenisation or collection of the composts.

To get the cumulative CO₂, CH₄ and N₂O emissions, daily fluxes from contiguous measurements were averaged and multiplied by the interval between sampling periods. In order to evaluate the effect of the different treatments on total GHG emissions, the measured emissions were converted into CO₂-equivalents according to the IPCC GWP factors of 28 and 265 times as much as CO₂ for CH₄ and N₂O, respectively (IPCC, 2013).

Statistical analyses

The results are presented as mean \pm standard deviation (SD) and all analytical determinations were made in quadruplicate. To evaluate the effects of each treatment (factor) at different sampling times (d) on physicochemical parameters, lignocellulosic composition and gas emissions, data were analysed by a two-way repeated measures analysis of variance. Differences between means were determined by the Tukey's honestly significant difference test at a significance level of 0.05. The relationship between total phenolic compounds, lignocellulosic compounds, and gaseous emissions were assessed by calculating Pearson's correlation r values. The referred statistical analyses were performed using the Statistix 10.0 software (Analytical Software, Tallahassee, USA). Prior to these tests, normality and homogeneity of the data were checked using Shapiro-Wilk and Levene tests.

Results and discussion

Evolution of physicochemical parameters during composting

Temperature

Regarding temperature variations with composting time, significant differences ($p < 0.05$) between treatments were observed. The highest temperature (54 °C) was reached with Broccoli, at a faster rate than with the other treatments, and was maintained for five consecutive days. Broccoli also resulted in the longer thermophilic phase and this may be explained by a greater content of easily degradable organic compounds in broccoli wastes, as compared to the other materials (Table 6). This pattern of temperature change during composting has also been observed with garden leaves (Kalamdhad et al., 2009), and horticultural wastes (Gavilanes-Terán et al., 2016).

The slowest increase in temperature was observed with Control, Chestnut, and Olive, probably because the wastes contained an organic matter more resistant to microbial degradation (Table 6). At the end of the composting process, the average temperature for all treatments had returned to or near ambient values (ca. 13 °C), an indication of the slowdown of microbial activities.

Physicochemical parameters

The physicochemical characteristics of the waste-straw mixtures at different times are displayed in Figure 12.

Except for WhiteGrape, the pH of the mixtures remained constant after 3 days, which could be attributed to lower microbial activities and the stabilisation of the composts. At the end of composting, final pH values ranged from 8.99 (WhiteGrape) to 9.26 (Chestnut) (Figure 12a). Although such high pH values might stimulate NH_3 losses (Lasaridi and Stentiford, 1998), taking into consideration other indices used to assess the performance of the composting process such as microbial degradations or agronomic tests, it is reasonable to say that all composts were safe for soil application, as attested by the production of lettuce plants fertilised with the same materials in a pot experiment (Santos et al., 2016).

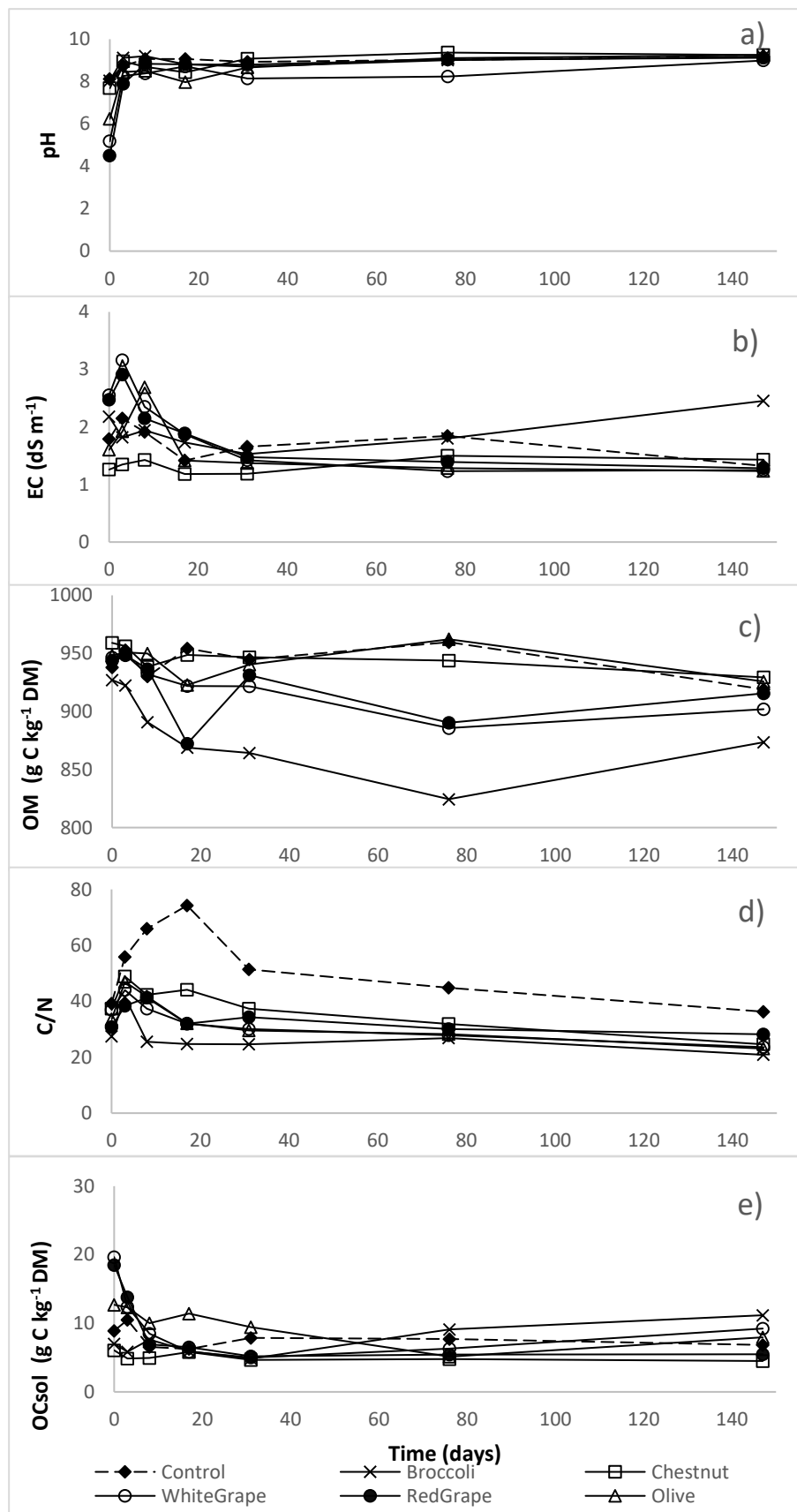


Figure 12: Evolution of main physicochemical characteristics of the waste-straw mixtures during composting. a) pH; b) Electrical conductivity; c) Organic matter; d) C/N ratio; e) Organic soluble ~Carbon

A strong increase in electrical conductivity (EC) values was observed during the first days of composting, corresponding to the thermophilic phase; and then a decrease as the composting process progressed, indicating accrued benefits in terms of final-product quality (Figure 12b). The only exception was with Broccoli, for which an opposite trend was observed.

Final EC values were in the range of 1.23 dS m⁻¹ for Olive and 2.45 dS m⁻¹ for Broccoli, which were all below the maximum EC value of 3 dSm⁻¹ recommended for compost application to soil (Solano et al., 2001).

The C/N ratio for all the mixtures (ca. 40) first increased, and then decreased with composting time to a final ratio ranged from 20.9 with Broccoli to 36.3 with Control (Figure 1d). The final C/N ratios exceeded the threshold limit of 20 recommended by Golueke (1981). However, the C/N ratio should be considered only as a relative indicator of compost maturation here since the bioavailability of organic C matters more than the total organic C content in high lignocellulosic materials (Sánchez-Monedero et al., 2001), such as those used in the present study.

The OM content decreased with composting time, and hence with mass reduction (Figure 1c). The highest OM content was observed with Chestnut, and it decreased with composting time from 959.0 g C kg⁻¹ DM to 929.4 g C kg⁻¹ DM. The lowest OM content was observed with

Broccoli, and it decreased from 927.1 g C kg⁻¹ to 873.4 g C kg⁻¹, with significant differences ($p < 0.05$) as compared to all other treatments (Figure 12c).

The same behaviour observed with OM was seen with OCsol, though with opposite directions of change. At the end of composting, the lowest OCsol content (4.50 g C kg⁻¹ DM) was observed with Chestnut, and the highest (11.18 g C kg⁻¹ DM) with Broccoli (Figure 12e). Differences ($p < 0.05$) between treatments could be explained by different turnover rates of labile and stable fractions of organic compounds. After 36 days, degradation of easily degradable compounds had already occurred in Control, Chestnut, and RedGrape. These treatments also exhibited stable OCsol contents until the end of the experiment (Figure 12e), indicating that equilibrium between microbial degradation of complex organic structures like cellulose and hemicellulose and production of soluble compounds (Serramiá et al., 2010) had been reached.

As a result of DM loss, the contents of all elements in the different materials increased during composting (Table 7).

Table 7: Evolution of mineral contents during composting.

Treatments	P (g kg ⁻¹ DM)	Ca (g kg ⁻¹ DM)	Mg (g kg ⁻¹ DM)	Fe (mg kg ⁻¹ DM)	Cu (mg kg ⁻¹ DM)	Zn (mg kg ⁻¹ DM)	Mn (mg kg ⁻¹ DM)
Day 0							
Control	1.02±0.46 b	6.01 ±2.70 a	0.64 ±0.12 a	203.2 ±133.53 a	2.85 ±0.51 a	7.50 ±1.29 b	28.50 ±1.29 bc
Broccoli	3.44 ±0.68 a	5.93 ±1.70 a	0.87 ±0.15 a	338.3 ±149.04 a	4.30 ±0.71 a	20.25 ±3.30 a	32.00 ±2.94 bc
Chestnut	0.82 ±0.35 a	3.91 ±0.77 a	0.54 ±0.07 a	146.2 ±54.83 a	3.25 ±0.47 a	7.25 ±1.26 b	23.25 ±1.26 c
WhiteGrape	1.27 ±0.33 a	4.18 ±0.30 a	0.78 ±0.18 a	128.5 ±29.27 a	3.40 ±0.76 a	10.50 ±2.38 b	74.00 ±15.49 a
RedGrape	1.29 ±0.25 a	4.34 ±0.56 a	0.77 ±0.17 a	167.0 ±25.07 a	5.35 ±0.87 a	12.25 ±2.87 b	54.75 ±13.72 ab
Olive	1.14 ±0.18 a	5.98 ±0.66 a	0.70 ±0.10 a	345.2 ±127.49 a	5.48 ± 1.75 a	11.25 ±1.50 b	32.00 ±4.08 bc
Day 147							
Control	1.59 ±0.32 b	9.86 ±4.78 ab	1.00 ±0.15 c	250.6 ±140.69 b	4.15 ±1.14 b	5.92 ±1.42 d	44.67 ±2.87 d
Broccoli	7.27 ±0.43 a	12.29 ±2.49 a	2.11 ±0.14 a	1638.3 ±477.39 a	11.23 ±0.66 a	40.75 ±2.87 a	79.08 ±35.62 bc
Chestnut	1.57 ±0.73 b	8.13 ±0.83 b	1.18 ±0.31 bc	293.5 ±88.77 b	6.44 ±0.89 b	9.42 ±3.37 cd	48.92 ±9.93 d
WhiteGrape	2.30 ±0.25 b	8.30 ±0.43 b	1.38 ±0.08 b	266.9 ±67.61 b	8.57 ±1.05 b	16.67 ±4.59 b	139.67 ±10.51 a
RedGrape	1.96 ±0.34 b	7.34 ±2.05 b	1.09 ±0.22 bc	236.7 ±34.74 b	7.67 ±0.60 b	14.08 ±2.79 bc	85.17 ±12.27 b
Olive	1.81 ±0.18 b	9.92 ±2.07 ab	1.26 ±0.14 bc	539.1 ±172.60 b	8.86 ±0.59 b	15.92 ±3.34 b	51.25 ±4.19 cd

Values within a column, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

Substantial amounts of mineral nutrients were present in all treatments, which for a fertilisation point of view, is an important quality for a final compost. Higher P, Mg, Fe and Zn contents were obtained with Broccoli, as compared with all other treatments ($p < 0.05$).

Broccoli compost was also found to be a rich source of Ca ($12.29 \text{ g kg}^{-1} \text{ DM}$), which is essential for plant nutrition. Previous studies have shown that micronutrients and heavy metals are present in significant amounts in vegetable wastes, and concurring with the results of this study, are increased during composting (Gavilanes-Terán et al., 2016).

Nitrogen contents

Figure 13 shows the time course of total nitrogen (Nt), soluble organic nitrogen (ONsol) ammonium (NH_4^+) and nitrate (NO_3^-) changes during composting.

Total nitrogen decreased at the beginning of composting and slightly increased as the process progressed, probably as a result of a concentration effect caused by weight loss associated with OM mineralisation (Senesi, 1989). At the end of the process, highest Nt ($24.12 \text{ g N kg}^{-1} \text{ DM}$) and ONsol ($3.03 \text{ g N kg}^{-1} \text{ DM}$) contents were obtained with Broccoli and were significantly different ($p < 0.05$) from the contents obtained with the other treatments (Figure 13a, b). Although the initial mixture of Broccoli was rich in Nt and ONsol, its initial content of OCsol was lower when compared with most of the other treatments (Table 6).

According to the data shown in Figure 13c, NH_4^+ contents were initially increased because of ammonifying activities. The highest NH_4^+ content was observed on day 3 ($1453.9 \text{ mg N kg}^{-1} \text{ DM}$ for Control), coinciding with a peak in soluble N contents. Thereafter, a significant decrease was observed with all treatments, mostly due to volatilisation of NH_3 and/or immobilisation or nitrification processes as seen in Figure 15. A slightly different trend of change was observed with Broccoli, with an initial decrease of NH_4^+ contents by day 3, followed by a sharp increase peaking at day 17, and a progressive decrease (Figure 13c). By the end of composting, NH_4^+ content was significantly lower ($p < 0.05$) with Broccoli than with the other treatments. The largest decrease in NH_4^+ contents observed with Broccoli (Figure 13c) as compared to its initial richness in NH_4^+ (Table 6) was probably related to higher Nt and ONsol contents. The final NH_4^+ contents in most piles were below the maximum recommended value for a mature compost, i.e., 400 mg kg^{-1} .

As observed for NH_4^+ , NO_3^- contents for all treatments increased sharply within the first days of composting (Figure 13d). The highest NO_3^- content was observed in the initial broccoli mixture (Table 6), which also led to significant N_2O emissions (see Table 9).

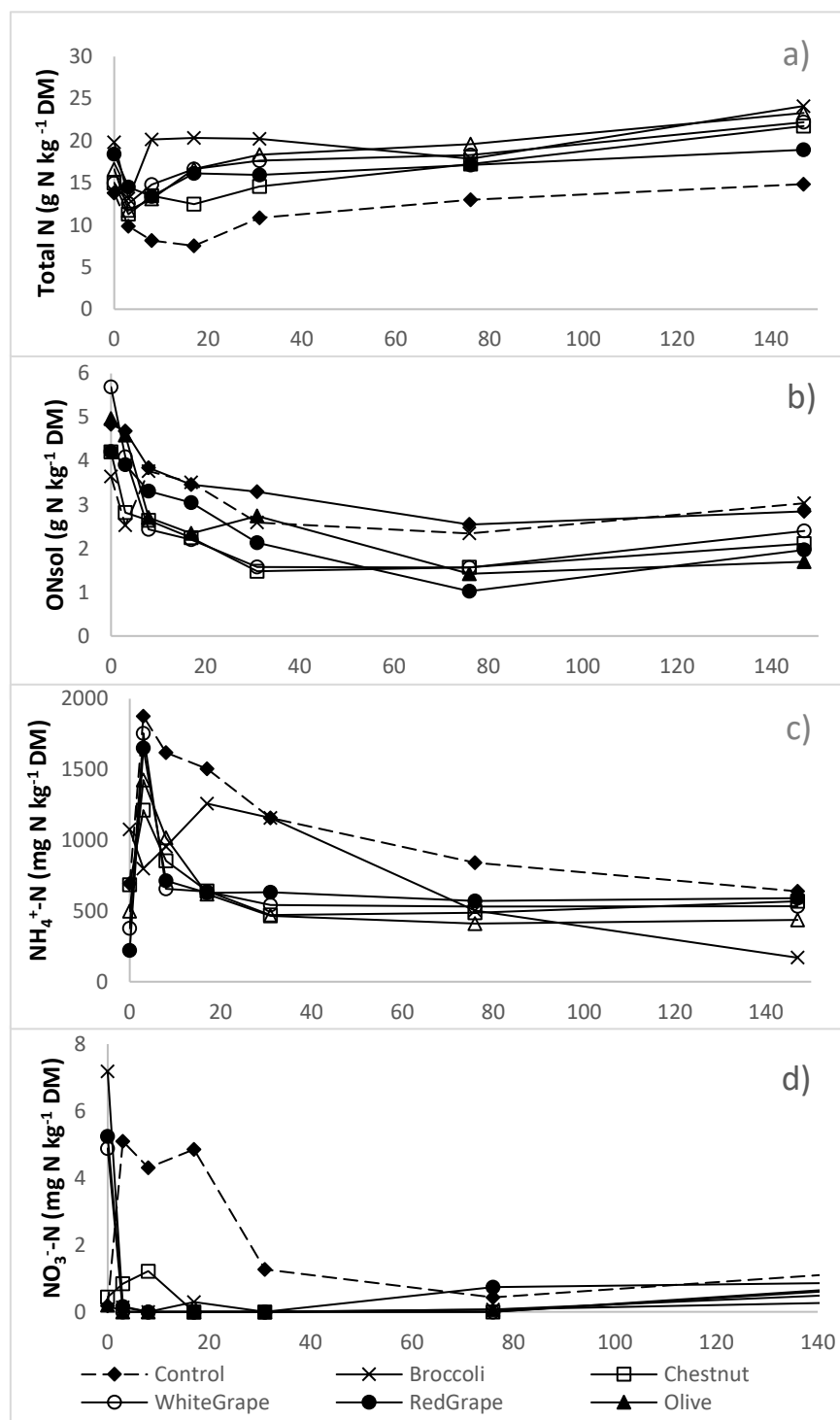


Figure 13: Evolution of the contents of N forms in different waste-straw mixtures during composting. a) total Nitrogen, b) Organic soluble Nitrogen; c) NH_4^+-N ; d) $\text{NO}_3^- - \text{N}$.

In previous studies using pig manure with straw (Szanto et al., 2007) and olive mill wastes with different agro-industrial by-products (Sánchez-Monedero et al., 2010), a correlation was found between N₂O emissions and NO₃⁻ contents during periods of intense nitrification.

Evolution of phenolic and lignocellulosic contents during composting

The contents of phenolics, hemicellulose, cellulose and lignin in the raw materials, waste-straw mixtures and final composts are presented in Table 8. Statistical analyses showed that time interacted with all the treatments to affect both the total phenolics and lignocelluloses.

The initial straw (3.75 mg g⁻¹ DM) and broccoli (6.61 mg g⁻¹ of DM) had the lowest contents of total phenolics, while olive (25.12 mg g⁻¹ DM) and white grape (30.04 mg g⁻¹ DM) had the highest contents. For all treatments, a significant decrease ($p < 0.05$) in phenolics occurred throughout the composting process, with final values ranging from 0.15 mg g⁻¹ DM with Olive to 1.18 mg g⁻¹ DM with Control (Table 2), indicating progressive degradation. It is possible that as soon as these phenolics were degraded and released, they were incorporated into biosynthetic pathways leading to the formation of humic substances (Stevenson, 1994).

The HPLC method used in this study for the separation of individual phenolics allowed the identification of the two tannins, namely gallic acid (in all treatments) and ellagic acid (in only the raw chestnut and initial chestnut-straw mixture). An increase in gallic acid contents occurred during composting (Table 8), reflecting a cumulative effect of straw added to wastes, but also the degradation of polyphenols into simple phenolics. Gallic acid exhibits a strong thermal stability and is metabolized only at temperatures above 60 °C (Volf et al., 2014). The maximum temperature reached during composting was 54 °C, which reasonably explains the presence of gallic acid in the composts at the end of the process.

Although beneficial at low levels, excess levels of water-soluble phenolics in composts may have adverse environmental impacts, mostly in inhibiting seed germination (Morthup et al., 1998) and limiting nitrogen immobilisation in soil (Bustamante et al., 2007).

Raw straw used as Control and raw chestnut had significantly higher ($p < 0.05$) contents of hemicellulose than the other materials, 386.3 and 358.4 g kg⁻¹ DM, respectively (Table 8). These contents remained relatively unchanged after mixing with the co-substrate but were significantly reduced ($p < 0.05$) by about 85 % by the end of composting. In the case of RedGrape, WhiteGrape, Olive and Broccoli, hemicellulose contents measured at the end of

composting were also much lower than those registered early in the process. Chestnut and straw, however, underwent the most intense degradation.

A more pronounced degradation of hemicellulose was observed than of cellulose, indicating that hemicellulose was the first cellulosic fraction used by microorganisms as C and energy source, as also reported by Serramiá et al. (2010).

Hemicellulose is a smaller branched carbohydrate compared to cellulose, which is made of very long unbranched fibrils held together by hydrogen bonding that prevents easy access by microorganisms and enzymes (Komiliis and Han, 2003).

The straw had the highest content of cellulose (413.0 g kg⁻¹ DM), while the raw Broccoli, Chestnut and Olive had the lowest contents (112.3, 143.3 and 150.1 g kg⁻¹ DM), with WhiteGrape and RedGrape in the middle range (ca. 200 g kg⁻¹ DM) (Table 2). By the end of composting, significantly higher ($p < 0.05$) reductions in cellulose contents were recorded with Olive (72 %) and Broccoli (76 %). The lowest reduction in cellulose content was observed for Chestnut (29 %), which also exhibited the highest reduction in hemicellulose content (Table 8).

As for lignin, its “relative proportion” increased as a consequence of the intense degradation of the holocellulose fraction (cellulose + hemicellulose). Olive, RedGrape and WhiteGrape were the wastes that contained more lignin (Table 8). These treatments took longer to react to temperature changes. Lignin acts as a protective factor for the cellulosic and hemicellulosic fractions and the biodegradation of lignin usually occurs tardily and at a very low rate (Malherbe and Cloete, 2002). At the end of the composting process, Chestnut, WhiteGrape and RedGrape were the treatments with highest lignin contents (Table 8).

The lignin/holocellulose ratio has been proposed as an index to monitor the biodegradability of the OM (Francou et al., 2008), and can be useful to understand a compost resistance to degradation after soil application. The lowest lignin/holocellulose ratio was with the Control and increased from an initial value of 0.06 to a final value of 0.84, which was significantly lower ($p < 0.05$) than the final highest value obtained with Olive i.e., 1.58 (Table 8). The increase of this ratio for all treatments indicates stabilisation of the OM.

All the composts obtained were found suitable for agricultural use and their suitability were in the order Chestnut > Olive > WhiteGrape > RedGrape > Broccoli. The broccoli compost in fact had lowest OM, C/N, lignin, NH₄⁺, NO₃⁻, and cellulose contents compared to the other composts.

Table 8: Evolution of the contents of total phenolics, gallic acid, lignocellulosic compounds (normalised to a constant ash content) and related ratio in the raw materials, initial straw-waste and final composts

	Total phenolics (mg g ⁻¹ DM)	Gallic acid (µg g ⁻¹ DM)	Hemicellulose (g kg ⁻¹ DM)	Cellulose (g kg ⁻¹ DM)	Lignin (g kg ⁻¹ DM)	Lignocellulose (g kg ⁻¹ DM)	Lignin/holocellulose ratio
Raw materials							
Straw	3.75 ± 0.69 e	26.16 ± 0.93 a	386.3 ± 12.7 a	413.0 ± 5.33 a	48.60 ± 1.50 b	847.9 ± 15.7 a	0.06 ± 0.00 c
Broccoli	6.61 ± 0.02 d	4.19 ± 0.03 c	32.03 ± 4.50 d	112.3 ± 17.8 c	43.40 ± 15.8 b	187.8 ± 2.31 d	0.31 ± 0.15 bc
Chestnut	14.99 ± 0.47 c	17.66 ± 0.67 b	358.4 ± 28.1 a	143.3 ± 12.0 c	167.2 ± 22.8 a	668.9 ± 16.3 b	0.33 ± 0.06 bc
White Grape	30.04 ± 0.73 a	11.53 ± 1.41 bc	69.38 ± 6.44 bc	208.0 ± 8.42 b	187.7 ± 15.5 a	465.0 ± 10.4 c	0.68 ± 0.08 ab
Red Grape	24.50 ± 0.61 b	14.37 ± 2.01 b	51.31 ± 8.09 cd	202.6 ± 2.65 b	197.3 ± 3.67 a	451.2 ± 9.60 c	0.78 ± 0.03 ab
Olive	25.12 ± 0.63 b	12.75 ± 2.06 b	97.81 ± 1.31 b	150.1 ± 4.79 c	225.8 ± 1.54 a	473.8 ± 4.09 c	0.91 ± 0.01 a
Initial straw-wastes mixtures							
Control	3.75 ± 0.69 d	26.16 ± 0.93 a	386.3 ± 12.7 a	413.0 ± 5.33 a	48.60 ± 1.50 b	847.9 ± 15.7 a	0.06 ± 0.00 a
Broccoli	4.29 ± 0.19 d	20.62 ± 1.27 a	280.0 ± 10.2 b	322.8 ± 5.05 b	47.04 ± 5.16 b	649.9 ± 11.7 d	0.08 ± 0.01 a
Chestnut	8.25 ± 0.59 c	23.66 ± 0.89 a	375.2 ± 16.4 a	305.1 ± 6.29 b	96.03 ± 9.64 ab	776.3 ± 2.96 b	0.14 ± 0.02 a
WhiteGrape	13.74 ± 0.61 a	21.32 ± 1.62 a	265.9 ± 9.88 b	335.1 ± 6.11 b	101.5 ± 6.50 ab	702.4 ± 11.9 c	0.17 ± 0.01 a
RedGrape	11.78 ± 0.37 b	22.35 ± 0.83 a	252.3 ± 4.53 b	328.9 ± 2.20 b	108.1 ± 2.29 ab	689.3 ± 5.87 cd	0.19 ± 0.00 a
Olive	12.30 ± 0.61 b	21.49 ± 0.88 a	270.9 ± 8.13 b	307.9 ± 3.06 b	119.5 ± 0.32 a	698.3 ± 7.81 c	0.21 ± 0.00 a
Final composts							
Control	1.18 ± 0.08 a	22.64 ± 0.92 a	55.65 ± 6.98 b	240.4 ± 3.73 a	248.1 ± 2.08 cd	544.2 ± 5.66 b	0.84 ± 0.01 b
Broccoli	1.17 ± 0.32 a	23.45 ± 0.81 a	115.5 ± 8.39 a	75.80 ± 3.99 c	205.9 ± 37.1 d	397.2 ± 33.4 d	1.16 ± 0.52 ab
Chestnut	1.07 ± 0.10 a	25.96 ± 1.87 a	58.95 ± 15.9 b	215.1 ± 17.5 ab	379.7 ± 18.4 a	653.8 ± 12.8 a	1.40 ± 0.23 a
WhiteGrape	1.02 ± 0.35 a	30.41 ± 6.58 a	108.9 ± 21.7 a	184.6 ± 46.9 b	352.0 ± 37.1 a	645.4 ± 32.3 a	1.27 ± 0.42 ab
RedGrape	0.74 ± 0.06 a	28.82 ± 2.73 a	131.4 ± 31.2 a	182.2 ± 2.95 b	324.5 ± 49.9 ab	638.1 ± 71.0 a	1.06 ± 0.38 ab
Olive	0.15 ± 0.00 a	30.02 ± 9.36 a	10.88 ± 4.12 a	86.00 ± 43.6 c	285.9 ± 40.3 bc	480.6 ± 139 c	1.58 ± 0.61 a

Values within a column, for the same day, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

Evolution of gaseous emissions during composting

Carbon dioxide

Throughout composting, CO₂ fluxes were significantly ($p < 0.05$) affected, regardless of the type of waste. Composting with Olive led to the maximum average emission of 125.6 g CO₂-C kg⁻¹ initial DM on day 9, while the lowest emission (34.6 g CO₂-C kg⁻¹ initial DM) was observed on day 16 on the WhiteGrape (Figure 14a). Major CO₂ fluxes were detected during the first 13 days of composting. After this initial period, a decrease in emissions occurred, with values equalising among the treatments towards the end of the experiment and leading to the accumulated CO₂-C emissions time pattern shown in Figure 14.

Only Olive led to emission values over 100 g CO₂-C kg⁻¹ initial DM. The final accumulated CO₂-C flux emissions (mg CO₂-C kg⁻¹ initial DM) are presented in Table 9. The highest emissions were observed with Olive and Chestnut, and the lowest emission with WhiteGrape. No significant correlation ($p < 0.05$) was found between final cumulative CO₂ emissions and the total phenolic and lignocellulosic contents of the initial waste-straw mixtures.

Methane

The production of CH₄ occurs in anaerobic micro-sites particles of compost materials, when degradation of fresh and easily available organic materials exhausts the oxygen (Epstein, 1997). CH₄ emissions were highest in the early phase of the composting process, mostly during the first 13 days, when microbial activities were intense and the demand for aeration high. These fluxes pattern are responsible for the accumulated CH₄-C emissions presented in Figure 14b. Statistical analyses indicated that CH₄ emissions differed ($p < 0.05$) among time, but not among waste types. Composting with Olive caused low CH₄ emissions during the first days of the process, with an average value of - 0.70 g CH₄-C kg⁻¹ initial DM, whereas the lowest values were obtained with WhiteGrape and RedGrape. A sharp decrease of CH₄ emissions occurred as composting progressed, leading to negligible emissions towards the end of the experiment (Figure 14b).

Accumulated CH₄ emissions (Table 9) correlated negatively with CO₂ emissions ($r = -0.756$; $p < 0.001$), but not with the total phenolic and lignocellulosic contents of the initial waste-straw mixtures.

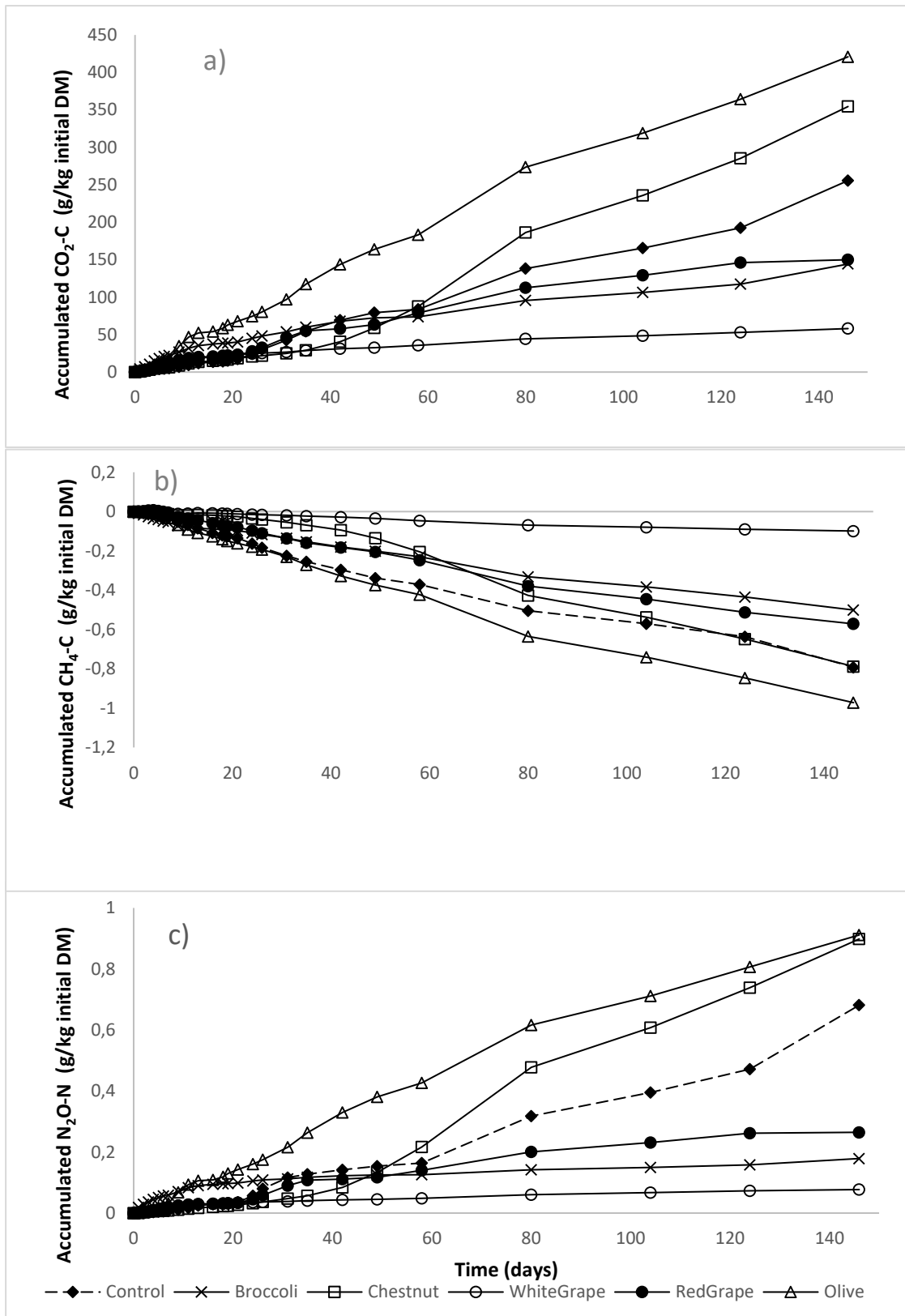


Figure 14: Evolution of accumulated GHG emissions during composting: (a) accumulated CO_2 (b) accumulated CH_4 (c) accumulated N_2O

Nitrous oxide

N₂O is formed through nitrification and/or denitrification, under both aerobic and anaerobic conditions (Williams et al., 1998). Results showed that N₂O emissions were affected ($p < 0.05$) by all treatments and time. N₂O emission rates were high in the first 10 days of composting, with a peak in emissions on day 7 recorded for Olive (0.80 g N₂O-N kg⁻¹ initial DM). The accumulated N₂O-N emissions during the composting process are illustrated in Figure 14c. Table 3 shows the final accumulated emissions values, which ranged from 0.08 g N₂O-N kg⁻¹ initial DM (WhiteGrape) to 0.91 g N₂O-N kg⁻¹ initial DM (Olive and Chestnut). Accumulated N₂O emissions in RedGrape, Broccoli and WhiteGrape treatments were 2.62, 3.78, and 8.50 times lower than in the Control. A significant correlation was found between cumulative N₂O emissions and the hemicellulose ($r = 0.522$; $p < 0.01$) and lignocellulose ($r = 0.506$; $p < 0.05$) contents of the initial waste-straw mixtures, but not with the total phenolic content.

Ammonia

The waste type, the time, and their interaction significantly affected ammonia emissions (Figure 15). Highest emissions occurred during the first 10 days of composting, corresponding to the most bioactive period of the process, with peak values of 0.003 (WhiteGrape) and 0.02 g NH₃-N kg⁻¹ initial DM (RedGrape) on day 6 (Figure 15a). The straw treatment (Control) emitted less NH₃ when compared with all the other treatments. After day 18, the emissions progressively decreased to null values, with no significant differences ($p < 0.05$) between treatments.

The lowest cumulative NH₃ emissions were calculated for Control and Chestnut and were significantly different ($p < 0.05$) from those calculated for the other treatments (Figure 3b). As a carbon-rich material, straw contains a substantial amount of degradable carbon, which stimulates immobilisation of ammonium on microbial biomass (Sommer et al., 2006), with subsequent increase in the C/N ratio (Table 1). The use of straw as bulking agent could improve compost porosity and increase oxygen concentrations (Chowdhury et al., 2015). Sánchez-Monedero et al. (2001) reported that the use of wastes with high lignocellulose contents led a reduction of about 25 % of N losses during the composting process. In agreement with the results of the present study, treatments with higher C/N ratios and lignocellulose contents (Control and Chestnut) led to lower cumulative NH₃ emissions.

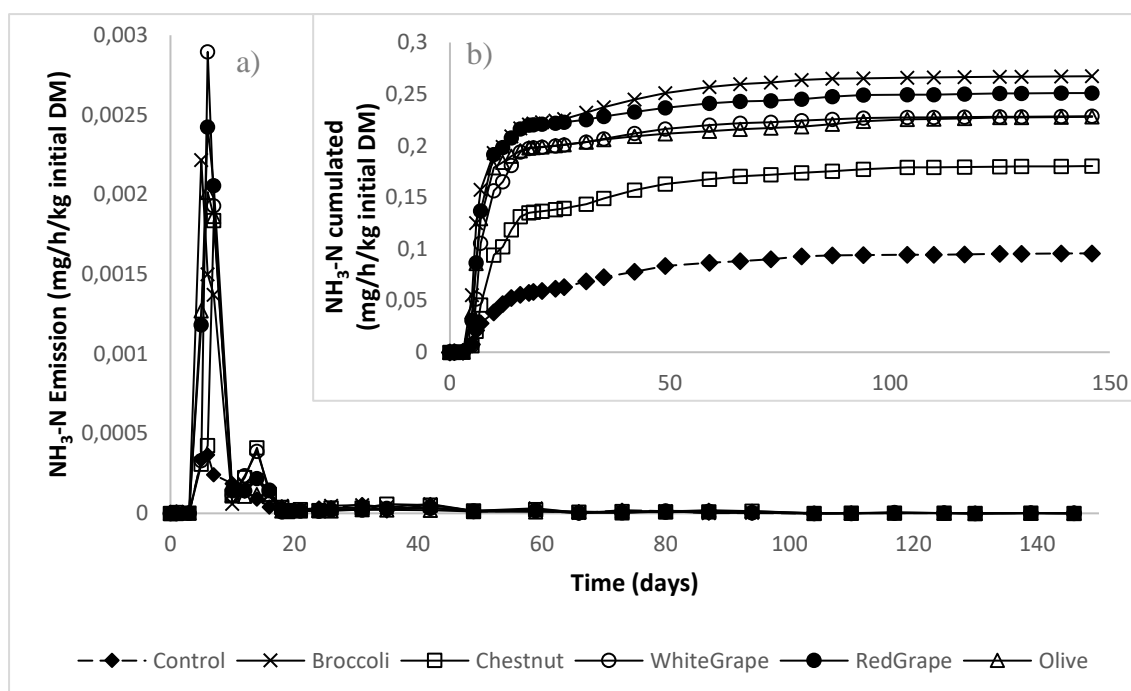


Figure 15: Evolution of instantaneous and accumulated NH₃ emissions during composting: (a) instantaneous ammonia emissions, (b) accumulated NH₃ emissions.

