applied sciences

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Biological Assessment of Green Waste and Dredged Sediment Co-Composting for Nursery Plant Cultivation

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Abstract: Co-composting efficiently reclaims dredged sediments (S) and green waste (GW), creating stable products for agricultural applications. However, the use of S-GW co-composts can be limited by legislative thresholds, especially for co-composts with a high S percentage. The evaluation of S-GW co-compost stability by biological assessment can allow for a better understanding of S and GW recycling, as well as the S-GW co-compost application. For this purpose, the microbial biomass, composition, respiration, and eco-enzyme stoichiometry (EST) were assessed, coupled with chemical analysis, in the co-composting of S and GW in different ratios. The *Photinia x fraseri* and *Viburnum tinus* L. growth was monitored in a plant trial, comparing the studied co-composts with a control substrate. The EST approach was applied as an indicator of the co-composting stability during the process and after the plant cultivation. The chemical and biological parameters confirmed the suitability of co-composting in the GW and S recovery and the EST approach highlighted a better stability for the 3S:1GW co-compost at the end of the process and after plant cultivation. *Viburnum tinus* showed a similar growth to the control, while *Photinia x fraseri* resulted in being more sensitive to the co-compost. The biological assessments were good indicators of the S-GW compost stability for their application in crop cultivation.

Keywords: compost stability; eco-enzyme stoichiometry; enzyme activity; fungi; microbial composition; microbial nutrient limitation; ornamental plants

Citation: Vannucchi, F.; Peruzzi, E.; Doni, S.; Manzi, D.; Azzini, L.; Pathan, S.I.; Pietramellara, G.; Arfaioli, P.; Nicese, F.; Masciandaro, G.; et al. Biological Assessment of Green Waste and Dredged Sediment Co-Composting for Nursery Plant Cultivation. *Appl. Sci.* **2024**, *14*, 5767. https://doi.org/10.3390/app14135767

Academic Editors: Dimitris P. Makris, Pablo Melgarejo Moreno, Dámaris Núñez-Gómez and Pilar Legua Murcia

Received: 24 May 2024
Revised: 21 June 2024
Accepted: 28 June 2024
Published: 1 July 2024

1. Introduction

Microorganisms play a key role in the feasibility of aerobic composting as microbial and fungal enzymatic activities that drive organic matter (OM) degradation and stabilization. In fact, bacteria and fungi mainly obtain carbon and nitrogen from organic matter to produce microbial biomass. Specifically, carbon is used as an energy source and nitrogen for protein synthesis (Azim et al. 2018) [1]. β-glucosidase (BG), acid phosphatase (AP), and N-acetyl-β-D-glucosaminidase (NAG) are among the main enzymes involved in OM degradation during co-composting. The BG catalyzes the hydrolysis of cellobiose residues in plant debris, producing glucose as a C energy source for microbial growth. AP allows the hydrolysis of esters and the anhydrides of phosphoric acid and NAG is an N-acquiring enzyme from chitin and peptidoglycan [2,3] (Moorhead et al. 2023; Adetunji et al. 2018). The operating conditions (e.g., temperature, pH) affect the proliferation and activity of

microbial communities in the composting process. Temperature is one of microbial activity indicators (Azim et al. 2018) [1]. The main microorganism groups involved in composting are mesophilic and thermophilic ones, including bacteria and fungi. The activities of microorganisms during composting affect the temperature, as a great amount of energy is produced but only a fraction (about 45%) is used for ATP production and the rest is lost as heat [4] (Nemet et al. 2021). At the beginning of composting, mesophilic bacteria colonize and degrade labile organic matter, increasing the temperature inside the pile. At temperatures above 45 °C (thermophilic phase), thermophilic fungi and bacteria enhance their activities and the further increase in the temperature allows the compost sterilization. In the thermophilic phase, the highest microbial diversity and variability in microbial community can occur, as seen in olive mill waste composting (Federici et al. 2011) [5]. Afterwards, the temperature drops allow mesophilic microorganisms to colonize the pile and the co-compost stability is reached when the microbial activities reduce [6,7] (Ayilara et al. 2020; Amuah et al. 2022). In addition, pH values around neutrality are optimal for microbial development during the co-composting and fungi in particular are more tolerant to pH variations (Azim et al. 2018) [1]. The properties of raw materials can also affect the microbial activities during co-composting, including the carbon-to-nitrogen ratio (C/N). Optimal values of 25 for C/N are commonly recommended in raw materials. In material with higher initial C/N ratio, such as lignocellulosic ones, the organic matter degradation is limited due to carbon chain complexity (Azim et al. 2018) [1]. The selection of raw materials and operation conditions can influence the bacteria and fungi abundance during the co-composting, affecting the co-compost quality. Hernández-Lara et al. (2022) [8] claimed that the combination of different waste materials (e.g., vineyard pruning, tomato waste) can favor the development of microorganisms with suppressive activities against pathogens, improving the co-compost quality.

