



# Insight in molecular degradation patterns and co-metabolism during rose waste co-composting

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**Abstract** Composting is recognized as a sustainable waste management strategy. However, little is known about green waste, and specifically rose waste, degradation patterns during composting. This study aimed (1) to gain insight in the underlying decomposition patterns during rose waste composting and (2) to identify co-metabolisms of ligneous material. Five different compost mixtures were tested ranging from pure rose waste to mixtures with tomato waste, kalanchoe waste or mature compost added. Samples were taken during a six-month experiment and analyzed by pyrolysis-GC/MS. The temporal trends in the relative abundance of 10 different compound groups were measured. Lignin and aliphatic compounds together accounted for  $\geq 50\%$  of the quantified

pyrolysis products, but with changing contributions during composting. The relative abundance of polysaccharides and terpenes strongly decreased with more than 60% in the first 2 months. The simultaneous decrease in relative abundance of lignin and polysaccharides during initial composting phase indicated co-metabolism of lignin. The results from this study showed that while the presence of lignin is commonly regarded as a challenge in composting, it actually undergoes degradation through distinct mechanisms at the various composting stages.

**Keywords** Pyrolysis-GC/MS · Composting · Co-metabolism · Lignin degradation · Biomarker

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## Introduction

Composting is globally gaining increasing interest within the horticultural sector as a sustainable management strategy to combat the waste challenge it faces (RVO 2019). Composting is a controlled degradation process in which organic matter (OM) is decomposed by microorganisms generating a stable OM which is referred to as compost and which can be used as soil amendment (Bernal et al. 2009; Ruiz et al. 2020). Worldwide rose farming is a crucial component of the horticultural sector, but re-use of rose waste as compost is currently severely limited due to its perceived unfavorable composting properties. Rose cultivation generates large amounts of green

waste through crop maintenance, rejected produce and grading (Idrovo-Novillo et al. 2018). This can average to 50 kg per ha per day, equivalent to 2000 kg of green waste per day for a medium-sized rose farm of 40 ha. These numbers make it an interesting prospect for improvement to this type of larger scale composting (de Nijs et al. 2023).

Rose waste, in particular the stems, are a type of lignocellulosic green waste, containing high concentrations of lignin that are assumed to hamper a rapid composting process (Idrovo-Novillo et al. 2018; Reyes-Torres et al. 2018). The degradation of OM is the crucial process by which waste transforms into compost. However, little is still known about the molecular decomposition patterns of horticultural green wastes over time during the composting process. Composting lignocellulose-rich waste, such as rose waste, is also challenging due to the potential inaccessibility to microorganisms and its molecular structure (Jurado et al. 2014; Wang et al. 2017; Yu et al. 2021). A better insight into the degradation mechanisms underlying composting of rose waste, and in particular the molecular transformations (or lack thereof) of lignin during the composting process would further advance our understanding enabling how to increase composting efficiency.

A well-established method to trace molecular conversions over time is Pyrolysis Gas Chromatography-mass Spectrometry (Py-GC/MS). Pyrolysis is a form of thermal degradation that releases a wide range of molecules, which allow for characterization of OM constituents (Alkalin and Karagöz, 2014; Derenne and Quéné, 2015; Kohl et al. 2023; Rueda et al. 2022; Šimkovic et al. 2023). The degradation patterns of lignocellulose-rich green wastes during composting processes have generally remained elusive with only a few studies examining the molecular changes occurring during composting. Dignac et al. (2005) used Py-GC/MS to examine the chemical composition of various fresh green wastes and composted wastes (*e.g.* garden- and municipal waste). Furthermore, they used ratios of peak areas for specific pyrolysis products to assess the origin and level of degradation of lignin (Dignac et al. 2005). Hayany et al. (2020) investigated the suitability of Py-GC/MS as tool for maturity evaluation of green waste and sewage sludge compost by monitoring 68 compounds. Two specific ratios of pyrolysis compounds were suggested to describe the biodegradation and evaluate the maturity of this