Broccoli with the lowest C/N ratio (Table 6) and lignocellulose content (Table 8) led to higher emissions. Cumulative NH₃ emissions were highly and negatively correlated with lignocellulose ($r = -0.873$; $p < 0.001$), and positively correlated with the total phenolic contents ($r = 0.462$; $p < 0.05$) of the initial waste-straw mixtures.

Nitric oxide

Nitric oxide (NO) is a precursor of tropospheric ozone, a greenhouse gas formed by photochemical reaction and which plays key roles in atmospheric chemistry (Williams et al., 1998). Few reports have measured NO emissions during composting trials. In the present study, time and waste type significantly ($p < 0.05$) affected NO emissions. In concordance with ammonia data, NO was emitted primarily during the first 10 days of composting (Figure 16a) with the highest peak values being recorded on day 6 for RedGrape (0.17 g NO-N kg⁻¹ initial DM) and WhiteGrape (0.16 g NO-N kg⁻¹ initial DM). After day 14, emissions started to decrease and remained close to zero until the end of the composting process. As with NH₃, the lowest cumulative NO emissions were calculated for Control and Chestnut (ca. 8.7 g NO-N kg⁻¹ initial DM at the end of composting); but the highest for Olive (15.8 g NO-N kg⁻¹ initial DM) rather than Broccoli (Figure 16b).

Cumulative NO emissions correlated negatively with lignocellulose ($r = -0.633$; $p < 0.001$) and hemicellulose ($r = -0.779$; $p < 0.001$) and positively with lignin ($r = 0.523$; $p < 0.01$) and total phenolics ($r = 0.626$; $p < 0.01$).

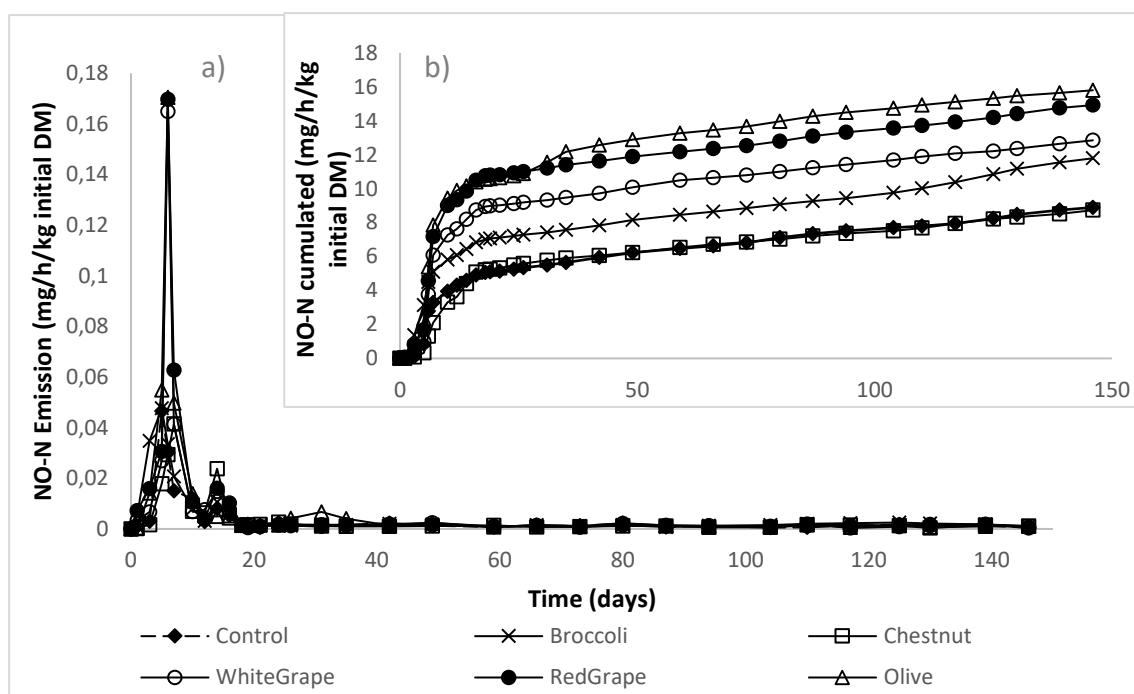


Figure 16: Evolution of instantaneous and accumulated NO emissions during composting: (a) instantaneous ammonia emissions, (b) accumulated NO emissions.

Greenhouse gas emissions

Emissions of N_2O , CO_2 , and CH_4 throughout the composting process were summed to get total GHG emissions, after their conversion into CO_2 -equivalents. The different treatments significantly ($p < 0.0001$) affected total GHG emissions, with values ranging from 79.9 (WhiteGrape) to 683.0 g CO_2 -eq. kg^{-1} initial DM (Olive) (Table 3). When CO_2 emissions were excluded from total GHG calculations, total GHG (without CO_2) emissions ranged from 21.8 to 262.2 g CO_2 -eq. kg^{-1} DM (Table 3). Of all the agro-food waste materials used in the experiment, it was clear that white grape's marc could effectively be transformed and reused as organic fertiliser, with the lowest negative environmental impacts.

Table 9: Final accumulated GHG emissions (CO₂, N₂O and CH₄) expressed as CO₂ equivalents.

	Accumulated emissions (g kg ⁻¹ Initial DM)			GHG emissions (g CO ₂ eq. kg ⁻¹ initial DM)	Contribution (%)			GHG emissions without CO ₂ (g CO ₂ eq. kg ⁻¹ initial DM)
	CO ₂	CH ₄	N ₂ O		CO ₂	CH ₄	N ₂ O	
Control	255.6 ± 35.5 ab	-0.79 ± 0.5 a	0.68 ± 0.1 a	450.2±63.0 ab	56.80 ± 1.2 a	- 3.68 ± 2.2 a	46.88 ± 1.8 a	194.6 ± 28.4 a
Broccoli	144.2 ± 60.9 bc	-0.50 ± 0.6 a	0.18 ± 0.1 b	189.1±81.1 bc	72.43 ± 13.1 a	-4.78 ± 3.4 a	32.36 ± 9.8 bc	44.9 ± 33.4 b
Chestnut	354.5 ± 34.3 a	-0.79 ± 0.5 a	0.90 ± 0.2 a	616.4±74.7 a	57.65 ± 2.0 a	-2.78 ± 1.3 a	44.93 ± 3.2 ab	261.9 ± 42.1 a
WhiteGrape	58.0 ± 9.3 c	-0.10 ± 0.0 a	0.08 ± 0.0 b	79.9 ±10.6 c	72.58 ± 5.3 a	-2.58 ± 0.4 a	30.00 ± 5.3 c	21.8 ± 5.0 b
RedGrape	150.0 ± 72.2 bc	-0.57 ± 0.8 a	0.26 ± 0.1 b	220.0±59.9 bc	64.77 ± 12.6 a	-4.03 ± 4.1 a	39.26 ± 9.7 abc	70.1 ± 26.3 b
Olive	420.8 ± 117.2 a	-0.97 ± 0.5 a	0.91 ± 0.4 a	683.0 ± 94.4 a	62.15 ± 3.0 a	-2.85 ± 0.9 a	40.70 ± 3.7 abc	262.2 ± 99.5 a

Values within a column, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

Conclusions

The findings presented in this study provide information on physicochemical changes and gaseous emissions (GHG, NH₃ and NO) during composting of agro-food wastes.

. Significant variations were observed over time and between the different wastes. White grape exhibited the lowest GHG emissions, with 58.0 CO₂, -0.10 CH₄, and 0.08 N₂O g kg⁻¹ initial DM, which corresponded to GHG emissions of 79.9 g CO₂ eq. kg⁻¹ initial DM. Broccoli contributed as much to the global warming potential as red grape (189.1 and 220.0 g CO₂ eq. kg⁻¹ initial DM), and chestnut and olive contributed thrice as much as the formers (616.4 and 683.0 g CO₂ eq. kg⁻¹ initial DM). A higher content of hemicelluloses in the wastes seemed to entail a higher emission of N₂O. The lowest NH₃ and NO emissions were registered during the intense degradation of chestnut and olive residues. Composting with chestnut lowered the emissions of NH₃ and NO up to levels like those found in wheat straw (< 0.01 g NH₃-N, 8.7 g NO-N kg⁻¹ initial DM). These changes were closely associated with the lignocellulosic composition of the wastes, a finding with significant implications for the choice of composting materials. These results suggest the potential use of wastes rich in lignocelluloses in composting for the purpose of reducing N losses by NH₃ and NO emissions. Although composting raised the pH and EC of the final composts, the increase in nutrient contents and lignin/holocellulose ratios indicates that a safe compost could be obtained under optimized conditions with negligible environmental impacts. These results can be helpful to investigators, producers and agro-industry companies in adopting best practices for valorisation of wastes, and in implementing mitigation strategies for GHG, NO and NH₃ emissions.

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Chapter 3. Effects of phytochemical composition of the composts on C and N cycles and gas emission

Greenhouse gas emissions and C and N mineralization of amended soils with different composted agro-industrial-wastes

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Abstract

Land application of organic residues constitute an effective way for recycling the wastes generated by the agro food industries and may at the same time be a good strategy to mitigate greenhouse gas (GHGs) emissions into the atmosphere. The effect of different composts and their chemical composition on soil C and N mineralisation and GHG emissions was explored by determining ammonium (NH_4^+) and nitrate (NO_3^-) dynamics and monitoring carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) fluxes from amended soils under laboratory conditions. Eight different composted agro-industrial wastes were incubated with a clay soil for 55 days at 20°C and 60% water holding capacity (WHC). Mineralisation of the C added to the soils was directly correlated to C/N, total phenolic/N, gallic acid/N and NH_4^+ and negatively correlated with total organic nitrogen (TON) and lignin content of the composts. Soils amended with straw compost presented the highest C mineralised with significantly higher CO_2 emissions throughout the incubation period. N mineralisation was only negatively correlated with C/N ratio and Straw compost exhibited a significantly and strong immobilisation when compared with the rest of treatments. The study demonstrates that the C/N ratio by itself is not suitable for predicting N mineralisation. All composts enhanced N_2O emissions and no significant differences in cumulative emissions were observed between treatments. The results revealed that composts with high initial soluble N contents and narrow C/N ratio, as in the case of Coffee grounds, produce higher N_2O emissions. With the exception of Mimosa (*Acacia*

dealbata) and Coffee grounds that behave as sources of methane, soils amended with composts displayed an overall sink effect for methane. These findings reveal that the effect of the different composts on C and N mineralisation and GHGs emissions depend essentially on their chemical composition.

Keywords: Chemical composition; Composts, GHG emissions, Mineralization kinetics, Waste biomass valorisation

Introduction

In recent decades, a decrease in soil organic matter (SOM) content of agricultural soils worldwide has been observed, including the Mediterranean areas, due to natural mineralisation processes and changes in the agricultural practices, resulting in a decrease in soil fertility. This SOM depletion associated with the intensification of agriculture is considered one of the foremost environmental threats in the EU (Marmo, 2008; C.E.C., 2002). Furthermore, it is well documented that agricultural activities had a pronounced effect on the global nitrogen (N) cycle which includes increased emissions of greenhouse gases (GHG) to the atmosphere (IPCC, 2007).

Due to these facts a more sustainable strategy has been proposed for increased recycling and utilisation of organic wastes, that potentially may enhance SOM and limit GHG emissions. This is supported by a considerable amount of research that has shown the benefits of using composts and other organic amendments to improve soil physical (water holding capacity, porosity, and bulk density), chemical (pH, electrical conductivity, and nutrient content), and biological properties such as soil microbial populations and diversity and plant growth. All this plays a key role in increasing crop productivity and resilience (Hargreaves et al., 2008; Stoffella and Kahn, 2001; Ghosh et al., 2012; Mohanty et al., 2013; Thangarajan et al., 2013).

However, it should be noted that organic residues, like composts, often exhibit different physicochemical properties affecting the soil ecosystem in a range of ways. For instance, residue type and their interaction with organic components of soil are recognised as an important factor controlling carbon (C) and N mineralisation and consequently nutrients release (Jensen et al., 2005; Singh et al., 2007; Mohanty et al., 2013). A study of Hadas (2004) revealed that biochemical composition, specially the soluble C content, determines the initial rate of

residue decomposition, while the more recalcitrant fractions like lignin content, regulate the medium to long-term decomposition of added C.

Previous studies have indicated that the total N concentration or C/N ratio of a plant residue will determine whether N mineralisation or immobilisation dominates during its decomposition (Jensen et al., 2005; Chaudhary et al., 2014; Redin et al., 2014) and residues with a high nitrogen (N) concentration and low C/N and lignin/N ratios often shown high mineralisation and decomposition rates (Raiesi, 2006). The application of organic wastes to soil usually results in an improvement in the nitrogen mineralisation potential of soils, at least in the longer term (Redin et al., 2014). However, the residue characteristics, rate, timing and method of application have an effect on the release of N available to plants in the shorter term (Cabrera et al, 2005).

Beside this, the application of organic amendments promotes the microbial degradation of organic matter and consequently the greenhouse gases (GHGs) production such as CH₄ and N₂O. Organic matter degradation can play an important role in the production of some of these GHGs by consuming the O₂ content of the soil and by giving the essential electrons for denitrification and methanogenesis (Lu, 2000). The increase in net carbon dioxide (CO₂) emission from soil to atmosphere due to declining SOM is a major challenge throughout the world, and this elevated CO₂ emission is attributed to the enhanced mineralisation of soil organic matter (Wu et al. 2010). In this context the application of organic amendments can play a crucial role in maintaining SOM and mitigating the effect of climate change.

To successfully manage nutrient cycling from organic amendments and to quantify the potential benefits of residues on soil quality and fertility it is therefore essential to predict the C and N mineralisation potentials and dynamics when applied to soil. In addition, this information will help avoiding possible contamination of groundwater by nitrate leaching and to know how the incorporation of these organic residues may influence the GHG emissions (Thangarajan et al., 2013).

The aim of the present work was therefore to evaluate the effects of the addition to soil of a range of composts with different chemical composition obtained from food-industry wastes such as broccoli, chestnut, olive leaves, wine grape marc's, mimosa (*Acacia dealbata*) and coffee grounds on their C and N mineralisation and the GHG production.

Materials and methods

Soil collection and characterization

The soil used for the incubation experiment was a clay soil collected from the topsoil layer (0-30 cm) of an arable field at Salvaterra de Magos (latitude: 39.20150 N, longitude: 8.442570 W, elevation: 18 m above sea level) in the central region of Portugal. The main physicochemical characteristics of the soil were: 55% clay, 28% silt, 17% sand, a water content of 30.8% at field capacity; initial pH_(H₂O) of 5.9, organic C and total N was 24.1 and 2.40 g kg⁻¹, respectively, C/N of 10.0.

The soil was air dried, sieved through a 2 mm mesh, adjusted to ca. 40% of field capacity (FC) and pre-incubated at room temperature (20°C), under aerobic conditions for 2 weeks prior to the beginning of the experiment.

Soil incubation with compost amendments

Eight treatments of compost amendments were used, including composts based on straw, wastes of broccoli (consisting in rejected flowering crests), wastes of chestnut (comprising peels and rejected fruits), olive leaves, red grapes marc's (*Vitis vinifera* L. var. Alfrocheiro), white grapes marc's (*Vitis vinifera* L. var. Moscatel) (obtained during winemaking process from a winery plant), mimosa (*Acacia dealbata* L.) and coffee grounds as composting feedstocks. The composts were produced at University of Trás-os-Montes e Alto Douro on 135L reactors in two distinct composting experiments with continuous forced air circulation provided by an air pump (KNF, model N010. KN.18) at a rate of 20 L h⁻¹ kg⁻¹ DM (dry matter) under the conditions described by Santos et al. (2017) and Santos et al. (2018). Briefly, composts were made by mixing straw as structural material with the different agro-industrial wastes to obtain an initial composting substrate with a C/N ratio of ca. 40. In the case of Coffee grounds compost, the structural material used was a mixture of straw and mimosa with an initial composting mixture C/N of ca. 20. When necessary water and N (as urea) were added to correct the C/N ratio and reach a moisture content in the range 45 % and 60 % (gravimetric basis). Composting was performed during 156 days in the case of Coffee grounds and 147 days for the other wastes. During the composting experiments, the maximum temperature registered in the case of Coffee grounds was 57.6 °C. In the second composting experiment, significant differences ($p < 0.05$) were observed between treatments with regard to temperature variations.

The highest temperature value was observed in Broccoli treatment (54°C) followed by white grape marc's (46°C). The main physicochemical properties of the composts produced are given on Table 10.

C and N mineralisation and the effect on GHG evolution of the different composts were measured by soil incubation. Plastic beakers with 100mL of volume were filled with 50 g DW soil and each compost (previously grounded into pieces of ca. 2mm) was homogeneously incorporated at a rate (DM) of 20 mg g⁻¹ equivalent to a field application rate of 60 t DM ha⁻¹ (assuming a bulk density of 1.2 and incorporation depth of 25 cm). An un-amended soil was introduced as a control. This procedure was repeated to achieve a total of 162 beakers - 18 beakers per treatment to perform 6 destructive sampling dates (days 0, 5, 12, 20, 40 and 55) with 3 replicates each. The same procedure was repeated to prepare another set of 36 beakers (9 treatments x 4 replicates) to be used for gas measurements (days, 0,1,2,3,4,5,6 and thereafter every 2 or 3 days until the end. The water content was then adjusted to 60% FC by addition of deionised water in all beakers. In order to ensure a proper solid fraction contact, the mixture was compacted gently with a cylinder in all beakers to achieve approximately the soil bulk density. Moisture levels were checked regularly by weighing and maintained constant by adding deionised water whenever necessary.

The incubation experiment comprised 9 treatments herein reported to as: Control, Straw, Broccoli, Chestnut, Olive, Redgrapes, Whitegrapes, Mimosa and Coffee grounds, and was carried out in constant temperature room (20 °C) in the dark for 55 days.

C and N mineralisation and GHG emissions measurement

For the determination of ammonium (NH₄⁺) and nitrate (NO₃⁻) concentrations, three replicate beakers were destructively sampled on days 0, 5, 12, 20, 40 and 55 in order to evaluate soil N dynamics (the net N mineralization/immobilization) of the composts amended to soil. Ammonium-N and nitrate-N in the soil samples were measured by shaking 20 g (DM) of each soil sub-sample with 80 mL 1M KCl for 45 min. After this, the suspension was filtered (Advantec 5C filter; Frisnette, Denmark) and frozen (-18°C) until analysis determined on a flow injection analyser (FIAS 300, Perkin Elmer, USA). N mineralization of the different added composts were calculated by subtracting the amount of N measured in the un-amended control soil from the amount of N measured in compost amended soil.

Table 10: Selected physicochemical composition of the agro-industrial composts used in the incubation experiment

	Straw	Broccoli	Chestnut	Olive	Redgrapes	Whitegrapes	Mimosa	Coffee grounds
pH	9.47 ± 0.31 ab	9.34 ± 0.14 ab	9.43 ± 0.30 ab	9.53 ± 0.14 a	9.01 ± 0.02 b	9.11 ± 0.06 ab	7.14 ± 0.05 c	7.05 ± 0.05 c
DM (%)	35.8 ± 0.60 ab	35.3 ± 6.10 ab	22.9 ± 0.41 d	38.3 ± 2.80 ab	33.3 ± 1.16 abc	30.2 ± 0.75 bcd	39.0 ± 3.83 a	25.9 ± 3.41 cd
NO₃⁻ (mg N kg⁻¹ DM)	0.39 ± 0.08 a	0.03 ± 0.01 a	0.25 ± 0.07 a	0.46 ± 0.11 a	0.70 ± 0.34 a	0.83 ± 0.27 a	0.00 ± 0.00 a	0.00 ± 0.00 a
NH₄⁺ (mg N kg⁻¹ DM)	727.7 ± 196.3 a	137.5 ± 24.7 d	602.7 ± 40.2 ab	447.1 ± 29.0 abcd	575.0 ± 22.2 abc	518.7 ± 15.7 abc	277.7 ± 72.2 cd	402.8 ± 23.6 bcd
N_{soluble} (g N kg⁻¹ DM)	2.22 ± 0.52 b	2.29 ± 0.89 b	2.26 ± 0.17 b	2.08 ± 0.22 b	1.87 ± 0.23 b	2.21 ± 0.14 b	1.75 ± 0.13 b	3.79 ± 0.06 a
C_{soluble} (g N kg⁻¹ DM)	6.22 ± 0.55 bc	12.6 ± 3.07 a	4.80 ± 0.36 bc	8.45 ± 0.86 ab	5.18 ± 0.41 bc	8.64 ± 0.19 ab	3.04 ± 0.03 c	5.96 ± 0.08 bc
TOC (g C kg⁻¹ DM)	527.1 ± 1.36 ab	479.7 ± 5.29 b	537.0 ± 3.51 a	533.2 ± 0.77 ab	523.0 ± 2.62 ab	518.9 ± 1.08 ab	507.0 ± 1.91 ab	495.4 ± 4.87 ab
TON (g N kg⁻¹ DM)	13.9 ± 1.65 e	22.9 ± 0.91 cd	22.1 ± 0.63 cd	24.4 ± 1.64 c	19.7 ± 2.59 d	23.3 ± 1.09 cd	41.0 ± 0.33 b	57.0 ± 1.15 a
C/N	38.4 ± 4.91 a	21.0 ± 2.78 b	24.3 ± 0.54 b	21.9 ± 1.52 b	26.9 ± 3.58 b	22.3 ± 0.99 b	12.4 ± 0.13 c	8.7 ± 0.15 c
Total Phenolics (mg g⁻¹ DM)	1.09 ± 0.07 ab	0.93 ± 0.14 bc	1.14 ± 0.04 ab	0.16 ± 0.00 d	0.67 ± 0.07 c	1.31 ± 0.20 a	1.15 ± 0.00 ab	0.93 ± 0.02 bc
Gallic acid (mg g⁻¹ DM)	23.3 ± 0.53 bc	24.1 ± 0.92 abc	27.1 ± 1.83 abc	23.2 ± 0.72 bc	31.9 ± 3.68 ab	35.6 ± 11.3 a	18.5 ± 1.57 c	27.8 ± 0.22 abc
Lignin (g kg⁻¹ DM)	247.1 ± 1.07 c	187.6 ± 7.69 d	372.2 ± 13.1 b	299.0 ± 37.5 c	373.9 ± 6.00 b	364.1 ± 34.4 b	472.0 ± 6.40 a	502.6 ± 17.7 a
Cellulose (g kg⁻¹ DM)	239.2 ± 3.40 a	90.9 ± 3.19 c	223.0 ± 9.27 ab	101.7 ± 3.70 c	183.3 ± 2.45 ab	167.3 ± 3.89 b	200.1 ± 5.03 ab	205.7 ± 13.6 ab
Hemicellulose (g kg⁻¹ DM)	58.7 ± 0.40 d	118.7 ± 0.66 a	63.8 ± 1.53 d	106.8 ± 0.18 ab	116.1 ± 0.72 a	101.0 ± 1.83 abc	70.5 ± 2.08 cd	73.5 ± 0.29 bcd

DM= Dry matter; TOC = total organic carbon; TON = total organic nitrogen

Values within a line, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

For gas emission determinations, four randomly chosen replicate beakers for each treatment were placed in Kilner Jars (1 L) and sealed. Gas fluxes were calculated based on changes in the headspace gas concentrations. Hence, the headspace air was sampled 0,30 min and 1 hour after jars closure. Four empty beakers placed in Kilner Jars were considered as blank and used to correct values for background concentrations.

Carbon dioxide (CO₂), N₂O and CH₄ fluxes were simultaneously measured using a gas chromatograph (Bruker 450-GC 2011) equipped with CO₂, CH₄ and N₂O detectors. A flame ionization detector at 300 °C and an electron capture detector at 350 °C detected the CH₄ and N₂O in the samples, respectively. The oven temperature was controlled at 50 °C, and helium (99.99%) and argon (99.99%) were used as the carrier gases for CH₄ and N₂O, respectively. Gas measurements were carried out on days, 0,1,2,3,4,5,6, thereafter every 2 or 3 days until day 30 and afterward every 5 days until the end (day 55).

To evaluate the effect of the different treatments on total GHG emissions (only considering CH₄ plus N₂O), the CO₂-equivalent (CO₂-eq) global warming potential (GWP) over a 100-year time for each of the treatments was calculated using the IPCC GWP factors for CH₄ and N₂O, which were 28 and 265 times as much as CO₂, respectively (IPCC, 2013).

Statistical analysis

All data are presented as mean ± standard deviation (SD) of three replicates for C and N mineralization data and four replicates for GHG emissions data. C mineralization of the different agro-industrial wastes composted was assessed by quantifying the CO₂ release. The C mineralization data were fitted to an exponential equation according to Stanford and Smith (1972) as follows:

$$C_{\min} = C_0 [1 - \exp^{(-kt)}]$$

Where, C_{min} is the amount of mineralized C; C₀ is the potentially mineralisable C pool; k is a mineralisation constant and t is the incubation time.

For statistical analysis, the data was submitted to analysis of variance (ANOVA), after testing data for normality and equality of variance using the Shapiro–Wilk test. The statistical significance of the mean differences was determined by comparison of means by the Tukey's test at the probability level of 0.05.

The relationship between the phenolic and lignocellulosic compounds with the C and N mineralization and gaseous emissions were assessed by calculating Pearson's correlation r values. We also used the interaction of polyphenols and lignocellulosic with the N compounds reported by TPhe/N (total phenolic/N), GA/N (gallic acid/N), lignin/N, cellulose/N and hemicelluloses/N to see if these ratios are better indices to predict C and N mineralization.

The mentioned statistical analyses were performed using the Statistix 10.0 software (Analytical Software, Tallahassee, USA).

Results and discussion

Soil inorganic nitrogen and net N mineralisation

At the start of the experiment NH_4^+ -N (Figure 17a) was the dominant form of inorganic N in all treatments, with Straw and Chestnut presenting the higher values. During the first 12 days a markedly decrease in NH_4^+ -N levels was observed and thereafter values were kept low and close to zero. This pattern indicates a loss of NH_4^+ -N probably due to nitrification since NO_3^- -N revealed a sharp increase.

Nitrate-N (Figure 17b) presented an increasing pattern during the first five days of incubation for all treatments without significantly differences between treatments. From this day, Control, Straw, Broccoli and Whitegrapes exhibited a decrease until day 12 while the other treatments exhibited an increase. From day 40, an increase in all treatments was observed up to the end of the incubation.

After day 12, Chestnut showed significant higher NO_3^- -N values, since the day 12 of the incubation experiment, indicating the dominance of nitrification and release of available NO_3^- -N in soil. The highest concentration of NO_3^- -N was observed at the end of incubation by Chestnut (91.8 mg kg⁻¹) followed by Coffee grounds (88.1 mg kg⁻¹) and Whitegrapes (87.3 mg kg⁻¹). Contrarily, NO_3^- -N concentration in soil amended with straw was significantly lower since day 20, being significantly different to all treatments in the last day (8.2 mg kg⁻¹). By the last sampling date, nitrate levels were increasing for all of the treatments, suggesting the possibility that these composts might continue to supply N for plant uptake over the long-term.

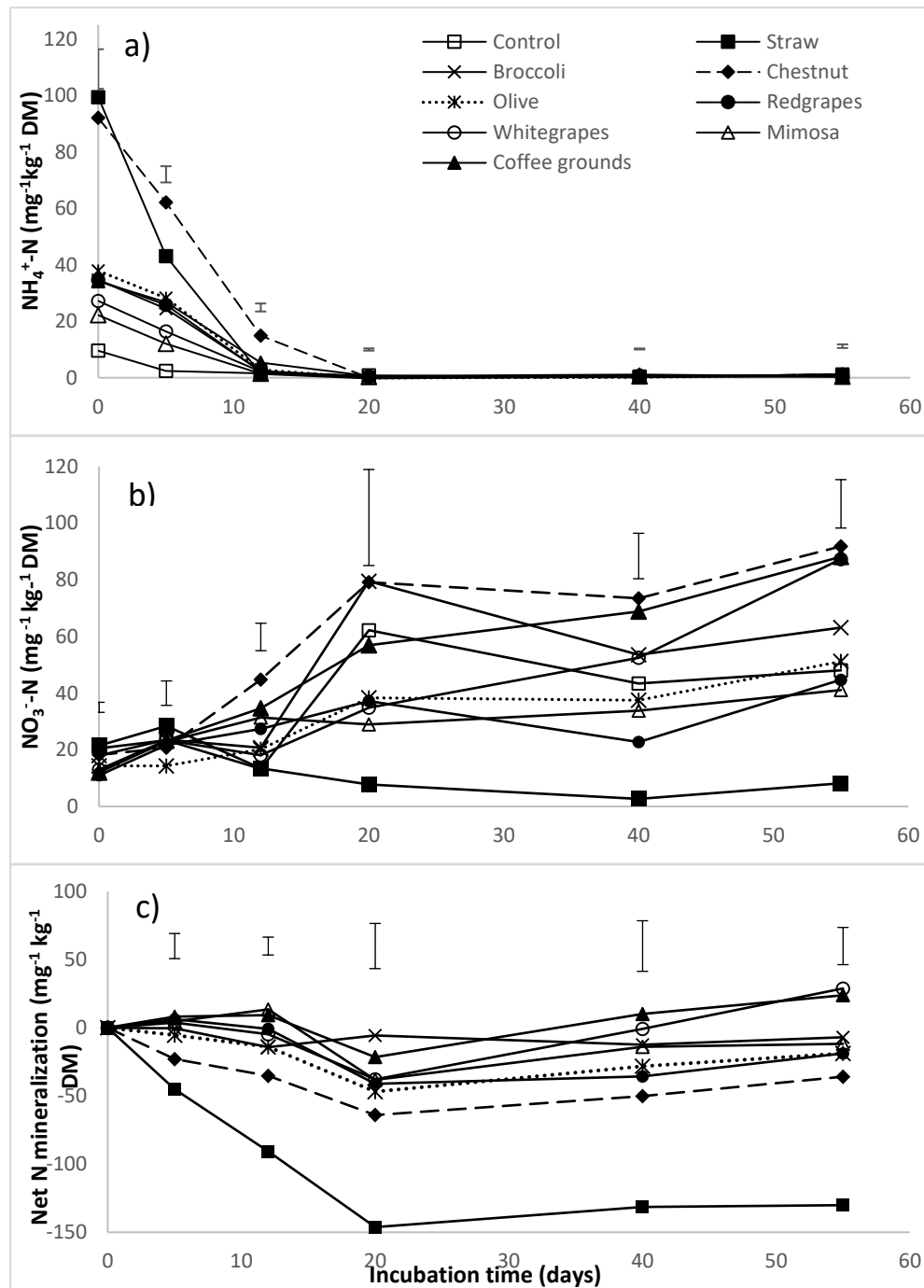


Figure 17: Evolution of $\text{NH}_4^+\text{-N}$ (a), $\text{NO}_3^-\text{-N}$, (b) and net N mineralization ($\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}$) (c) after agro-industrial wastes composts addition along a 55-day incubation period. Control have been subtracted. The vertical bars represent the maximum standard error observed in each day (n = 3).

Net N mineralisation (Figure 17c) decreased for Straw, Chestnut and Olive from the beginning of the experiment until day 20, that indicate a complete immobilisation of N forms. Straw amendment exhibited significantly and more accentuated N immobilisation than the rest of the treatments including Mimosa.

These results are in accordance with a previous work that refer that leaves and stems of Poaceae family (straw) had gradual and stronger N immobilisation than exhibited by Fabaceae (mimosa) being this behaviour imputed to the presence of higher cellulose and hemicellulose contents, low concentration of soluble compounds but also to low N content associated with limited N availability in the soil (Birouste et al. 2012). The difference on N mineralisation patterns is typically due to the different composition of the organic amendments, since N mineralisation depends on total and inorganic N content, C/N ratio and biochemical composition. Some studies have identified groups such as polyphenols, proteins, hemicellulose-like, cellulose-like and lignin compounds and use them for predict N mineralisation (Wang WJ, 2004; Thuries et al., 2002). In these treatments the availability of N in the initial mixtures, in particular the N-NH₄ differences, seem to rise the microbial N immobilization, reducing the net accumulation of mineral N in the soil. This trend was also found in a study that investigate the effect of mixing stem and leaf litters from 25 species of crops and cover crops on subsequent carbon (C) and nitrogen (N) (Redin et al., 2014).

It has been reported that crop residues with a C/N ratio of less than 25 will provide N mineralisation (Paul and Clark, 1989) and are designated as good quality residues, while residues with C/N ratio higher than 25 favour immobilisation and are designated as poor-quality residues. In this study, Straw with a C/N of 38, present a strong immobilisation during the first 20 days, conversely to Coffee grounds and Mimosa (C/N ratios of 8.7 and 12.4 respectively), which is in accordance with Abbasi and Khalia (2016), that reported that wheat straw residues promote net N immobilisation in initial stages, releasing N at larger stages.

However, Chestnut (C/N = 24.3) and Broccoli (C/N = 21.0) with similar C/N ratios presented different patterns, which indicate that C/N ratio by itself cannot explain all differences in net N mineralisation as the C/N reveals little on the availability of C and N to microorganisms (Kumar and Goh, 2000). In this study the net N mineralisation was only negatively correlated with C/N ratio ($r = -0.48$, $P < 0.05$). There was no effect of either phenolic and lignocellulosic contents or the initial N content on net mineralised N of the different amendments.

CO₂-C production and cumulative carbon mineralisation

The addition of the agro-industrial waste composts induced an increase in respiration rates (CO₂-C flux) throughout the incubation experiment (Figure 18).

The fluxes of CO₂-C from the compost treatments ranged from 0.09 to 6.0 mg h⁻¹ kg⁻¹, whereas for the un-amended soil always remained lower. Nevertheless, only the Straw treatment showed significant differences ($P < 0.05$) from the Control. In general, after a high initial flux a decrease was observed during the first 2-3 days of incubation, after which the CO₂ fluxes increased to reach maximum values between day 15 and 23 and thereafter decreased slowly until the end of the incubation period.

Straw showed the largest peak of CO₂ emission, observed on day 15 with a flux of 6.0 mg kg⁻¹. This high emission is expected to be linked to the degradation of the easiest mineralisable carbon fraction in Straw. From day 50 to 55, the soil amended with Mimosa and Coffee grounds showed significant lower values than the other treatments, which can be related with the lower content of soluble C present in these initial composts and the significantly greater lignin content (472.0 and 502.6 g kg⁻¹ DM, respectively).

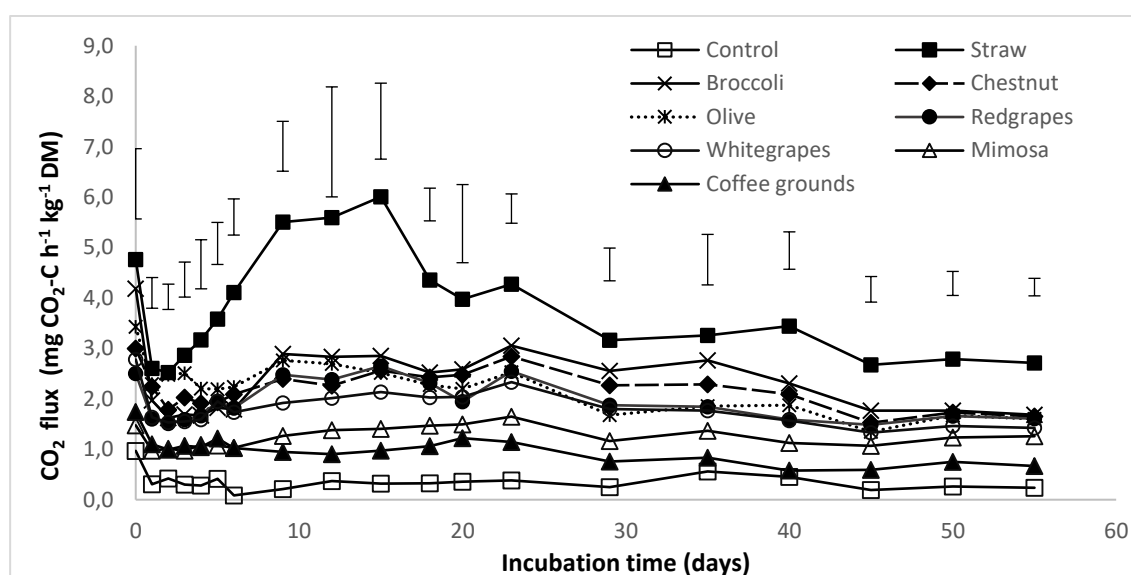


Figure 18: CO₂ flux emissions from soil amended with different agro-industrial wastes composts along a 55-day incubation period. The vertical bars represent the maximum standard error observed in each day ($n = 4$).

A first-order kinetic model previously introduced was fitted to the cumulative CO₂-C evolved from the agro-industrial wastes composts (Figure 19). Cumulative C mineralisation in Straw was significantly higher and at the end of the experiment was ca 6x higher than Coffee grounds. The last treatment showed the lower cumulative C mineralisation of all the treatments although was not significantly different ($P > 0.05$) from Mimosa.

The accumulated mineralised C at the end of incubation was positively correlated with the initial C/N ratio ($r = 0.90$; $P < 0.001$), TPhe/N, ($r = 0.75$; $P < 0.001$), GA/N ($r = 0.70$; $P < 0.001$) and NH_4^+ ($r = 0.46$; $P < 0.05$) and negatively correlated with TON ($r = -0.80$; $P < 0.005$) and lignin ($r = -0.78$; $P < 0.001$) of the amendments. Thus, all these parameters showed a strong correlation with the amounts of C mineralised ($P < 0.001$)

There was no effect of total phenolic, gallic acid, cellulose, hemicelluloses and holocellulose (cellulose + hemicellulose) contrary with what was observed in previous findings using annual crop residues (Jensen et al., 2005; Redin et al., 2014). There was no relation of the C mineralised with the initial concentrations in TOC and C_{sol} which means that neither the organic carbon content, neither the soluble fraction is the main responsible for the amount of mineralised carbon.