The efficiency of aerobic composting in organic waste recovery and recycling has been widely demonstrated, producing stable fertilizers [9] (Xu et al. 2023). However, the performance of composting depends on the initial material properties. In green waste (GW), the lignocellulosic biomass affects the composting process, due to the recalcitrance of lignin that wraps cellulose and hemicellulose in the plant structure. In the early stage, the cellulose is the main carbon source for bacteria, but their ability to degrade lignin is limited. In fact, the lignin degradation is mainly driven by fungi. The low biodegradability of lignocellulosic biomass restrains the GW recovery through composting. To overcome this problem, chemical and biological pre-treatments are applied to enhance the composting feasibility [6,10,11], but they are often expensive, such as nitrogen-rich additives or woody fraction removal [12,13] (Inghels et al., 2016; Reyes-Torres et al. 2018; Ayilara et al. 2020; Wu et al. 2023; Liu et al., 2023). The co-composting of GW with other matrices can enhance GW recycling and management, simultaneously allowing different wastes to be recovered. Sediment (S), dredged from water bodies, has been successfully co-composted with GW such as municipal [14,15] (Mattei et al. 2016; Feng and Zhang 2022), agricultural [16,17] (Macci et al. 2022 and 2023) and marine [18] (Peruzzi et al. 2020) plant residues. In particular, the S addition to GW can improve the water-holding capacity, electrical conductivity and nutrient availability of the final product [15] (Feng and Zhang 2022). In addition, the S-GW co-composting is effective in the reduction in organic pollutants, of which S are rich [14,17] (Mattei et al. 2016; Macci et al., 2023). However, previous studies demonstrate some limitations in S-GW co-composting, that can reduce the field of application of the final products. The restricted thermophilic phase has been observed in 0.200 m^3 piles with 1S:1GW and 3S:1GW (v–v) ratios, affecting the sterilization of the co-composts [14] (Mattei et al. 2016). In addition, the high pH of the co-composts limits their application as growing media, especially in agriculture [14] (Mattei et al., 2016). Peruzzi et al. [18] (2020) noticed an organic carbon content in S-GW-based co-composts that did not comply with the local normative limits for agricultural applications. Changes in S and GW ratios can affect the co-composting process as well as the maturity of co-composts [19] (Zhang et al. 2023). In particular, GW can enhance the microbial diversity during the co-

composting thanks to its role as an energy source [20] (Mattei et al. 2017). Macci et al. [17] (2023) demonstrate that the addition of a high proportion of GW in the co-compost pile improves the S-GW co-composting and the quality of the final products, while the co-compost with a high proportion of S (3S:1GW) shows some limitation for its agricultural applicability, according to national law [17] (Macci et al. 2023).

S-GW co-composting allows the production of sustainable growing media that can replace peat for agricultural production [17,21] (Macci et al. 2023; Nicese et al. 2024). In fact, peat is the main non-renewable growing media used for plant cultivation and the research of alternative growing media is of pressing interest. The use of peat implies high costs for its extraction and transport as well as the negative environmental impact for peatland exploitation [22,23] (Pascual et al. 2018; Räsänen et al. 2023). The application of S-GW co-compost as growing media for peat replacement has been previously investigated for ornamental plants, with positive effects on plant performance [20,21,24] (Mattei et al. 2017; Vannucchi et al. 2022; Nicese et al. 2024). Although the species-specific effects of S-GW co-compost on the plant growth has been observed, the use of S-GW co-compost as growing media leads to a reduction in GHG emissions around 11.66–23.1% [21] (Nicese et al. 2024). In addition, the use of co-compost, derived from the S-GW co-composting, can improve the plant tolerance to abiotic stress and the nutritional status, as seen in ornamental plants growing on co-compost, composed of S and *Posidonia oceanica* residues [24] (Vannucchi et al. 2022)