specific compost (*i.e.* 4-methyl- $\alpha$ -methylstyrene/benzene). El Fels et al. (2014) monitored the biotransformation of 11 main pyrolysis products of lignin over time for sewage sludge and palm waste. These components were divided into two groups based on whether the concentrations of these components decreased or increased during composting, were components of the latter are likely released during lignin degradation. San-Emeterio et al. (2021) observed, for maize biomass composting, an initial increase in the lignin-to-polysaccharides ratio together with a relative increase of syringyl lignin (S) structures, suggesting that cellulose in maize waste is preferentially degraded over lignin. The ratio between syringyl lignin and guaiacyl (G) structures is often used as proxy to identify vegetation sources, *e.g.* coniferous or deciduous trees (Dignac et al. 2005; Altmann et al. 2021a, b). More recently the validity of biomarker analysis to identify OM origin has been debated. In a 1-year litter incubation experiment, vegetation biomarker indicators changed over time without a linear trend (Altmann et al. 2021a, b). This variability has also been observed during green waste composting, where the S/G ratio decreased substantially between fresh green waste and the matured compost (Dignac et al. 2005; San-Emeterio et al. 2021). None of the mentioned studies monitored molecular degradation in lignocellulosic waste in detail during the complete composting process, and none focused on rose waste.

Molecular degradation of OM is controlled by an interplay of both intrinsic characteristics and specific environmental conditions and often considered after incorporation into the soil matrix (Amelung et al. 2008; Schmidt et al. 2011). The current study focused on degradation patterns of fresh plant material during composting. This is prior to incorporation into the soil where the macroscopic structure of the OM may play an important role as previously indicated by Huang et al. (1997). In the past, the stability of OM in soils was mainly attributed to recalcitrance of specific molecules leading to the formation of stable ‘humic substances’ (Schmidt et al. 2011). However, this view has shifted over the past decades to one where context dependent physical or chemical (in)accessibility of OM for decomposers is seen as key regulating mechanism of decomposition (Schmidt et al. 2011; Lehmann and Kleber 2015). Specifically, the susceptibility of OM to degradation is thought to be regulated by a combination of the following prerequisites: the accessibility

of the OM, the presence of decomposers and suitable abiotic conditions such as pH, temperature and moisture (Doetterl et al. 2016). The same conditions apply for an efficient composting process. This implies that under suitable conditions, the complex molecular structure of lignin molecules does not prevent degradation in early decomposition stages when easily degradable compounds, such as polysaccharides, are still prevalent as energy source for decomposers. Klotzbücher et al. (2011) demonstrated this phenomenon through a litter study conducted in a forest ecosystem, showing that the degradation of lignin was regulated by the availability of dissolved organic C. The presence of easily available C was the prerequisite for lignin degradation, a process called co-metabolism. Although composting is a controlled degradation process, composting can be compared to an accelerated version of the first stages of decomposition in a natural environment, such as litterbag experiments in the litter layer. To date, most molecular studies on the fate of OM are focused on the role of molecular composition once the OM has entered the soil system (Gleixner et al. 2002; Angst et al. 2017; Kögel-Knabner 2017; Roth et al. 2019).

Therefore, our study was aimed (1) to gain insight into the temporal degradation patterns of rose waste upon composting at a molecular level, and (2) to identify potential co-metabolism compounds of lignocellulosic waste during composting. It was hypothesized that degradation patterns similar to those occurring in the litter layer occur during composting and that the easily available C during the initial composting phase would enable the co-composting of lignin. The focus on the process of lignin degradation during the various stages of composting and how this relates to the traditional view regarding the fate of lignin during OM degradation. Molecular changes during the composting process were monitored by Py-GC/MS. For this, an novel extensive Py-GC/MS processing method was specifically developed to monitor lignocellulosic-rich green waste composting. Five different co-composting mixtures were followed for a 6-month period to elucidate degradation patterns.

## Materials and methods

### Composting experiment

Five different compost mixtures of pure rose waste and rose waste mixed with other green wastes or with mature rose compost were composted. Rose, tomato and kalanchoe waste were collected respectively from a rose distribution center, tomato and kalanchoe farm in the Netherlands. Mature rose compost was obtained from a rose farm located in Kenya. Each mixture was prepared in triplicate and 6 kg of material was composted for 6 months in buckets modified with air-holes. The following mixtures were prepared on fresh weight basis: pure rose waste (R), rose waste with 20% additions of either tomato cuttings (T) or kalanchoe plant waste (K), and rose waste with a 20% addition of mature rose compost (C) and one mixture with an altered leaf-to-stem ratio for the rose waste (CL). On a fresh weight basis, the composite rose waste consisted of 20% flowers, 50% stems and 30% leaves. In the CL mixture, leaf and stem proportions were reversed. The temperature was regularly monitored and the moisture content was adjusted when needed. The mixtures were turned manually at each sampling moment to ensure aerobic conditions. Composite samples ( $n=5$ ) from the whole profile of approximately 80 g were collected on day 0, 7, 15, 23, 35, 58, 90 and 181. Samples were immediately air-dried at 40 °C for 72 h and ground to 0.5 mm prior to Py-GC/MS analysis (Retsch ultra centrifugal mill ZM 200).