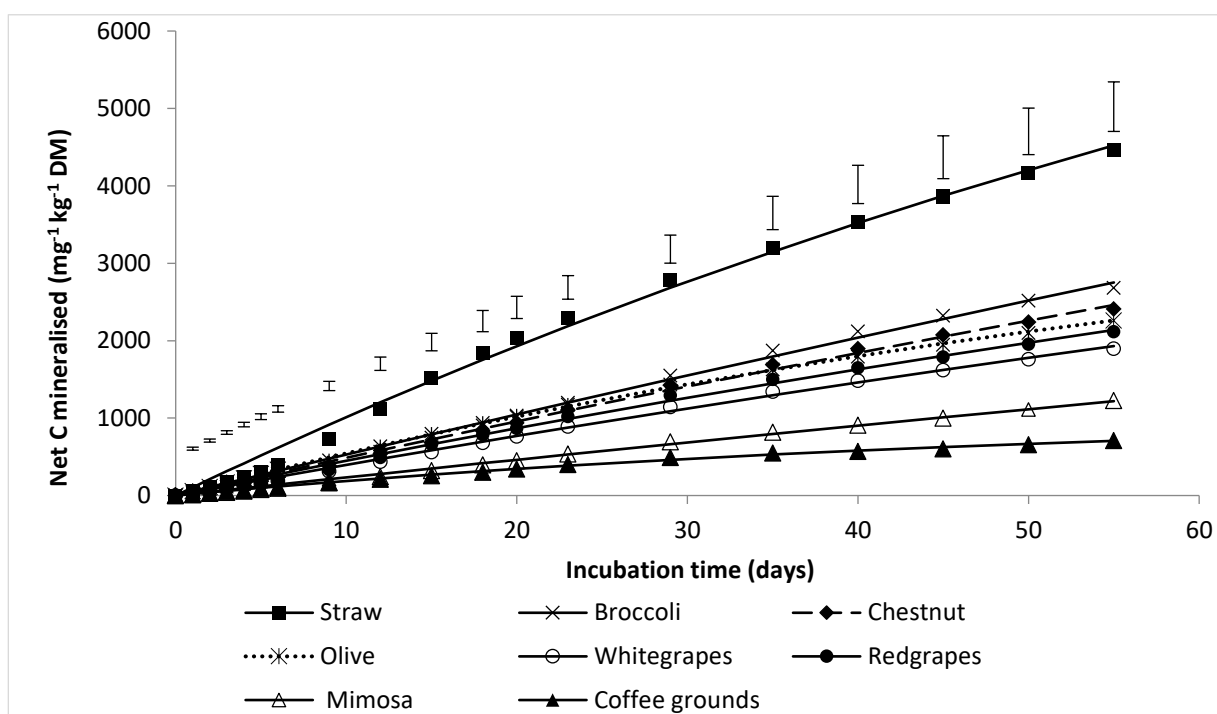


Figure 19: Cumulative C mineralisation from soil amended with different agro-industrial wastes composts after agro-industrial wastes composts addition along a 55-day incubation period. Control have been subtracted. Symbols represent the observed values and the lines the expected C mineralisation applying the exponential equation from Stanford and Smith (1972). The vertical bars represent the maximum standard error observed in each day ($n = 4$).

N₂O and CH₄ production

Flux emissions and cumulative emission of N₂O-N within the 55-day incubation for the treatments are represented in Figures 20a and 20b, respectively. Soil amendment with composted agro-industrial wastes enhanced N₂O emissions, which is in accordance with the results from Redin et al. (2014).

The Straw treatment exhibited the largest and significant emissions of nitrous oxide on days 5, 6 and 9, probably linked to the large nitrification activity in this period (Figure 17). Nitrous oxide emissions in the soil amended with coffee grounds increased from day 40, and at the end of experiment (day 55) a significant higher emission was observed comparing with the rest of treatments.

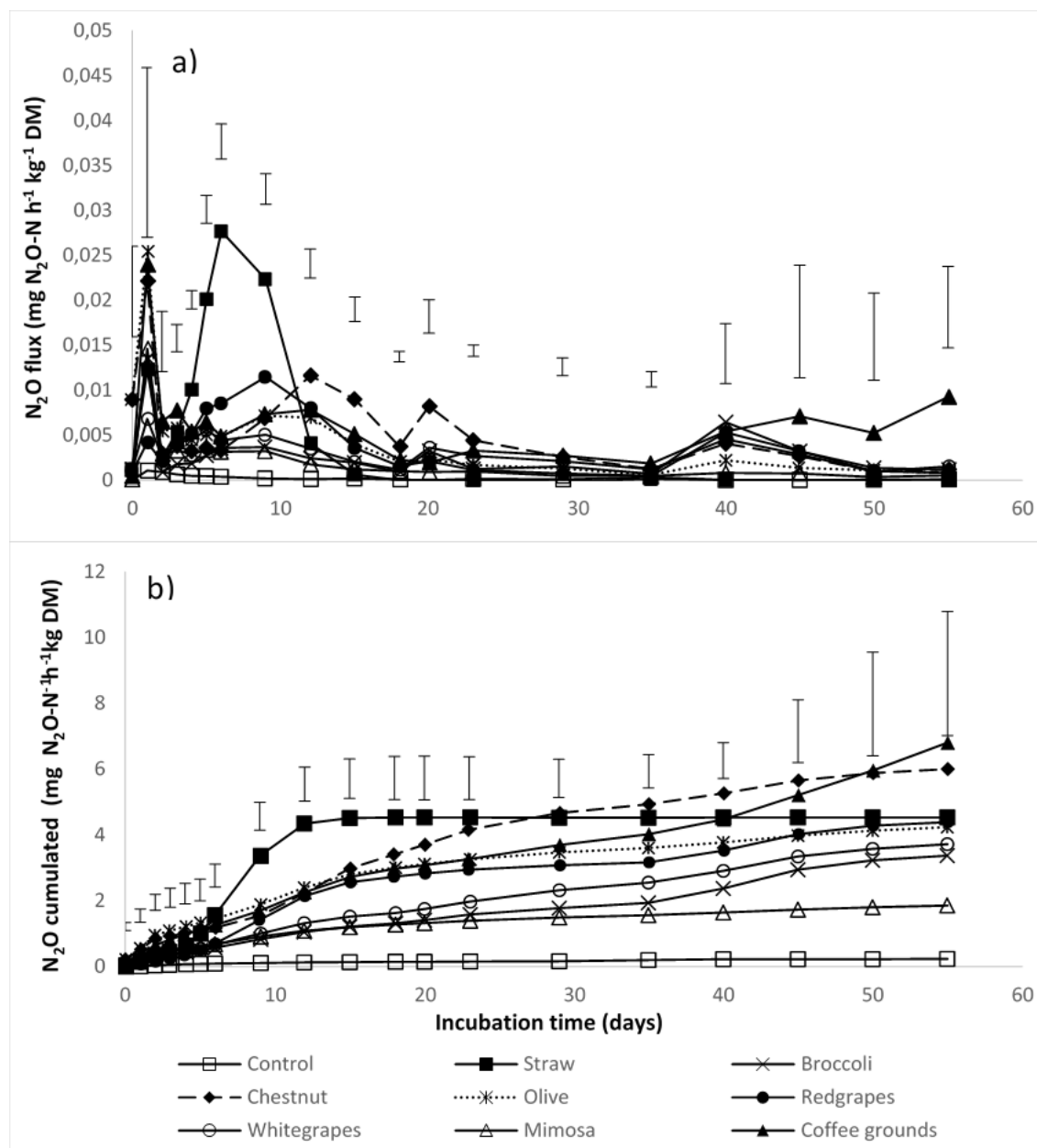


Figure 20: Flux emissions (a) and cumulative emissions (b) of N_2O from soil amended with different agro-industrial wastes composts along a 55-day incubation period. The vertical bars represent the maximum standard error observed in each day ($n = 4$).

After 55 days, cumulative emissions of N₂O were not significantly different between treatments excepting for the Control. Cumulative emission of N₂O on Coffee grounds increase almost four times when compared with Mimosa. This fact could be related with the initial high N content and narrow/low C/N ratio presented by Coffee grounds. Wastes with these properties result in faster N mineralisation rates and consequently increasing soil N₂O emissions (Raiesi, 2006).

Contrarily, wastes with low N content and high C/N ratio result in N immobilisation and in little impact on soil N₂O emissions (Huang et al., 2004). It was assumed that wastes with a high availability of labile C and N can induce to great emissions of CO₂ and N₂O (Nguyen et al., 2014)

A positive correlation was found between cumulative N₂O emission and initial N content: TON ($r = 0.80$; $P < 0.001$) and N_{soluble} ($r = 0.78$; $P < 0.001$). Other biochemical composition of residues also affected N₂O emissions; nitrous oxide emissions increased with the content of lignin ($r = 0.56$; $P < 0.01$) but a negative correlation was found with C/N ($r = -0.59$; $P < 0.01$).

Previous studies also reported similar correlation between soil N₂O emissions and C/N ratio following cover-crop residues application that may be associated with the slower decomposition and transitory N immobilisation of soil N in cells of microorganism (Chen et al., 2013; Nguyen et al., 2014; Pimentel et al., 2015).

When the interaction of polyphenols and lignocellulosic compounds with the N contents were expressed (TPhe/N), GA/N, lignin/N, hemicelluloses/N), cumulative N₂O emissions decreased as these ratios increased. Pimentel et al. (2015) also observed this trend that probably is linked to the recalcitrant nature of polyphenols and lignin to microbial decomposition. These indices can be used as a tool for the choice of wastes with the minimal impact on soil N₂O emissions.

In our study, soil NH₄⁺ concentration was close to zero (Figure 17a) and did not vary since day 20. Contrarily, NO₃⁻ is the most available N form and their concentration increased through the incubation period (Figure 17b). So, it's reasonable to say that nitrification is the main N₂O production process, since N-NO₃ was frequently reported as being associated with N₂O emissions (Alvarez et al., 2012; Cosentino et al., 2013). Beside this explanation, it is commonly acknowledged that at 30-60% WFPS and at aerobic conditions, nitrification is the main source of N₂O (Chen et al., 2013).

Flux emission and cumulative emissions of CH₄-C within the 55-day incubation for the treatments are represented in Figures 21a and 21b, respectively. It's possible to observe that peaks of methane emissions were largely coincident or close to the days of water addition.

In general, very low or negative fluxes were produced in all treatments during the incubation experiment. Even so, Straw was the treatment that present the lowest methane emission.

Coffee grounds treatment presented a significantly higher cumulative CH_4 emission at the end with a value of 0.32 mg kg^{-1} (Figure 21b).

A positive correlation was found between cumulative CH_4 emission and lignin ($r = 0.64$; $P < 0.001$), TON ($r = 0.81$; $P < 0.001$) and $\text{N}_{\text{soluble}}$ ($r = 0.50$; $P < 0.05$).

The cumulative CH_4 emission was negatively correlated with C/N ratio ($r = -0.75$, $P < 0.001$), TPhe/N ratio ($r = -0.75$; $P < 0.001$) and GA/N ratio ($r = -0.64$; $P < 0.001$).

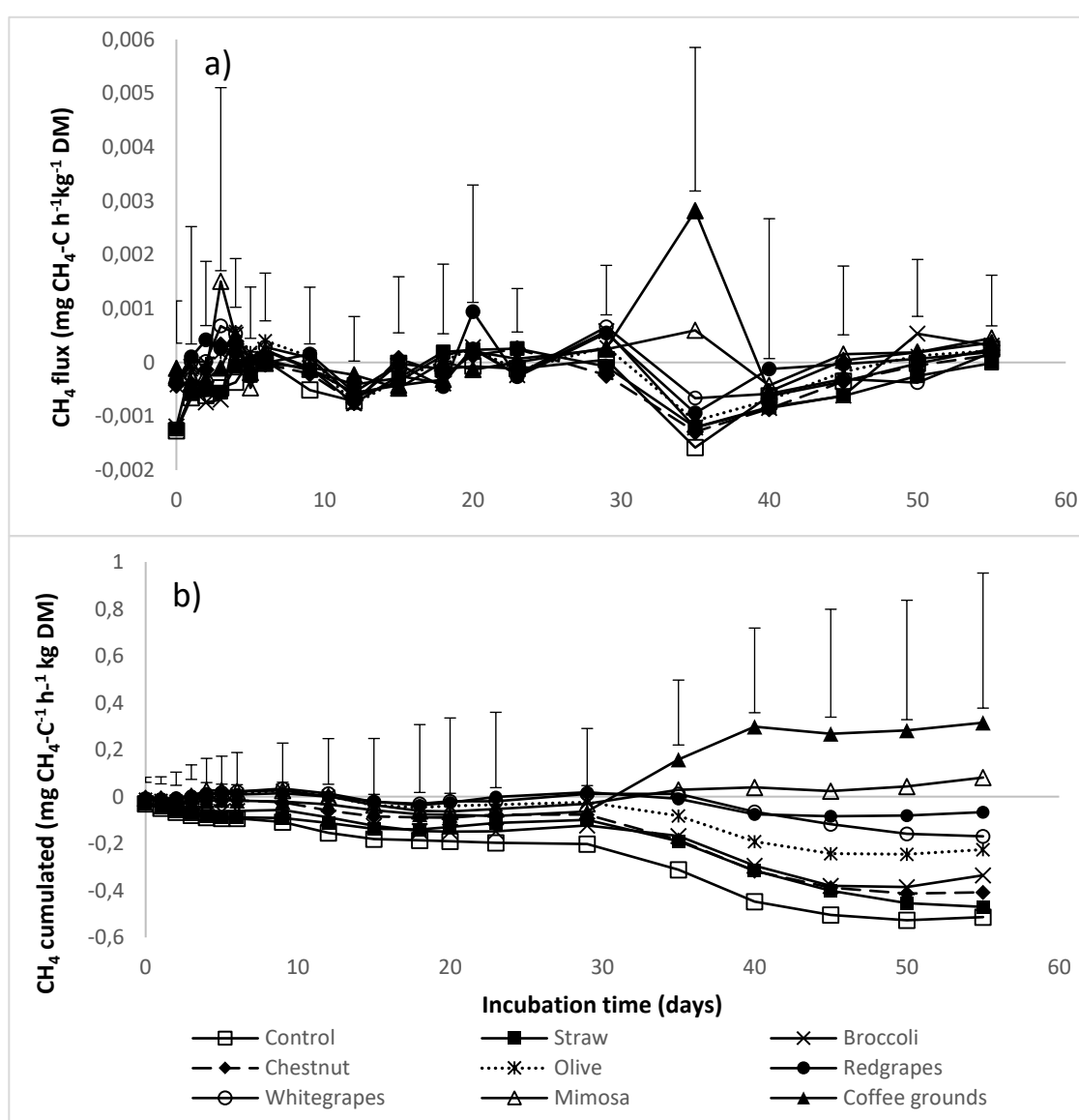


Figure 21: Flux emissions (a) and cumulative emissions (b) of CH_4 from soil amended with different agro-industrial wastes composts along a 55-day incubation period. The vertical bars represent the maximum standard error observed in each day ($n=4$).

Greenhouse gas emissions

The global warming potential is a comparative measure that reflects the impact of the emission of a gas relative to one unit of the reference gas, carbon dioxide. The conversion of N₂O and CH₄ emissions to CO₂ equivalents allows a better understanding of the impact of the different amendments in terms of GHG emissions to the atmosphere by considering the contribution of each gaseous compound to the greenhouse effect. In this study the CO₂ emissions from the amended composts were considered as a fast cycling C process and thereafter not included in the calculation of GWP.

The cumulative GHG emissions of the treatments are summarized in Table 11 with values ranging from 0.45 g CO₂ eq. kg⁻¹ initial DM (Mimosa) to 1.76 g CO₂ eq. kg⁻¹ initial DM (Coffee grounds), following the sequence: Coffee grounds > Chestnut > Straw > Redgrapes > Olive > Whitegrapes > Broccoli > Mimosa.

The lowest GHGs emission were observed from Mimosa which was significantly lower than all other treatments, however, the highest contribution of CH₄ were observed on this treatment.

N₂O was the main contributor to total GHG emission with values ranged by 96.2 % (Mimosa) to 99.9 (Whitegrapes).

Table 11: Final cumulative GHG emissions (N₂O and CH₄) expressed as CO₂ equivalents and relative contribution of N₂O and CH₄ from soil amended with different agro-industrial wastes composts along a 55-day incubation period.

Amendments	GHG emissions g CO ₂ eq. kg ⁻¹ DM amendment	Contribution (%)	
		N ₂ O	CH ₄
Straw	1.14 ± 0.04 bc	99.4 a	0.6 b
Broccoli	0.84 ± 0.11 cd	99.8 a	0.2 b
Chestnut	1.53 ± 0.12 ab	98.6 a	1.4 b
Olive	1.07 ± 0.12 ab	99.2 a	0.8 b
Redgrapes	1.11 ± 0.19 bc	98.9 a	1.1 b
Whitegrapes	0.93 ± 0.16 cd	99.9 a	0.1 b
Mimosa	0.45 ± 0.02 d	96.2 b	3.8 a
Coffee grounds	1.76 ± 0.49 a	99.0 a	1.0 b

Values within a column, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

From this it can be infer that Mimosa is likely to be a better treatment in terms of GHG mitigation. Although the higher contribution of CH₄ from this compost, the emissions are considered negligible. Coffee grounds compost revealed not be the better strategy comparing with the rest of treatments, since promote the higher N₂O emissions.

Conclusions

Our laboratory experiment showed the influence that the initial characteristics of the organic composts used have on the GHG emission and C and N mineralisation. The incorporation of organic composts resulted in higher cumulative CO₂, N₂O and CH₄ emissions when compared with the control (un-amended soil).

The results suggest that the effect of compost incorporation on GHG emissions are dependent on their chemical composition. Composts with higher TON and lignin content, as the case of Coffee grounds and Mimosa, displayed lower C mineralisation and hence low CO₂ emissions. The interaction of polyphenols and gallic acid with the N compounds such as TPhe/N and GA/N demonstrated to be appropriated for establish a relation with C mineralisation.

Composts with greater TON, N_{sol} and lignin initial contents, presented faster N mineralisation and produced higher N₂O and CH₄ emissions. When the interaction of polyphenols and lignocellulosic compounds with the N contents were expressed, TPhe/N, GA/N, lignin/N, hemicelluloses/N cumulative a decreasing on N₂O emissions occurred as these ratios increased. We can state that these indices can be used as an appliance for the choice of organic materials with the least impact on soil N₂O emissions.

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Chapter 4. Effects of the produced composts on soil-borne pathogens

Bio-pesticide potential of composted lignocellulosic agro-industry-wastes to suppress phytopathogenic fungi: *in vivo* and *in vitro* studies

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Abstract

Composting is considered a reliable way for recycling and reusing organic wastes, hence addressing the increasing public concern about environmental pollution. Besides their fertilizing effects, composts have been reported to have disease-suppressive properties and could be used in strategies for managing soil-borne diseases. The aim of this study was to test the disease suppressive potential of four composts made from chestnut peels and shells, coffee grounds, grape marc and olive leaves. The suppressive capacity of the compost water extracts was first evaluated *in vitro* against six soil-borne fungi using the macrodilution broth method. *In vivo* bioassays involved growing potted lettuces on 5% and 10% composted media inoculated either with *Botrytis cinerea* or *Fusarium oxysporum*. The determination of minimal inhibitory concentrations indicated that none of the composts inhibited the mycelium growth of the pathogens. *In vivo* however, the coffee-based compost at 5% and the grape-based compost at 5% and 10% also exhibited fertilizing effects but increased the severity and the incidence of the diseases, by 50%. A suppressive effect was noticed for the chestnut- and olive-based composts, with disease severity indexes of 1, and disease incidences of 0%, indicating an absence of symptoms; moreover, highest total dry matter yields were measured for plants grown on the

two composts, with no differences found between the results obtained using 5% and 10% doses. The beneficial fertilizing and biocontrol effects of the chestnut and olive composts could be exploited in strategies aimed at managing agro-food wastes and reducing reliance on synthetic chemicals in agriculture.

Keywords: Antifungal activity/suppressive effect, Biological control, Lignocellulosic composts, Macrodilution assay, Soil-borne diseases

Introduction

During the last century, a decline in soil organic matter, soil fertility and an increase in pathogen resistance occurred worldwide; these changes are largely attributed to an unreasonable application of synthetic fertilizers and pesticides, and the introduction of new plant varieties resistant to diseases (Hoitink and Boehm, 1999; Bailey and Lazarovits, 2003; Mehta et al., 2014). A number of research works have been carried out with the aim of finding ways to reduce the use of synthetic fertilisers and pesticides, improve soil organic matter, and ultimately create an eco-sustainable and efficient agriculture (Martin, 2003; Bonanomi et al., 2010). In this regard, the use of composts has been reported to be advantageous for both recycling of wastes and replenishing of soil fertility (Mehta et al., 2014). Moreover, application of composts to soils is seen as a natural tool for managing soil-borne diseases and reducing the need of pesticides (Mehta et al., 2014). It's important to highlight the reliability of this alternative to fertilisers and pesticides, since the main requirement for a safe application of composts is their stability, maturity and absence of pathogens. Composts usually contain low levels of heavy metals and phytotoxicity is mainly observed with immature composted materials (Zucconi et al., 1981; Widmer et al., 2007) applied at elevated rates (Erhart et al., 1999; Szczech and Smolinska, 2001).

Hoitink et al. (1975) first proposed the use of composts for disease control. Since then, numerous studies have been published on the topic. Bonanomi et al. (2010) analysed 250 articles reporting 2423 experiments on the use of several types of organic amendments for disease control, and concluded that composts were the most suppressive amendments, with more than 50% of experiments resulting in effective disease control. Diseases caused by soilborne fungi are among the major factors limiting the productivity of agroecosystems,

resulting in significant economic losses (Metha et al, 2014). The suppressive effect of composts on soilborne fungi has been demonstrated with *Botrytis* sp. (Borrero et al., 2013), *Fusarium* sp. (Morales et al., 2016), *Pythium* sp. (Vestberg et al., 2014), *Rhizoctonia* sp. (Mengesha et al., 2017), *Alternaria* sp (Pane et al., 2012) and *Sclerotinia* sp. (Pane et al., 2011).

The disease suppressive capacity of composts comprises an intricate set of mechanisms, imputed to their biotic and/or abiotic properties (Hadar and Papadopoulou 2012, Vestberg et al., 2014). The following physical and chemical mechanisms of composts are known: competition for carbon and nutrients, formation of humic and fulvic acids, and release of toxic compounds during the decomposition of organic matter (Smolinska, 2000; Tenuta and Lazarovits, 2007). Reported biological mechanisms of composts include antagonism through antibiosis (Hoitink and Boehm, 1999), parasitism, incremented competition for resources leading to, fungistasis (Lockwood, 1990), antibiosis, and systemic induced resistance in host plants (Zhang et al., 2007; Pharand et al., 2007).

The suppressive properties of organic amendments have been primarily attributed to biotic effects. However, the profile of bacterial and fungal communities seems to be influenced by the chemical composition of the raw material from which the compost is made (Blaya et al. 2013). Hence, the effectiveness of disease control might also depend on the compost type, which is related to the chemical and physical characteristics of the raw materials (Termorshuizen et al., 2006; Galitskaya et al., 2015), storage conditions before composting, compost maturity, and mode, timing and rate of application (Blaya, 2013; Suárez-Estrella et al., 2013). For example, nutrients, humic, phenolic or bioactive compounds present in the composts are reported to protect plants against diseases through enhanced nutritional status of the soil, direct toxicity on pathogens, and/or induced systemic resistance in plants (Hoitink et al., 1997; Spatafora, 2012). The suppressive ability of composts also varies depending on the pathogen species. Studies have demonstrated that a compost can suppress one pathogen, but be ineffective against or beneficial to another pathogen (Bonanomi et al., 2007).

The objective of this work was to test the *in vitro* antifungal activity of four composted agro-food wastes against different soilborne plant pathogens, and their suppressive ability against *Botrytis cinerea* and *Fusarium oxysporum* *in vivo* in lettuce. This study fits squarely into the circular economy concept, which advocates finding means for achieving a sustainable growth. Interesting data were obtained that could be used as support in developing biological control strategies against soilborne diseases, allowing a reduced use of chemical fungicides and a recycling option for agroindustrial wastes.

Materials and methods

Preparation of composts

The composts used in this study were produced at a specialised pilot composting plant under aerobic conditions. Composting was carried out in two distinct experiments depending of the raw materials. The following raw materials were collected from agro-food industries in the north of Portugal: a) Chestnut peels and shells; b) Coffee grounds; c) Grape marc from *Vitis vinifera* L. var. Alfrocheiro and d) Olive leaves. Wheat straw and silver wattle (*Acacia dealbata*) leaves were used as bulking agents. The composting procedures were as described by Santos et al. (2017) and Santos et al. (2018). Moisture was maintained between 45 and 60% by occasional watering. A complete temperature stabilisation occurred after 156 days in the case of coffee grounds and 147 days for the other raw materials, at which time the composting process was stopped. The physicochemical characteristics of the composts were determined and are shown in Table 12. These composts had good levels of nutrients, phenolic and lignocellulosic compounds (Santos et al., 2017; Santos et al., 2018), a high degree of humification and no phytotoxic effect on the germination of seeds from several species (data not shown).

Table 12: General characteristics of the composts used in the study on a dry weight (DW) basis

	TOC (g C kg ⁻¹)	TN (g N kg ⁻¹)	C/N	pH	EC
Chestnut	537.0 ± 3.51 a	22.1 ± 0.63 bc	24.3 ± 0.54 a	9.43 ± 0.30 ab	1.43 ± 0.19 a
Coffee grounds	495.4 ± 4.87 b	57.0 ± 4.87 a	8.7 ± 0.15 c	7.05 ± 0.05 c	0.78 ± 0.07 b
Grape marc	523.0 ± 2.62 ab	19.7 ± 2.59 c	19.7 ± 2.59 b	9.01 ± 0.02 b	1.28 ± 0.13 a
Olive leaves	533.2 ± 0.77 ab	24.4 ± 1.64 b	21.9 ± 1.52 a	9.53 ± 0.14 a	1.23 ± 0.12 a

Values (mean ± standard deviation of four replicates) within a column, with the same letter are not significantly different ($P < 0.05$).

TOC - total organic carbon, TN - total nitrogen., EC - electrical conductivity.

Phytopathogenic fungi

The suppressive ability of the composts was tested against some economically relevant phytopathogenic fungi, namely *Botrytis cinerea* ATCC RW003, *Fusarium oxysporum* ATCC MYA-3072, *Sclerotinia sclerotiorum* ATCC 18015, *Rhizoctonia solani* ATCC 56612, and some *Alternaria* and *Pythium* sp. *Botrytis cinerea* ATCC RW003, *Fusarium oxysporum* ATCC MYA-3072, *Sclerotinia sclerotiorum* ATCC 18015 and *Rhizoctonia solani* ATCC 56612 were supplied by the American Type Culture Collection (ATCC). *Alternaria* sp. and *Pythium* sp. were isolated from infected lettuce plants grown in the Trás-os-Montes e Alto Douro Region of Portugal. Root, stem and leaf sections of the lettuces with evident lesions were cut and placed on a potato dextrose agar (PDA) medium after surface sterilisation. A pure fungal culture was obtained, and the pathogens were maintained on PDA in a culture chamber at 25°C.

***In vitro* antifungal activity using the macrobroth dilution method**

The composts' water extracts were prepared by adding 250 g of grounded compost (2 mm sieve) with 1 L of sterile distilled water (1:4 w:v). The mixtures were placed on an orbital shaker (200 rpm) in the dark at 25°C. After 72 h of incubation, the solid fraction was removed by filtration and the water extracts were stored at 4°C until used (St. Martin and Brathwaite, 2012)

The *in vitro* antifungal activity of the composts was evaluated against all the phytopathogenic fungi above mentioned, using the macrodilution broth method, and specifically the protocol M38-A2 of the National Committee for Clinical Laboratory Standards (CLSI, 2008).

Two-fold serial dilutions of the composts' extracts were made and five concentrations ranging from 1.6 to 200 µL/mL were prepared from the dilutions. Fresh cultures of each phytopathogenic fungus were used to prepare cell suspensions of $1-2 \times 10^4$ cells/mL, measured using a hemacytometer. Test tubes with Roswell Park Memorial Institute RPMI 1640 medium were inoculated with 80 µL of inoculum suspension and added with 80 µL of composts' extract or amphotericin B as positive control. Negative control tubes contained the inoculum suspension and 80 µL of water. The tubes were incubated at 30°C and were observed daily for 7 d, at which time minimal inhibitory concentrations (MICs) were registered. From the tubes in which no visual fungal growth was detected, the MLCs were determined by plating 100 µL of the broth, onto Sabouraud dextrose agar plates. MLC plates were then incubated under the same conditions described above. In all cases, the tests were carried out in duplicate.

***In vivo* experiment using potted lettuce**

The *in vivo* experiment was carried out in a greenhouse using the lettuce (*Lactuca sativa*) variety Maravilha dos Invernos. *Botrytis cinerea* ATCC RW003 and *Fusarium oxysporum* ATCC MYA-3072 were chosen for infecting plants because of their devastating impact on lettuce. Seeds were first germinated on plastic trays (41 L × 41 l × 5 H cm) filled with peat in a darkened growing chamber at 28°C for 24 h. Then, the seeds were transferred to the greenhouse where they were grown at 25–30°C (day)/15–18°C (night) and 60-75% relative humidity for 12 d until the second leaf development stage.

A clay soil with 17% sand, 28% silt and 55% clay was collected from the topsoil layer (0-30) in the central region of Portugal (latitude: 392.20150 N, longitude: 844.2570 W), sieved and introduced into 2-L plastic pots. To estimate compost's suppressiveness, the soil in pots was inoculated with *F. oxysporum* and *B. cinerea* (10⁴ spores/g) and left at 25°C. In control pots, water was added instead of the spore suspension (no inocula treatment). After 12 d of incubation, the coffee-, chestnut-, grape-, and olive-based composts were added to the pots at doses of 5 and 10 % (w/w) and mixed with the top-layer of the soil, constituting 27 treatments: [(2 fungi + no inocula) × 4 composts × 2 composts' doses) + (2 fungi without compost + no inocula without compost)]. The 27 treatments were incubated at 25°C for 5 d to allow complete infection of the growing media.

Afterward, the lettuce seedlings were transplanted with one seedling per pot. In the case of *B. cinerea* treatments, basal leaves were gently covered with soil to promote plant infection. The pots were distributed in the greenhouse using a complete randomized design with three factors (compost × doses × inoculum) and four replicates per treatment. The plants were grown under natural daylight conditions and were irrigated when needed. Thirty days after transplantation, the pot experiment was ended. Disease severity was assessed using the following scale: (1) no symptoms; (2) leaf yellowing; (3) stem wilting; (4) plant dead (Suárez-Estrella et al., 2013; Blaya et al., 2013). The shoots and roots of plants were harvested, and the number of infected plants was recorded i.e., plants with visible rotten leaf, roots or stems or with blackening of the roots. Disease incidence (DI) was calculated as the percentage of infected plants. The plant parts were weighted, dried for 72 h at 80 °C, and the dry weights recorded.

Statistical analyses

Results are presented as mean \pm standard deviation (SD, $n = 4$). Data were transformed using the arcsine square root ($x/100$) for disease incidence and weight, and \ln for disease severity. Data were analysed by ANOVA using Statistix 10.0 (Analytical Software, Tallahassee, USA). When the effect of the factors or their interactions was significant ($P < 0.05$), the Tukey's test was used to separate the means at the probability level of 0.05.

Results and discussion

In vitro antifungal activity of composts against phytopathogenic fungi

In the present work, the antifungal activity of four composts was tested *in vitro* against six soilborne fungi. None of the composts' extracts exhibited significant inhibitory activities against the pathogens, no matter their concentrations. Mycelium growth was observed for all fungi isolates over the period between 3 and 5 days of incubation.

In vitro tests using macrodilution to assess fungal susceptibility to potential control substances are already standardized and extensively used (Balouri et al., 2016). Such tests, however, have been rarely applied in selecting composts that could be used as natural fungicides. An absence of inhibitory effect by the composts in the present study could not be attributed to the macrodilution broth method used. In fact, amphotericin B used as positive control showed minimal inhibitory concentrations (MIC) $\geq 0.25 \mu\text{g/mL}$ depending of the tested soilborne pathogen, attesting to the reliability of the method. Moreover, MIC values of amphotericin B in relation to *Fusarium sp.*, and *Botrytis sp.*, were within the ranges of those reported by other authors using the macrodilution broth method (CLSI, 2008).

In vivo antifungal activity of composts against phytopathogenic fungi

Lettuce plants were grown in composted media inoculated with *Botrytis cinerea* ATCC RW003 and *Fusarium oxysporum* ATCC MYA-3072. Disease severity indexes following plant grow on two proportions (5% and 10%) of the media are shown in Figure 22. No disease symptom, in the case of *F. oxysporum*, was observed on non-inoculated plants. Plants grown on the chestnut- and olive-based composts did not exhibit any disease symptoms, showing the effectiveness of these composts in controlling *B. cinerea* and *F. oxysporum*. Plants grown on the coffee- and grape-based composts on the other hand were severely affected by both wilt

diseases, showing leaf yellowing, stem wilting, and even dead (Figure 22). In fact, 5% coffee-based composts and 5% and 10% grape-based composts, with respect to the control (soil), increased the severity of the two diseases. Borrero et al. (2013) analysed the disease suppressive capacity of different composts and found the grape marc-based compost to be the most effective in controlling *F. oxysporum* on tomato and carnation plants. The discrepancy between Borrero et al. (2013) and the results of the present study might be explained by differences in plant species used, and fungi isolates tested.

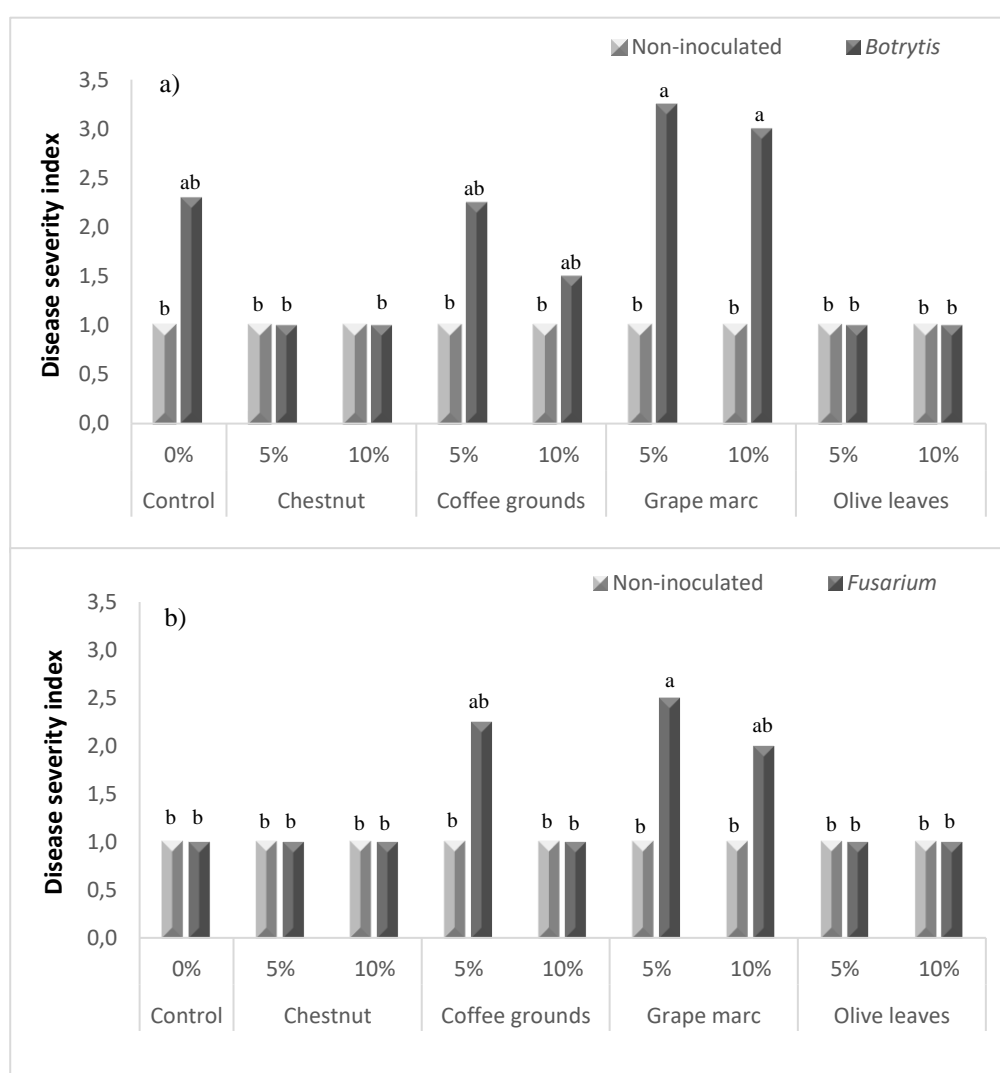


Figure 22: Effect of composts at two different doses (5% and 10%) on the severity of *Botrytis cinerea* ATCC RW003 (a) and *Fusarium oxysporum* ATCC MYA-3072 (b) on lettuce plants. The disease severity index is rated using a semi-quantitative scale from 1 to 4 where: 1= no symptoms; 2 = leaf yellowing; 3 = stem wilting and 4 = plant dead. Different letters above the bars indicate significant differences according to the ANOVA/Tukey's test ($P < 0.05$).

In the case of coffee-based compost, the dose of 5% increased disease severity with respect to the control, while the dose of 10% decreased the severity of *B. cinerea* and had no effect on that of *F. oxysporum*. The dose of 10% was also more effective than 5% in limiting the spread of both diseases in the case of the grape-based compost, although no significant difference ($P < 0.05$) was found (Figure 22). The dose-dependent effect of composts has been demonstrated in several other studies (e.g., Tuitert et al. 1998; Galitskaya et al. 2016), many of which have demonstrated that doses ranging from 10 to 25% are sufficient to incite disease suppression (Raviv, 2008).

The disease incidence of *Botrytis cinerea* and *Fusarium oxysporum* in lettuce plants grown on composts are shown in Table 13.