The biological assessment of the S-GW co-compost stability can allow one to better assess their recycling and their application, especially for co-compost with high percentage of S. The co-compost stability is related to microbial activities that reduce during the co-composting, along with the organic matter degradation degree. However, the microbial nutrient limitation can occur after the application of the co-compost as a growing media. The instability of the growing media leads to a further decomposition of organic material and the uptake of nutrients by a microorganism causes a plant nutrient deficiency [25] (Barret et al. 2016). The eco-enzyme stoichiometry theory (EST) can allow one to better assess the biological stability of the co-composts as it links the enzyme activities and microbial nutrient availability. In nutrient and energy-limited conditions, an imbalance amongst enzyme activities occurs, and thus there is a deviation from the expected enzyme ratio (BG:NAG:AP = 1:1:1). This enzyme unbalance leads to a higher production of enzymes for a specific nutrient and/or energy source [26,27] (Hill et al. 2014; Sinsabaugh et al. 2012), affecting the stability of growing media. EST has already been applied to assess the efficiency of different biological technologies for the recovery of soil [28,29] (Xu et al. 2020; Yadav et al. 2022) and sediments [30] (Macci et al. 2021). To our knowledge, EST has not been applied in the evaluation of co-compost stability.

The hypothesis of this study was that the microbial community composition as well as the enzyme activities and ratios are good indicators to better understand the stability of the S-GW co-composts and their suitability for agricultural uses, especially for co-compost with high S percentage. For this purpose, the EST approach was applied in the evaluation of the S-GW co-composting feasibility and the stability of the final co-composts before and after plant cultivation. In addition, the effect of different ratios of S and GW on microbial biomass, respiration and abundance was evaluated during the co-composting. To do so, in the framework of LIFE AGRISED project (LIFE17 ENV/IT/269), S-GW co-composting was carried out for 100 days and the main chemical properties were monitored to evaluate the suitability of the process. The obtained co-composts were tested as growing media for the cultivation of ornamental container-grown shrubs (*Photinia x fraseri* and *Viburnum tinus* L.), ones of the commonly cultivated plants in the Italian nursery sector.

2. Materials and Methods

2.1. Green Waste and Dredged Sediment Collection

The sediments were dredged from the Navicelli Canal in Pisa (Italy), a navigable canal which connects Pisa to the sea at the Port of Livorno (length: 16 km, width: 32 m, depth: 3 m). Prior to the co-composting process, the dredged sediments (S) were stored in a basin along the Navicelli canal for about 1 month, which was necessary in order to lose a large percentage of water. The green waste (GW) was provided by Agrobios (Pistoia, Italy), an Agricultural Cooperative specialized in the recovery and enhancement of agricultural by-products. GW was derived from the ornamental nursery sector and was composed of both a herbaceous and woody component. GW was crushed and screened to obtain particles of 3–10 cm, ensuring air diffusion. In Table 1, DS and GW characteristics are reported. The heavy metal concentrations were below the law limits for Italian legislation for the application in agriculture [31] (D.lgs. 75/2010).

Table 1. Properties of the dredged sediments (S) and green waste (GW) used in the co-composting.

Chemical Parameters		S	GW
pH		7.68 ± 0.04	6.51 ± 0.01
EC	dS/m	4.20 ± 0.02	0.17 ± 0.01
TOC	%	1.41 ± 0.03	34.1 ± 5.7
TN	%	0.18 ± 0.01	1.64 ± 0.02
TOC/TN		7.83	20.8
Organic contamination			
C > 12	mg kg^{-1}	133.5 ± 10.2	
PCB		<LOD	
Heavy metals			
Cu	mg kg^{-1}	56.6 ± 4.7	35.3 ± 5.6
Zn		108 ± 1.0	24.6 ± 2.4
Cd		<LOD	<LOD
Ni		50.8 ± 1.0	7.02 ± 0.30
Pb		16.6 ± 0.7	3.11 ± 0.91
Cr		58.5 ± 5.6	19.3 ± 3.1

EC = electrical conductivity; TOC = total organic carbon; TN = total nitrogen; C > 12 = hydrocarbons C > 12; PCB = total polychloro biphenyls; LOD = limit of detection.