All mixtures reached the thermophilic phase ( $>40$  °C) within 5 days which was sustained for 5 to 8 days. Maturation phase started after approximately 20 days and temperatures remained close to ambient for the remaining composting time (Fig. S1). Table 1 shows the initial and final characteristics of the different compost mixtures. Further details on the set-up and the evolution of the extensive set of physicochemical parameters during the composting experiment have been described in detail in de Nijs et al. (2023).

### Analytical methods

Organic matter (OM) content was determined by loss on ignition at 550 °C for 16 h (Nelson and Sommers 1996).

**Table 1** Initial and final physicochemical characteristics of the matured compost mixtures (adapted from de Nijs et al. 2023)

	Compost mixtures <sup>a</sup>									
	R		T		K		C		CL	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	5.6	6.8	5.5	7.5	5.6	7.2	5.9	6.6	5.8	7.1
EC (dS m <sup>-1</sup> )	1.9	5.9	2.2	7.2	2.3	6.4	2.0	4.0	2.2	4.2
OM (%)	94.7	81.9	93.9	76.1	93.9	78.5	78.0	48.4	79.2	46.0
C/N	25.3	8.6	21.9	8.0	23.9	8.0	18.3	7.5	15.5	6.6

<sup>a</sup>Mean, dry weight basis,  $n=3$

To monitor molecular composition during composting, samples were analyzed on a multi-shot furnace pyrolyser EGA/Py-3030D (Frontier Lab, Japan) that was coupled to a Thermo Scientific Trace 1300 GC (Italy) and equipped with a Rtx-1 column (Restek: 30m; 0.25 mm i.d.), nonpolar column coated with cross bonded polydimethylsiloxane (100% dimethylpolysiloxane, 0.5  $\mu$ m film thickness). The samples were introduced to the pyrolyser by an AS-1020E Auto-shot sampler (Frontier lab, Japan), using the experimental triplicates as independent replicates. To establish an efficient set of measurement configurations, several test runs were conducted and evaluated based on signal quality. The signal was evaluated for minimal noise and optimal recovery. The pyrolyzer temperature setting was tested ranging between 350 and 550 °C with 50 °C increments followed by pyrolysis time from 0.2 to 0.8 min with 0.1 min increments. Finally, the optimal amount of material to be pyrolyzed was tested, ranging from 50 to 400  $\mu$ g per sample with steps of 50–100  $\mu$ g. Final pyrolysis temperature was set at 400 °C for 0.4 min with sample weights of approximately 250  $\mu$ g. Helium was used as carrier gas at a constant flow of 1 mL/min. The GC was coupled to a Thermo Scientific ISQ 7000 Single Quadrupole MS (Italy) ( $m/z$ : 40–500; ionization energy: 70 eV). The injection temperature of the GC (split mode: 1:100) was set at 250 °C. Temperature of the GC oven increased from 40 °C (hold time: 1 min, heating rate: 7.0 °C/min) to a final temperature of 320 °C (hold time: 10 min). Chromatograms illustrating the composition of one of the R mixture samples at the beginning and end of the composting process are provided in figure S2.

## Data analysis

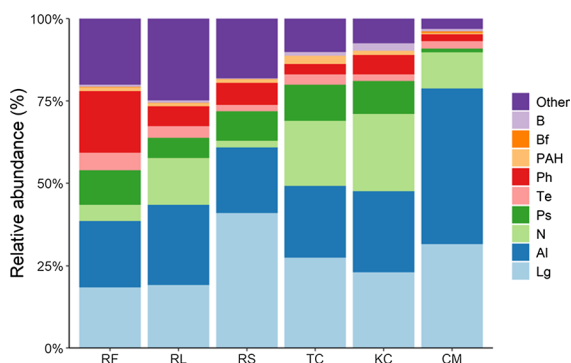
The Py-GC/MS data was analyzed using Chromeleon Studio v7 (Fisher Scientific), and pyrolysis products were identified using the NIST MS search 2.3 Library version 17.

The processing method was developed based on non-targeted screening of fresh rose waste and mature (rose) composts. The dominant compounds were identified and added to the processing method. This was complemented with findings from previous Py-GC/MS studies with relevant compounds in lignin (Van Erven et al. 2017), plant and wood material (Ralph and Hatfield, 1991; Brock et al. 2020) and various composts, sewage sludge, green- and municipal wastes (Dignac et al. 2005; El Fels et al. 2014; Hayany et al. 2020). A total of 143 compounds were retrieved by Py-GC/MS (Table S1).