Table 13: Effect of composts at two different doses (5% and 10%, v:v) on the disease incidence of *Botrytis cinerea* ATCC RW003 and *Fusarium oxysporum* ATCC MYA-3072 in lettuce

	Dose	Non-inoculated	<i>Botrytis</i>	<i>Fusarium</i>
Control	0%	n.i	25	n.i
Chestnut	5%	n.i	n.i	n.i
	10%	n.i	n.i	n.i
Coffee grounds	5%	n.i	50	50
	10%	n.i	25	n.i
Grape marc	5%	n.i	50	100
	10%	n.i	75	50
Olive leaves	5%	n.i	n.i	n.i
	10%	n.i	n.i	n.i

Disease incidence was calculated as percentage of diseased plants of four replicates. Different letters indicate significant differences according to the ANOVA Tukey's test ($P < 0.05$). n.i – no incidence

Disease incidence results were generally in agreement with disease severity results: no disease in plants grown on the chestnut- and olive-based composts; *Botrytis* and *Fusarium* wilt incidences of 50% in plants grown on 5% coffee- and grape-based composts, respectively; *Botrytis* wilt incidence of 75% and *Fusarium* wilt incidence of 50% in plants grown on 10% grape-based compost. However, the coffee-based compost at 10% was effective in reducing the incidence of *F. oxysporum* in respect to the control (Table 13).

Taken together, these results indicate the potential use of the chestnut- and olive-based composts at 5 and 10% for controlling *B. cinerea* and *F. oxysporum* on lettuce plants. The grape- and coffee-based composts on the other hand enhanced disease severity and incidence, though the effect depended on the dose for the later compost. These results can largely be

explained by the physicochemical characteristics of the composts used. It is also reported that the microbial community of a media is influenced by the characteristics of the raw materials used in composting (Avilés et al., 2011).

It is widely recognized that pH conditions affect the microbial community of composts. For example, the population of Actinomycetes increases with increasing pH, and this is reportedly related to the capacity of these bacteria to generate antibiotics and chitinases with a suppressive effect on *F. oxysporum* (Ntougias et al., 2008; Patel et al., 2010). Morales et al. (2016) grew muskmelon seedlings on composts produced from fruits and vegetables wastes and found a negative correlation between the compost pH and *F. oxysporum* f.sp. melonis severity. A similar correlation was found in the present study (-0.70 ; $P < 0.05$). Indeed, the coffee- and grape-based composts had lower pH values than the other composts (Table 12), and their use led to higher disease incidences.

Several studies have demonstrated the importance of the availability of the growth media's nutrients for controlling diseases e.g., Mn, Zn, Fe, and Cu for Fusarium wilt biocontrol (Cotxarrera et al., 2002; Borrero et al., 2007; Segarra et al., 2010). It was reported that low amounts or unavailability of nutrients create a competition among microorganisms relying on the same nutrients, which in situations of competition disadvantage limits the sporulation, growth and pathogenicity of *F. oxysporum* (Cotxarrera et al. 2002, Blaya et al. 2013). The composts used in the present study had good amounts of nutrients. Although the chestnut- and olive-based composts had lower contents of Mn than the other composts, no significant differences were found between the composts with respect to P, Ca, Mg, Fe, Cu and Zn (Santos et al., 2017; Santos et al., 2018). Therefore, differences in disease severity and incidence shown in Figure 1 and Table 2 could not be explained by differences in the availability of nutrients.

The dry weights of shoots and roots of lettuce plants grown on the composted media were recorded and the total dry weight calculated as shown in Figure 23. For the same treatment, plants grown in non-inoculated pots had in general higher mean total dry weights than plants grown in inoculated pots. There were no significant differences ($P < 0.05$) between the dry weights of Botrytis-inoculated plants and those of Fusarium-inoculated plants. With respect to the control, all treatments increased the dry weights of plants, with the exception of 5% coffee-based compost. The lowest values of dry weights were calculated for 10% coffee-based compost, and 5% and 10% grape-based composts. This was an interesting finding as it showed that despite increasing disease severity and disease incidence (Figure 1 and Table 2), the coffee- and grape-based composts were beneficial to plant growth. The chestnut- and olive-based media led to highest total dry weights, with the top values observed for the dose of 10%.

Santos et al. (2016) have also reported the potential use of the same chestnut- and olive-based composts studied in this study, as natural fertilizers. In their study using the lettuce varieties Maravilha dos Invernos and Quatro Estações, higher yields were observed with the two composts, as compared to the grape- and broccoli-based composts; moreover, the former composts led to increased antioxidant activities and phenolic contents in lettuces (Santos et al. 2016). The production of phenolic compounds by plant represents an important line of defence against infections and their accumulation in lettuces grown on the composted media could also explain the low susceptibility to *B. cinerea* and *F. oxysporum*.

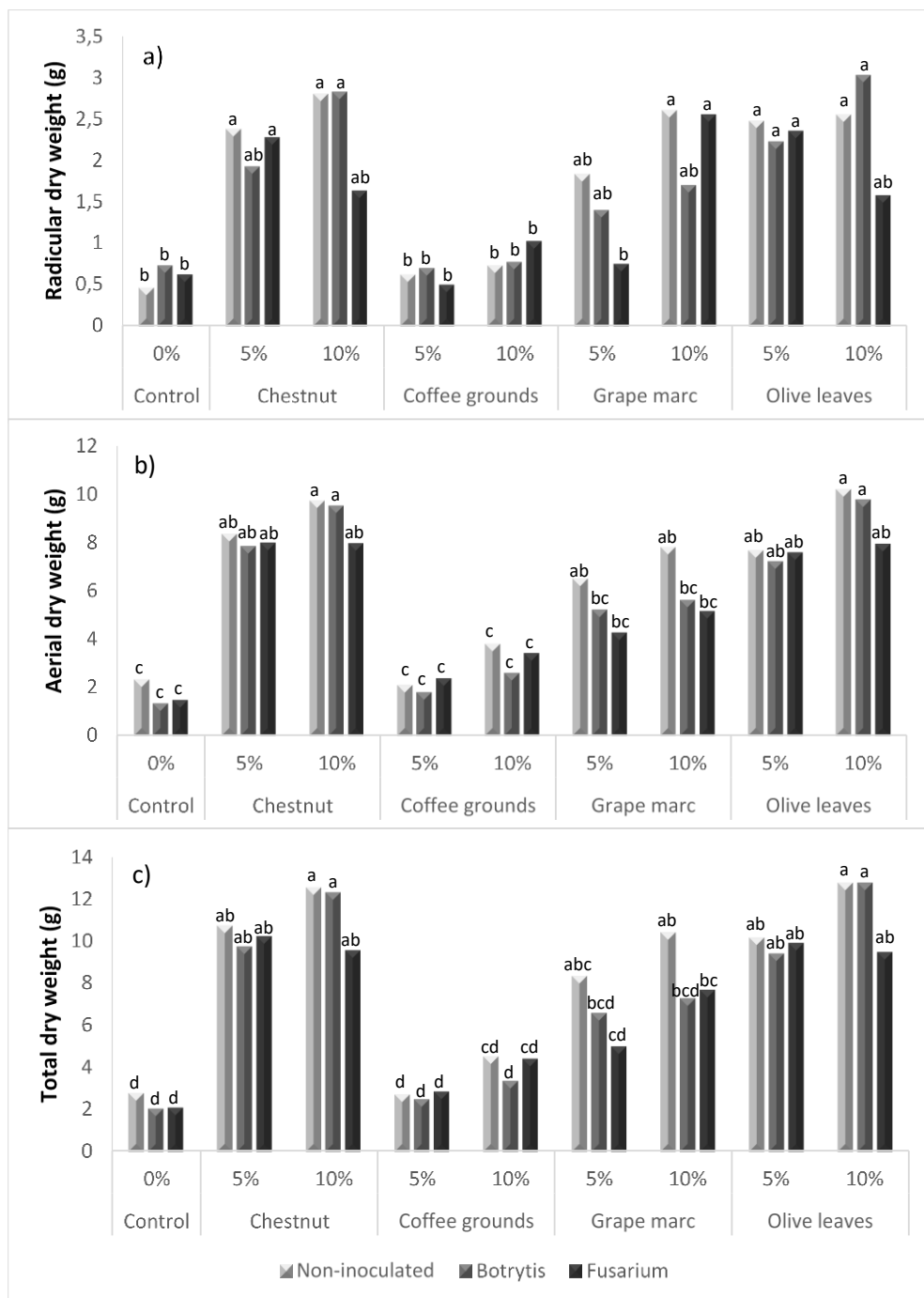


Figure 23: Effect of composts at two different doses (5% and 10%) on a) root weight (g DM), b) shoot weight (g DM) and total plant weight (g DM) of lettuce exposed to *Botrytis cinerea* ATCC RW003 and *Fusarium oxysporum* ATCC MYA-3072 for 30 days.

Conclusions

In this study, the disease suppressive capacity of four composts on lettuces was evaluated in *in vitro* and *in vivo* bioassays. It was found that chestnut and olive-based composts were effective in controlling soilborne diseases caused by *B. cinerea* and *F. oxysporum*, resulting in improved lettuce growth. No differences were found between the results obtained using 5% and 10% doses, denoting the possibility of saving production costs by using low amounts of the composts. Despite increasing disease severity and disease incidence, the coffee- and grape-based composts were also found to be beneficial to plant growth, as compared to the control. Further studies are required to (i) define the best application mode for the composts, (ii) select the dose allowing better disease control and optimum lettuce yield, and (iii) analyse the long-term impacts of the tested composts in lettuce cultivation.

Acknowledgments

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Chapter 5. General discussion and conclusions

General discussion

Despite composting be a process extensively studied and with characteristics already standardized, there is a wide range of conditions that makes each composting process run in a very particular way. For this reason, the study of this technology should always be carried out and evaluated taking into account, at least the relationships between the physicochemical properties of the materials used, GHG emissions and C and N kinetics.

The main objective of this PhD work was to study and provide more information about how the chemical composition of agroindustrial residues influence the composting process and simultaneously evaluate the end-product quality characteristics for the application to the soil. The target is to practice a more sustainable agriculture and to meet the premises for a circular economy.

Characteristics of the composts and their progress during composting

With exception of the coffee grounds, the residues used in this study, namely grape, olive, broccoli and chestnut were chosen due to their presence and importance in the Portuguese agro-industrial activities. The spent coffee grounds were chosen due to the large amounts of this solid residues generated and their weak commercial value and applicability.

The main results obtained from the papers presented in Chapters 2, 3 and 4 are now generically discussed and related.

The temperature behaviour observed in the two composting trials (Chapter 2.1 and 2.2) were within the average parameters of the composting process. The highest temperature was reached with Coffee grounds (57.6°C) followed by Broccoli (54 °C), both with the longer thermophilic phase (over 40°): 8 days for Coffee grounds (at 20 and 40% of total DM) and 5 days for Broccoli. At phytosanitary level, it can be said that the composts produced from Coffee grounds and Broccoli will be the most suitable for soil application.

Regarding the electrical conductivity, with the exception of the Broccoli compost (2.45 dS m⁻¹), the values in the final composts produced ranged from 0.78 to 1.62, indicating an efficient composting process and attests to the usefulness of the compost produced as crop amendment according to the majority of authors. Solano et al. (2001), proposed EC values up to a maximum of 3 dS m⁻¹ for compost application to soil which also makes possible the

application of the compost produced from broccoli residues to the soil. However, when compared to the rest of the treatments it is shown to be the least attractive.

Towards the pH analysis of final composts, the Coffee grounds presents a final pH near to 7.0 indicative of a mature compost and then, suitable for most cultivated crops (Lasaridi and Stentiford, 1998). However, in the remaining residues the pH values at the end of composting ranged from 8.99 (WhiteGrape) to 9.26 (Chestnut). Although such high pH values might stimulate NH_3 losses (Lasaridi and Stentiford, 1998), the Chestnut compost revealed the lower NH_3 emissions. Taking into consideration the pot experiment tests performed later (Chapter 4 and Annex 3), it is reasonable to say that all composts were safe for soil application.

In the present work, the final C/N ratios of Coffee grounds composts ranged between 8.6-12.1, suggesting a greater decomposition of organic matter (Liu and Price, 2011), and thus, adopting the criteria set by Morais and Queda (2003), we can state that at the end of the experiment the Coffee grounds composts were mature. In the rest of the compost residues, the final C/N ratio ranged from 20.9 with Broccoli to 36.3 with Straw what exceeded the threshold limit of 20 recommended by Golueke (1981). However, the C/N ratio should be considered only as a relative indicator of compost maturation here since the bioavailability of organic C matters more than the total organic C content in high lignocellulosic materials (Sánchez-Monedero et al., 2001), such as those used.

At the end of the two composting experiments, the lowest OCsol content ($4.50 \text{ g C kg}^{-1} \text{ DM}$) was observed with Chestnut, and the highest ($11.18 \text{ g C kg}^{-1} \text{ DM}$) with Broccoli.

The highest Nt ($59.7 \pm 1.4 \text{ g N kg}^{-1} \text{ DM}$) and ONsol ($3.03 \text{ g N kg}^{-1} \text{ DM}$) contents were obtained with Coffee grounds and Broccoli, respectively.

Substantial amounts of mineral nutrients were detected in all treatments, which from a fertilisation point of view, is an important quality for a final compost. Higher P, Fe and Zn contents were obtained with Broccoli, as compared with all other treatments ($p < 0.05$). Besides, Broccoli compost was also found to be a rich source of Ca ($12.29 \text{ g kg}^{-1} \text{ DM}$).

These results show the greater presence of soluble compounds (C and N) and micronutrientes in the Broccoli compost, which is essential for plant nutrition and a benefit for its possible application as a fertilizer.

During the composting process for both trials, a significant decrease ($p < 0.05$) in phenolics occurred throughout the composting process, except for gallic acid, indicating a progressive degradation. It is possible that as soon as these phenolics were degraded and released, they were incorporated into biosynthetic pathways leading to the formation of humic substances (Stevenson, 1994).

Greenhouse gases emissions

Concerning the emissions that occurred during the composting process, the highest CO₂ emissions were observed with Olive and Chestnut, and the lowest emission with WhiteGrape. Through our results, no relation ($p < 0.05$) was found between final cumulative CO₂ emissions and the total phenolic and lignocellulosic contents of the initial waste-straw mixtures. This result was also observed when the composts were applied to the soil (Chapter 3), where total phenolic, gallic acid, cellulose, hemicelluloses and holocellulose (cellulose + hemicellulose) of the composts revealed no relation with the accumulated mineralized C at the end of incubation. In the other hand it's possible to see that the accumulated mineralized C showed a strong correlation ($P < 0.001$) with TPhe/N, ($r = 0.75$), GA/N ($r = 0.70$) and lignin ($r = -0.78$). In this experiment (Chapter 3), Straw showed the largest peak of CO₂ emission and Mimosa and Coffee grounds presented the lower values.

Composting Olive residues caused the lowest CH₄ emissions and Whitegrape the highest. During the soil incubation experiment (Chapter 3), flux emission of CH₄-C were very low or negative fluxes in all treatments. Straw was the treatment that present the lowest methane emission. In opposition, Coffee grounds treatment presented a significantly higher cumulative CH₄ emission at the end of the experiment.

In the second composting experiment, the highest final accumulated N₂O emissions values were observed in Olive and Chestnut treatments (0.91 g N₂O-N kg⁻¹ initial DM) and a significant correlation was found between cumulative N₂O emissions and the hemicellulose ($r = 0.522$; $p < 0.01$) and lignocellulose ($r = 0.506$; $p < 0.05$) contents of the initial waste-straw mixtures. Soil amendment with composted agro-industrial residues (Chapter 3) enhanced N₂O emissions. Coffee grounds and Chestnut compost exhibited the largest cumulative N₂O emissions at the end of the incubation experiments. It was found that N₂O emissions increased with the content of lignin ($r = 0.56$; $P < 0.01$).

To better understand the impact of the different amendments in terms of GHG emissions to the atmosphere, the conversion of N₂O and CH₄ emissions to CO₂ equivalents were made (Chapter 2.2 and 3). The global warming potential is a comparative measure that reflects the impact of the emission of a gas relative to one unit of the reference gas, carbon dioxide.

Through the results of Chapter 2.2, the cumulative GHG emissions following the sequence: Olive > Chestnut > Straw > Redgrapes > Broccoli > Whitegrapes. It was clear that from all the agro-food residues materials used in the experiment, white grape's marc could

effectively be transformed and reused as organic fertiliser, with the lowest negative environmental impacts.

Through the results of Chapter 3, the cumulative GHG emissions after soil amendment with the composts, following the sequence: Coffee grounds > Chestnut > Straw > Redgrapes > Olive > Whitegrapes > Broccoli > Mimosa. The lowest GHGs emission were observed from Mimosa which was significantly lower than all other treatments, however, the highest contribution of CH₄ were observed on this treatment. From this it can be infer that Mimosa is likely to be a better treatment in terms of GHG mitigation. Although the higher contribution of CH₄ from this compost comparing to the others, the emissions were considered negligible. Coffee grounds compost revealed not be the better strategy comparing with the rest of treatments, since promote higher N₂O emissions.

NH₃ and NO emissions were also measured in the second composting experiment (Chapter 2.2). NH₃ and NO emissions occurred mainly in the most bioactive period with peak values presented by WhiteGrape (0.003 g NH₃-N kg⁻¹; 0.16 g NO-N kg⁻¹ initial DM), and for RedGrape (0.02 g NH₃-N kg⁻¹; 0.17 g NO-N kg⁻¹ initial DM). Straw was the treatment that emitted less NH₃ when compared with all the other treatments.

The lowest cumulative NH₃ and NO emissions were calculated for Straw and Chestnut. Straw contains a substantial amount of degradable carbon, which stimulates immobilization of ammonium on microbial biomass (Sommer et al., 2006), with subsequent increase in the C/N ratio. Sánchez-Monedero et al. (2001) reported that the use of wastes with high lignocellulose contents, led to a reduction of about 25 % of N losses during the composting process. In agreement with the results of the present study, treatments with higher C/N ratios and lignocellulose contents (Straw and Chestnut) led to lower cumulative NH₃ emissions, and also for NO cumulative emissions.

Broccoli with the lowest C/N ratio and lignocellulose content led to higher NH₃ emissions comparing to the other treatments. Olive presented the highest NO emissions. Cumulative NH₃ emissions were highly and negatively correlated with lignocellulose ($r = -0.873$; $p < 0.001$), and positively correlated with the total phenolic contents ($r = 0.462$; $p < 0.05$) of the initial waste-straw mixtures. Cumulative NO emissions correlated negatively with lignocellulose ($r = -0.633$; $p < 0.001$) and hemicellulose ($r = -0.779$; $p < 0.001$) and positively with lignin ($r = 0.523$; $p < 0.01$) and total phenolics ($r = 0.626$; $p < 0.01$).

Composts agronomic value and fungi-suppressive properties

By the last sampling date of the soil incubation experiment, nitrate levels were increasing for all the treatments, suggesting the possibility that these composts might continue to supply N for plant uptake over the long-term. The highest concentration of NO_3^- -N was observed at the end of incubation by Chestnut (91.8 mg kg^{-1}) followed by Coffee grounds (88.1 mg kg^{-1}) and Whitegrapes (87.3 mg kg^{-1}).

The use of composts has been reported to be advantageous for both recycling of wastes and replenishing of soil fertility (Mehta et al., 2014). Moreover, composts have been reported to have disease-suppressive properties and could be used in strategies for managing soil-borne diseases, reducing the need of pesticide application (Martin, 2003, Bonanomi et al., 2010, Mehta et al., 2014).

In this regard, through the results provided for the pot experiment performed on Chapter 4, we observed that plants grown on the chestnut- and olive-based composts did not exhibit any disease symptoms, showing their effectiveness in controlling *Botrytis cinerea* and *Fusarium oxysporum*. Plants grown on the coffee- and grape-based composts on the other hand were severely affected by both wilt diseases.

The dose did not prove to be significant in the severity or incidence of the diseases, however the dose of 10% was also more effective than 5% in limiting the spread of both diseases in the case of the grape-based compost. These results indicate the potential use of the chestnut- and olive-based composts at 5 and 10% doses for controlling *Botrytis cinerea* and *Fusarium oxysporum* on lettuce plants. Chestnut- and olive-based composts presented the higher pH values, leading to lower disease incidences. This fact may be related with the compost community, since it is widely recognized that pH conditions affect the compost microbial community (Ntougias et al., 2008; Patel et al., 2010; Morales et al., 2016).

With respect to the control, all treatments increased the dry weights of the plants, apart from the coffee-based compost at 5% dose. The lowest values of dry weights were observed for 10% coffee-based compost, and 5% and 10% grape-based composts. This was an interesting finding as it showed that despite increasing disease severity and disease incidence the coffee- and grape-based composts were beneficial to plant growth. The chestnut- and olive-based media led to highest total dry weights, with the highest values observed for the dose of 10%.

Final remarks

- Along the composting of mixtures containing different proportions of spent coffee grounds a substantial decrease in total phenolics and total tannins occurred and gallic acid increase;
- For the Coffee composts we did not find any relation between the chemical composition of the initial residue and gaseous emission;
- Emissions of greenhouse gases, CO₂, N₂O and CH₄ were very low and without significant difference between the treatments;
- We demonstrate that the optimal proportion of coffee grounds was 40 % which led to better conditions for composting (via moisture levels), quality end product (via EC, OM, dissolved C and N contents) and lower gaseous emissions;
- Through the composting residues of broccoli, olive, chestnut, white and red grape we observed that the lignocellulose composition of the raw materials seemed to entail a higher emission of N₂O; No relation was found between the phenolic fraction of the initial wastes and gaseous emission;
- Composts produced from white grape marcs exhibited the lowest GHG emission and the higher GHG occurred in broccoli wastes;
- The lowest NH₃ and NO emissions were registered during the intense degradation of chestnut and olive residues;
- The results provided by laboratory incubation experiment suggest that the effect of compost incorporation on GHG emissions are dependent on their chemical composition;
- The incorporation of organic composts resulted in higher cumulative CO₂, N₂O and CH₄ emissions when compared with the control (unamended soil).
- Composts with higher TON and lignin content, as the case of Coffee grounds and Mimosa, displayed lower C mineralization and hence small CO₂ emissions;
- No relation was found between initial compost concentration of total phenolic, gallic acid, cellulose and hemicellulose on C mineralization. However, the interaction of polyphenols and gallic acid with the N compounds such as TPhe/N and GA/N demonstrated to be appropriated for establish a relation with C mineralization;
- Mimosa and Coffee grounds treatments revealed to be potential sources of methane, but the other composts used behaved as a sink;

- When the compost suppressive effect was tested it was concluded that Chestnut and Olive with a dose of 10% are the more effective treatments for disease suppression in soils spiked with *Botrytis* and *F. oxysporum* promoting a better lettuce growth.

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Annexes

Annex 1. Paper: Effect of different rates of spent coffee grounds (SCG) on composting process, gaseous emissions and quality of end-product



Effect of different rates of spent coffee grounds (SCG) on composting process, gaseous emissions and quality of end-product



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ABSTRACT

The use of spent coffee grounds (SCG) in composting for organic farming is a viable way of valorising these agro-industrial residues. In the present study, four treatments with different amounts of spent coffee grounds (SCG) were established, namely, C₀ (Control), C₁₀, C₂₀ and C₄₀, containing 0, 10, 20 and 40% of SCG (DM), respectively; and their effects on the composting process and the end-product quality characteristics were evaluated. The mixtures were completed with *Acacia dealbata* L. shoots and wheat straw. At different time intervals during composting, carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) emissions were measured and selected physicochemical characteristics of the composts were evaluated.

During the composting process, all treatments showed a substantial decrease in total phenolics and total tannins, and an important increase in gallic acid. Emissions of greenhouse gases were very low and no significant difference between the treatments was registered. The results indicated that SCG may be successfully composted in all proportions. However C₄₀, was the treatment which combined better conditions of composting, lower GHG emissions and better quality of end product.

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1. Introduction

Coffee is one of the most popular beverages in the world and the second largest traded commodity after petroleum (Daglia et al., 2000), as attested by the over 8.5 million tonnes produced in 2014 (www.ico.org).

During the production of espresso or hydrosoluble coffee, large amounts of solid residues (550–670 g kg⁻¹ coffee beans) known as spent coffee grounds (SCG) are generated. Generally, these SCG do not have any commercial value or substantial application (Panusa et al., 2013). However, recently, different authors have reported some potential interesting uses of SCG, such as additives for bio-diesel (Kondamudi et al., 2008) and as a source of natural phenolic antioxidants and bioactive compounds with great interest for pharmaceutical and cosmetic industries (Acevedo et al., 2013; Panusa et al., 2013). Furthermore, SCG have shown potential as fertilizers for agriculture and even as pellets for burning (Kondamudi et al., 2008; Limousy et al., 2013). However, burning of SCG is very controversial due to greenhouse gases emissions and their building up in the atmosphere (Limousy et al., 2013) which results in

increased air pollution. The growing concern about the negative impacts of SCG on the environment and the increased social pressure to reduce pollution have forced researchers to look for new and effective recycling alternatives. Moreover, the findings about the high content of organic matter; the presence of bioactive compounds such as polyphenols; and other types of phytochemicals in SCG have led to new approaches. In this direction the bio-conversion of SCG into high added-value products seems to be a valid and promissory solution (Panusa et al., 2013).

Another alternative under investigation is the incorporation of SCG in soil as amendments. Soil amendment with organic residues is a common practice improving physical and nutritional soil properties. However, due to their chemical composition, SCG can be very toxic to many life processes. The incorporation of organic wastes in soil without complete degradation can immobilize plant nutrients causing phytotoxicity (Butler et al., 2001; Ros et al., 2006). To ensure that a compost respects the previous assumptions, compost's maturity can be assessed by different methods, based on physical, chemical and biological characteristics (Lasaridi and Stentiford, 1998). The most well-worn biological test, used standards plants and microorganisms as models. However, organisms from other trophic levels are increasingly used in order to integrate all information and to access to the real environmental

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hazard. Pivato et al. (2016) tested solid and leaching composts to access toxic effects on different organisms: Plant *Lepidium sativum*, earthworm *Eisenia foetida*, aquatic crustacean *Daphnia magna* and bacteria *Vibrio fischeri*. Thus, prior to soil incorporation, SCG needs to be properly treated in order to eliminate or reduce any compounds that may have toxicity in the soil, and in that regard, composting has been considered effective. Composting has been a common practice for recycling biowastes and it is considered a simple and efficient way of transforming agro-industrial wastes into a stable, non-toxic, pathogen free and plant nutrient-rich product, suitable for use as soil conditioner and plant fertilizer (Ros et al., 2006). Although composting is usually considered environment friendly, its effects can be seen as beneficial or harmful, depending on the associated gases' emissions.

Composting consumes oxygen (O_2) and releases into the atmosphere carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ammonia (NH_3) and other biogenic volatile compounds. Methane (CH_4) and nitrous oxide (N_2O) are of the highest importance since they are considered strong greenhouse gases (GHG) highly associated with global warming potential (GWP), thereby contributing to climate change (IPCC, 2013). The Fifth Assessment Report of the International Panel on Climate Change (IPCC) states that the GWP of CH_4 and N_2O , on a 100-year time frame excluding climate-carbon feedbacks, is 28 and 265 times higher than that of CO_2 , respectively. Ammonia is not considered to be a direct GHG, but contributes to global warming because once deposited in the soil, it may be converted into N_2O after nitrification and denitrification reactions. Due to its high GWP, N_2O can contribute strongly to the carbon footprint, thus it is important to understand its formation during the composting process. The role of CO_2 emissions from composting activities emerges as less relevant since these emissions are considered as part of the C short cycle and therefore usually accounted as neutral (i.e. the GWP of CO_2 is considered zero) (Christensen et al., 2009). The quantification of GHG emissions from composting facilities is thus a very important step towards the improvement of composting emission models, and the development of environmental friendly technologies for GHG mitigation. In addition, available quantitative data on country-level GHG emissions from composting facilities can increase consistency and precision of data reported by different national and international databases (Andersen et al., 2010).

Composting of lignocellulosic wastes such as wheat straw and *A. dealbata* may be used together for supply extra carbon for microbial activity, balance the moisture and C/N ratio and avoid compaction of the composting materials. Furthermore, Sánchez-Monedero et al., 2001 demonstrated that the inclusion in the mixtures of wastes with a high lignocellulosic content reduced N losses during the composting process.

Acacia dealbata represents one of the most critical invaders in some European countries such as Portugal, Italy, France and Spain (Sheppard et al., 2006). The plant affects the sustainability and biodiversity of agro and natural ecosystems through modifications of some soil properties, nutrient cycles, as well as physical-chemical properties beneath plant canopy and the control of its spread is a key-issue in above mentioned countries. (Fried et al., 2014). Apart from *A. dealbata*, wheat straw is a great feedstock for composting considering its characteristics and since it is the second largest biomass in the world (Talebnaia et al., 2010) with a low commercial value.

Therefore, the main objectives of this study were: (1) to study, during the composting process, the changes of physical and chemical characteristics of mixtures of SCG at different rates with *A. dealbata* and wheat straw (2) to measure the emissions of methane, carbon dioxide and nitrous oxide during composting and assess its environmental significance, and; (3) to evaluate the end-product quality characteristics and the efficiency of the entire process.

2. Material and methods

2.1. Treatments and composting procedure

The study was conducted at University of Trás-os-Montes e Alto Douro (UTAD) campus, N 41°17'7.28"; W 7°44'36.83". The compost was produced in 135 L insulated reactors with continuous forced air circulation; the composting system was developed to allow the measurement of gases emissions during the process (Fig. 1). Aeration was achieved by blowing air using an air pump (KNF, model N010. KN.18) connected to a punctured plastic plate located at the bottom of the reactor. A flowmeter equipped with a needle valve (Aalborg, model P, 5 L min⁻¹) was used to control the aeration rate at 20 L h⁻¹ kg⁻¹ DM (dry matter) of the composting mixture. This rate was chosen to create an optimum aeration level that allows simultaneously reducing the CH_4 , N_2O and NH_3 losses. Jiang et al. (2011) demonstrated that values of lower aeration rates exhibited longer emission periods and higher emission rates. In the opposite, higher aeration rates reduced the CH_4 emission but increase the N_2O and NH_3 emissions. The structural materials used in the current study were *A. dealbata* and wheat straw. *A. dealbata* shoots were collected in the UTAD campus and were grinded using a shredder (Viking model GB 370 S); the wheat straw was grinded using a knife mill (Yike, 9FQ-360 straw hammer mill). Four different treatments were then established, and they consisted of four different rates of SCG co-composted with *A. dealbata* and wheat straw. The four rates of SCG considered were 0, 10, 20 and 40% of total DM, and are referred in all the study as treatments C₀, C₁₀, C₂₀ and C₄₀, respectively. In all treatments, the proportion (dry weight basis) of *A. dealbata* and straw in the portion of structural material was kept at 35:65, respectively. Each treatment was done in triplicate and the treatment C₀ was prepared with only *A. dealbata* and straw, and was considered as control.

The coffee grounds were supplied by a private company and dried in a laboratory oven (Memmert, UL 60) at 60 °C to remove all the humidity and inhibit microbial processes. After, these materials were mixed and used in composting according to their properties (Table 1) to achieve a mixture with a C/N ratio of ca. 20, the idea being to obtain a compost rich in nitrogen for plant fertilization.

About 10 kg DM of each mixture was composted over 156 days. The moisture was maintained in the range 45–60% of water content by adding water when necessary. The mixture was homogenized manually revolving each mixture every week during the most active phase of composting, and then every 15 days throughout the maturation period. The temperature in the centre of each reactor was measured daily during the 10 first days, and thereafter at increasing intervals until day 105 of the composting period, using a digital thermometer (model Prima Long).

Samples were taken on days 0, 3, 9, 22, 41, 64, 105 and 156, mixing subsamples from different areas of each reactor. Each sample was divided into 3 parts: one part was frozen and stored for determination of physical and chemical properties; the second part was used to determine DM; and the third was dried at 40 °C for phytochemicals determination. Samples from the period with highest gases emissions i.e., first six weeks (day 0, 3, 9, 22 and 41), were analysed for mineral N.

2.2. Physical and chemical analysis

For physical and chemical properties analyses, the samples were ground with a coffee mill and sieved (particle size <1 mm). The following analysis were carried out in fresh samples: electrical conductivity (EC), pH and Kjeldahl N were determined by standard procedures (CEN, 1999); Soluble Organic Carbon (Soluble OC) and

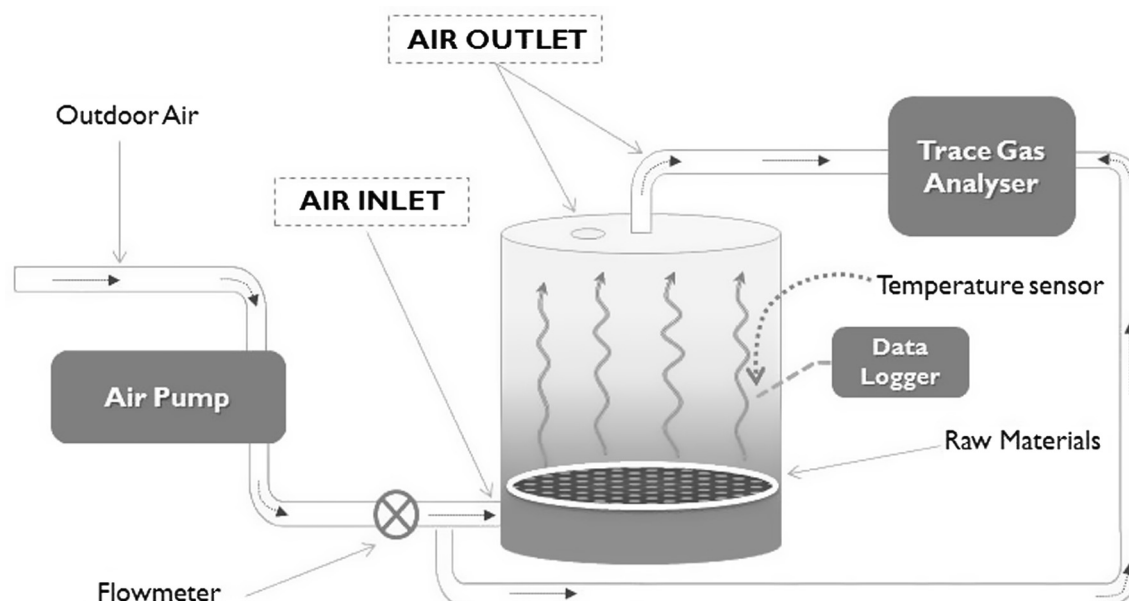


Fig. 1. Schematic representation of the composting system and gas measurements.

Table 1
Main characteristics of the raw materials.

	TOC (g C kg ⁻¹ of DM)	TN (g N kg ⁻¹ of DM)	C/N
Wheat straw	467.6 ± 5.59	5.06 ± 0.20	92.5 ± 2.51
<i>A. dealbata</i>	500.7 ± 3.10	33.5 ± 4.33	15.1 ± 1.86
Coffee grounds	512.1 ± 0.99	25.4 ± 0.06	20.2 ± 0.09

Soluble Organic Nitrogen (Soluble ON) were determined by an elemental analyser (Formac, Skalar) by NIRD and chemiluminescence detection, respectively, after extraction with CaCl₂ 0.01 M (1:10 w/v); ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) were extracted by shaking 3 g of sample with 30 ml KCl 1 M and concentrations were determined in filtered extracts by molecular absorption spectrophotometry (Houba et al., 1995). The concentration of the elements Ca, Mg and K were measured in dry samples by atomic absorption spectroscopy after nitric-perchloric acid digestion and total P was measured by flame photometry (CEN, 1999); total organic carbon and total organic nitrogen were determined also in dry samples by NIRD (near infrared detector) and thermic conductivity, respectively. Then, the C/N ratio was calculated.

The determination of polyphenols included the extraction and quantification of total phenolics, simple phenolics, total tannins, and individual phenolics. The total and simple phenolics content were determined by spectrophotometric methods prior to extraction with the Folin-Ciocalteu reagent, according to the method of Javanmardi et al. (2003). The total tannin content was calculated by the difference between the average content of total and simple phenolics. Quantification was done using a calibration curve of gallic acid (Sigma-Aldrich, Taufkirchen, Germany) as commercial standard, and all the data were expressed as mg gallic acid equivalent (GAE) g⁻¹ DM.

The individual polyphenol profile of each sample was evaluated by a HPLC-DAD system, with a mobile phase of water with 1% of trichloroacetic acid (TFA) (solvent A) and acetonitrile with 1% TFA (solvent B), at a flow rate of 1 mL min⁻¹. The separation was done using a C18 column (250 × 46 mm, 5 µm). The gradient used started with 100% of water, injection volume of 10 µL, and chromatograms were recorded at 280, 320, 370 and 520 nm. Polyphenols were identified using peak retention times, UV spectra and

UV maximum absorbance bands, compared with those found in literature and with external commercial standards. The quantification was performed using internal (naringin, Sigma-Aldrich, Germany) and external standards. The external standards gallic acid, (+)(-) catechin, (+) epicatechin, ellagic acid, chlorogenic acid, protocatechuic acid, caffeic acid, ferulic acid, and luteolin-7-O-glucoside were purchased from Extrasynthese (Cedex, France). All standards were freshly prepared in 70% methanol (methanol:water) at 1.0 mg mL⁻¹ and ran in HPLC-DAD before the samples. Methanol and acetonitrile were purchased from Panreac chemistry (Lisbon, Portugal) and Sigma-Aldrich (Taufkirchen, Germany), respectively. The aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore). All data were expressed as µg g⁻¹ DM.

2.3. Gas measurement

The measurements of CO₂, CH₄ and N₂O emissions were performed using a photo-acoustic analyzer (1412 Photoacoustic Field Gas Monitor, Innova AirTech Instruments, Ballerup, Denmark) with internal filters for small particles and water vapor, and optical filters for CO₂ (filter UA0982), N₂O (filter UA0985) and CH₄ (filter UA0969). The photo-acoustic analyzer was connected to Teflon tubes placed on air inlet and outlet entries of each composter (Fig. 1). The CO₂, CH₄ and N₂O detection limits of the analyzer were 1.5, 0.4 and 0.03 ppm, respectively. Gas measurements were carried out daily during the first 10 days of composting and thereafter on days 12, 14, 16, 19, 22, 26, 29, 36, 41, 43, 49, 57, 64, 70 and 93. The flux of CO₂, CH₄ and N₂O in each sampling period was calculated using the gas concentration in the outlet air of each composter and discounting the concentration of each gas in the inlet air. The accumulated gas emissions between sampling periods were calculated as the average of the flux between successive measurements multiplied by the time interval between sampling periods.