2.2. Co-Composting Set Up and Monitoring

The co-composting was carried out at "Gorini Piante" facilities (Pistoia, Italy), where three piles (6 m^3 each) were designed as follows (*v–v*): A = 3S:1GW; B = 1S:1GW; C = 1S:3GW. Urea (1 kg per m^3) was added to each pile, to facilitate the composting process in its initial phase. The piles were regularly homogenized, and the temperature was monitored as reported in Macci et al. [17] (2023). The temperatures were measured during co-composting using a thermometer (Checktemp®1, Hanna Instruments, Padova, Italy). The probe was inserted at least 1 m deep inside each pile to obtain a representative measure of the entire pile mass. Samples from each pile were collected before starting the co-composting and after 4 (T4), 30 (T30), 60 (T60), and 100 (T100) days, until the reduction in the total enzyme activities occurred as observed in Macci et al. [17] (2023). At each sampling time, the pH, electrical conductivity (EC), total nitrogen (TN), total organic carbon (TOC), TOC/TN, and humic acids (HA) were assessed. The germination index (GI) was only measured at T100.

One hour of water extraction (1:5 *v–v*) was carried out at room temperature for EC and pH determination. Afterwards, specific electrodes were used (EC: Hanna Instruments, Padova, Italy; pH: SevenMulti, Mettler Toledo, Milano, Italy). TOC and TN were determined by dry combustion using a FlashSmart elemental analyzer (Thermo Fisher

Scientific, Milan, Italy). HAs were determined on co-compost samples (1 g) by extraction with 0.05 M sodium pyrophosphate (pH = 9) overnight. H_2SO_4 was added to the surnatant and titration with 0.5 N Mohr salt was carried out [32] (Senesi et al. 1996). The hydrocarbon (C > 12) concentrations were determined using a gas chromatography–mass spectrometry (GC–MS) [33] (EPA 8270 E, 2018). Trace 1300 instrument with AS 3000 autosampler (Thermo Scientific, Waltham, MA, USA), following the official method [34] (UNI EN 14039, 2005). The GI was determined on seedling roots and seed of *Lepidium sativum* incubated for 72 h at 25 °C on co-compost water extract (1:5, *v–v*) in Petri dishes [35] (Hoekstra et al. 2002).

2.3. Ecoenzyme Activities and Stoichiometry

The methods proposed by Marx et al. [36] (2001) and Vepsäläinen et al. [37] (2001) were used for the determination of hydrolytic enzyme activities with fluorogenic methylumbelliferyl (MUF) substrates. The enzymes analyzed were β-glucosidase (BG; EC 3.2.1.21), acid phosphatase (AP; EC 3.1.3.2), and *N*-acetylglutamate synthase (NAG; EC 3.2.1.14). Each enzyme activities were measured after 0, 30, 60, 120, and 180 min of incubation, with an automated fluorimetric plate-reader (excitation 360 nm; emission 450 nm) (Infinite F200 pro TECAN). The ecoezyme stoichiometry approach was applied at T4, T60 and T100 as well as before and after the plant cultivation. The enzyme activities (BG, AP and NAG) were normalized to TOC and the ratio ln(NAG)/ln(BG), and ln(NAG)/ln(AP) were calculated, according to method proposed by Sinsabaugh et al. [27,38] (2008) and (2012). The results were reported in a scatterplot of eco-enzymatic stoichiometry, according to the method of Hill et al. [26] (2012).