The processing method was used to process the data, peaks were identified based on quantifying and confirming ion together with retention time. Peak identification and integration was checked manually. Ground and dried Oak (*Quercus sp.*) root was measured every nine samples and served as a systematic quality control. The peak areas of all identified compounds per sample were summed and set at 100%. This was used to calculate the relative abundance of the different compounds. All identified compounds were assigned to 10 different chemical compound groups, which were based on previous pyrolytic studies (Schellekens et al. 2017; Brock et al. 2020). These groups were used for further processing: aliphatics (Al), benzenes (B), benzofurans (Bf), lignin phenols (Lg), nitrogen-containing compounds (N), polyaromatic hydrocarbons (PAH), phenols (Ph), polysaccharides (Ps), terpenes

(Te) and other compounds. The lignin phenols were further subdivided in syringyl (S) and guaiacyl (G) units to calculate S/G ratio according to van Erven et al. (2017).

Data analyses and visualizations were performed using R version 4.0.5, visualizations were created using ggplot2 (Wickham 2016). Line- and bar graphs are shown with error bars representing the standard error of the mean.



**Fig. 1** Raw waste composition of RF=rose flower, RL=rose leaf, RS=rose stem, TC=tomato cuttings, KC=kalanchoe, CM=mature compost. Chemical groups: Other, B=benzenes, Bf=benzofurans, PAH=Polyaromatic hydrocarbons, Ph=phenols, Te=terpenes, Ps=polysaccharides, N=N-containing compounds, Al=aliphatics, Lg=lignin phenols

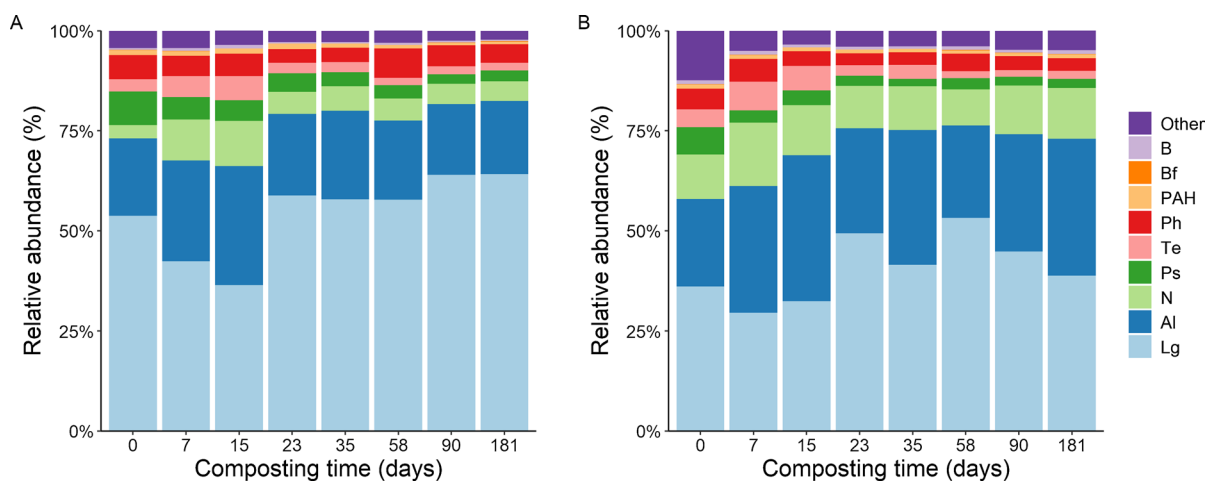
## Results

### Raw waste composition

A total of 143 of compounds were identified in the raw waste materials and throughout the composting process and divided over 10 chemical groups (Table S1). The differences in composition of the different raw waste materials are presented in Fig. 1. Rose stems contained relatively more Lg whereas the flowers had the largest contribution of Ph and Te, and rose leaves contained more N-containing compounds compared to the other rose components (Fig. 1). Tomato cuttings and kalanchoe were similar in contribution of easily degradable Ps compounds whereas the mature compost had the lowest contribution (Fig. 1).

### Molecular composition during composting

The evolution of different compound groups during composting was monitored for the five different mixtures over time. Figure 2 shows the dynamics of the relative abundance of these groups for mixture R (roses) and CL (roses with compost and altered leaf-to-stem ratio) during the 6-months period of composting. Changes in molecular composition in terms of relative abundance during composting of all mixtures can be found in table S2.