2.4. Statistical analysis

All the experiments were conducted in triplicate, and the data are presented as mean values ± standard error mean (SEM). A

Two-way repeated measures ANOVA analysis was performed and the differences between the means were separated by the Tukey's test at the probability level of 0.05. Posteriorly, the mean separation was performed using a software routine that allow analyzing simple main effects of one factor (treatment) limited to a particular level of other factor (day). Pearson's correlation tests were computed for the variables to assess their linear association. Principal Components Analysis (PCA) was conducted as a mean to identify the best composts and variables that best described the separations between treatments. All these analyses were done using the software Statistix 10.0 (Analytical Software, Tallahassee, USA).

3. Results and discussion

3.1. Temperature

It is largely accepted that each stage of fermentation is characterized by a different temperature and different types of microorganism activities, and hence the names, i.e., mesophilic, thermophilic, and cure stages. As a consequence, each stage is characterized by a specific group of chemical and physical properties. Temperature is considered one of the most important tools for monitoring the quality of the composting process, since it can help to define the actual stage, the type of microbial activities going on, and consequently, the final quality of the compost (Tiquia et al., 1996; Kulcu and Yaldiz, 2014).

According to our temperature results presented in Fig. 2, a similar trend of variation in all treatments occurred. We noted, a quick sharp increment of temperature, reaching a peak of 57.6 °C in the third day of composting, followed by a fall to near 54 °C on day 4 (Fig. 2). The decrease in temperatures after day 4 was more accentuated for the treatments C₀ and C₁₀ for which the thermophilic phase (over 40 °C) ended by day 6. For the treatments C₂₀ and C₄₀, the duration of the thermophilic phase was longer (8 days). The sharp rise in temperatures in the first days of fermentation can be explained by the increment of microbial metabolic activities (Cunha-Queda et al., 2007), mainly resulting from the regular aeration of the medium, but also from the intrinsic nature of the raw material, particularly its level of moisture, organic matter and C/N ratio. In fact, at the beginning of the composting process, there is higher amount of readily degradable compounds such as sugars, fatty acids, pectins, amino acids, among others, boosting the microbial metabolic activity and thus, sharply incrementing the

temperatures. On the opposite side, the temperature decrease during the curing period is due to the decay of microbiological activities caused by depletion of easily degradable organic materials (Caceres et al., 2006). Although some authors have reported that aeration can delay the fermentation process through release of heat from fermentation piles (Himanen and Hanninen, 2011), our results from small-scale reactors seems to contradict this, as aeration, at least at the beginning of the process, seems to have helped incrementing microbial activities through increasing the oxygen flow, and causing a rise in temperatures. Since there was a very short period with high temperatures to guarantee *Acacia* seed destruction, it is important that this feedstock do not include viable seeds. Simple management approaches, as the harvest of *Acacia* shoots before flowering, must be taken into account to avoid collection of seeds.

After 14 days of experiment, the average temperature returned gradually to a mesophilic phase of around 22 °C in all treatments. The return of temperatures to values near the ambient conditions at this stage is a good indicator that the most active fermentation process was almost complete and the thermophilic phase and respective microbial activities have slowed down.

3.2. Physic-chemical characteristics of the compost

The maturity, stability and quality of any compost can be assessed by determining changes in chemical and physical properties. The changes in physic-chemical characteristics of the mixtures during the composting process are presented in Table 2. The interaction between treatment x composting time period showed significant effects on all the parameters with the exception of C/N ratio and TOC.

Moisture loss during the first phases of composting can be used as an index of decomposition rate (Epstein, 1997) because the heat generation from microbial activities drove water evaporation from soluble solids. Despite this initial loss, the moisture content remained in the optimal range for the composting process to proceed (Richard et al., 2002), and since water content was adjusted frequently, no significant differences ($P > 0.05$) were observed in moisture levels between treatments.

The electrical conductivity (EC) measures the salinity of the compost and it is also a good indicator of its maturity. The values between 0.75 and 1.99 dS m⁻¹ are considered the most acceptable for the germination of seeds and for seedling establishment

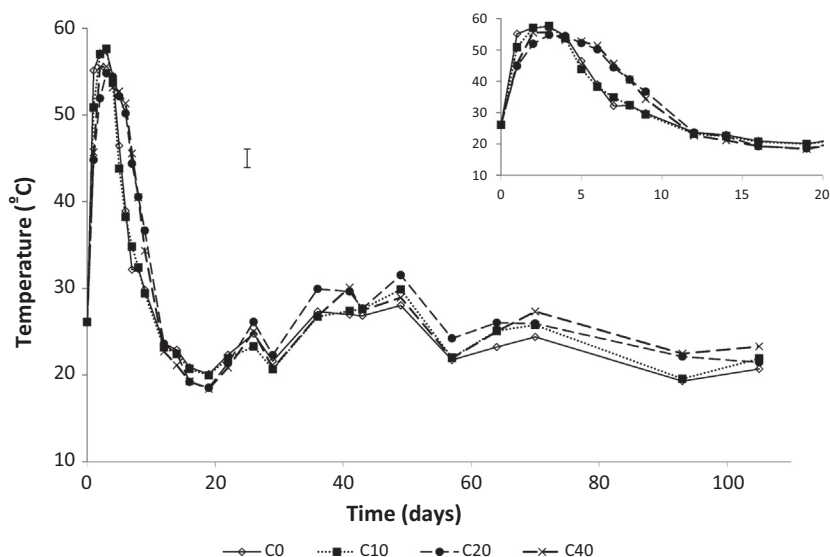


Fig. 2. Temperature profile during the composting period. The inner figure represents the temperature profile in the first 20 days.

Table 2
Evolution of physico-chemical parameters during composting.^{A,B,C}

	Moisture (%)	EC (dS m ⁻¹) (water 1:5)	pH	Soluble OC (g C kg ⁻¹ of DM)	TOC (g C kg ⁻¹ of DM)	Soluble ON (mg N kg ⁻¹ of DM)	TN (g N kg ⁻¹ of DM)	NH ₄ ⁺ -N (mg N kg ⁻¹ of DM)	NO ₃ ⁻ -N (mg N kg ⁻¹ of DM)	C/N
<i>Day 0</i>										
C ₀	60.5 ± 0.3 ^a	1.40 ± 0.04 ^a	5.7 ± 0.0 ^a	11.56 ± 0.00 ^{bc}	535.9 ± 12.3	3.79 ± 0.09 ^b	24.2 ± 0.4 ^a	878.9 ± 25.3 ^a	2.1 ± 0.2 ^b	22.2 ± 0.9
C ₁₀	61.3 ± 0.4 ^a	1.38 ± 0.02 ^a	5.8 ± 0.1 ^a	13.12 ± 0.10 ^a	536.7 ± 9.9	4.82 ± 0.11 ^a	25.2 ± 0.6 ^a	674.6 ± 16.4 ^{ab}	1.9 ± 0.1 ^b	21.5 ± 1.9
C ₂₀	60.1 ± 0.4 ^a	1.26 ± 0.02 ^a	5.8 ± 0.0 ^a	12.27 ± 0.11 ^{ab}	493.1 ± 4.0	4.65 ± 0.07 ^{ab}	25.4 ± 0.6 ^a	558.6 ± 16.8 ^b	7.0 ± 0.1 ^a	19.4 ± 0.3
C ₄₀	52.0 ± 0.1 ^a	1.24 ± 0.01 ^a	5.9 ± 0.1 ^a	10.11 ± 0.00 ^c	496.0 ± 7.5	4.12 ± 0.10 ^{ab}	26.5 ± 0.8 ^a	622.5 ± 14.9 ^{ab}	8.7 ± 0.2 ^a	18.7 ± 0.7
<i>Day 3</i>										
C ₀	68.6 ± 1.1 ^a	0.91 ± 0.13 ^a	7.2 ± 0.1 ^a	6.60 ± 0.07 ^c	486.7 ± 2.3	2.42 ± 0.18 ^c	30.1 ± 1.3 ^a	444.1 ± 92.4 ^b	2.7 ± 1.0 ^b	16.4 ± 1.5
C ₁₀	64.2 ± 2.2 ^a	1.05 ± 0.16 ^a	6.5 ± 0.3 ^b	6.43 ± 0.48 ^c	487.4 ± 9.8	2.53 ± 0.25 ^{bc}	28.3 ± 0.8 ^a	533.4 ± 54.2 ^{ab}	2.5 ± 0.5 ^b	17.4 ± 1.5
C ₂₀	66.5 ± 0.3 ^a	1.11 ± 0.08 ^a	5.5 ± 0.2 ^c	8.71 ± 0.53 ^b	509.8 ± 8.0	3.17 ± 0.27 ^b	28.1 ± 0.4 ^a	579.4 ± 38.1 ^{ab}	3.8 ± 0.3 ^{ab}	18.2 ± 0.7
C ₄₀	56.2 ± 0.3 ^a	1.31 ± 0.08 ^a	5.3 ± 0.1 ^c	10.51 ± 0.18 ^a	521.3 ± 2.7	4.25 ± 0.06 ^a	25.7 ± 0.9 ^a	744.6 ± 23.4 ^a	5.2 ± 0.1 ^a	20.6 ± 1.8
<i>Day 9</i>										
C ₀	64.4 ± 2.0 ^{ab}	2.11 ± 0.25 ^a	7.1 ± 0.1 ^a	23.69 ± 2.55 ^a	519.3 ± 9.2	3.53 ± 0.49 ^a	30.8 ± 1.6 ^a	353.1 ± 56.0 ^a	n.d.	17.2 ± 1.7
C ₁₀	67.6 ± 0.2 ^a	1.63 ± 0.04 ^a	7.1 ± 0.0 ^a	15.04 ± 0.66 ^a	521.6 ± 2.8	2.59 ± 0.11 ^a	24.2 ± 2.8 ^a	394.9 ± 61.2 ^a	n.d.	25.0 ± 3.7
C ₂₀	69.0 ± 1.6 ^a	1.79 ± 0.24 ^a	7.1 ± 0.1 ^a	18.53 ± 0.82 ^a	539.9 ± 32.8	3.96 ± 0.19 ^a	30.6 ± 2.9 ^a	426.4 ± 36.1 ^a	n.d.	19.5 ± 1.3
C ₄₀	52.3 ± 2.2 ^b	1.80 ± 0.16 ^a	6.5 ± 0.1 ^b	22.36 ± 2.35 ^a	459.8 ± 47.6	5.27 ± 0.62 ^a	23.4 ± 1.4 ^a	520.2 ± 69.7 ^a	n.d.	19.3 ± 0.3
<i>Day 22</i>										
C ₀	56.3 ± 2.4 ^a	2.59 ± 0.19 ^a	6.5 ± 0.2 ^a	24.95 ± 0.40 ^a	544.7 ± 2.2	4.68 ± 0.07 ^a	29.9 ± 1.2 ^a	262.0 ± 36.6 ^a	n.d.	18.4 ± 1.5
C ₁₀	52.7 ± 1.3 ^a	2.53 ± 0.08 ^{ab}	6.7 ± 0.1 ^a	27.22 ± 1.47 ^a	551.6 ± 1.8	4.74 ± 0.22 ^a	26.3 ± 0.4 ^a	256.5 ± 16.0 ^a	n.d.	21.0 ± 0.7
C ₂₀	46.0 ± 1.5 ^a	2.64 ± 0.13 ^a	6.6 ± 0.0 ^a	24.20 ± 0.88 ^a	542.8 ± 9.1	4.45 ± 0.18 ^a	25.8 ± 1.0 ^a	273.5 ± 21.5 ^a	n.d.	21.2 ± 1.7
C ₄₀	55.3 ± 3.0 ^a	1.96 ± 0.23 ^b	6.3 ± 0.0 ^a	25.39 ± 1.69 ^a	541.1 ± 3.8	4.80 ± 0.37 ^a	30.7 ± 0.6 ^a	295.8 ± 42.9 ^a	n.d.	17.7 ± 0.7
<i>Day 41</i>										
C ₀	64.7 ± 1.2 ^a	2.20 ± 0.07 ^a	7.3 ± 0.1 ^a	22.39 ± 1.13 ^a	514.4 ± 7.9	5.42 ± 0.29 ^a	27.3 ± 0.5 ^a	459.9 ± 32.7 ^a	n.d.	18.9 ± 0.5
C ₁₀	59.6 ± 1.2 ^a	2.14 ± 0.04 ^a	6.9 ± 0.1 ^a	22.59 ± 1.11 ^a	486.5 ± 3.3	5.26 ± 0.19 ^a	20.7 ± 2.3 ^a	428.4 ± 46.2 ^a	n.d.	26.1 ± 3.1
C ₂₀	65.8 ± 1.9 ^a	1.78 ± 0.13 ^{ab}	7.1 ± 0.1 ^a	20.32 ± 0.31 ^a	491.7 ± 3.6	5.12 ± 0.04 ^a	20.1 ± 0.5 ^a	318.8 ± 20.2 ^a	n.d.	24.5 ± 0.4
C ₄₀	71.8 ± 0.5 ^a	1.44 ± 0.08 ^b	7.0 ± 0.0 ^a	24.68 ± 0.77 ^a	507.6 ± 13.2	6.61 ± 0.22 ^a	23.2 ± 1.5 ^a	281.6 ± 18.9 ^a	n.d.	22.6 ± 1.6
<i>Day 64</i>										
C ₀	62.8 ± 0.8 ^a	2.35 ± 0.11 ^{ab}	6.9 ± 0.0 ^a	18.64 ± 0.25 ^a	560.7 ± 4.4	4.75 ± 0.03 ^a	34.1 ± 1.6 ^a	n/a	n/a	16.7 ± 0.8
C ₁₀	59.0 ± 1.2 ^a	2.45 ± 0.09 ^{ab}	6.6 ± 0.1 ^{ab}	20.74 ± 0.12 ^a	552.0 ± 17.0	5.21 ± 0.11 ^a	31.9 ± 1.4 ^a	n/a	n/a	18.9 ± 1.0
C ₂₀	60.8 ± 1.7 ^a	2.53 ± 0.03 ^a	6.5 ± 0.1 ^b	24.10 ± 0.31 ^a	550.2 ± 5.3	5.87 ± 0.15 ^a	32.7 ± 0.6 ^a	n/a	n/a	16.9 ± 0.8
C ₄₀	67.0 ± 0.7 ^a	1.94 ± 0.01 ^b	6.5 ± 0.0 ^b	25.41 ± 0.43 ^a	548.9 ± 6.0	5.67 ± 0.04 ^a	30.6 ± 0.4 ^a	n/a	n/a	18.0 ± 0.8
<i>Day 105</i>										
C ₀	62.3 ± 1.5 ^{ab}	2.65 ± 0.01 ^a	6.8 ± 0.1 ^a	18.65 ± 1.50 ^a	555.8 ± 20.0	4.17 ± 0.28 ^a	35.9 ± 1.9 ^{ab}	n/a	n/a	16.6 ± 0.7
C ₁₀	49.1 ± 2.2 ^b	2.52 ± 0.16 ^{ab}	6.6 ± 0.1 ^a	17.71 ± 1.16 ^a	432.3 ± 33.1	4.39 ± 0.31 ^a	30.3 ± 2.1 ^b	n/a	n/a	14.2 ± 0.4
C ₂₀	55.6 ± 4.9 ^{ab}	2.05 ± 0.05 ^{bc}	6.8 ± 0.0 ^a	21.61 ± 2.88 ^a	492.9 ± 63.0	4.83 ± 0.62 ^a	37.9 ± 3.6 ^{ab}	n/a	n/a	12.7 ± 0.8
C ₄₀	67.6 ± 2.5 ^a	1.79 ± 0.09 ^c	6.6 ± 0.1 ^a	30.13 ± 3.63 ^a	497.7 ± 39.5	6.98 ± 0.96 ^a	44.5 ± 2.6 ^a	n/a	n/a	11.1 ± 0.5
<i>Day 156 (final composts)</i>										
C ₀	61.0 ± 1.1 ^{ab}	1.33 ± 0.17 ^{ab}	7.0 ± 0.1 ^a	3.22 ± 0.09 ^b	509.5 ± 1.4	1.33 ± 0.07 ^b	42.4 ± 0.7 ^b	n/a	n/a	12.1 ± 0.5
C ₁₀	55.5 ± 3.1 ^b	1.53 ± 0.35 ^a	6.8 ± 0.1 ^a	3.72 ± 0.23 ^b	510.2 ± 10.8	1.84 ± 0.07 ^b	41.8 ± 2.1 ^b	n/a	n/a	12.5 ± 0.8
C ₂₀	62.7 ± 2.9 ^{ab}	1.62 ± 0.40 ^a	7.0 ± 0.2 ^a	3.98 ± 0.12 ^b	484.4 ± 16.9	2.03 ± 0.07 ^b	46.0 ± 0.6 ^b	n/a	n/a	10.6 ± 0.5
C ₄₀	74.1 ± 1.0 ^a	0.78 ± 0.07 ^b	7.0 ± 0.1 ^a	5.48 ± 0.24 ^a	507.6 ± 6.3	2.84 ± 0.25 ^a	59.7 ± 1.4 ^a	n/a	n/a	8.6 ± 0.6
ANOVA										
Treatment	0.457	0.000	0.000	0.003	0.812	0.002	0.232	0.843	0.012	0.474
Day	0.000	0.000	0.000	0.000	0.117	0.000	0.000	0.000	0.000	0.000
Treatment × Day	0.000	0.005	0.000	0.000	0.689	0.000	0.024	0.001	0.000	0.364

Values within a column, for the same day, with the same letter are not significantly different. The columns without any letter did not shown significant differences.

^A Values are expressed as mean ± SEM (standard error of the mean) of three replicates.

^B n.d. – not detected.

^C n/a – not analyzed.

(Epstein, 1997). In this study, the EC values in final composts ranged from 0.78 to 1.62 which indicates an efficient composting process and attests to the usefulness of the compost produced as crop amendment.

Altogether, a slightly decrease in EC was seen in the final stage of composting and this could be related with the precipitation of minerals or the volatilization of ammonia (Epstein, 1997). The variations in the EC values can also be explained, in part, by the variations in water moisture, given that a negative correlation (data not

shown) was observed between the moisture content and the EC ($r = -0.46$; $p < 0.01$). Thus, the lower value of EC measured at the end of composting for the treatment C₄₀ (0.78 ± 0.04 dS m⁻¹) may be related with the highest value of moisture ($74.1\% \pm 1.0$) observed at that specific time period (Table 2).

The pH analysis of composted samples from the different treatments showed a gradual increment, from slightly acidic to neutral, probably due (i) to the mineralization of proteins, peptides and amino acids into ammonia, (ii) to the degradation of acidic

compounds, such as phenolic acids, organic acids, and carboxylic groups during the thermophilic phase (Hachicha et al., 2009), and/or (iii) to the production of humic substances which can act as buffers (Zenjari et al., 2006) and attenuate extra pH changes due to their neutralizing capacity. The final pH, near to 7.0 in all treatments, indicates a mature compost suitable for most cultivated crops (Lasaridi and Stentiford, 1998). In addition, this pH reflects a classical evolution of the compost properties in aerobic conditions, suggesting the formation of humic substances (Hachicha et al., 2009).

The evolution of total organic carbon (TOC) during composting is another parameter that can be used as an index of compost stability and maturity, allowing the monitoring of the decomposition throughout the process. No significant differences were observed in TOC along the composting process, although a tendency for a decreasing trend was noticed. This is related with the mineralization of labile organic compounds. Throughout the composting process, the organic compounds are being lost, increasing the proportion of the conservative compounds. As a consequence, TOC decrease slowly during the process.

We observed a significant ($p < 0.001$) difference between treatments for soluble organic carbon (soluble OC), particularly at the beginning and end of the composting process. At the beginning of the experiment, the treatment C_{10} showed the highest content of soluble OC ($13.12 \pm 0.1 \text{ g C kg}^{-1} \text{ DM}$) and the treatment C_{40} presented the lowest soluble OC content ($10.11 \pm 0.0 \text{ g C kg}^{-1} \text{ DM}$); but as the experiment progressed, this tendency changed. For example, on day 3, the C_{10} treatment presented soluble OC values significantly lower than those of the C_{20} and C_{40} treatments. These results are probably due to the fact that the former treatment contained lower levels of coffee grounds and so, the carbon sources were rapidly depleted. At the end of the maturation period, the C_{40} treatment showed the highest values of soluble OC ($5.48 \pm 0.24 \text{ g C kg}^{-1} \text{ DM}$) while the C_0 treatment instead showed the lowest values ($3.22 \pm 0.09 \text{ g C kg}^{-1} \text{ DM}$). A similar tendency was observed for the soluble organic nitrogen (soluble ON) fraction. We noted significant differences ($p < 0.01$) among treatments in the initial and final periods of the process. On day 3, the soluble ON in treatment C_{40} ($4.25 \pm 0.06 \text{ g N kg}^{-1} \text{ DM}$) was significantly higher ($p < 0.05$) than the values found for C_0 , C_{10} and C_{20} treatments. After this day, all treatments presented similar values of soluble ON throughout the experiment until day 156 when the C_{40} treatment showed the highest value of ON ($2.84 \pm 0.25 \text{ g N kg}^{-1} \text{ DM}$) and the C_0 treatment showed the lowest value ($1.33 \pm 0.07 \text{ g N kg}^{-1} \text{ DM}$).

Total nitrogen (TN) increased during composting for all treatments. At the end of the experiment (day 156), the C_{40} treatment ($59.7 \pm 1.4 \text{ g N kg}^{-1} \text{ DM}$) has a significantly higher value of TN ($p < 0.05$) than the other treatments. From these results, we can state that the addition of 40% of SCG in the C_{40} treatment resulted in a compost with up to 40% more TN than that of the C_0 treatment.

The dynamics of mineral N (NH_4^+ and NO_3^-) in the first six weeks are presented in Table 2. The NH_4^+ content in all treatments decreased throughout the 41 days; however, the decrease was without significant differences among treatments, except for treatment C_{40} on day 41, which showed a lower value ($281.6 \pm 9.5 \text{ mg NH}_4^+\text{-N kg}^{-1} \text{ DM}$) compared to the other treatments ($318.8\text{--}459.9 \text{ mg NH}_4^+\text{-N kg}^{-1} \text{ DM}$). The highest NO_3^- concentration was observed on day 1 in C_{20} ($7.0 \pm 0.0 \text{ mg NO}_3^-\text{-N kg}^{-1} \text{ DM}$) and C_{40} ($8.7 \pm 0.1 \text{ mg N kg}^{-1} \text{ DM}$) treatments. From day 9 to day 41, we observed a total absence of NO_3^- (values below detection limit), probably due to the oxidation of NH_4^+ .

The C/N ratio is usually used as a parameter for the determination of the compost maturity. A compost with a C/N ratio below 20 is considered acceptable and labelled as "mature". Nonetheless, it's preferable to achieve a C/N ratio of 15 or lower (Morais and Queda,

2003). Although, in the present work, the initial C/N ratios of the mixtures were already low, this parameter decreased from the initial values of 18.7–22.2 to the final values of 8.6–12.1 (Table 2); suggesting a greater decomposition of organic matter (Liu and Price, 2011), and thus, adopting the criteria set by Morais and Queda (2003), we can state that at the end of the experiment our compost was mature. The decrease of the C/N ratio can be explained by higher losses of carbon compared to nitrogen, resulting from microbial activities during the fermentation process. Consequently, as discussed before, TN concentration increase in the final composts.

Regarding the mineral composition (Table 3), at the end of the experiment no significant differences ($p > 0.05$) were found between treatments for P and Ca. Regarding K and Mg, C_{40} presented the lower values. However, an increase in all concentrations was observed in the final compost, as a result of absolute carbon losses of the materials during the process, as have been previously reported in the literature (Kulcu and Yaldiz, 2014; Liu and Price, 2011).

3.3. Phenolic compounds

The results for the quantitative determination and qualitative composition of polyphenols, at the beginning and at the end of the experiment are presented in Table 4. Different types of polyphenols were found in the material used to produce the composting matrix. The coffee grains presented the highest diversity of phenolic compounds (6 compounds), whilst *A. dealbata* showed lesser diversity, but higher contents, particularly of total tannins. Our results are in agreement with Anjos et al. (2014), who recently reported high contents of the flavonoid luteolin and gallotannins such as gallic acid and its respective isomers in different *A. dealbata* accessions. The wheat straw exhibited the lowest contents in polyphenols. The compounds catechins, epicatechins and its respective isomers were not found in the current study; this is in agreement with findings from several others authors, which showed low contents of such compounds in coffee beans, probably resulting from degradation during the course of industrial treatments such as roasting, composting or fermentation (Verzelloni et al., 2011). After the mixing of initial materials with different proportions of SCG, the polyphenol content varied in all treatments due to the differing contribution of each material, and in general the differences were statistically significant ($p < 0.05$) (Table 3). After composting, except for gallic acid, the contents of all polyphenols decreased sharply. These decreases could be associated with microbial activities during the composting process. Ghaly et al. (2012) reported a reduction of 73.9% of phenolics during the composting process. However, Volf et al. (2014) contradicted some of these findings and stated that any degradation of phenolic compounds will be strongly dependent on the occurrence of three conditions: temperature, type of phenolics and thermal stability of phenolics. In addition, according to the same authors, gallotannins like gallic acid, ellagic acid and vanillic acid, have strong thermal stability and are only metabolized when temperatures reaches values above 60°C , which was not the case in the current study. The maximum temperature reached during the composting process was 57°C , lower than 60°C , and thus, this seems to explain the similar contents of gallic acid in the beginning and at the end of the experiment.

3.4. Gas emissions

3.4.1. Carbon dioxide (CO_2) emissions

The measurement of CO_2 emissions during the composting process is important because of the correlation between CO_2 levels and microbial activities, which can be used as a direct method

Table 3
Chemical analysis of macronutrients at selected composting times.^A

Treatments	P (g kg ⁻¹ DM)	K (g kg ⁻¹ DM)	Ca (g kg ⁻¹ DM)	Mg (g kg ⁻¹ DM)
<i>Day 0</i>				
C ₀	0.95 ± 0.03	7.75 ± 0.04 ^a	0.56 ± 0.05 ^a	8.82 ± 0.33 ^a
C ₁₀	1.13 ± 0.05	5.43 ± 0.40 ^{ab}	0.74 ± 0.07 ^a	9.98 ± 0.42 ^a
C ₂₀	1.09 ± 0.02	4.51 ± 0.02 ^b	0.54 ± 0.01 ^a	9.14 ± 0.06 ^a
C ₄₀	1.02 ± 0.03	3.62 ± 0.03 ^b	0.51 ± 0.05 ^a	10.08 ± 0.45 ^a
<i>Day 3</i>				
C ₀	0.94 ± 0.07	5.31 ± 0.49 ^a	0.97 ± 0.12 ^a	9.66 ± 1.67 ^a
C ₁₀	1.22 ± 0.08	6.33 ± 0.56 ^a	1.51 ± 0.29 ^a	9.45 ± 0.21 ^a
C ₂₀	1.11 ± 0.07	4.91 ± 0.12 ^a	1.22 ± 0.08 ^a	9.17 ± 0.07 ^a
C ₄₀	1.24 ± 0.05	5.47 ± 0.92 ^a	1.19 ± 0.02 ^a	8.26 ± 0.14 ^a
<i>Day 22</i>				
C ₀	1.39 ± 0.28	7.85 ± 1.23 ^a	1.46 ± 0.22 ^a	12.88 ± 0.81 ^a
C ₁₀	1.17 ± 0.04	6.61 ± 0.20 ^a	1.43 ± 0.03 ^a	10.57 ± 0.28 ^{ab}
C ₂₀	1.46 ± 0.13	7.14 ± 0.66 ^a	1.67 ± 0.43 ^a	10.64 ± 0.60 ^{ab}
C ₄₀	1.46 ± 0.11	5.46 ± 0.13 ^a	0.79 ± 0.02 ^b	9.52 ± 0.31 ^b
<i>Day 156 (final composts)</i>				
C ₀	2.17 ± 0.06	19.88 ± 0.71 ^a	2.38 ± 0.86 ^a	10.96 ± 0.17 ^a
C ₁₀	2.11 ± 0.13	19.53 ± 0.85 ^{ab}	2.55 ± 0.51 ^a	11.14 ± 0.10 ^a
C ₂₀	1.77 ± 0.40	16.8 ± 1.64 ^{bc}	2.45 ± 0.53 ^a	9.73 ± 0.09 ^{ab}
C ₄₀	2.33 ± 0.04	16.17 ± 1.11 ^c	2.38 ± 0.39 ^a	8.23 ± 0.09 ^b
<i>ANOVA</i>				
<i>Treatment</i>	0.543	0.006	0.012	0.045
<i>Day</i>	0.000	0.000	0.000	0.001
<i>Treatment × Day</i>	0.188	0.111	0.232	0.023

Values within a column, for the same day, with the same letter are not significantly different. The columns without any letter did not shown significant differences.

^A Values are expressed as mean ± SEM (standard error of the mean) of three replicates.

Table 4
Average content of polyphenols in raw materials and at the beginning and at the end of the composting process in each treatment.^{A,B}

Treatments	Total phenolics (mg g ⁻¹ DM)	Simple phenolics (mg g ⁻¹ DM)	Total tannins (mg g ⁻¹ DM)	Tannins (μg g ⁻¹ DM) Gallic acid	Phenolic acids (μg g ⁻¹ DM)				Flavonoids (μg g ⁻¹ DM)
					Chlorogenic acid	Protocatechuic acid	Caffeic acid	Ferulic acid	Luteolin-7-O- glucoside
Initial material									
Straw	8.73 ± 0.27 ^b	0.01 ± 0.00	8.72 ± 0.27 ^b	23.99 ± 1.79 ^a	20.75 ± 6.86 ^a	n.d.	n.d.	n.d.	n.d.
<i>A. dealbata</i>	63.65 ± 2.41 ^a	0.05 ± 0.00	63.60 ± 2.41 ^a	24.16 ± 1.18 ^a	n.d.	n.d.	n.d.	n.d.	83.74 ± 11.87 ^a
SCG	12.02 ± 0.10 ^b	0.01 ± 0.00	12.01 ± 0.09 ^b	23.83 ± 6.09 ^a	31.83 ± 2.36 ^a	172.41 ± 3.82 ^a	14.52 ± 0.95 ^a	12.18 ± 0.82 ^a	n.d.
Initial composts									
C ₀	44.43 ± 1.66 ^a	0.03 ± 0.00	44.40 ± 1.66 ^a	24.10 ± 1.19 ^a	7.26 ± 2.40 ^b	n.d.	n.d.	n.d.	54.43 ± 7.71 ^a
C ₁₀	41.46 ± 1.50 ^{ab}	0.03 ± 0.00	41.43 ± 1.50 ^{ab}	24.07 ± 0.58 ^a	9.61 ± 1.89 ^b	17.24 ± 0.38 ^c	1.45 ± 0.09 ^c	1.22 ± 0.08 ^c	49.41 ± 7.00 ^{ab}
C ₂₀	38.50 ± 1.34 ^b	0.03 ± 0.00	38.47 ± 1.34 ^b	24.04 ± 0.54 ^a	11.97 ± 1.38 ^{ab}	34.48 ± 0.76 ^b	2.90 ± 0.19 ^b	2.44 ± 0.16 ^b	44.38 ± 6.29 ^{ab}
C ₄₀	32.57 ± 1.02 ^c	0.03 ± 0.00	32.54 ± 1.02 ^c	23.99 ± 1.81 ^a	16.68 ± 0.37 ^a	68.96 ± 1.53 ^a	5.81 ± 0.38 ^a	4.87 ± 0.33 ^a	34.33 ± 4.87 ^b
Final composts									
C ₀	1.18 ± 0.03 ^a	0.00 ± 0.00	1.17 ± 0.03 ^a	18.43 ± 0.91 ^c	n.d.	n.d.	n.d.	n.d.	n.d.
C ₁₀	1.47 ± 0.20 ^a	0.01 ± 0.00	1.46 ± 0.20 ^a	24.14 ± 2.64 ^{bc}	n.d.	n.d.	n.d.	n.d.	n.d.
C ₂₀	0.61 ± 0.04 ^a	0.01 ± 0.00	0.60 ± 0.04 ^a	44.15 ± 3.04 ^a	n.d.	n.d.	n.d.	n.d.	n.d.
C ₄₀	0.89 ± 0.04 ^a	0.00 ± 0.00	0.89 ± 0.04 ^a	28.02 ± 1.06 ^b	n.d.	n.d.	n.d.	n.d.	n.d.

Values within a column, for the same day, with the same letter are not significantly different. The columns without any letter did not shown significant differences.

^A Values are expressed as mean ± SEM (standard error of the mean) of three replicates.

^B n.d. – not detected.

for assessing the stability of the final compost (Kalamdhad et al., 2008). Moreover, CO₂ emissions measurements are surrogates for mineralization and full degradation of organic matter measurements; and composts can be considered “very stable” when the CO₂ fluxes are lower than 2 mg CO₂-C g⁻¹ day⁻¹. The results of CO₂-C emissions (mg h⁻¹ kg⁻¹ initial DM) during the composting process are shown in Fig. 3. Adopting the criteria of Epstein (1997), we can state that the final composts obtained were very stable since the average values detected were always below the

limit of 2 mg CO₂-C g⁻¹ day⁻¹. In addition, there were no significant differences ($p > 0.05$) between treatments over time. The highest emission value was observed during the first day (444.16 ± 50.34 mg CO₂-C h⁻¹ kg⁻¹ initial DM) of the experiment (C₁₀ treatment, Fig. 3); and then, a slow decrease was observed during the first 4 days, followed by a sharp decrease from day 4 until day 9 (Fig. 3). After day 9 and until the end of the experiment, the values were maintained below 100 mg CO₂-C h⁻¹ kg⁻¹ initial DM.

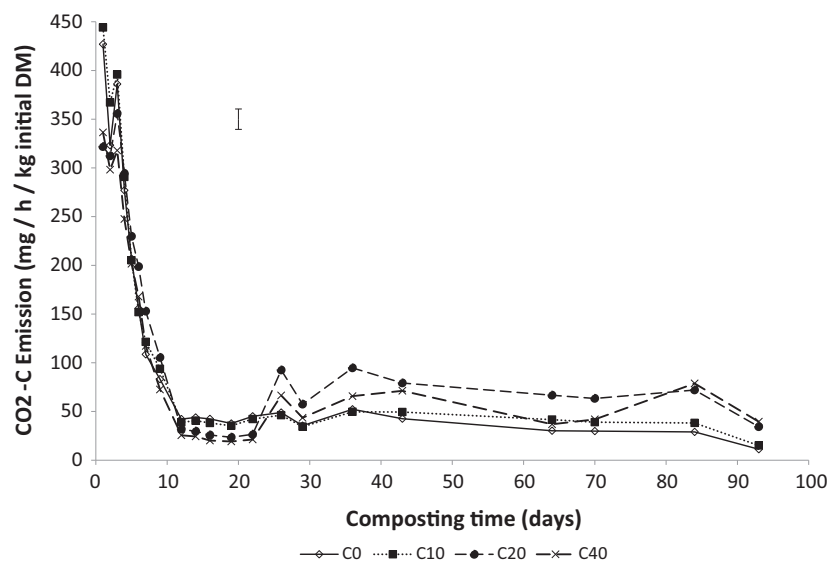


Fig. 3. Emission patterns of CO₂-C during the composting period. The vertical bar represents the maximum standard error observed.

An increase in CO₂ emissions is associated with high microbial activities during the bio-oxidation of organic residues, and this can be used to state a thermophilic phase since the more labile organic matter is degraded, leading to high temperatures. On the other hand, a decrease in CO₂ levels indicates a lower microbial activity, a more stable composted organic matter, a lower temperature, and thus a mesophilic phase (Ros et al., 2006; Liu and Price, 2011). According to our data (Figs. 2 and 3), the temperature and CO₂ emissions followed the same trends of change, which is in agreement with the previous sentence. Thus, we can state that the evolution of temperatures and CO₂ emissions during the composting process of our samples are highly correlated with each other ($r = 0.92$; $p < 0.001$). The mesophilic phase was extremely short and corresponded to the first day of composting. Afterwards, the highest CO₂ emissions were reached, corresponding to the thermophilic phase of the composting process with temperatures over 40 °C, which lasted approximately until day 6. Later, the analysis of our data suggested two main periods, the first one characterized by a still active composting phase that occurs until day 12 with high emissions of CO₂, and the second one with slightly

constant temperatures corresponding to the phase of maturation with reduced CO₂ emissions.

3.4.2. Methane (CH₄) emissions

Methane is formed by the deoxidization of CO₂/H and acetic acid by methanogens under anaerobic conditions (Epstein, 1997). The results from CH₄-C emissions (mg h⁻¹ kg⁻¹ initial DM) measured in different treatments during the composting process are shown in Fig. 4. Statistical analysis showed that CH₄ emissions were only affected by the sampling date ($p < 0.001$). Although the average values of CH₄ emissions were different between treatments, the trend of change of CH₄ emissions was very similar in all treatments (Fig. 4). In the first 3 days of composting, we observed the highest emissions of CH₄ and then a sharp decrease to negative or near zero values until the end of the experiment. On day 1, CH₄ emissions were significantly different between treatments, with lower emissions for the treatment C₄₀ (-0.32 ± 0.09 CH₄-C h⁻¹ kg⁻¹ initial DM) compared to C₀ (0.61 ± 0.21 CH₄-C h⁻¹ kg⁻¹ initial DM) and C₂₀ treatments (0.85 ± 0.18 CH₄-C h⁻¹ kg⁻¹ initial DM). The highest CH₄ emission

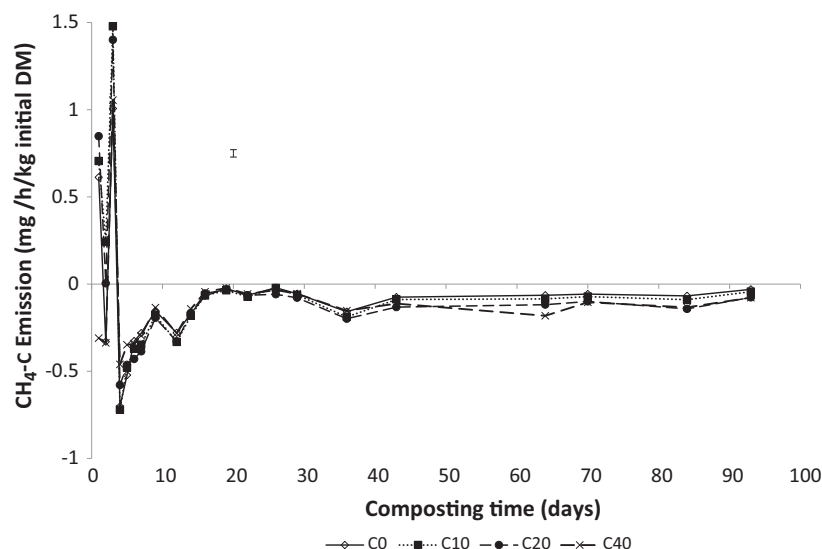


Fig. 4. Emission patterns of CH₄-C during the composting period. The vertical bar represents the maximum standard error observed.

was recorded on day 3 for the C₁₀ treatment (1.48 ± 0.29 mg CH₄-C h⁻¹ kg⁻¹ initial DM).