2.4. Microbial Biomass, Respiration, and Abundance

During the co-composting (T0, T4, T8, T12, T16, T20, T30, T40, T60, T70, and T100), the soil microbial biomass and respiration were monitored. The soil microbial biomass was detected, according to the method proposed by Fornasier et al. [39] (2014). The microbial respiration was measured by the alkali titration method [40] (Anderson and Domsch, 1978). Specifically, 20 g of soil were placed in glass beakers, then the beaker with soil and a beaker with 4 mL of 1M NaOH were incubated in air-tight flasks in the dark at 25 °C for 3 days. After incubation, the beakers with NaOH were removed from flasks, 8 mL of 0.75 N $BaCl_2$ were added, and phenolphthalein indicator (1% solution in 95% ethanol *v*/*v*), and the solution was titrated against 0.1M HCl. The microbial diversity in terms of the abundance of bacteria and fungi was assessed by extracting the total DNA followed by real-time PCR method at T0 and T100. The total DNA was extracted using the Fast DNA™SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA). The yield of the co-compost-extracted DNA was checked by 2% agarose gel electrophoresis. The quality control and concentration of DNA was measured by Picodrop Microliter UV/Vis Spectrophotometer (Picodrop limited, Hinxton, UK). Real-time PCR was performed to quantify 16S rRNA (bacteria) and 18S rRNA (fungal) gene copies in soil DNA extracts. The 16S rRNA gene copies were determined with a primer set Eub341F [41] (Muyzer et al., 1993)/Eub515R [42] (Simmons et al., 2007), and 18S rRNA gene copies were determined with a set of primers FF390/ FR1 [43] (Prévost-Bouré et al., 2011).

2.5. Plant Trial

In April 2021, the one-year-old plants of two ornamental evergreen shrubs, Fraser Photinia (*Photinia x fraseri*), and Laurustinus (*Viburnum tinus*) were potted in 10 L (24 cm Ø) plastic containers with compost-based substrates, to which 4.5 g/L of Basacote® Plus (12M; 15N-15P_2O_5-15K_2O) was added. The three co-composted mixes, A (3S:1GW), B (1S:1GW), and C (1S:3GW), were used as they were, without adding any other components, while the control treatment substrate was obtained with a mix of coir peat and coco fiber (70:30). A total of 96 plants (48 for each species) were placed in a plant nursery in a

randomized complete block design with 12 replicates per treatment. The plants were spaced in a metal support iron grid, drip irrigated, and then, weed control was performed, covering the pots surface with natural coco coir fiber discs. At the end of the growing season (November 2021), four plants/treatment were planted out, roots were washed free of media, and the total height and fresh weight of the plants measured, as well as their dry weight, the shoot–root (S/R) and dry/fresh weight (DW/FW) ratio. This allowed us to evaluate the effect of the different substrates on the growth of the plants tested.

2.6. Statistical Analysis

Statistical analysis was performed R Statistical Environment (R development Core Team 2008). One-way ANOVA, followed by the HSD Tukey's test ($p < 0.05$), was carried out within each time and within each co-compost type.

3. Results

3.1. Co-Compost Maturity and Stability

The maximum temperatures reached in the thermophilic phase during the co-composting were 34.1, 44.5 and 64.2 °C in pila A, B, and C, respectively. The Pile C showed a higher temperature than the other piles and the peaks of temperatures were in correspondence with pile homogenization (Figure 1). Table 2 reported the monitoring of the main parameters during co-composting. At the beginning of co-composting, the piles significantly differed in pH ($p < 0.001$), EC ($p = 0.01$), TOC ($p < 0.001$), TN ($p < 0.001$), TOC/TN ($p = 0.02$) and HA ($p = 0.07$) (Table 2). In particular, the pH had the following trend in the piles A > B > C, while for EC and HA, the trend was A > B = C. Instead, the trend of A < B < C was observed for TOC and TN. During the composting, the pH significantly decreased in pile B ($p < 0.001$), while in pile C, a significant decrease was observed for EC ($p < 0.001$), TOC and TN ($p < 0.001$). An increase in TOC/TN ($p < 0.001$) was observed in piles A and C. At T4, the HA was lower in pile B then A and C ($p < 0.001$), and it remained constant during the co-composting. Generally, no variation was also observed in C for HA. Instead, the HA decreased in pile A during the process ($p < 0.001$). At the end of co-composting, the three co-composts differed in the chemical properties, as seen at the beginning. In particular, the co-compost C showed the highest TOC, TN, and the lowest EC for $p < 0.001$ (Table 2). As a result, the compost did not conform to Italian law [31] for its use as a mixed growing medium for TOC (>4%) in co-compost A and for EC (<1 dS m^{-1}) in co-composts A and C.