**Fig. 2** Distribution of relative abundance of distinguished compounds during the composting process of **a** R mixture—100% rose waste and **b** CL mixture—80% rose waste

with altered leaf-to-stem ratio and 20% mature compost. See Fig. 1 for chemical compound group definition

Overall, similar patterns of relative contributions were observed for all mixtures except CL (Fig. 2A, Table S2). In all mixtures Lg and Al compounds together accounted for  $\geq 50\%$  of the quantified pyrolysis products throughout the composting process. The relative abundance of Lg initially decreased or remained stable. However, after 3 weeks the relative abundance of Lg compounds started increasing and finally contributed more than half of the identified compounds in all mixtures. The contribution of Ps and Te compounds decreased during the composting process, with the strongest reduction observed during the active phase (first 3 weeks).

In the CL mixture, leaf and stem proportions were reversed, resulting in more leaves and less stems compared to the other mixtures. As a result, the CL mixture had a higher proportion of N-containing compounds, which are more prevalent in leaves (Fig. 2, Table S2). Furthermore, the CL mixture had a lower initial contribution of Lg originating from the stems, compared to the other mixtures (Fig. 2B). Notably, for the CL mixture the relative contribution of N-containing compounds remained relatively stable during composting. This indicates a similar degradation rate as that of the total OM content of the compost (Fig. S3), whereas the other mixtures showed an erratic pattern. The degradation of Ps, on the other hand, did show similar reduction in relative contribution during composting for all mixtures. The contribution of Al

compounds remained relatively stable during composting for all mixtures.

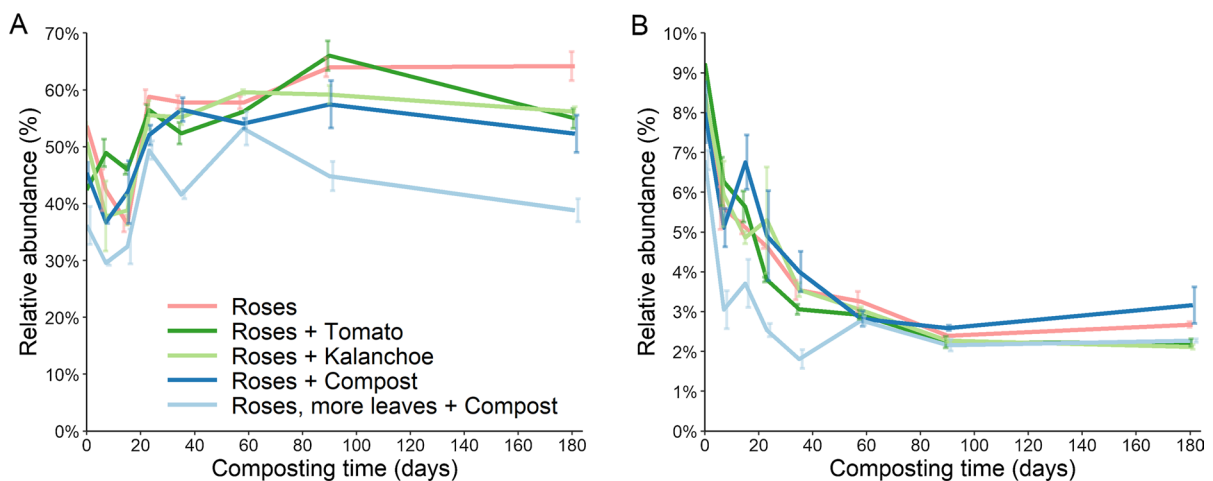
#### Patterns of Lg and Ps

In all mixtures except T the relative contribution of Lg decreased during the first weeks of composting (Fig. 3A). This correlated with a strong decrease of Ps, known as easily degradable compounds (Brock et al. 2020) (Fig. 3B). Hereafter the relative contribution of Lg erratically increased for all mixtures. During maturation Lg decreased a second time for most mixtures (Fig. 3A). The addition of mature compost (C & CL mixture) increased the overall rate of Lg degradation whereas increasing the leaf-to-stem ratio enhanced total Lg degradation (Fig. 3A).