Methane emissions from the treatments in our study occurred mainly in the thermophilic phase, which is in agreement with the observations made by Li et al. (2013) using different organic materials and food wastes for composting. The largest emission of CH₄ in the first three days occurred probably due to the large amounts of nutrients and easily degradable organic compounds that stimulated microbial activities, thereby reducing available oxygen and promoting optimum conditions for methanogenic bacteria.

From the third day till the end of composting, there was a stable trend in the emissions in all the treatments and during that period, the composts acted as CH₄ sinks. Furthermore, the negative fluxes of CH₄ seem to indicate an absence of CH₄ emissions, probably due to their oxidation as a consequence of the constant aeration performed during the composting process. In addition, we believe that the pronounced decrease of CH₄ emissions can also be related with the quick exhaustion of carbon compounds by microbes in the composting materials (Table 2).

3.4.3. Nitrous oxide (N₂O) emissions

The formation of N₂O occurs during the incomplete nitrification/denitrification processes that normally convert NH₄⁺ into N₂, a non-polluting gas. During denitrification, N₂O can be synthesized where there is a lack of O₂ and/or nitrate (or nitrite) accumulation (Philippe et al., 2012). During nitrification, N₂O is produced in the presence of O₂ and/or low availability of degradable carbohydrates (Epstein, 1997). Therefore, N₂O can be produced under both aerobic and anaerobic conditions.

The results for nitrogen emissions in the form of nitrous oxide (mg N₂O-N h⁻¹ kg⁻¹ initial DM) are shown in Fig. 5. Emissions of N₂O were only affected by the sampling date ($p > 0.05$), with the results being very similar in all treatments except in the first two days of composting. The highest value of N₂O was recorded on day 2 in C₁₀ treatment (0.65 ± 0.11 mg NO₂-N h⁻¹ kg⁻¹ initial DM), followed by C₀ treatment (0.64 ± 0.12 mg NO₂-N h⁻¹ kg⁻¹ initial DM), and C₄₀ treatment (0.41 ± 0.04 mg NO₂-N h⁻¹ kg⁻¹ initial DM). The same trend was observed on day 1 where the emissions were significantly higher for the treatments C₀ (0.59 ± 0.12 mg NO₂-N h⁻¹ kg⁻¹ initial DM) and C₁₀ (0.49 ± 0.04 mg NO₂-N h⁻¹ kg⁻¹ initial DM) compared to C₂₀ (0.26 ± 0.08 mg NO₂-N h⁻¹ kg⁻¹ initial DM) and C₄₀ treatments (0.26 ± 0.11 mg NO₂-N h⁻¹ kg⁻¹ initial

DM). After day 9 and until the end of the experiment, N₂O emissions were similar and the values remained close to zero.

These results indicate that N₂O production is greatly dependent of the composting materials; the specific characteristics of SCG and *A. dealbata* have determined N transformations, thereby promoting the activity of soil microbes, increasing nitrification and denitrification rates and subsequently N₂O emissions. Treatments C₀ and C₁₀ with lower rates of SCG, showed higher N₂O emissions; these emissions occurred with ammonia oxidation and depletion of nitrates as may be concluded by the evolution of mineral N content in the treatments (Table 2) simultaneously with an high composting activity, suggestion that denitrification may have been the main source of N₂O.

As with CH₄ emissions, we also noted that N₂O in all treatments were mostly emitted during the thermophilic phase; these results are similar to the emissions patterns previously reported by other authors (Sommer and Møller, 2000), but contrary to the assumption of Thompson et al. (2004) that the activity of nitrifiers would be inhibited by high temperatures (>40 °C) and N₂O would be unable to be produced in the thermophilic phase. Additionally, emissions of N₂O during aerobic conditions may be caused by the transportation (denitrification) of NO₂⁻-NO₃⁻ from aerobic portions to low O₂ concentration microsites in the middle of composting mixtures. Thus, high emissions of N₂O at the beginning of the composting process seem to be related with the composition of the materials, the temperature, and the intensive aeration and its effects, as described above.

3.5. Effect of the different treatments on composting process, gaseous emissions and quality of end products

Finally, an overview of changes observed during composting was displayed using PCA. Plotting (Fig. 6) the first two PC and calculating the distances between C₀, C₁₀, C₂₀, and C₄₀ on the PC1 axis clearly showed that there was an influence of the treatments on the combined measured parameters, which was stronger with increasing spent-coffee ground concentrations.

C₀ composts tended to cluster together with C₁₀ composts; this was mainly reflected in the CO₂ and N₂O emissions which remained similar between the two treatments, and decreased with increasing SCG concentrations, indicating that C₀ and C₁₀ were the least desirable treatments in terms of gaseous emissions.

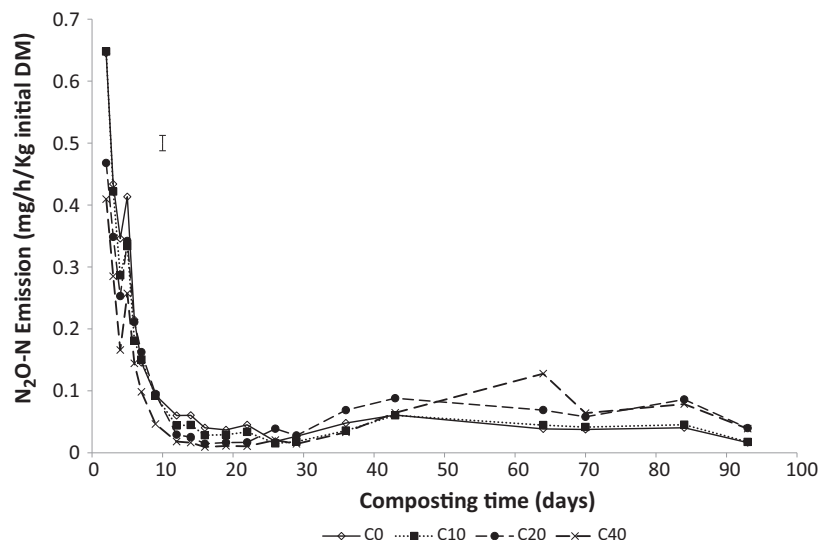


Fig. 5. Emission patterns of N₂O-N during the composting period. The vertical bar represents the maximum standard error observed.

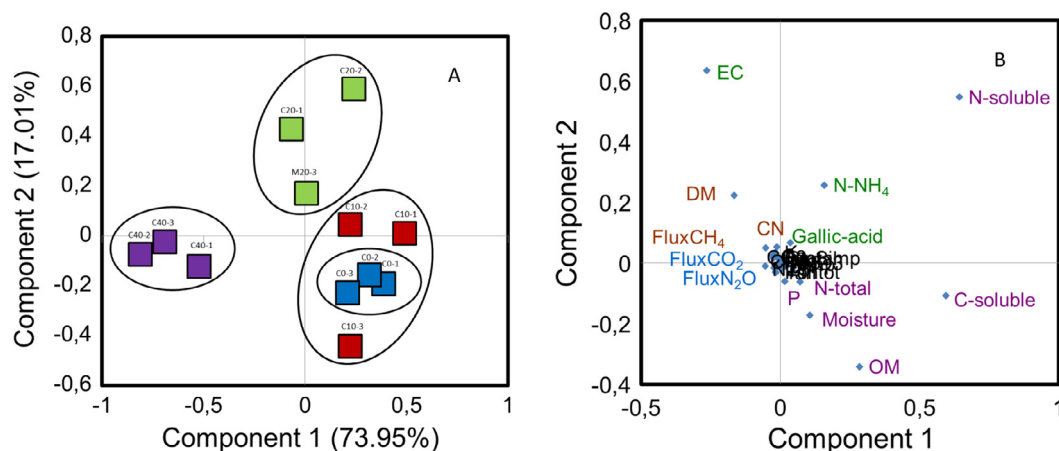


Fig. 6. PCA biplots showing the effect of the different treatments on all parameters obtained: (A) separation of treatments and (B) distribution of all parameters in relation to the treatments. Parameters near the centre of plot B are temperature, pH, N-NO₃, TOC, K, Ca, Mg, total phenolics, simple phenolics and total tannins.

In general, however, the coefficient of variation of the PC scores was higher for C₁₀ than for C₀ (i.e., C₁₀ data sets were more dispersed on the PCA plots), which was related to higher C/N, DM and CH₄ contents in C₁₀ compared to all other treatments. Therefore, if composts with low C/N (denoting a more complete maturity state) and CH₄ emissions (denoting lower environmental impacts) are sought, C₁₀ is definitely not the ideal choice.

The C₂₀ set of samples formed a distinct cluster near to C₁₀, but on the positive coordinates of the PC, in agreement with the less homogeneity of the responses. For example, EC increased from C₀ to C₁₀, peaked at C₂₀, and then decreased at C₄₀. The N-NH₄ content on the other hand increased at C₁₀, and then decreased at C₂₀ to peak at C₄₀. Although C₁₀ favoured higher gallic acid contents, its high EC and the observed variability in the responses as compared with other treatments could disqualify its use in agriculture.

Changes observed were greater and more beneficial when using C₄₀, whose replicates formed a closer cluster some 1.25 distance away from C₀ on the PC1 axis. Differences were primarily caused by larger amounts of dissolved C (15%, 23%, and 70% from C₀ to C₁₀, C₂₀, and C₄₀ respectively) and N (37%, 47%, and 103% from C₀ to C₁₀, C₂₀, and C₄₀ respectively) as the concentrations of SCG increased. Minor contributors were OM, moisture, total N and P contents, which remained unchanged between C₀ and C₁₀, but increased by up to 19% with C₂₀ and up to 54% with C₄₀.

4. Conclusions

During the composting process of mixtures containing different proportions of spent coffee grounds (0–40%) an increase in pH and TN and a decrease in C/N ratio and TOC were observed. A substantial decrease in total phenolics and total tannins, and an important increase in gallic acid occurred.

Emissions of greenhouse gases, CO₂, N₂O and CH₄ were very low and no significant difference between the treatments was registered. Overall, it is possible to conclude that all SCG treatments are suitable for composting. However, C₄₀ which led to better conditions for composting (via moisture levels), quality end product (via EC, OM, dissolved C and N contents) and lower gaseous emissions, would be desirable compared with the other treatments.

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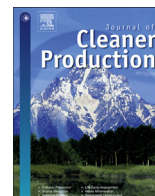
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Annex 2. Paper: Effect of lignocellulosic and phenolic compounds on ammonia, nitric oxide and greenhouse gas emissions during composting



Effect of lignocellulosic and phenolic compounds on ammonia, nitric oxide and greenhouse gas emissions during composting

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ABSTRACT

Composting is recognised a promising technology for recycling and adding value to agro-food wastes. There are, however, potential environmental risks associated with composting, such as the emission of greenhouse gases (GHGs). The aim of this study was to quantify the emissions of carbon dioxide, methane, nitrous oxide, nitric oxide and ammonia during composting of five agro-food wastes originated from the broccoli, chestnut, olive and grape industries, and to study the relationship between the emissions and the lignin, cellulose, hemicellulose and phenolic contents of the wastes. According to physicochemical indicators monitored during the process, all agro-food residues tested were suitable for composting, with chestnut in the top and broccoli in the bottom of the range. Composting of chestnut and olive led to higher carbon dioxide, methane and nitrous oxide emissions, whereas lower emissions were observed with white grape. A positive correlation was found between phenolics and nitric oxide ($r = 0.63$; $p < 0.01$), but not with the other gases. Lignocellulose showed a positive correlation with nitrous oxide ($r = 0.51$; $p < 0.05$), but not with carbon dioxide and methane. The accumulation of lignin was highest in the chestnut compost, which was associated with lower nitric oxide and ammonia emissions relative to the other waste materials. The results show that wastes with high lignocellulose can be managed using composting with additional benefits on the environment in relation to mitigating nitrogen losses.

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1. Introduction

Large amounts of agro-food wastes, generated as a consequence of rapid population growth and subsequent increase in food production and consumption, nowadays pose serious environmental and economic risks (Mirabella et al., 2014). The agro-food sector contributes to the loss of an abundant pool of organic valuable materials that could be used as a substitute for mineral fertilisers and pesticides, thereby promoting a more sustainable agriculture.

Crop residues represent a substantial portion of organic wastes. In southern Europe, grape, olive and chestnut crops are widely cultivated and wineries, chestnut and olive oil mills constitute the

dominant agro-industrial activities in the region (Salgado et al., 2014). The winery industry for example, reportedly produces 14.5 Mt of grape byproducts in the region (Pinelo et al., 2006).

Direct soil application of agro-food wastes, especially those rich in bioactive compounds (e.g., polyphenols), is cautiously recommended due to toxic effects on microorganisms and plants (Medina et al., 2015). For these reasons, transformation and stabilisation of these wastes before their application to soil are imperative; composting provides a viable and secure technology option for achieving that goal. Composting can be used to effectively counter the toxicity of wastes while yielding a pathogens-free material. The composting process also reduces the waste volume and weight by about 50% (Senesi, 1989) with a low investment cost compared with other waste treatment technologies (Lim et al., 2016); the reduction in weight ensures a stable final product which can be applied to improve the chemical and physical properties of soils

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(Bustamante et al., 2011), including the performance of plants (Santos et al., 2016).

Composting could lead to secondary pollution involving ammonia (NH_3), nitric oxide (NO) and greenhouse gases (GHG) emissions (Ermolaev et al., 2015), which are known drivers of global warming and stratospheric ozone depletion. Although composting is considered to have a lesser environmental impact compared to other waste treatments (Saer et al., 2013), its advantages can be negated if the composition process is not optimized. Productivity gains following compost application should also be weighed against the amount of gases released during the composting process (Sanchez-Garcia et al., 2015). Quantitative studies on gaseous emissions during the composting process are needed to make such comparisons possible and to develop models; emission models would be useful for environmental assessments involving different conditions such as the waste type, chemical composition, the bulking agents and the processing unit airflow.

The last decade has seen a growing interest in GHG emissions during composting. For example, Sánchez-Monedero et al. (2001) evaluated the effect of olive mill wastes mixed with different agroindustrial by-products on GHG emissions; Cayuela et al. (2012) studied GHG emissions during composting of a lignocellulosic mixture added with different N sources. GHG emissions have also been addressed in composting studies involving varying experimental units and operation schemes: Amlinger et al. (2008) evaluated the influence of turning frequency and different aeration rates on GHG, NH_3 and N_2O emissions; Ermolaev et al. (2015) studied N_2O and CH_4 emissions at different temperatures. These studies collectively demonstrate that the rate of gas production, diffusion and emission from composting piles are highly dependent on the physical characteristics of the starting material, such as its temperature, and its microbial population. There is, however, a lack of information on the relationship between the physical properties of the wastes and GHG emissions during composting. It is reasonable to assume that chemical compounds, such as lignocelluloses and phenolics which are characterised by a low degradation rate, would highly influence the mechanisms involved in GHG emissions during composting.

The objectives of this work were to (i) assess the reuse/recycling potential of broccoli, chestnut, grape and olive wastes as composts, with consequent environmental implications regarding gaseous emissions during the composting process, and (ii) assess the relationship between the mechanisms involved in CO_2 , CH_4 , N_2O , NO and NH_3 emissions and the lignin, cellulose, hemicellulose and phenolic contents of the wastes. This information would be crucial for disposing and/or valorising agro-food wastes based on sound environmental decision criteria, and through implementation of improved management practices.

2. Materials and methods

2.1. Agro-food wastes and experimental design

The composting experiment was carried out at the Universidade de Trás-os-Montes e Alto Douro (N 41°17'7.28"; W 7°44'36.83"), and lasted ca. 5 months. The compost was produced in 135-L insulated reactors. The air was supplied continuously with a diaphragm pump (KNF, model N010.KN.18, Neuberger GmbH, Freiburg, Germany) from the underside of the reactor, where a perforated plate was positioned between the base and the composting mixture. The photoacoustic and chemiluminescent N oxide analysers used to measure gas concentrations were connected to the reactor with Teflon tubes positioned on the air inlets and outlets. Additional information about the composting system can be found in Santos et al. (2017). Aeration of the composting mixture

was at the rate of $20 \text{ L h}^{-1} \text{ kg}^{-1} \text{ DW}$ (dry weight) during the first 15 d, and $10 \text{ L h}^{-1} \text{ kg}^{-1} \text{ DW}$ thereafter, using a flowmeter equipped with a needle valve (Aalborg, model P, Orangeburg, USA) operating at 5 L min^{-1} .

Five types of agro-food wastes were used in the composting experiments: (i) broccoli; (ii) chestnut; (iii) white grape marc from *Vitis vinifera* L. var. Moscatel; (iv) red grape marc from *Vitis vinifera* L. var. Alfrocheiro; and (v) olive. Broccoli wastes consisted of rejected flowering inflorescences; chestnut wastes comprised of peels and rejected fruits; grape marc were collected during the grape stripping step of the winemaking process; and olive leaves were obtained from an olive oil extraction plant. Dried wheat straw previously milled (2–4 cm) using a knife mill (Yike, 9FQ-360 straw hammer mill, Zhengzhou, China) was used as structural material. A control treatment was prepared with wheat straw complemented with urea to adjust the carbon-to-nitrogen (C/N) ratio to 40.

Twenty-four (24) hours before the composting experiment starts, agro-food raw materials were grinded into pieces of ca. 1 cm in length using a shredder (Viking model GB370S, Langkampfen, Austria) and stored fresh at 4°C until use.

Four reactors were used per treatment, representing the replicates. Each reactor was filled with 10 kg dry matter (DM) of the waste-straw mixture, in a proportion allowing an initial C/N ratio of ca. 40, as calculated from the elemental contents of the raw materials (Table 1). Treatments consisted of a mixture of the wastes with wheat straw and urea (except for broccoli) and are herein referred to as: "Control", "Broccoli", "Chestnut", "WhiteGrape", "RedGrape" and "Olive". Moisture was maintained approximately between 45% and 60% by adding water when necessary. The temperature was measured using TM1 temperature probes (Delta-T Devices, Cambridge, UK) positioned inside the reactors and in the centre of the composting mixture, where the highest temperatures were recorded. The mixture was turned manually once a week during the most biooxidative phase and then every 15 d till the end of the maturation period.

A representative sample of each compost was taken on days 0, 3, 8, 17, 30, 76 and 147, by mixing sub-samples from six different locations in the reactor and at depths between 20 and 80 cm. Each composite sample was disassembled into 3 subsamples, one of which was ground (particle size $< 1 \text{ mm}$), frozen and stored for chemical and physical analyses; another subsample was dried at 40°C for phenolic and lignocellulosic determination; the last subsample was oven dried at 65°C during 48 h to determine the DM content.

2.2. Measurements of physicochemical properties of the composts

Fresh composts were analysed for their pH, electrical conductivity (EC), and content of organic matter (OM), using standard procedures (CEN, 1999).

Soluble organic nitrogen (ONSol) and soluble organic carbon (OCsol) were determined after extraction of dry samples with 0.01 M CaCl_2 (1:10 w/v) in an elemental analyser (Formac, Skalar, Analytical B.V., Breda, Netherlands), followed by chemiluminescence and near infrared (NIR) detection. Total organic N (Nt) and total organic C (Ct) were evaluated by thermoconductivity and NIR detection in a Skalar Primacs SNC-100 and the C/N ratio calculated.

Ammonium N ($\text{NH}_4^+\text{-N}$) and nitrate N ($\text{NO}_3^-\text{-N}$) concentrations were determined in filtered extracts by molecular absorption spectrophotometry with a segmented flow system (SanPlus, Skalar) prior to extraction of samples with 1 M KCl (1:10 v/v) (Houba et al., 1995). Iron (Fe), Zn, Ca, Cu, Mn and Mg were measured by atomic absorption spectroscopy, after nitric-perchloric acid digestion of dry samples. Total P was determined by molecular absorption

Table 1

General characterisation of the initial waste-straw mixtures used for composting.

	pH	EC (dS m ⁻¹) (water 1:5)	OCsol (g C kg ⁻¹ of DM)	OM (g C kg ⁻¹ of DM)	ONsol (g N kg ⁻¹ of DM)	Nt (g N kg ⁻¹ of DM)	NH ₄ ⁺ -N (mg N kg ⁻¹ of DM)	NO ₃ ⁻ -N (mg N kg ⁻¹ of DM)
Control	8.11 ± 0.48 a	1.79 ± 0.07 bc	8.89 ± 1.84 cd	938.0 ± 24.0 a	4.84 ± 2.00 a	13.8 ± 0.5 a	693.4 ± 167.9 ab	0.18 ± 0.06 c
Broccoli	8.04 ± 0.24 a	2.18 ± 0.27 ab	6.99 ± 1.03 cd	927.1 ± 19.6 a	4.06 ± 0.28 a	19.8 ± 3.2 a	1075.5 ± 156.4 a	7.18 ± 2.77 a
Chestnut	7.69 ± 0.55 a	1.26 ± 0.21 d	6.06 ± 1.40 d	959.0 ± 4.7 a	4.21 ± 0.92 a	15.0 ± 2.2 a	686.0 ± 295.1 ab	0.44 ± 0.10 bc
WhiteGrape	5.18 ± 0.39 c	2.55 ± 0.22 a	19.65 ± 3.67 a	946.6 ± 4.8 a	5.69 ± 1.25 a	15.0 ± 2.2 a	378.4 ± 151.7 b	4.88 ± 1.03 ab
RedGrape	4.51 ± 0.34 c	2.47 ± 0.28 a	18.51 ± 5.29 ab	943.8 ± 5.6 a	4.97 ± 0.74 a	16.1 ± 1.1 a	222.2 ± 63.3 b	5.24 ± 1.14 a
Olive	6.24 ± 0.63 b	1.61 ± 0.16 cd	12.70 ± 1.20 bc	948.2 ± 6.8 a	4.21 ± 1.02 a	16.6 ± 2.8 a	280.0 ± 127.1 b	0.20 ± 0.09 c

EC = electrical conductivity; OCsol = soluble organic carbon; OM = organic matter; ONsol = soluble organic nitrogen; Nt = total nitrogen.

Values within a column, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

spectrophotometry with the Skalar segmented flow system.

Phytochemical analyses consisted of the extraction and quantification of total phenolics and gallic acid. Total phenolics were extracted using the Folin-Ciocalteu reagent, and detected by spectrophotometry, as described by Javanmardi et al. (2003). A calibration curve with different gallic acid concentrations (Sigma-Aldrich, Taufkirchen, Germany) was constructed and all data quantified as mg gallic acid equivalent (GAE) g⁻¹ DM. Gallic acid was determined by high performance liquid chromatography as reported by Santos et al. (2017).

The lignocellulosic fractions were determined in the raw materials, waste-straw mixtures and final composts. Neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined by the sequential method of Van Soest et al. (1991). Hemicellulose and cellulose were calculated as the difference between NDF and ADF, and between ADF and ADL. All analyses were done in four replicates.

2.3. Measurements of gas emissions

Emissions of CO₂, CH₄ and N₂O were measured using a 1412 Photoacoustic Field Gas Monitor (Innova AirTech Instruments, Ballerup, Denmark), with internal filters for water vapour, and particulate and optical filters for CO₂ (UA0982), CH₄ (UA0969) and N₂O (UA0985). The CO₂, CH₄ and N₂O detection limits specified for the analyser were 1.5, 0.4 and 0.03 ppm, respectively. Emissions of NO and NH₃ were performed with a chemiluminescent N oxide analyser (SIR MODEL S-5012, Madrid, Spain) with a detection limit of 0.5 ppb. Emission rates at the different events were determined after discounting the concentration of each gas in the inlet air to the outlet air in INNOVA and SIR data.

All gases were sampled once a day during the first 8 d of composting, every two days for the following three weeks and then on days 31, 36, 42, 49, 59. Ammonia (NH₃) and NO were measured on days 66, 73, 80, 87, 94, 104, 110, 117, 125, 130; and CO₂, N₂O and CH₄ on days 80, 104, 117 and 147. To minimise the influence of ambient air on the gas concentrations, measurements were always done before homogenisation or collection of the composts.

To get the cumulative CO₂, CH₄ and CO₂ emissions, daily fluxes from contiguous measurements were averaged and multiplied by the interval between sampling periods. In order to evaluate the effect of the different treatments on total GHG emissions, the measured emissions were converted into CO₂-equivalents according to the IPCC GWP factors of 28 and 265 times as much as CO₂ for CH₄ and N₂O, respectively (IPCC, 2013).

2.4. Statistical analyses

The results are presented as mean ± standard deviation (SD) and all analytical determinations were made in quadruplicate. To evaluate the effects of each treatment (factor) at different sampling

times (d) on physicochemical parameters, lignocellulosic composition and gas emissions, data were analysed by a two-way repeated measures analysis of variance. Differences between means were determined by the Tukey's honestly significant difference test at a significance level of 0.05. The relationship between total phenolic compounds, lignocellulosic compounds, and gaseous emissions were assessed by calculating Pearson's correlation r values. The referred statistical analyses were performed using the Statistix 10.0 software (Analytical Software, Tallahassee, USA). Prior to these tests, normality and homogeneity of the data were checked using Shapiro-Wilk and Levene tests.

3. Results and discussion

3.1. Evolution of physicochemical parameters during composting

3.1.1. Temperature

With regard to temperature variations with composting time, significant differences ($p < 0.05$) between treatments were observed. The highest temperature (54 °C) was reached with Broccoli – at a faster rate than with the other treatments –, and was maintained for five consecutive days. Broccoli also resulted in the longer thermophilic phase and this may be explained by a greater content of easily degradable organic compounds in broccoli wastes, as compared to the other materials (Table 1). This pattern of temperature change during composting has also been observed with garden leaves (Kalamdhad et al., 2009), and horticultural wastes (Gavilanes-Terán et al., 2016).

The slowest increase in temperature was observed with Control, Chestnut, and Olive, probably because the wastes contained an organic matter more resistant to microbial degradation (Table 1). At the end of the composting process, the average temperature for all treatments had returned to or near ambient values (ca. 13 °C), an indication of the slowdown of microbial activities.

3.1.2. Physicochemical parameters

The physicochemical characteristics of the waste-straw mixtures at different times are displayed in Fig. 1. With the exception of WhiteGrape, the pH of the mixtures remained constant after 3 days, which could be attributed to lower microbial activities and the stabilisation of the composts. At the end of composting, final pH values ranged from 8.99 (WhiteGrape) to 9.26 (Chestnut) (Fig. 1a). Although such high pH values might stimulate NH₃ losses (Lasaridi and Stentiford, 1998), taking into consideration other indices used to assess the performance of the composting process such as microbial degradations or agronomic tests, it is reasonable to say that all composts were safe for soil application, as attested by the production of lettuce plants fertilised with the same materials in a pot experiment (Santos et al., 2016).

A strong increase in electrical conductivity (EC) values was observed during the first days of composting, corresponding to the

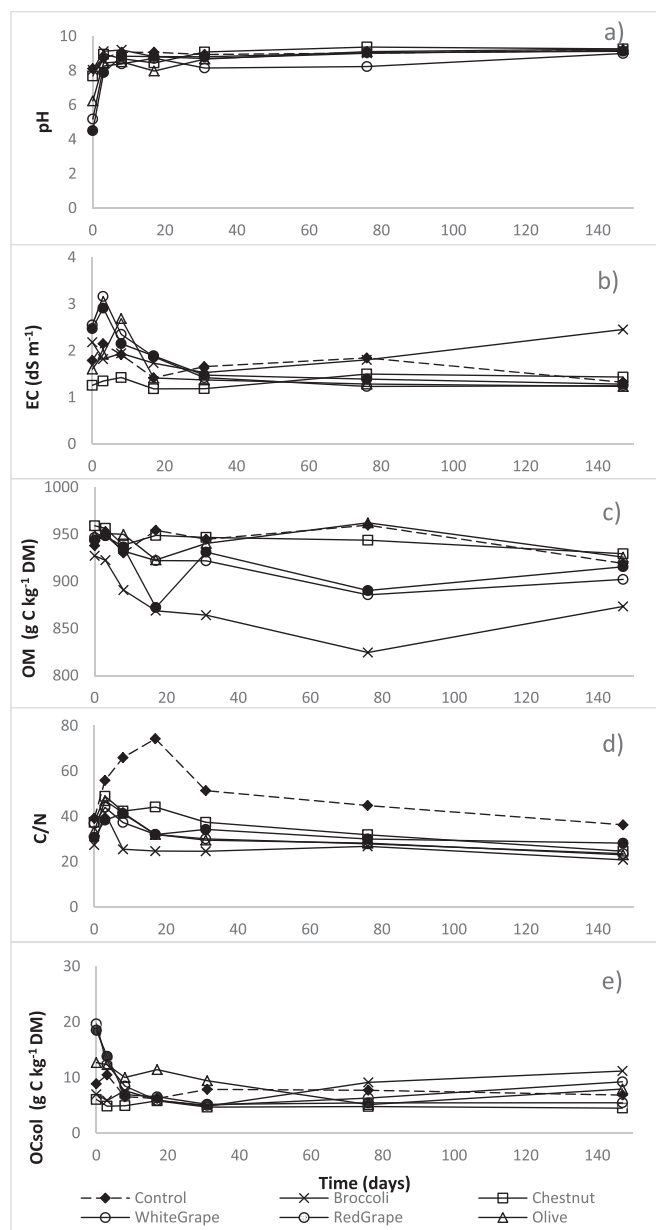


Fig. 1. Evolution of main physicochemical characteristics of the waste-straw mixtures during composting. a) pH; b) Electrical conductivity; c) Organic matter; d) C/N ratio; e) Organic soluble Nitrogen.

thermophilic phase; and then a decrease as the composting process progressed, indicating accrued benefits in terms of final-product quality (Fig. 1b). The only exception was with Broccoli, for which an opposite trend was observed. Final EC values were in the range of 1.23 dS m⁻¹ for Olive and 2.45 dS m⁻¹ for Broccoli, which were all below the maximum EC value of 3 dS m⁻¹ recommended for compost application to soil (Solano et al., 2001).

The C/N ratio for all the mixtures (ca. 40) first increased, and then decreased with composting time to a final ratio ranged from 20.9 with Broccoli to 36.3 with Control (Fig. 1d). The final C/N ratios exceeded the threshold limit of 20 recommended by Golueke (1981). The C/N ratio should be considered only as a relative indicator of compost maturation here since the bioavailability of organic C matters more than the total organic C content in high lignocellulosic materials (Sánchez-Monedero et al., 2001), such as those used in the present study.

The OM content decreased with composting time, and hence with mass reduction (Fig. 1c). The highest OM content was observed with Chestnut, and it decreased with composting time from 959.0 g C kg⁻¹ DM to 929.4 g C kg⁻¹ DM. The lowest OM content was observed with Broccoli, and it decreased from 927.1 g C kg⁻¹ to 873.4 g C kg⁻¹, with significant differences ($p < 0.05$) as compared to all other treatments (Fig. 1c).

The same behaviour observed with OM was seen with OCsol, though with opposite directions of change. At the end of composting, the lowest OCsol content (4.50 g C kg⁻¹ DM) was observed with Chestnut, and the highest (11.18 g C kg⁻¹ DM) with Broccoli (Fig. 1e). Differences ($p < 0.05$) between treatments could be explained by different turnover rates of labile and stable fractions of organic compounds. After 36 days, degradation of easily degradable compounds had already occurred in Control, Chestnut, and Red-Grape. These treatments also exhibited stable OCsol contents until the end of the experiment (Fig. 1e), indicating that equilibrium between microbial degradation of complex organic structures like cellulose and hemicellulose and production of soluble compounds (Serramiá et al., 2010) had been reached.

As a result of DM loss, the contents of all elements in the different materials increased during composting (Supplementary Figure S1). Substantial amounts of mineral nutrients were present in all treatments, which for a fertilisation point of view, is an important quality for a final compost. Higher P, Mg, Fe and Zn contents were obtained with Broccoli, as compared with all other treatments ($p < 0.05$). Broccoli compost was also found to be a rich source of Ca (12.29 g kg⁻¹ DM), which is essential for plant nutrition. Previous studies have shown that micronutrients and heavy metals are present in significant amounts in vegetable wastes, and concurring with the results of this study, are increased during composting (Gavilanes-Terán et al., 2016).

3.1.3. Nitrogen contents

Fig. 2 shows the time course of total nitrogen (Nt), soluble organic nitrogen (ONSol) ammonium (NH₄⁺) and nitrate (NO₃⁻) changes during composting.

Total nitrogen decreased at the beginning of composting and slightly increased as the process progressed, probably as a result of a concentration effect caused by weight loss associated with OM mineralisation (Senesi, 1989). At the end of the process, highest Nt (24.12 g N kg⁻¹ DM) and ONSol (3.03 g N kg⁻¹ DM) contents were obtained with Broccoli, and were significantly different ($p < 0.05$) from the contents obtained with the other treatments (Fig. 2a and b). Although the initial mixture of Broccoli was rich in Nt and ONSol, its initial content of OCsol was lower when compared with most of the other treatments (Table 1).

According to the data shown in Fig. 2c, NH₄⁺ contents were initially increased as a consequence of ammonifying activities. The highest NH₄⁺ content was observed on day 3 (1453.9 mg N kg⁻¹ DM for Control), coinciding with a peak in soluble N contents. A significant decrease was observed with all treatments, mostly due to volatilisation of NH₃ and/or immobilisation or nitrification processes as seen in Fig. 3. A slightly different trend of change was observed with Broccoli, with an initial decrease of NH₄⁺ contents by day 3, followed by a sharp increase peaking at day 17, and a progressive decrease (Fig. 2c). By the end of composting, NH₄⁺ content was significantly lower ($p < 0.05$) with Broccoli than with the other treatments. The largest decrease in NH₄⁺ contents observed with Broccoli (Fig. 2c) as compared to its initial richness in NH₄⁺ (Table 1) was probably related to higher Nt and ONSol contents. The final NH₄⁺ contents in most piles were below the maximum recommended value for a mature compost, i.e., 400 mg kg⁻¹.

As observed for NH₄⁺, NO₃⁻ contents for all treatments increased sharply within the first days of composting (Fig. 2d). The highest

NO_3^- content was observed in the initial broccoli mixture (Table 1), which also led to significant N_2O emissions (see Table 3). In previous studies using pig manure with straw (Szanto et al., 2007) and olive mill wastes with different agro-industrial by-products (Sánchez-Monedero et al., 2010), a correlation was found between N_2O emissions and NO_3^- contents during periods of intense nitrification.

3.2. Evolution of phenolic and lignocellulosic contents during composting

The contents of phenolics, hemicellulose, cellulose and lignin in the raw materials, waste-straw mixtures and final composts are presented in Table 2. Statistical analyses showed that time interacted with all the treatments to affect both the total phenolics and lignocelluloses.

The initial straw ($3.75 \text{ mg g}^{-1} \text{ DM}$) and broccoli ($6.61 \text{ mg g}^{-1} \text{ DM}$) had the lowest contents of total phenolics, while olive ($25.12 \text{ mg g}^{-1} \text{ DM}$) and white grape ($30.04 \text{ mg g}^{-1} \text{ DM}$) had the highest contents. For all treatments, a significant decrease ($p < 0.05$) in phenolics occurred throughout the composting process, with final values ranging from $0.15 \text{ mg g}^{-1} \text{ DM}$ with Olive to $1.18 \text{ mg g}^{-1} \text{ DM}$ with Control (Table 2), indicating progressive degradation. It is possible that as soon as these phenolics were degraded and released, they were incorporated into biosynthetic pathways leading to the formation of humic substances (Stevenson, 1994).

The HPLC method used in this study for the separation of individual phenolics allowed the identification of the two tannins, namely gallic acid (in all treatments) and ellagic acid (in only the raw chestnut and initial chestnut-straw mixture). An increase in gallic acid contents occurred during composting (Table 2), reflecting a cumulative effect of straw added to wastes, but also the degradation of polyphenols into simple phenolics. Gallic acid exhibits a strong thermal stability and is metabolised only at temperatures above 60°C (Volf et al., 2014). The maximum temperature reached during composting was 54°C , which reasonably explains the presence of gallic acid in the composts at the end of the process.

Although beneficial at low levels, excess levels of water-soluble phenolics in composts may have adverse environmental impacts, mostly in inhibiting seed germination (Morthup et al., 1998) and limiting nitrogen immobilisation in soil (Bustamante et al., 2007).

Raw straw used as Control and raw chestnut had significantly higher ($p < 0.05$) contents of hemicellulose than the other materials, 386.3 and $358.4 \text{ g kg}^{-1} \text{ DM}$, respectively (Table 2). These contents remained relatively unchanged after mixing with the co-substrate, but were significantly reduced ($p < 0.05$) by about 85% by the end of composting. In the case of RedGrape, WhiteGrape, Olive and Broccoli, hemicellulose contents measured at the end of composting were also much lower than those registered early in the process. Chestnut and straw, however, underwent the most intense degradation.

A more pronounced degradation of hemicellulose was observed than of cellulose, indicating that hemicellulose was the first cellulosic fraction used by microorganisms as C and energy source, as also reported by Serramiá et al. (2010). Hemicellulose is a smaller branched carbohydrate compared to cellulose, which is made of very long unbranched fibrils held together by hydrogen bonding that prevents easy access by microorganisms and enzymes (Komilis and Ham, 2003).