#### Lignin S/G ratio changes during composting

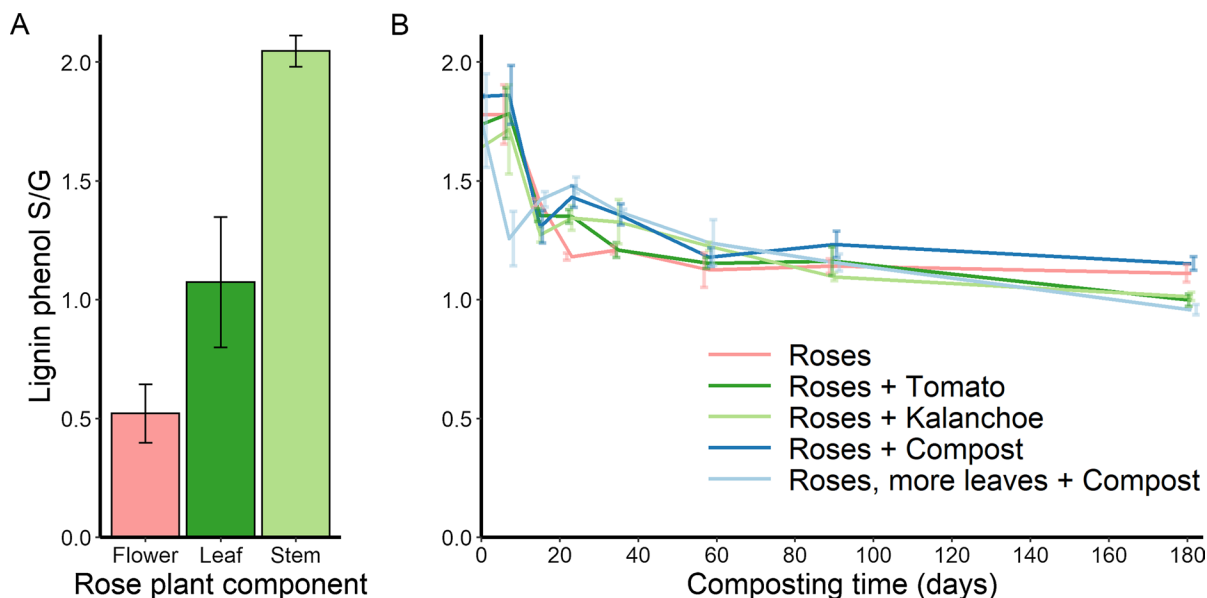
Lignin phenols were divided over the three main structure types: p-hydroxypheny (H), syringyl (S) and guaiacyl (G) (Van Erven et al. 2017). The S/G ratio differed significantly between the different rose waste components, ranging from 0.5 for flowers to 1.1 for leaves and over 2 for stems (Fig. 4A). S/G ratio for the other green wastes were all below 1, indicating a larger proportion of G units compared to S units (Fig. S4).

The S/G ratio altered strongly during the active phase of the composting process from above 1.5



**Fig. 3** Relative abundance (mean  $\pm$  SE) of **a** lignin phenols (Lg) and **b** polysaccharides (Ps) per gram organic matter during composting





**Fig. 4** Lignin S to G unit ratio (mean  $\pm$  SE) of **a** the different rose waste components and **b** the five different compost mixtures over time

to around 1.0 for all mixtures (Fig. 4B). The largest reduction in S/G ratio was observed during the first weeks of composting. A subsequent gradual decrease was observed during the remainder of the active composting phase (first 3 months). The S/G ratio remained stable or slightly decreased during the maturation phase. The final S/G ratio values were similar to the S/G ratio of 1.1 of the mature compost previously obtained from Kenya (Fig. S4). The S/G ratio evolution patterns between the different mixtures did not vary substantially.

## Discussion

### Raw rose waste composition

The composition of the initial raw wastes showed large differences in the relative contribution of the distinguished chemical compound groups (Fig. 1). This is supported by Dignac et al. (2005) who found that the composition of pyrolysis products varied with the nature of the composted waste. Dignac et al. (2005) showed that the main pyrolysis products of green waste composts originated from Lg and Ps, with for Lg a mixture of G and S type. For the green wastes used in this study Lg and Al contributed most

to the pyrolysis products. Dignac et al. (2005) found low amounts of Al in both fresh wastes and other composts. Rose-based mature compost, which was used to kickstart the composting process in the C and CL mixture, consisted for almost 90% of the more persistent compounds groups Al, Lg and N (Fig. 2). This does not imply that these components did not undergo degradation during composting, it only indicates that they are comparatively less susceptible to degradation in comparison to *e.g.* Ps (Brock et al. 2020). As a result, they constitute the majority of the mature compost composition (Fig. 2). The large variations in molecular composition of rose components (Fig. 1), *i.e.*, high lignin in the stems and high Ph contribution in the flowers, were reflected in the composition of the different mixtures, as can be seen for the composition of the R and CL mixture at day zero (Fig. 2).