The straw had the highest content of cellulose ($413.0 \text{ g kg}^{-1} \text{ DM}$), while the raw Broccoli, Chestnut and Olive had the lowest contents (112.3 , 143.3 and $150.1 \text{ g kg}^{-1} \text{ DM}$), with WhiteGrape and RedGrape in the middle range (ca. $200 \text{ g kg}^{-1} \text{ DM}$) (Table 2). By the

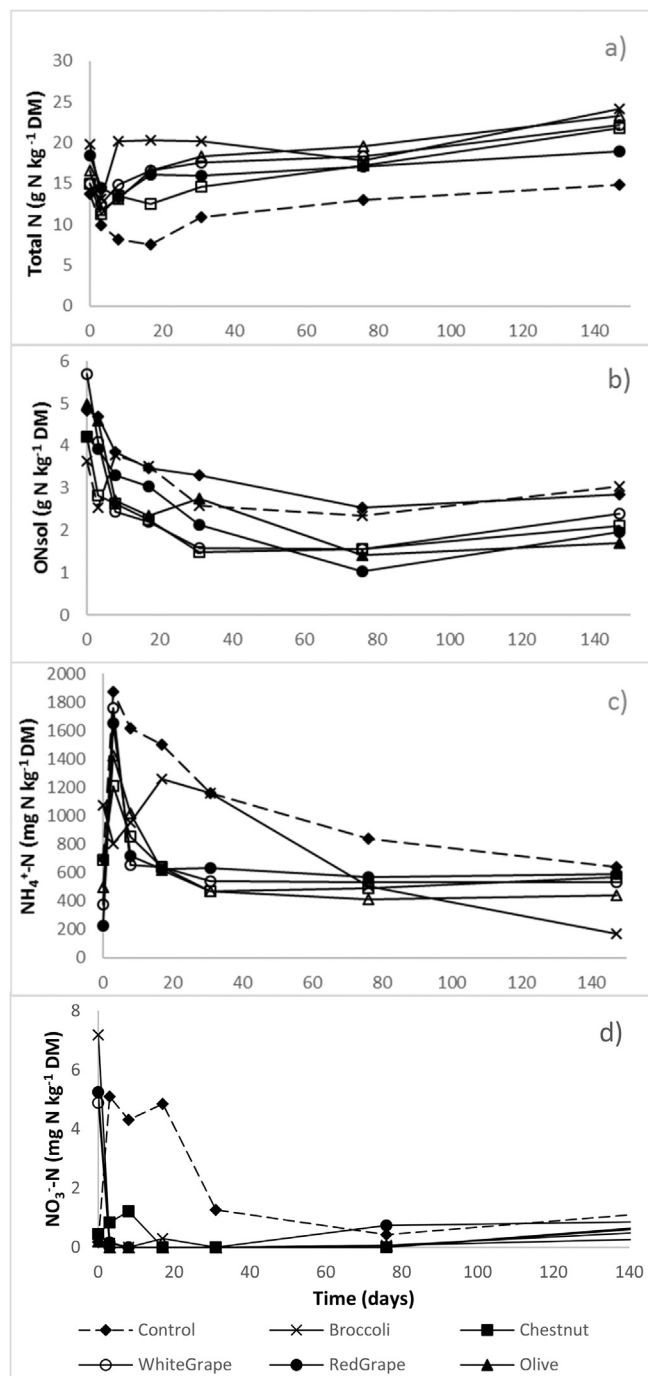


Fig. 2. Evolution of the contents of N forms in different waste-straw mixtures during composting. a) total Nitrogen, b) Organic soluble Nitrogen; c) $\text{NH}_4^+\text{-N}$; d) $\text{NO}_3^-\text{-N}$.

end of composting, significantly higher ($p < 0.05$) reductions in cellulose contents were recorded with Olive (72%) and Broccoli (76%). The lowest reduction in cellulose content was observed for Chestnut (29%), which also exhibited the highest reduction in hemicellulose content (Table 2).

As for lignin, its “relative proportion” increased as a consequence of the intense degradation of the holocellulose fraction (cellulose + hemicellulose). Olive, RedGrape and WhiteGrape were the wastes that contained more lignin (Table 2). These treatments took longer to react to temperature changes. Lignin acts as a protective factor for the cellulosic and hemicellulosic fractions and the

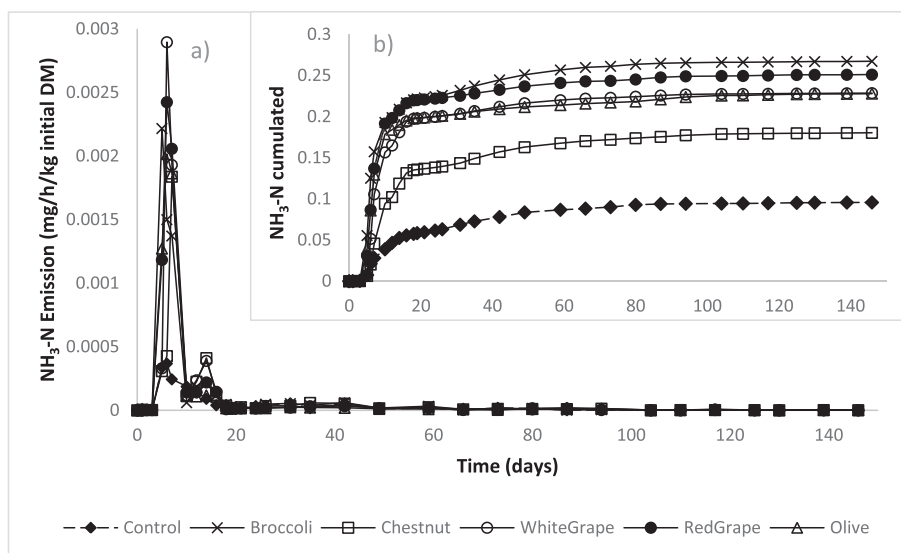


Fig. 3. Evolution of instantaneous and accumulated NH₃ emissions during composting: (a) instantaneous ammonia emissions, (b) accumulated NH₃ emissions.

Table 2

Evolution of the contents of total phenolics, gallic acid, lignocellulosic compounds (normalised to a constant ash content) and related ratio in the raw materials, initial straw-waste mixtures and final composts.

	Total phenolics (mg g ⁻¹ DM)	Gallic acid (μg g ⁻¹ DM)	Hemicellulose (g kg ⁻¹ DM)	Cellulose (g kg ⁻¹ DM)	Lignin (g kg ⁻¹ DM)	Lignocellulose (g kg ⁻¹ DM)	Lignin/holocellulose ratio
Raw materials							
Straw	3.75 ± 0.69 e	26.16 ± 0.93 a	386.3 ± 12.7 a	413.0 ± 5.33 a	48.60 ± 1.50 b	847.9 ± 15.7 a	0.06 ± 0.00 c
Broccoli	6.61 ± 0.02 d	4.19 ± 0.03 c	32.03 ± 4.50 d	112.3 ± 17.8 c	43.40 ± 15.8 b	187.8 ± 2.31 d	0.31 ± 0.15 bc
Chestnut	14.99 ± 0.47 c	17.66 ± 0.67 b	358.4 ± 28.1 a	143.3 ± 12.0 c	167.2 ± 22.8 a	668.9 ± 16.3 b	0.33 ± 0.06 bc
White Grape	30.04 ± 0.73 a	11.53 ± 1.41 bc	69.38 ± 6.44 bc	208.0 ± 8.42 b	187.7 ± 15.5 a	465.0 ± 10.4 c	0.68 ± 0.08 ab
Red Grape	24.50 ± 0.61 b	14.37 ± 2.01 b	51.31 ± 8.09 cd	202.6 ± 2.65 b	197.3 ± 3.67 a	451.2 ± 9.60 c	0.78 ± 0.03 ab
Olive	25.12 ± 0.63 b	12.75 ± 2.06 b	97.81 ± 1.31 b	150.1 ± 4.79 c	225.8 ± 1.54 a	473.8 ± 4.09 c	0.91 ± 0.01 a
Initial straw-wastes mixtures							
Control	3.75 ± 0.69 d	26.16 ± 0.93 a	386.3 ± 12.7 a	413.0 ± 5.33 a	48.60 ± 1.50 b	847.9 ± 15.7 a	0.06 ± 0.00 a
Broccoli	4.29 ± 0.19 d	20.62 ± 1.27 a	280.0 ± 10.2 b	322.8 ± 5.05 b	47.04 ± 5.16 b	649.9 ± 11.7 d	0.08 ± 0.01 a
Chestnut	8.25 ± 0.59 c	23.66 ± 0.89 a	375.2 ± 16.4 a	305.1 ± 6.29 b	96.03 ± 9.64 ab	776.3 ± 2.96 b	0.14 ± 0.02 a
WhiteGrape	13.74 ± 0.61 a	21.32 ± 1.62 a	265.9 ± 9.88 b	335.1 ± 6.11 b	101.5 ± 6.50 ab	702.4 ± 11.9 c	0.17 ± 0.01 a
RedGrape	11.78 ± 0.37 b	22.35 ± 0.83 a	252.3 ± 4.53 b	328.9 ± 2.20 b	108.1 ± 2.29 ab	689.3 ± 5.87 cd	0.19 ± 0.00 a
Olive	12.30 ± 0.61 b	21.49 ± 0.88 a	270.9 ± 8.13 b	307.9 ± 3.06 b	119.5 ± 0.32 a	698.3 ± 7.81 c	0.21 ± 0.00 a
Final composts							
Control	1.18 ± 0.08 a	22.64 ± 0.92 a	55.65 ± 6.98 b	240.4 ± 3.73 a	248.1 ± 2.08 cd	544.2 ± 5.66 b	0.84 ± 0.01 b
Broccoli	1.17 ± 0.32 a	23.45 ± 0.81 a	115.5 ± 8.39 a	75.80 ± 3.99 c	205.9 ± 37.1 d	397.2 ± 33.4 d	1.16 ± 0.52 ab
Chestnut	1.07 ± 0.10 a	25.96 ± 1.87 a	58.95 ± 15.9 b	215.1 ± 17.5 ab	379.7 ± 18.4 a	653.8 ± 12.8 a	1.40 ± 0.23 a
WhiteGrape	1.02 ± 0.35 a	30.41 ± 6.58 a	108.9 ± 21.7 a	184.6 ± 46.9 b	352.0 ± 37.1 a	645.4 ± 32.3 a	1.27 ± 0.42 ab
RedGrape	0.74 ± 0.06 a	28.82 ± 2.73 a	131.4 ± 31.2 a	182.2 ± 2.95 b	324.5 ± 49.9 ab	638.1 ± 71.0 a	1.06 ± 0.38 ab
Olive	0.15 ± 0.00 a	30.02 ± 9.36 a	10.88 ± 4.12 a	86.00 ± 43.6 c	285.9 ± 40.3 bc	480.6 ± 139 c	1.58 ± 0.61 a

Values within a column, for the same day, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

biodegradation of lignin usually occurs tardily and at a very low rate (Malherbe and Cloete, 2002). At the end of the composting process, Chestnut, WhiteGrape and RedGrape were the treatments with highest lignin contents (Table 2).

The lignin/holocellulose ratio has been proposed as an index to monitor the biodegradability of the OM (Francou et al., 2008), and can be useful to understand a compost resistance to degradation after soil application. The lowest lignin/holocellulose ratio was with the Control, and increased from an initial value of 0.06 to a final value of 0.84, which was significantly lower ($p < 0.05$) than the final highest value obtained with Olive i.e., 1.58 (Table 2). The increase of this ratio for all treatments indicates stabilisation of the OM.

All the composts obtained were found suitable for agricultural use though the Broccoli had lowest OM, C/N, lignin, NH₄⁺, NO₃⁻, and cellulose contents compared to the other composts.

3.3. Evolution of gaseous emissions during composting

3.3.1. Carbon dioxide

Throughout composting, CO₂ fluxes were significantly ($p < 0.05$) affected, regardless of the type of waste. Composting with Olive led to the maximum average emission of 125.6 g CO₂-C kg⁻¹ initial DM on day 9, while the lowest emission (34.6 g CO₂-C kg⁻¹ initial DM) was observed on day 16 on the WhiteGrape (Supplementary Figure S1a). Major CO₂ fluxes were detected during the first 13 days of composting. After this initial period, a decrease in emissions occurred, with values equalising among the treatments towards the end of the experiment and leading to the accumulated CO₂-C emissions time pattern shown in Supplementary Figure S1a). Only Olive led to emission values over 100 g CO₂-C kg⁻¹ initial DM. The final accumulated CO₂-C flux emissions (mg CO₂-C kg⁻¹ initial

DM) are presented in Table 3. The highest emissions were observed with Olive and Chestnut, and the lowest emission with WhiteGrape. No significant correlation ($p < 0.05$) was found between final cumulative CO₂ emissions and the total phenolic and lignocellulosic contents of the initial waste-straw mixtures.

3.3.2. Methane

The production of CH₄ occurs in anaerobic micro-sites particles of compost materials, when degradation of fresh and easily available organic materials exhausts the oxygen (Epstein, 1997).

CH₄ emissions were highest in the early phase of the composting process, mostly during the first 13 days, when microbial activities were intense and the demand for aeration high. These fluxes pattern are responsible for the accumulated CH₄-C emissions presented in Supplementary Figure S1b. Statistical analyses indicated that CH₄ emissions differed ($p < 0.05$) among time, but not among waste types. Composting with Olive caused low CH₄ emissions during the first days of the process, with an average value of $-0.70 \text{ g CH}_4\text{-C kg}^{-1}$ initial DM, whereas the lowest values were obtained with WhiteGrape and RedGrape. A sharp decrease of CH₄ emissions occurred as composting progressed, leading to negligible emissions towards the end of the experiment (Supplementary Figure S1b).

Accumulated CH₄ emissions (Table 3) correlated negatively with CO₂ emissions ($r = -0.756$; $p < 0.001$), but not with the total phenolic and lignocellulosic contents of the initial waste-straw mixtures.

3.3.3. Nitrous oxide

N₂O is formed through nitrification and/or denitrification, under both aerobic and anaerobic conditions (Williams et al., 1998). Results showed that N₂O emissions were affected ($p < 0.05$) by all treatments and time. N₂O emission rates were high in the first 10 days of composting, with a peak in emissions on day 7 recorded for Olive ($0.80 \text{ g N}_2\text{O-N kg}^{-1}$ initial DM). The accumulated N₂O-N emissions during the composting process are illustrated in Supplementary Figure S1c. Table 3 shows the final accumulated emissions values, which ranged from $0.08 \text{ g N}_2\text{O-N kg}^{-1}$ initial DM (WhiteGrape) to $0.91 \text{ g N}_2\text{O-N kg}^{-1}$ initial DM (Olive and Chestnut). Accumulated N₂O emissions in RedGrape, Broccoli and WhiteGrape treatments were 2.62, 3.78, and 8.50 times lower than in the Control. A significant correlation was found between cumulative N₂O emissions and the hemicellulose ($r = 0.522$; $p < 0.01$) and lignocellulose ($r = 0.506$; $p < 0.05$) contents of the initial waste-straw mixtures, but not with the total phenolic content.

3.3.4. Ammonia

The waste type, the time, and their interaction significantly affected ammonia emissions (Fig. 3). Highest emissions occurred during the first 10 days of composting, corresponding to the most

bioactive period of the process, with peak values of 0.003 (WhiteGrape) and $0.02 \text{ g NH}_3\text{-N kg}^{-1}$ initial DM (RedGrape) on day 6 (Fig. 3a). The straw treatment (Control) emitted less NH₃ when compared with all the other treatments. After day 18, the emissions progressively decreased to null values, with no significant differences ($p < 0.05$) between treatments.

The lowest cumulative NH₃ emissions were calculated for Control and Chestnut, and were significantly different ($p < 0.05$) from those calculated for the other treatments (Fig. 3b). As a carbon-rich material, straw contains a substantial amount of degradable carbon, which stimulates immobilisation of ammonium on microbial biomass (Sommer et al., 2006), with subsequent increase in the C/N ratio (Table 1). The use of straw as bulking agent could improve compost porosity and increase oxygen concentrations (Chowdhury et al., 2015). Sánchez-Monedero et al. (2001) reported that the use of wastes with high lignocellulose contents led a reduction of about 25% of N losses during the composting process. In agreement with the results of the present study, treatments with higher C/N ratios and lignocellulose contents (Control and Chestnut) led to lower cumulative NH₃ emissions. Broccoli with the lowest C/N ratio (Table 1) and lignocellulose content (Table 2) led to higher emissions. Cumulative NH₃ emissions were highly and negatively correlated with lignocellulose ($r = -0.873$; $p < 0.001$), and positively correlated with the total phenolic contents ($r = 0.462$; $p < 0.05$) of the initial waste-straw mixtures.

3.3.5. Nitric oxide

Nitric oxide (NO) is a precursor of tropospheric ozone, a greenhouse gas formed by photochemical reaction and which plays key roles in atmospheric chemistry (Williams et al., 1998). Few reports have measured NO emissions during composting trials. In the present study, time and waste type significantly ($p < 0.05$) affected NO emissions. In concordance with ammonia data, NO was emitted primarily during the first 10 days of composting (Fig. 4a) with the highest peak values being recorded on day 6 for RedGrape ($0.17 \text{ g NO-N kg}^{-1}$ initial DM) and WhiteGrape ($0.16 \text{ g NO-N kg}^{-1}$ initial DM). After day 14, emissions started to decrease and remained close to zero until the end of the composting process. As with NH₃, the lowest cumulative NO emissions were calculated for Control and Chestnut (ca. $8.7 \text{ g NO-N kg}^{-1}$ initial DM at the end of composting); but the highest for Olive ($15.8 \text{ g NO-N kg}^{-1}$ initial DM) rather than Broccoli (Fig. 4b). Cumulative NO emissions correlated negatively with lignocellulose ($r = -0.633$; $p < 0.001$) and hemicellulose ($r = -0.779$; $p < 0.001$) and positively with lignin ($r = 0.523$; $p < 0.01$) and total phenolics ($r = 0.626$; $p < 0.01$).

3.3.6. Greenhouse gas emissions

Emissions of N₂O, CO₂, and CH₄ throughout the composting process were summed to get total GHG emissions, after their conversion into CO₂-equivalents. The different treatments significantly

Table 3
Final accumulated GHG emissions (CO₂, N₂O and CH₄) expressed as CO₂ equivalents.

	Accumulated emissions (g kg ⁻¹ Initial DM)			GHG emissions (g CO ₂ eq. kg ⁻¹ initial DM)	Contribution (%)			GHG emissions without CO ₂ (g CO ₂ eq. kg ⁻¹ initial DM)
	CO ₂	CH ₄	N ₂ O		CO ₂	CH ₄	N ₂ O	
Control	255.6 ± 35.5 ab	-0.79 ± 0.5 a	0.68 ± 0.1 a	450.2 ± 63.0 ab	56.80 ± 1.2 a	-3.68 ± 2.2 a	46.88 ± 1.8 a	194.6 ± 28.4 a
Broccoli	144.2 ± 60.9 bc	-0.50 ± 0.6 a	0.18 ± 0.1 b	189.1 ± 81.1 bc	72.43 ± 13.1 a	-4.78 ± 3.4 a	32.36 ± 9.8 bc	44.9 ± 33.4 b
Chestnut	354.5 ± 34.3 a	-0.79 ± 0.5 a	0.90 ± 0.2 a	616.4 ± 74.7 a	57.65 ± 2.0 a	-2.78 ± 1.3 a	44.93 ± 3.2 ab	261.9 ± 42.1 a
WhiteGrape	58.0 ± 9.3 c	-0.10 ± 0.0 a	0.08 ± 0.0 b	79.9 ± 10.6 c	72.58 ± 5.3 a	-2.58 ± 0.4 a	30.00 ± 5.3 c	21.8 ± 5.0 b
RedGrape	150.0 ± 72.2 bc	-0.57 ± 0.8 a	0.26 ± 0.1 b	220.0 ± 59.9 bc	64.77 ± 12.6 a	-4.03 ± 4.1 a	39.26 ± 9.7 abc	70.1 ± 26.3 b
Olive	420.8 ± 117.2 a	-0.97 ± 0.5 a	0.91 ± 0.4 a	683.0 ± 94.4 a	62.15 ± 3.0 a	-2.85 ± 0.9 a	40.70 ± 3.7 abc	262.2 ± 99.5 a

Values within a column, with the same letter are not significantly different ($p < 0.05$). Values are expressed as mean ± SD (standard deviation) of four replicates.

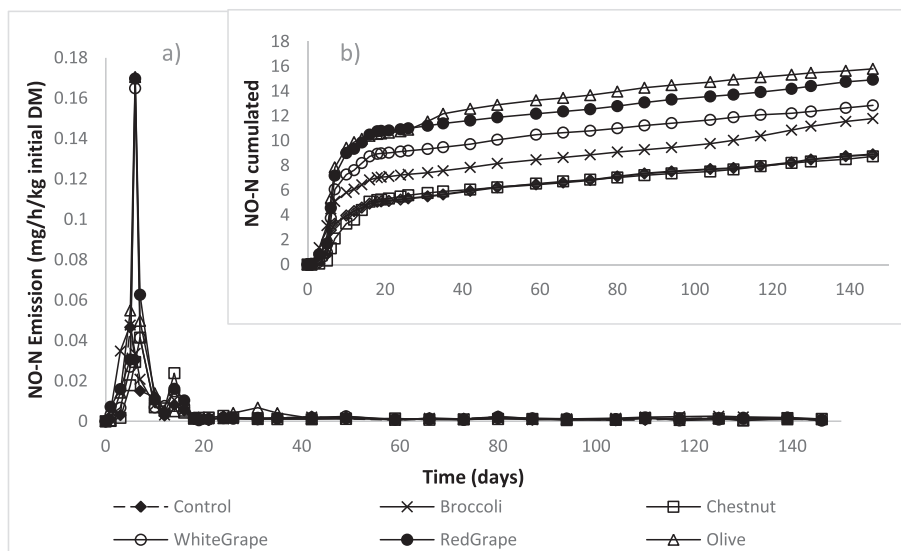


Fig. 4. Evolution of instantaneous and accumulated NO emissions during composting: (a) instantaneous ammonia emissions, (b) accumulated NO emissions.

($p < 0.0001$) affected total GHG emissions, with values ranging from 79.9 (WhiteGrape) to 683.0 g CO₂-eq. kg⁻¹ initial DM (Olive) (Table 3). When CO₂ emissions were excluded from total GHG calculations, total GHG (without CO₂) emissions ranged from 21.8 to 262.2 g CO₂-eq. kg⁻¹ DM (Table 3). Of all the agro-food waste materials used in the experiment, it was clear that white grape's marc could effectively be transformed and reused as organic fertiliser, with the lowest negative environmental impacts.

4. Conclusions

The findings presented in this study provide information on physicochemical changes and gaseous emissions (GHG, NH₃ and NO) during composting of agro-food wastes.

Carbon dioxide, CH₄, N₂O, NH₃ and NO were quantified over five months of composting using mixture containing broccoli, olive, chestnut, and white or red grape wastes. Significant variations were observed over time and between the different wastes. White grape exhibited the lowest GHG emissions, with 58.0 CO₂, -0.10 CH₄, and 0.08 N₂O g kg⁻¹ initial DM, which corresponded to GHG emissions of 79.9 g CO₂ eq. kg⁻¹ initial DM. Broccoli contributed as much to the global warming potential as red grape (189.1 and 220.0 g CO₂ eq. kg⁻¹ initial DM), and chestnut and olive contributed thrice as much as the formers (616.4 and 683.0 g CO₂ eq. kg⁻¹ initial DM). A higher content of hemicelluloses in the wastes seemed to entail a higher emission of N₂O. The lowest NH₃ and NO emissions were registered during the intense degradation of chestnut and olive residues. Composting with chestnut lowered the emissions of NH₃ and NO up to levels similar to those found in wheat straw (~ 0.01 g NH₃-N, 8.7 g NO-N kg⁻¹ initial DM). These changes were closely associated with the lignocellulosic composition of the wastes, a finding with significant implications for the choice of composting materials. These results suggest the potential use of wastes rich in lignocelluloses in composting for the purpose of reducing N losses by NH₃ and NO emissions. Although composting raised the pH and EC of the final composts, the increase in nutrient contents and lignin/holocellulose ratios indicates that a safe compost could be obtained under optimized conditions with negligible environmental impacts. These results can be helpful to investigators, producers and agro-industry companies in adopting best practices for valorisation of wastes, and in implementing mitigation strategies for GHG, NO and NH₃ emissions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jclepro.2017.10.050>.

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Annex 3. Paper: Comparison of five agro-industrial waste-based composts growing media for lettuce: Effect on yield, phenolic compounds and vitamin C.



Comparison of five agro-industrial waste-based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C



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ABSTRACT

Overall phenolic content in plants is on average higher in organic farming, including when renewable resources such as composts are used as soil amendments. In most cases, however, the composting process needs to be optimized to reach the desired outcome. Using composts obtained from chestnut, red and white grapes, olive and broccoli wastes, the relative antioxidative abilities of lettuces cultivated in greenhouse were examined. Results clearly coupled high phenolic levels with high yield in lettuce grown on the chestnut-based compost. A huge accumulation of phenolics was observed with the white grape-based compost, but this coincided with low yield. Three compounds were identified as discriminating factors between treated samples, namely quercetin 3-*O*-glucoside, luteolin 7-*O*-glucoside, and cyanidin 3-*O*-(6''-malonyl)- β -*D*-glucoside; these are also some of the compounds receiving health claims on lettuce consumption. On a negative note, all composts led to decreased vitamin C levels. Collectively, the data suggest that compost amendments can help add value to lettuce by increasing its antioxidant activity as compared to other organic resources.

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1. Introduction

Lettuce (*Lactuca sativa* L.) farming practices have become much larger in scale in terms of growth facilities, growth seasons, and inputs. Nowadays, lettuce is one of the main crops grown in greenhouses worldwide; open-air-grown lettuce, however, still holds the highest portion of the market (Becker, Klaering, Kroh, & Krumbein, 2014; Durazzo et al., 2014; Li, Zhao, Sandhu, & Gu, 2010). Several varieties have been bred for different climates (Baslam, Morales, Garmendia, & Goicoechea, 2013; Nicolle et al., 2004), making lettuce available over the whole year. In terms of input source, conventionally – as opposed to organically-grown lettuce is still dominant (Heimler, Vignolini, Arfaio, Isolani, & Romani, 2012; Liu et al., 2007). However, the share of organic land in lettuce production worldwide has steadily increased during the last decades.

The concept of organic agriculture was popularized because of the need to avoid synthetic chemical residues in foods (Smith-Spangler et al., 2012). There have been two other major arguments

put forward for promoting organic agriculture i.e., more nutrients in the food produced and lower negative environmental impacts. There is robust evidence supporting the perception that the risk for contamination with pesticide residues is lower among organic than conventional produces. However, that appears to be nutritionally irrelevant since the levels found do not exceed maximum limits set by environmental protection agencies (Nicoletto, Santagata, Zanin, & Sambo, 2014). The long-held belief that organic foods are significantly more nutritious than conventional foods has been challenged by recent meta-analyses. Besides phenolics which were significantly higher in organic produces, no major differences were found in the nutrients contents of organic and conventional plant foods (Smith-Spangler et al., 2012). As for the third reason, it seems that organic agriculture is more environmental-friendly than its conventional counterpart; the concept of friendliness concerning two main aspects, which are soil management and greenhouse gases emissions (Pereira & Trindade, 2015).

As a consequence of the recognition of organic agriculture as an instrument of environmental policy, application of organic supplements is now widely promoted. A number of potential organic fertilizers have been identified in efforts to achieve more sustainable agriculture, and composts have proved to be solid alternatives to

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synthetic chemicals (Montemurro et al., 2015). With the increasing popularity of organic foods, strategies to improve the organic cultivation system using advanced technologies have also been developed. In the case of lettuce, growing evidence suggests that it is now possible under optimized conditions to achieve yields close to average conventional agriculture, as observed with olive mill waste – (Kelepesi & Tzortzakis, 2009), fruits/dregs distillery residues – (Nicoletto et al., 2014), olive pomace – (Montemurro et al., 2015), and posidonia-based composts (Grassi et al., 2015).

In addition, the quality of lettuce in response to compost amendments has been reported, with substantial proportions of studies showing either lower or higher nutrients contents compared with the conventional field. For example, both protein decreases (Montemurro et al., 2015) and increases (Nicoletto et al., 2014) have been observed. Lettuce cultivated with composted spent-coffee grounds showed enhanced carotenoids, but decreased vitamin E (Cruz et al., 2014). In the study by Nicoletto et al. (2014), the vitamin C content of lettuce was higher for all compost treatments.

While yield and nutrients still dominate lettuce improvement efforts in the composting industry, selection for antioxidant properties is slowly emerging. In the few studies published on the subject, garden-based composts were shown to increase phenolics in lettuce (Heimler et al., 2012), while the response varied depending on the compost concentration for fruits/dregs distillery residues – (Nicoletto et al., 2014), and spent-coffee grounds-based composts (Cruz et al., 2014). While the existing scientific evidence regarding a primary role for phenolics in imparting health-benefits on lettuce consumption is still unclear, scattered studies on dietary supplementation with phenolic-rich extracts from lettuce have demonstrated improvements in lipid and antioxidant profiles in neuronal PC-12 cells (Im et al., 2010), Caco-2 cells (Durazzo et al., 2014), J774A.1 monocyte/macrophage cells (Pepe et al., 2015), rodents (Cheng et al., 2014) and healthy humans (Serafini et al., 2002). It has been hypothesized that as the health effects of lettuce phenolics are better understood and established, contents of these compounds may become part of the requirements for organic products (Cheng et al., 2014).

Although lettuce reportedly provides relatively low levels of antioxidative phytochemicals (Caldwell, 2003), its high per capita consumption makes it a considerable contributor to the amount of antioxidants in the diet. On the other hand, studies have highlighted the importance of variation in factors such as cultivar, agronomic practices, climatic conditions, and storage conditions, as a key tool to obtain healthful and more nutritious food crops (Buer, Imin, & Djordjevic, 2010; Queiroz, Morais, & Nascimento, 2002). In that regard, composting could become a major component of strategies aiming at maximizing the levels of bioactive molecules in lettuce.

In this study, it was hypothesized that the contents of phenolics in the compost may have roles in the transport and synthesis of antioxidative compounds in lettuce. There is precedence for considering that possibility, as it has been recently demonstrated that flavonoids are selectively taken up from the roots and are capable of long-distance movement within the plant (Buer et al., 2010). To test that hypothesis, five plant food-based wastes coming from the olive, chestnut, grape, and broccoli industries were selected for composting. In Mediterranean countries, these industries generate large amounts of wastes, which are routinely dumped in landfill sites or incinerated, causing serious environmental concerns due to their high organic load (Kelepesi & Tzortzakis, 2009). It follows that a parallel environmental issue is the disposal of these agricultural wastes; and their use in composting clearly represents a profitable recycling approach.

The objectives of this study were to (i) evaluate the five plant food-based composts as medium amendments in greenhouse

organic lettuce production, and as alternatives to non-renewable organic substrates such as peat, and (ii) assess the impact of compost polyphenols on the phenolic composition, vitamin C and carotenoids contents of lettuce.

2. Materials and methods

2.1. Agro-industrial plant food-based wastes and composting set-up

In order to provide contrasting growing conditions for lettuce, five raw materials were used for composting. Each initial batch was assembled with wastes collected from privately owned industries located in the Vila Real area in Portugal: (i) broccoli stems and florets were collected because of their allegedly bio-fumigant properties; (ii) white grape rachis were obtained after separation of berries of *Vitis vinifera* L. var. Moscatel for fermentation; as was (iii) red grape rachis from *Vitis vinifera* L. var. Alfrocheiro; (iv) olive leaves, and (v) chestnut shells and peels were discarded from a 3-phase centrifugation mill, and an agro-food company, respectively. Wheat straw was provided by a neighbouring farm and was used as bulking agent during composting. After manually removing the non-biodegradable coarse part of the materials, the remaining matrix was crushed using a shredder (Yike 9FQ-360 straw hammer mill, Zhengzhou, China), and passed through a 40 mm sieve.

Composting was done at a specialised pilot composting plant built at UTAD. The composting process was optimized to have a final product containing close to 20 g/kg nitrogen. A windrow of 20 kg was prepared by piling the wastes with wheat straw in a 135-L reactor in the proportions 30:70 for broccoli and 40:60 for the other materials (dry matter – DM basis). A treatment was also prepared with only wheat straw. Mechanical aeration was done by air injection through a pump. Moisture was controlled weekly, and was maintained around 45–60% by adding water. The windrow was turned mechanically once a week during the most active biooxidative phase, and then every 15 days throughout the maturation period. The composting process was held until complete temperature stabilization ca. 5.5 months. One week after the end of the process, the compost was removed from the reactor and stored at 4 °C until used. In all cases, the final product showed a high degree of humification, and no phytotoxic effect on seed germination. The nitrogen contents measured were 23.48, 22.59, 23.77, 20.10, and 20.73 g/kg for broccoli, white grape, olive, red grape, and chestnut, respectively (data not shown), with no statistical differences, except between olive and red grape ($P < 0.05$). Portions of raw materials and composts were homogenized in a Tecator Cyclotec 1093 Mill (Foss, Hoganas, Sweden) to a 0.2 mm particle size, freeze-dried and submitted to phenolic measurements.

2.2. Design of the experiment and growth conditions

The composts were carefully mixed with sand in a proportion allowing an amendment equivalent to 15 t DM/ha in 1 L plastic pots. Potted organic lettuce is widely grown in substrates that consist of peat and inorganic materials such as sand, perlite or vermiculite; therefore, the treatment with peat and sand was used as control. The experiment was conducted during the winter of 2014 in a greenhouse in Vila Real (N 41°17'7.28"; W 7°44'36.83"), first because of the expanding growth of winter greenhouse-grown lettuce in the region, second because cool-cultivated lettuce reportedly contain higher levels of phenolics than warm-cultivated ones (Becker et al., 2014).

Two lettuces were used for nursery tests. Maravilha Inverno, extensively cultivated in greenhouses during winter, is a bright

green lettuce. Quatro Estações has a red pigmentation, especially in the borders of the most ruffled leaves, and is adapted to be grown during all year. Both lettuces develop a round, dense head, with very broad leaves and a consistent crisp texture. The two varieties have undergone repeated field selections for pests and diseases and shown polygenic resistance to several diseases such as bottom rot and downy mildew, justifying their use in organic farming. The seeds were purchased from a local shop, and sown on November 28th in pots filled with the composts (three seeds per pot and 4 pots per treatment); the treatments are herein referred to as CONTROL, BROCCOLI, WHITEGRAPE, OLIVE, REDGRAPE, and CHESTNUT. When seedlings had emerged, they were thinned to 1 plant per pot. The experiment was carried out in a completely randomized design. Weather conditions were recorded in the greenhouse as follow: 12.9/5.8 °C day/night temperatures, 68.6/87.4% day/night relative humidity and photosynthetic photon flux of 300–400 $\mu\text{mol}/\text{m}^2/\text{s}$ during a 10 h photoperiod. The plants were manually irrigated according to plant needs based on the Reference Evapotranspiration value of lettuce, using the same amount of water in all pots. Weeding was done by hand when needed.

2.3. Harvest and measurement of morphological traits

When an acceptable leaf size and growing shape was attained, plants were harvested (90 days after sowing) by uprooting and cutting the above-ground parts; the roots were washed with water, blotted between two layers of paper towel, and all materials were transported to the laboratory within 5 min. The following morphological parameters were determined: fresh weight of leaves and roots (by weighing), plant height (with a ruler), number of leaves (by counting), root length (with a rhizometer), and stem diameter (with a caliper). Since no injured leaves were found, the whole leaves and roots were frozen with liquid nitrogen, freeze-dried, and the moisture losses recorded. Freeze-dried samples were blended into powder and used for analytical determinations.

2.4. Extraction and chromatographic separation of phenolic compounds

Samples (40 mg) were added with 1 mL of 70% methanol (Panreac Quimica; Barcelona, Spain), incubated at room temperature for 30 min with vortexing every 5 min, and then centrifuged (13,000, 15 min, 25 °C). The supernatant was collected and passed through a 0.2 μm filter (Spartan 13/0.2 RC; Whatman, Dassel, Germany) using a syringe. Ultra pure water was used throughout the study.

Individual phenolics were determined by high-performance liquid chromatography (HPLC) using a Gilson system (Villers-le-bel, France) equipped with a Finnigan Surveyor photo diode array detector (DAD 81401; Thermo Electron, San Jose, USA), and the software Xcalibur 2.0 (Thermo Fisher Scientific, Waltham, USA), which generated a 3-dimensional data set (absorbance, retention time, and wavelength). Phenolics in the injected extracts (10 μL) were separated using a C18 column (5 μm , 250 \times 4.5 mm; Sigma/Aldrich, Steinheim, Germany) enclosed in an oven maintained at 25 °C. The mobile phase consisted of (A) 0.1% trifluoroacetic acid in water and (B) 0.1% trifluoroacetic acid in acetonitrile (all from Sigma/Aldrich, Steinheim, Germany), using a linear gradient starting with 100% A for 5 min, decreasing to 80% A at 15 min, 50% A at 30 min, 0% A at 45 min, and then reverting to 100% A at 55 till 60 min. The flow rate was constant at 1 mL/min. DAD data acquisition was set in the range of 200–400 nm, and peak areas were registered at the maximum absorbance of the compounds of interest i.e., 320 nm for phenolic acids, 370 nm for flavonoids and 520 nm for anthocyanins (Llorach, Martínez-Sánchez, Tomás-Barberán,

Gil, & Ferreres, 2008; Ribas-Agustí, Gratacós-Cubarsí, Sárraga, García-Regueiro, & Castellari, 2011).

The chromatographic peaks obtained were identified by matching samples with standards on the basis of their retention times, UV spectra, and co-chromatography. Seven compounds were identified with some certainty, including caftaric, chlorogenic, and chicoric acids, quercetin 3-O-(6''-O-malonyl)- β -D-glucoside, quercetin 3-O-glucoside, luteolin 7-O-glucoside, and cyanidin 3-O-(6''-malonyl)- β -D-glucoside. The quantitation of these compounds was done using a six-point regression curve ($r \geq 0.989$) for each standard. The four first standards + caffeic acid, gallic acid and trolox were from Sigma/Aldrich (Steinheim, Germany), and the two others + cyanidin 3-O-glucoside and quercetin 3-O-rutinoside from Extrasynthese (Lyon, France). Several peaks did not agree with any of the standards available in the laboratory; in those cases, bibliographic data was used for their identification. Tentatively identified compounds were quantified as equivalents of the most similar compound i.e., caffeic acid for caffeoylmalic acid and caffeic acid derivatives, cyanidin 3-O-glucoside for cyanidin 3-O-(6''-malonyl)- β -D-glucoside, and quercetin 3-O-rutinoside for the unidentified flavonoid. Since lettuce is consumed fresh, the results are expressed in mg/g fresh weight (FW) after normalization with the moisture content.

2.5. Other analytical methods

2.5.1. HPLC determination of vitamin C in lettuce

For the extraction of ascorbic acid, 0.2 g sample in a dark tube was homogenized with an Ultra-Turrax T25 (IKA, Staufen, Germany) for 30 s on an ice bath with 5 mL of extraction medium (3% metaphosphoric acid +8% acetic acid added with 1 mM tert-butylhydroquinone) (Llorach et al., 2008; Nicolle et al., 2004). All chemicals were from Panreac Quimica (Barcelona, Spain). The homogenate was centrifuged (4000 rpm, 2 min, 4 °C) and the supernatant was recovered, filtered through a 0.45 μm Spartan filter, and immediately injected into the HPLC equipment, column and DAD described above for phenolics. The mobile phase was 0.2% orthophosphoric acid with an isocratic elution. The injection volume and the flow rate were 20 μL , and 1.2 mL/min, respectively. Vitamin C was quantified on the basis of the chromatograms obtained at 440 nm, using standard ascorbic acid (Sigma-Aldrich, Buchs, Switzerland).

2.5.2. Spectrophotometric determination of other phytochemicals in lettuce

The total phenolics content (TPC) was measured in the extract obtained in 2.4. by a modified Folin-Ciocalteu method (Cheng et al., 2014) and results expressed as mg gallic acid equivalent (GAE)/g FW. A microplate reader method based on the conventional method described by Lakhdar et al. (2011) was used for the total flavonoids content (TFC) and results expressed as mg catechin equivalent (CAE)/g FW. The ability of the extracts to scavenge DPPH was measured and expressed in mg trolox equivalent (TE)/g FW as described by Li, Zhao, Sandhu, and Gu (2010). The total monomeric and anthocyanins (TAC) contents were measured by the pH differential assay (Baslam, Morales, Garmendia, & Goicoechea, 2013), calculated with the equations reported by Li et al. (2010) and expressed as cyanidin 3-O-glucoside equivalent (mg CGE/g FW). Chlorophylls and carotenoids were extracted and calculated (mg/g FW) using the equations established by Hartmut Lichtenthaler and reported by Li et al. (2010).

2.5.3. Determination of phenolics in the raw materials and composts

Total phenolics and simple phenolics were extracted (0.2 g sample; 10 mL of 70% methanol) as described by Queiroz et al. (2002),

and expressed in GAE mass basis. Gallic acid was quantified as in 2.4.

2.6. Statistical analyses

The results are presented as mean \pm standard deviation (SD, $n = 4$). All analyses were done with compost type, lettuce variety, and measured parameters as class variables in the statistical models. Dependent variables were first studied with ANOVA and the Tukey's honestly significant difference (HSD) procedure applied for means comparison at a 5% significance level. Pearson's correlation tests were then established between all morphological traits; between TPC, TFC and TAC determined by chromatography and spectrophotometry; between compost phenolics and lettuce phenolics; and between yield and phytochemicals. Principal components analysis (PCA) was finally applied to single out composts which performed best in increasing the phenolic content of lettuce, and to identify phenolics which adequately summarised the effects observed. All analyses were performed using SPSS 15.0 (Chicago, USA).

3. Results and discussion

In this study, the possibility of using composts as organic mineral fertilizer in lettuce production was investigated, with an emphasis on the contents of antioxidant compounds.

3.1. Effect of composts on growth parameters

As compared to CONTROL, two of the composts were clearly beneficial to lettuce growth, namely CHESTNUT and OLIVE (Fig. 1). CHESTNUT increased the yield of Quatro Estações by 31% ($P < 0.05$). The second highest yield means were with CONTROL and OLIVE. With Maravilha Inverno, CHESTNUT also resulted in the highest yield mean, although it was statistically similar with the yield obtained with CONTROL and OLIVE, a pattern also seen with root yield (Supporting Information 1). WHITEGRAPE, REDGRAPE, and BROCCOLI showed 1.5–2.4 times lower yields than

CONTROL, with BROCCOLI at the lower end of the range (Fig. 1). Several studies have emphasized the importance of plant food-based composts as sources of minerals for vegetable production (Grassi et al., 2015; Lakhdar et al., 2011). Given that all the composts had the same nitrogen content, the intrinsic nature of some of the composts predictably retarded mineral absorption, thus ensuring a lower crop production. For example, although having much more nutrients compared to peats, composts often compact a phenomenon which impedes the movement of nutrients. Furthermore, the availability of minerals in the composts depends on its interaction with the media microorganisms and weeds, whereas peat is usually sterile with no weed seeds. The current observation of lower yield with composts is not isolated. In fact, Montemurro et al. (2015) found that depending on the stage of maturity, olive pomace-based composts could lead to lower lettuce yield as compared with the unfertilized control.

A negative correlation was found between lettuce yield and moisture ($r = -0.76$). Fig. 1 indicated that the tissues of lettuce grown on WHITEGRAPE and BROCCOLI were more hydrated than those of lettuces grown on the other media, despite all plants grew under optimal water regime. Other morphological traits affected included alterations in the number of leaves, plant height, stem diameter and root length. However, the differences in yields seem to be mostly related to differences in the number of leaves produced ($r = 0.92$). Lettuce grown on CHESTNUT developed 1–2 more leaves per plant than the other lettuces (Fig. 1). Although the same trends were observed with the other parameters, no significant differences were found among treatments, other than BROCCOLI with plant height (Fig. 1), and WHITEGRAPE with root length in Maravilha Inverno (Supporting Information 1).

3.2. Identification of phenolic compounds in lettuce

The HPLC method used in this study allowed the separation of 15 peaks. Interferences of other matrix components were minimised and besides peaks 9 and 10 which co-eluted in the conditions selected, a satisfactory resolution between the target phenolics was observed (Fig. 2). UV-vis spectra showed three

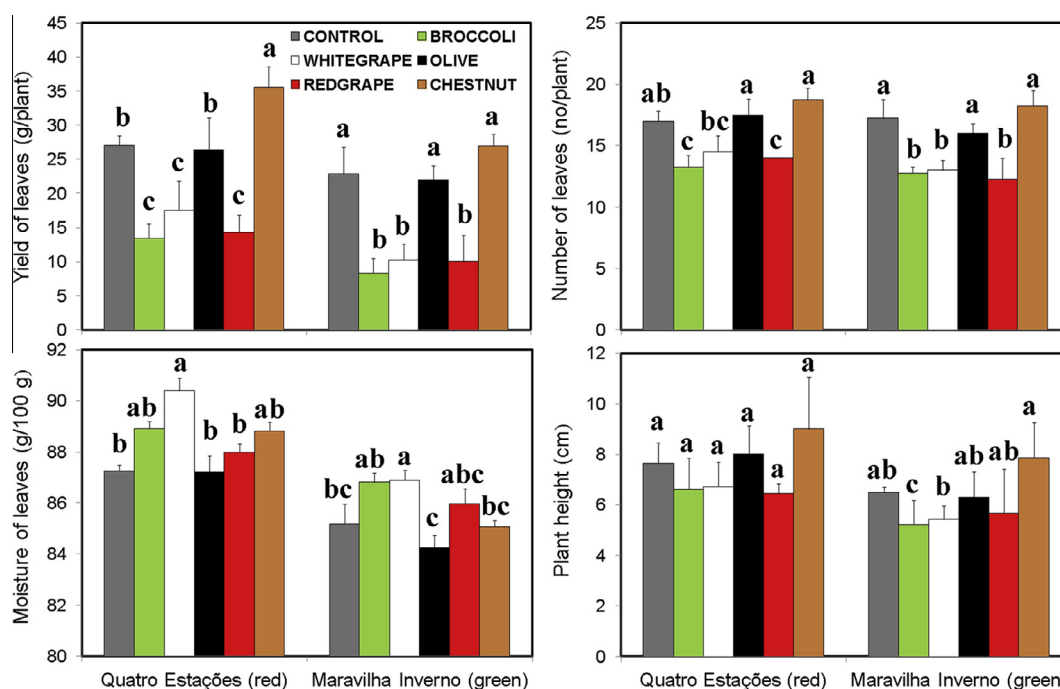


Fig. 1. Changes in aboveground traits of lettuce grown in media with composts; Means and SD as error bars with the same letter do not differ (Turkey's HSD test; $P < 0.05$).

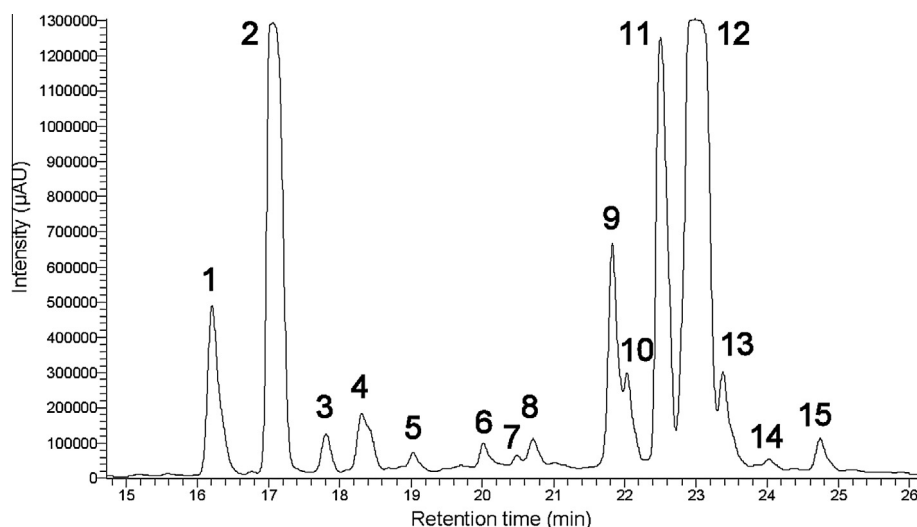


Fig. 2. Typical HPLC-DAD chromatogram of lettuce extract recorded at λ 320 nm. Compounds correspond to those in Table 1 and Supporting Information 2. (1) Caftaric acid, (2) Chlorogenic acid, (3) Unidentified flavonol, (4) Caffeoylmalic acid, (5) Caffeic acid derivative 1, (6) Caffeic acid derivative 2, (7) Caffeic acid derivative 3, (8) Cyanidin 3-O-(6''-malonyl)- β -D-glucoside, (9) Luteolin 7-O-glucoside, (10) Quercetin 3-O-glucoside, (11) Quercetin 3-O-(6''-O-malonyl)- β -D-glucoside, (12) Chicoric acid, (13) Caffeic acid derivative 4, (14) Caffeic acid derivative 5, (15) Caffeic acid derivative 6.

spectrum typologies, corresponding to: (i) 3 known, 1 tentatively identified (caffeoylmalic acid) and 6 unidentified phenolic acids (caffeic acid derivatives 1–6) with maximum bands at λ 311–330 nm; (ii) 3 known and 1 unidentified flavonoid with maximum bands at λ 352–367 nm; and (iii) 1 tentatively identified anthocyanin (cyanidin 3-O-(6''-malonyl)- β -D-glucoside) with a maximum band at λ 516 nm.

The two lettuces showed both quantitative and qualitative differences regarding their composition of phenolics. As expected (Li et al., 2010), cyanidin 3-O-(6''-malonyl)- β -D-glucoside was identified in only the red Quatro Estações (Table 1). Quatro Estações was also unusual in that it contained no detectable amount of caffeic acid derivative 3 (Supporting Information 2). When separated

by colour, the red lettuce possessed higher phenolic acids and flavonoids than the green one, reflecting a 1.14, and 1.23-fold difference, respectively.

Using commercial standards for quantification, phenolic contents in lettuce (mg/g FW) were observed in the following order: chicoric acid (2.48) > chlorogenic acid (2.15) > quercetin 3-O-(6''-O-malonyl)- β -D-glucoside (0.92) > luteolin 7-O-glucoside (0.51) > quercetin 3-O-glucoside (0.27) > caftaric acid (0.19). Next was cyanidin 3-O-(6''-malonyl)- β -D-glucoside which was quantified as CGE (Table 1). These major phenolic constituents were similar to those reported in other studies, notably showing chicoric acid and quercetin 3-O-(6''-O-malonyl)- β -D-glucoside as the main phenolic acid and flavonoid in lettuce, respectively (Becker et al.,

Table 1

Effect of composts on lettuce phenolics (mean \pm SD; mg/g FW). Caffeoylmalic acid and cyanidin 3-O-(6''-malonyl)- β -D-glucoside are tentatively identified and expressed as caffeic acid, and cyanidin 3-O-glucoside equivalent, respectively.

No ^a	Varieties/compounds	CONTROL	BROCCOLI	WHITEGRAPE	OLIVE	REDGRAPE	CHESTNUT
<i>Quatro Estações (red)</i>							
1	Caftaric acid	0.28 \pm 0.06a	0.23 \pm 0.04a	0.04 \pm 0.03b	0.19 \pm 0.04ab	0.31 \pm 0.08a	0.29 \pm 0.04a
2	Chlorogenic acid	1.68 \pm 0.19b	2.43 \pm 0.21b	2.31 \pm 0.34b	1.98 \pm 0.22b	2.00 \pm 0.49b	2.64 \pm 0.34a
4	Caffeoylmalic acid	0.03 \pm 0.00ab	0.03 \pm 0.0ab	0.03 \pm 0.01ab	0.02 \pm 0.01b	0.02 \pm 0.01b	0.05 \pm 0.01a
12	Chicoric acid	2.43 \pm 0.46a	2.73 \pm 0.24a	1.14 \pm 0.10b	2.83 \pm 0.37a	3.01 \pm 0.41a	3.35 \pm 0.48a
8	Cyanidin 3-O-(6''-malonyl)- β -D-glucoside	0.14 \pm 0.05b	0.15 \pm 0.04b	0.25 \pm 0.07ab	0.15 \pm 0.02b	0.15 \pm 0.09b	0.30 \pm 0.03a
9	Luteolin 7-O-glucoside	0.37 \pm 0.05ab	0.26 \pm 0.03b	0.62 \pm 0.15a	0.33 \pm 0.07ab	0.44 \pm 0.20ab	0.67 \pm 0.09a
10	Quercetin 3-O-glucoside	0.17 \pm 0.05b	0.14 \pm 0.02b	0.44 \pm 0.23a	0.18 \pm 0.04ab	0.29 \pm 0.06ab	0.39 \pm 0.04a
11	Quercetin 3-O-(6''-O-malonyl)- β -D-glucoside	1.05 \pm 0.13ab	1.14 \pm 0.11ab	0.87 \pm 0.19b	1.20 \pm 0.12ab	1.25 \pm 0.28ab	1.59 \pm 0.33a
	Total flavonoids (TFC)	1.66 \pm 0.21b	1.61 \pm 0.16b	2.03 \pm 0.36ab	1.78 \pm 0.17b	2.05 \pm 0.49ab	2.76 \pm 0.33a
	Total phenolics (TPC)	6.37 \pm 0.88b	7.32 \pm 0.64b	6.00 \pm 0.72b	7.07 \pm 0.50b	7.83 \pm 1.68ab	9.58 \pm 0.61a
<i>Maravilha Inverno (green)</i>							
1	Caftaric acid	0.18 \pm 0.03a	0.11 \pm 0.04a	0.16 \pm 0.01a	0.18 \pm 0.02a	0.16 \pm 0.02a	0.12 \pm 0.01a
2	Chlorogenic acid	1.65 \pm 0.05 cd	1.60 \pm 0.29d	2.97 \pm 0.11a	2.60 \pm 0.26ab	2.43 \pm 0.32abc	1.63 \pm 0.53 cd
4	Caffeoylmalic acid	0.01 \pm 0.00a	0.01 \pm 0.00a	0.03 \pm 0.01a	0.04 \pm 0.02a	0.02 \pm 0.00a	0.02 \pm 0.00a
12	Chicoric acid	2.51 \pm 0.20a	1.90 \pm 0.23a	2.37 \pm 1.04a	2.70 \pm 0.22a	2.82 \pm 0.79a	2.16 \pm 0.15a
8	Cyanidin 3-O-(6''-malonyl)- β -D-glucoside	ND	ND	ND	ND	ND	ND
9	Luteolin 7-O-glucoside	0.50 \pm 0.07bc	0.26 \pm 0.06d	0.94 \pm 0.06a	0.62 \pm 0.03bc	0.57 \pm 0.10bc	0.68 \pm 0.07b
10	Quercetin 3-O-glucoside	0.19 \pm 0.03b	0.18 \pm 0.09b	0.66 \pm 0.12a	0.24 \pm 0.01b	0.26 \pm 0.07b	0.35 \pm 0.05b
11	Quercetin 3-O-(6''-O-malonyl)- β -D-glucoside	0.67 \pm 0.15a	0.40 \pm 0.08b	0.62 \pm 0.20a	0.68 \pm 0.07a	0.72 \pm 0.13a	0.62 \pm 0.07a
	Total flavonoids (TFC)	1.44 \pm 0.23b	0.88 \pm 0.19c	2.33 \pm 0.16a	1.62 \pm 0.09b	1.63 \pm 0.23b	1.77 \pm 0.20b
	Total phenolics (TPC)	5.95 \pm 0.44bc	4.61 \pm 0.32c	8.04 \pm 1.12a	7.37 \pm 0.54ab	7.24 \pm 1.27ab	5.85 \pm 0.87bc

Row values with no letter in common differ at $P < 0.05$ (Tukey's HSD test).

ND = non detected.

^a Letters refers to compounds in Fig. 2.

2014; Llorach et al., 2008;). The high content of chlorogenic acid found in this study was in agreement with Li et al. (2010), and Ribas-Agustí et al. (2011), but differed from Caldwell (2003), and Pepe et al. (2015), which observed the compound in low amounts. Several studies (Liu et al., 2007; Llorach et al., 2008) have pointed out discrepancies on the occurrence and relative abundance of several phenolics in lettuce, which is likely due to variety, but also to extraction and quantification methods.

3.3. Effect of composts on lettuce phenolics

The response of phenolics to composts was strongly genotype-specific; therefore data from the two lettuces were separately submitted to PCA to discriminate between the composts and better decipher the compounds accounting for the effects observed.

For Quatro Estações, higher levels of phenolic acids, flavonoids and anthocyanins in CHESTNUT-grown lettuce versus CONTROL were found (Fig. 3A). Only CHESTNUT and WHITEGRAPE were effective in increasing the cyanidin 3-*O*-(6''-malonyl)- β -D-glucoside content. For flavonoids, moderate performances were observed with WHITEGRAPE and REDGRAPE (Table 1). The impact of individual phenolics to the overall variance in lettuce was visualized on the loading plot of PCA (Fig. 3B); although all compounds had higher mean values with CHESTNUT, only few were truly responsible for the effect observed. The contents of all flavonoids and cyanidin 3-*O*-(6''-malonyl)- β -D-glucoside were highly sensitive to the growing media; in the case of phenolic acids, the differences induced by CHESTNUT were most clearly seen with caffeic acid

derivative 1, caffeoylmalic acid, and chlorogenic acid (Fig. 3B). All these caffeic acid derivatives contain one or two caffeoyl moieties, which are important structures involved in antiperoxidative and radical scavenging activities (Caldwell, 2003; Nicolle et al., 2004); and therefore changes in their contents could have health consequences. Isochlorogenic acid is interesting in this regard as it has been identified as the main phenolic involved in the neuroprotective effect of lettuce extracts (Im et al., 2010). WHITEGRAPE significantly ($P < 0.05$) induced the accumulation of caffeic acid derivative 1, unidentified flavonol, quercetin 3-*O*-glucoside, and luteolin 7-*O*-glucoside, but that was not sufficient to produce a major effect as with CHESTNUT, because of decreases in chicoric acid, caftaric acid, and quercetin 3-*O*-(6''-*O*-malonyl)- β -D-glucoside contents (Table 1; Supporting Information 2).

On the other hand, Maravilha Inverno grown on WHITEGRAPE contained significantly ($P < 0.05$) higher levels of flavonoids and phenolic acids. This was followed by CHESTNUT although the effect observed was not as pronounced as with Quatro Estações (Fig. 3C). In fact, only WHITEGRAPE led to values higher than CONTROL with respect to flavonoids (Table 1). In all cases, BROCCOLI induced significantly ($P < 0.05$) lower accumulation of phenolics (Fig. 3C). Four phenolics formed a cluster on the PCA loading plot and their accumulation in Maravilha Inverno seems to be responsible for the effect observed with WHITEGRAPE: quercetin 3-*O*-glucoside, luteolin 7-*O*-glucoside, unidentified flavonol and caffeic acid derivative 6 (Fig. 3D). Levels of most other compounds were not affected by any of the growing media (Table 1; Supporting Information 2). Consistent with what was observed with CHESTNUT in Quatro

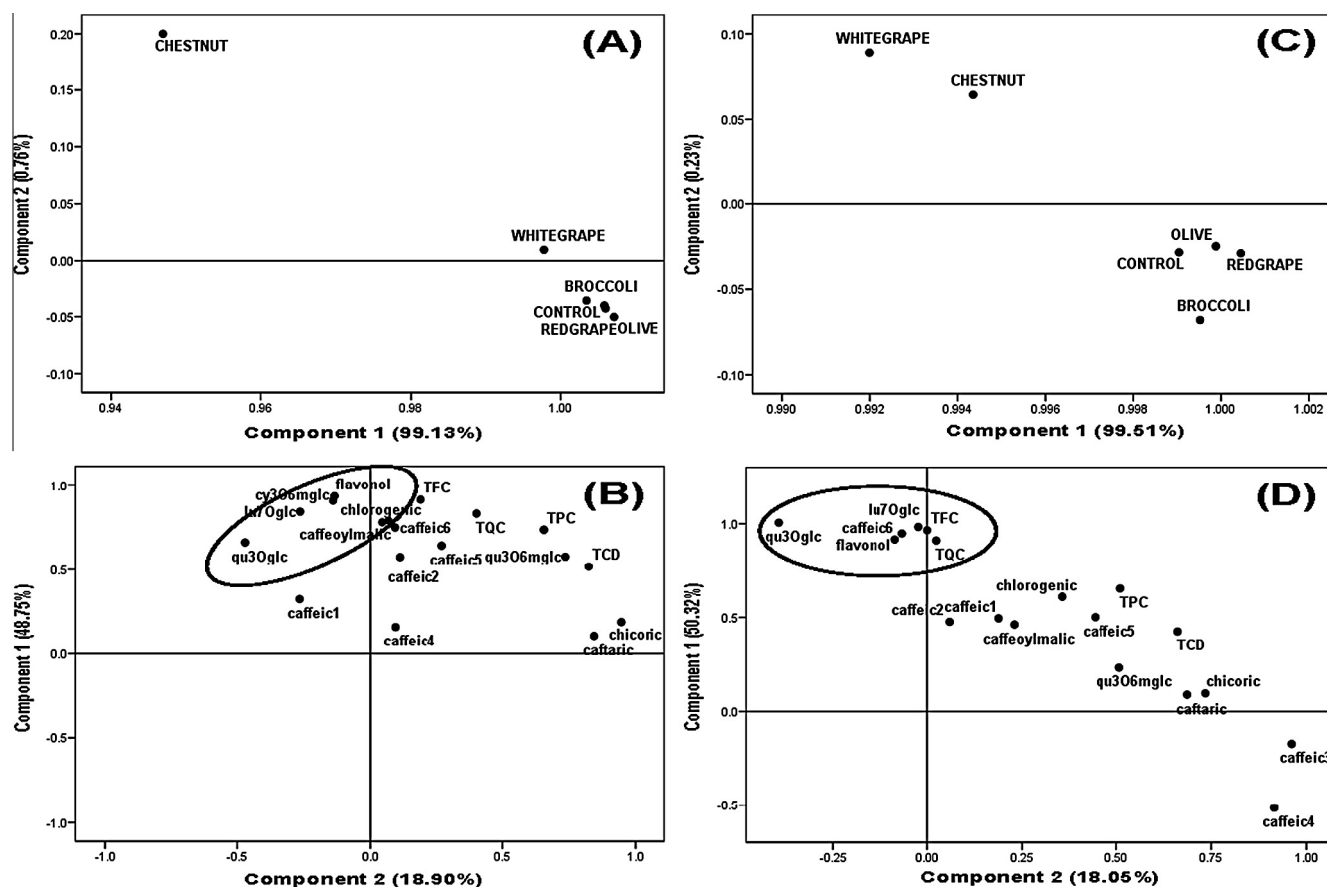


Fig. 3. PCA biplots showing the influence of composts on phenolics: (A) separation of composts in red Quatro Estações, (B) distribution of phenolics in relation to the treatments in Quatro Estações (C) separation of composts in green Maravilha Inverno, and (D) distribution of phenolics in relation to the treatments in Maravilha Inverno. For abbreviations, see Table 1 and Supporting Information 2.

Estações, flavonoids contributed the most to the improvements observed with WHITEGRAPE in Maravilha Inverno. In the study by Caldwell (2003), the decreasing order of peroxyl radical scavenging activities for flavonoids was cyanidin 3-O-(6''-malonyl)- β -D-glucoside > quercetin 3-O-glucoside > quercetin 3-O-(6''-O-malonyl)- β -D-glucoside. That ranking was used by the authors to suggest certain health consequences of increased consumption of lettuce. Some of the best supporting evidence for the role of lettuce flavonoids in diabetes prevention have recently been reported, and shown cyanidin 3-O-(6''-malonyl)- β -D-glucoside and quercetin 3-O-(6''-O-malonyl)- β -D-glucoside to be associated with the reduction of hyperglycemia in mice (Cheng et al., 2014).

Although this study was not specifically designed to compare organically and conventionally-grown lettuces, it is important to state that polyphenol contents are significantly lower under conventional farming (Grassi et al., 2015; Heimler et al., 2012; Lakhdar et al., 2011); and even though it is not a universal phenomenon (Durazzo et al., 2014), that support the prediction that when cultivated in the presence of CHESTNUT and WHITEGRAPE applied under appropriate conditions versus chemical fertilizers, the lettuce plant could be enriched in bioactive compounds.

3.4. Effect of composts on DPPH, TPC, TFC, and TAC

The DPPH assay was selected to investigate if changes in lettuce phenolics with compost treatments could translate into improved antioxidant capacities. DPPH had a significant ($P < 0.05$) positive correlation with total phenolics/flavonoids in Quatro Estações ($r = 0.73$) and in Maravilha Inverno ($r = 0.86$); and high phenolic/flavonoid contents in lettuces from CHESTNUT and WHITEGRAPE were associated with high reducing capacities in Quatro Estações and in Maravilha Inverno, respectively (Fig. 4; Table 1). OLIVE and REDGRAPE in Maravilha Inverno also led to high DPPH values. Determination of the actual relevance of these changes will require consideration of cell or in vivo studies.

Marked differences existed between TPC and TFC measured spectrophotometrically, and those obtained by HPLC in Quatro Estações: the spectrophotometric TPC was not affected ($P < 0.05$) by any of the treatments, while highest HPLC TPC were obtained with CHESTNUT AND REDGRAPE; the highest spectrophotometric TFC and the lowest HPLC TFC were both obtained with BROCOLI (Fig. 4; Table 1). When compared in a linear correlation model, there was only a loose correlation ($r = 0.58$ for TFC; $r = 0.61$ for TPC) between the two methods in Maravilha Inverno. Although the Folin-Ciocalteu and aluminum chloride assays are regularly used to indirectly estimate total polyphenols contents in plants, these methods are not completely specific for the target substances, and not all phenolics exhibit the same level of activity in the assays (Liu et al., 2007); therefore depending on the vegetable matrix, their use should be carefully reconsidered.

Spectrophotometric measurements found TAC of 0.30 and 0.08 mg CGE/g FW in the red and green lettuces, respectively (Fig. 4), although cyanidin 3-O-(6''-malonyl)- β -D-glucoside was only detected in the red lettuce. In most cases, lettuce is stripped of its outer wrapped leaves before analyses, which was not the case in this study. A darker coloration was found toward the extremities of the outer leaves of Maravilha Inverno; since this was clearly not related to leaves senescence, it could explain the level of anthocyanins measured. In fact, it was demonstrated that anthocyanin and vitamin C contents of lettuce are 10 times as high in the green outer leaves as in the inner light-coloured ones (Baslam et al., 2013). In contrast with the TPC and TFC, spectrophotometric TAC results were comparable to HPLC ones ($r = 0.70$), notably showing CHESTNUT and WHITEGRAPE as effective in increasing anthocyanin levels.

3.5. Effect of composts on vitamin C and carotenoids

Compost as amendments significantly decreased the content of vitamin C in the lettuces. The CONTROL on average had 1.29 mg/g FW vitamin C; the next highest value was with Quatro Estações grown on REDGRAPE, followed by Maravilha Inverno grown on WHITEGRAPE (Fig. 4). Although no strong correlation was observed between DPPH and vitamin C (Supporting Information 3), recent studies have reaffirmed a positive link between vitamin C and antioxidant capacities in lettuce (Llorach et al., 2008; Nicolle et al., 2004; Serafini et al., 2002).

The total chlorophylls and carotenoids contents of lettuces grown with the composts were mostly statistically similar to CONTROL (Supporting Information 4). However, the total carotenoids content is not the main factor to take into account as specific carotenoids may be affected to varying degrees. Lettuce carotenoids have been identified as lutein, β -carotene, neoxanthin, violaxanthin and lactucaxanthin (Baslam et al., 2013; Durazzo et al., 2014; Nicolle et al., 2004); and at least one study has shown increments in their levels in lettuce grown on compost (Cruz et al., 2014).

3.6. Correlation between compost and lettuce phenolics

The second objective of this study was to investigate if changes in lettuce antioxidant profiles with compost treatments fit the picture of phenolics uptake from roots.

A major challenge faced by the composting industry is to alleviate toxicity concerns associated with the use of raw wastes. Agro-industrial-based wastes are known to be rich in high MW phenolics, with the potential for production of phytotoxic leachates as they decompose (Keleş & Tzortzakis, 2009; Lakhdar et al., 2011). On the other hand, these chemicals at lower concentrations may elicit stimulatory effects on different plant organs. Therefore, their contents need to be manipulated to counter the polluting effect and ensure efficient plant growth. Composting the five wastes with wheat straw vastly reduced total (94%) and simple phenolics (85%) contents, but increased gallic acid content by 187% on average (Table 2). At the end of the composting process, the compost stability was closely related to polyphenol contents, denoting a high degree of maturity (data not shown).

From Tables 1 and 2, it seems that simple phenolics in the compost – not total phenolics – determined the contents of phenolics in lettuce. This tendency was verified by Pearson's correlation analysis; a positive correlation between the compost gallic acid content and Maravilha Inverno phenolics was established ($r = 0.48$ – 0.62), but this was less pronounced than the highly linear relationship ($r = 0.57$ – 0.67) observed between all simple phenolics in the compost, and the TPC, TFC, and DPPH in Quatro Estações (Supporting Information 3). Although correlation does not imply causation, the fact that high levels of simple phenolics and gallic acid in CHESTNUT and WHITEGRAPE, respectively, were associated with high levels of polyphenols in lettuce grown on these media, suggests a cause-effect relationship. It is possible that specific non-planar flavonoids produced during composting may have acted as signal factors, prompting root-to-shoot phenolic uptake by protein transporters as recently observed in *Arabidopsis* (Buer et al., 2010), resulting in an enhanced accumulation and synthesis of these compounds in lettuce.

4. Conclusions

On the whole, type of compost has a marked influence on both yield and phenolics in lettuce; and genetics seems to play a large role in the ability of lettuce to respond to the growing media. By

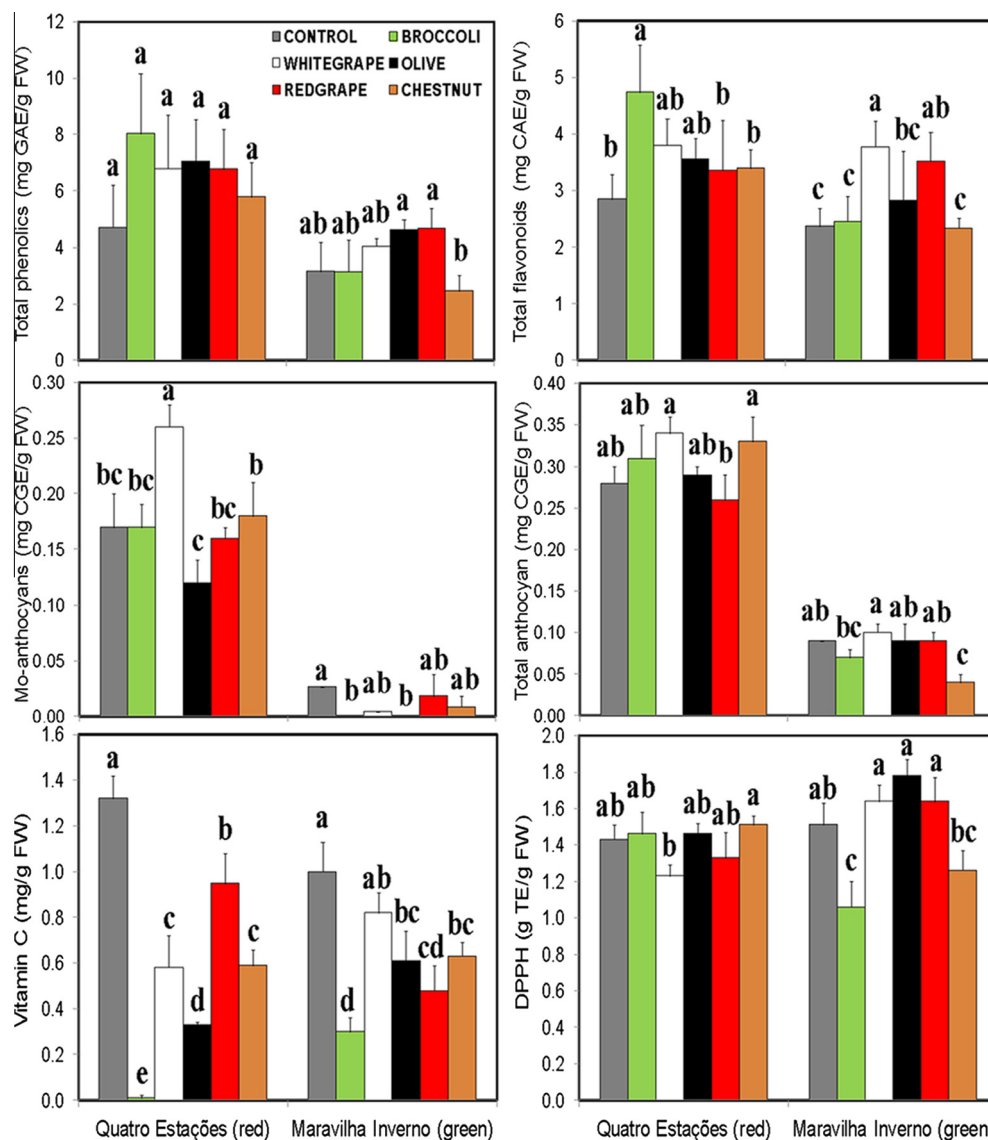


Fig. 4. Total flavonoids, phenolics, and anthocyanins, vitamin C and DPPH radical scavenging activity of lettuce cultivated with composts. Means and SD as error bars with the same letter do not differ (Turkey's HSD test; $P < 0.05$).

Table 2

Phenolic composition (mean \pm SD; DW) of the raw material and composts used in the study.

	Total phenolics content (g GAE/kg)	Simple phenolics content (mg GAE/kg)	Galic acid (μ g/kg)
<i>Raw material</i>			
Wheat straw ^a	3.83 \pm 0.79e	2.29 \pm 0.05f	27.71 \pm 2.37a
BROCCOLI	6.65 \pm 0.05d	7.08 \pm 0.05e	4.17 \pm 0.07d
WHITEGRAPE	29.57 \pm 1.88a	25.04 \pm 1.14a	12.60 \pm 3.43c
OLIVE	25.52 \pm 1.06b	20.93 \pm 0.31b	13.41 \pm 4.20c
REDGRAPE	25.31 \pm 1.25b	18.68 \pm 1.37c	13.17 \pm 2.96c
CHESTNUT	15.22 \pm 0.53c	11.17 \pm 0.41d	17.95 \pm 1.52b
<i>Compost</i>			
Wheat straw	1.14 \pm 0.11a	1.05 \pm 0.01d	22.89 \pm 0.91b
BROCCOLI	1.08 \pm 0.32a	1.15 \pm 0.12d	23.79 \pm 0.95b
WHITEGRAPE	1.15 \pm 0.38a	1.18 \pm 0.16d	34.37 \pm 9.57a
OLIVE	0.16 \pm 0.01c	2.72 \pm 0.13b	28.18 \pm 8.48ab
REDGRAPE	0.70 \pm 0.09b	2.30 \pm 0.23c	30.54 \pm 4.09ab
CHESTNUT	1.10 \pm 0.09a	3.46 \pm 0.15a	26.53 \pm 1.91ab

Different letters in a column show significant mean differences ($P < 0.05$; Turkey's HSD test).

^a Wheat straw was used as co-substrate during composting.

developing a parallelism between yield and antioxidants, five main lessons could be learned: (i) Quatro Estações grown on CHESTNUT exhibited increased yield and antioxidant capacity; yield and polyphenol pools were positively correlated on average ($r = 0.60$), suggesting that factors that promoted growth might have also enhanced phenolic synthesis. Lettuce is mostly consumed fresh; thus these modifications can have significant direct nutritional impacts; (ii) Maravilha Inverno grown on WHITEGRAPE exhibited increased production of phenolics; however, that seems to act in direct competition for assimilated carbon to the plants' growth processes, given the low yield recorded. Provided growers decide to mainly select for increased antioxidant activity, there is scope to consider WHITEGRAPE for that variety; (iii) CHESTNUT also increased the yield of Maravilha Inverno without adversely affecting its phenolic content. A cooperative action between CHESTNUT and WHITEGRAPE could be beneficial in enhancing the properties of the latter, and this may be the focus of a future research direction; (iv) Lettuces grown on BROCCOLI exhibited the worst yields and phenolic levels. Therefore, it seems better to avoid their application in organic horticulture, unless adequate composting strategies are implemented to improve their fertilizing efficiency;

(v) With respect to vitamin C, all the composts led to decreased contents in lettuces, which is undesirable from a nutritional point of view, but does not necessarily denote an overall negative outcome. It is obvious that composting not only provides an adequate disposal approach for plant food-based wastes, but composts can be used as substitutes for non-renewable resources such as peat in organic lettuce production. The data show that if lettuces with high yield and polyphenol contents are desired, the chestnut-made compost developed in the course of this study and available at UTAD can be used for their cultivation.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.04.087>.

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