### Evolution of pyrolysis products

The large diversity of quantified pyrolysis products resulted in an assignment approach via 10 main compound groups based on chemical similarity (Schellekens et al. 2017; Hayany et al. 2020). The absolute distribution of the compound groups and their evolution throughout composting varied between the different

mixtures. However, they all followed similar patterns of relative enrichment and reduction, where the green waste mixtures were more alike than the mixtures with mature compost added. Only for the Lg group was a clear distinction between mixtures observed (Fig. 3A). Its relative contribution remained stable for the green waste mixtures during the maturation phase whereas it substantially decreased for the mixtures with mature compost added. Similar trends of increased degradation efficiency with the addition of mature compost have been shown for a wide range of physicochemical parameters (Yang and Zhang 2022). The relative contribution strongly decreased throughout the composting process for B, PAH, Ps, Te, N and the other compounds category (Fig. 2). This strong decrease primarily occurred during the initial 20 days of composting, indicating ease of degradation which is associated with high microbial activity (Lim et al. 2016). Al, Bf, Lg and Ph compounds fluctuated over time and made up the majority of the final compost composition. Bf and Ph are both lignin constituents that are released in parallel with Lg degradation as was previously observed by El Fels et al. (2014). The Ph contribution spiked around the onset of the maturation phase indicating increased degradation of Lg. An increase in Ph levels was also observed by Rueda et al. (2022) for olive mill pomace compost mixtures. This is in accordance with previous studies stating that lignin is preferentially degraded by ligninolytic fungi that colonize the compost during the maturation phase (Vargas-García et al. 2010; Cáceres et al. 2018). A comparison of the pyrolysis products of fresh green waste and mature compost by Dignac et al. (2005) further support these findings. That study found a higher relative abundance of N and Ps compared to Lg, suggesting an accelerated degradation rate of Lg in comparison to these compounds during composting. The contribution of N-containing compounds spiked during the first week. This suggested the breakdown of proteins and polypeptides, which subsequently underwent rapid degradation (San-Emeterio et al. 2021).

#### Potential co-metabolism of lignin

This study also focused on the process of Lg degradation during the various stages of composting and specifically explored the occurrence of co-metabolism by using patterns of Lg and Ps evolution. During the

composting process, ligneous materials are traditionally believed to be degraded primarily during the maturation phase, when temperatures decrease and easily degradable substrates have been consumed (Albrecht et al. 2010; Idrovo-Novillo et al. 2018). The decreased microbial activity during the maturation phases results in a prolonged composting process for lignocellulosic waste types, such as rose waste.

A decrease in Lg contribution can be due to either transformations of lignin molecules into other compounds or eventually complete mineralization (Klotzbücher et al. 2011). Contrary to expectations, this study found that the relative contribution of Lg decreased in most of the tested mixtures during the initial composting phase (Fig. 3A). Hereafter, a relative enrichment of Lg was observed followed by a second decrease during the maturation phase. This initial decrease coincided with the strongest observed decrease of Ps, an easy decomposable C source (Fig. 3B). These patterns are supported by previous findings of San-Emeterio et al. (2021) where a significant decrease in lignocellulosic compounds was observed during the first month of composting followed by a relative increase during the maturation phase. The observed simultaneous reduction of Lg and Ps contribution could be attributed to the occurrence of co-metabolism. This was previously described for a forest ecosystem by Klotzbücher et al. (2011) who concluded that the ample availability of C for microorganisms gives them the energy to produce Lg degrading metabolites. After the initial phase, abundance of Ps became too low to keep this energy supply sufficient for the simultaneous degradation of Lg and thus a relative enrichment of Lg was observed. Limited Lg degradation is attributed to low energy production in the absence of available C sources. This phenomenon has been noted before in cases where minimal or no easily accessible C was present (Klotzbücher et al. 2011). Since ligninolytic fungi are known for their colonization during the maturation phase is likely to be the main driver behind the second decrease of Lg contribution (Albrecht et al. 2010; Vargas-García et al. 2010; Van Erven et al. 2017).

Furthermore, the addition of mature compost to the starting mixtures and changes in stem-to-leaf ratio increased the overall rate of Lg degradation (Fig. 3A). This effect was more pronounced during the last 3 months of composting, *i.e.* the maturation



phase and could be attributed to the inoculation with suitable microorganisms from the mature compost and provision of easily available C sources. Enhanced degradation with microorganism inoculation to promote biodegradation has been shown for green waste composting under various circumstances (Awasthi et al. 2014; Wang et al. 2019; Yang and Zhang 2022). The importance of microorganisms for the turnover of lignocellulosic waste was also observed by Jurado et al. (2015), who observed a 25% increase in the degradation rate when inoculating the waste piles. Interestingly, their findings showed that it was not necessarily the specialists that triggered degradation, but instead, the overall rise in microorganism growth and activity caused by the inoculation. This finding further emphasizes the idea that efficient turnover of lignocellulosic waste in the absence of mineral material requires an enhanced microbial activity. Thereby confirming that when the availability of polysaccharides is sufficient, co-metabolism of lignin occurs.

#### Patterns of lignin S/G ratio

Combining the relative Lg contributions with the changes in S/G ratio during composting gives insight in patterns of preferential degradation (San-Emeterio et al. 2021). The S/G ratio strongly decreased during the initial, *i.e.*, active phase, of composting for all mixtures (Fig. 4B). This was followed by a gradual decrease in ratio, which eventually stabilized during the maturation phase. Dignac et al. (2005) also found a decrease in S/G ratio during the transformation from fresh green waste to compost, with the largest decrease in the first weeks. S/G ratio composition of different rose waste components shows that Lg from the stems is preferentially degraded in those first weeks. This can be concluded because only the stems have an S/G ratio higher than 2 and they contain most Lg (Figs. 4A, S4). This further supports the occurrence of co-metabolic character of Lg degradation during composting.

The decrease in S/G ratio indicates that there is a preferential degradation of S structures over G structures (Lucejko et al. 2020). This phenomenon can partly be attributed to the fact that G chemical structures have more cross linkages compared to S chemical structures, making them more resistant to degradation (Dignac et al. 2005). Another potential explanation is that S structures have additional methoxy groups on the

aromatic ring compared. Decrease in S structure contribution might thus also be caused by this extra side chain oxidation done by generalist microorganisms to obtain C as energy source (Lucejko et al. 2020). This depolymerization and C translocation of side chain molecules was previously also observed by San-Emeterio et al. (2021) for a composting process. During the later stages of composting, when specialists colonize the compost, the aromatic rings might be cleaved causing a concurrent decrease of both structure types and resulting in an overall decrease of lignin contribution. However, without insight in the evolution of microorganism communities throughout the composting process it is not possible to discriminate between these potential processes.

The S/G ratio, often used as biomarker to distinguish plant species (*e.g.* Campbell and Sederoff 1996; Moldoveanu 2021), has raised debates on its suitability for identifying the origin of organic materials (Altmann et al. 2021a, b). Nierop and Filley (2007) found that only the S/G ratio remained rather stable among various biomarkers. Furthermore, Altmann et al. (2021a, b) and San-Emeterio et al. (2021) observed changing biomarker values over 1 to 2 year periods, especially during the first months. The results from the current composting study align with these findings, showing an notable decrease in S/G ratio in the initial 3 weeks of composting, followed by a relatively stable pattern for the remaining time. Apparently, there is a preferential degradation of Lg S units during specific circumstances, such as initial composting phases, causing a shift in S/G ratio.

Further research is required to assess the potential of Lg co-metabolism across different compost compositions and operational scales. Additionally, it is crucial to integrate molecular insights on co-metabolism with detailed data on microorganism community colonization during composting. This integration will allow for directly coupling of the degradation of different molecular groups with the abundance of specific microbial communities, thereby enhancing our understanding of the key degradation mechanisms at play during composting.

#### Conclusion

Temporal trends in the relative abundance of 10 different compound groups showed similar patterns

across the various tested rose waste compost mixtures, except when there was an increase in the contribution of easily degradable material. This study demonstrated the occurrence of co-metabolism of Lg during the initial stages of the lignocellulosic rose waste composting, as evidenced by shifts in S/G ratio. Given that Lg degradation is often perceived as a limiting factor in composting efficiency, the findings of this study contribute to the development of practical improvements for the composting of lignocellulosic wastes. In practice, the degradation rate of Lg can be enhanced by ensuring an adequate supply of easily available C sources, thereby optimizing the composting process.

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**Author contributions** All authors contributed to the conceptualization of this study. Sample preparation, data collection and analysis were performed by EAdN, BJ and SA. The first draft of the manuscript was written by EAdN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability** The datasets generated during the current study are not publicly available but are available from the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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