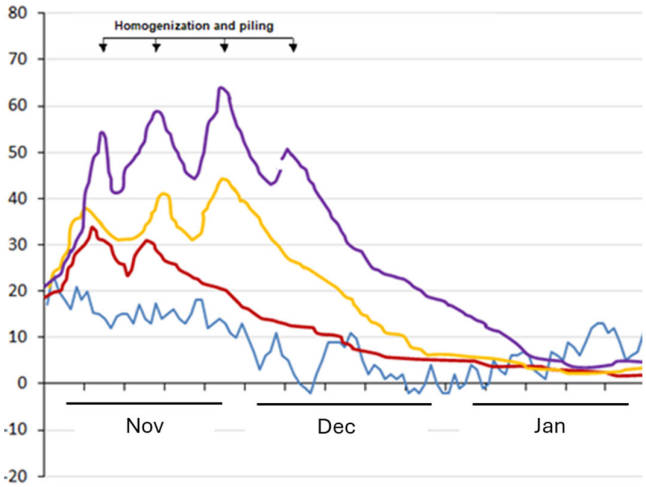

Figure 1. The time-course of temperature (°C) during the co-composting in pile A (red line), pile B (yellow line), and pile C (violet line). The ambient temperature is also reported (blue line).

Table 2. Monitoring of the co-composting chemical properties in 3S:1GW (A), 1S:1GW (B) and 1S:3GW (C) piles (±standard deviation). Different lowercase letters indicate statistically different values (time effect) within each co-compost. Different uppercase letters indicate statistically different values (treatment effect) within each time.

Pile		Sampling Time			
		4	**30**	**60**	**100**
pH	A	8.2 ± 0.13 Cb	7.9 ± 0.23 Bb	7.2 ± 0.15 Aa	7.4 ± 0.08 ABa
	B	7.7 ± 0.13 Bb	7.5 ± 0.03 Aa	7.4 ± 0.04 Ba	7.5 ± 0.03 Ba
	C	7.3 ± 0.16 Aa	7.4 ± 0.00 Aa	7.3 ± 0.06 ABa	7.3 ± 0.15 Aa
EC	A	3.0 ± 0.29 Ba	3.0 ± 0.33 Ca	2.9 ± 0.21 Ca	2.7 ± 0.2 Ca
	B	2.3 ± 0.16 Aa	2.6 ± 0.06 Ba	2.4 ± 0.30 Ba	2.4 ± 0.15 Ba
	C	2.5 ± 0.30 Ac	1.8 ± 0.14 Ab	1.3 ± 0.09 Aa	1.2 ± 0.00 Aa
TOC	A	1.72 ± 0.63 Aa	1.72 ± 0.40 Aa	1.65 ± 0.05 Aa	1.86 ± 0.06 Aa
	B	4.33 ± 1.45 Bab	4.07 ± 0.28 Ba	5.78 ± 0.84 Bb	3.54 ± 0.62 Ba
	C	11.25 ± 0.52 Cc	10.49 ± 0.10 Cb	11.66 ± 0.49 Cc	9.39 ± 0.25 Ca
TN	A	0.22 ± 0.09 Aa	0.14 ± 0.01 Aa	0.13 ± 0.01 Aa	0.15 ± 0.01 Aa
	B	0.36 ± 0.07 Bab	0.31 ± 0.03 Ba	0.38 ± 0.01 Bb	0.31 ± 0.02 Ba
	C	1.17 ± 0.08 Cd	0.74 ± 0.02 Cb	0.71 ± 0.00 Cc	0.58 ± 0.02 Ca
TOC/TN	A	8.0 ± 7.19 Aa	12.2 ± 2.73 Ab	12.4 ± 2.87 Ab	12.7 ± 4.84 Ab
	B	11.8 ± 4.76 Ba	13.3 ± 4.21 ABab	15.1 ± 4.48 Bb	11.4 ± 3.96 Aa
	C	9.6 ± 5.69 Aa	14.2 ± 5.08 Bb	16.4 ± 4.12 Cc	16.2 ± 5.20 Bc
HA	A	71.9 ± 10.17 Bc	27.3 ± 3.86 Aa	28.7 ± 4.06 Aa	48.4 ± 6.84 ABb
	B	47.6 ± 6.73 Aa	42.1 ± 5.95 Ba	44.8 ± 6.34 Ba	39.6 ± 5.60 Aa
	C	56.9 ± 8.05 Ab	50.8 ± 7.18 Bab	41.2 ± 5.83 Ba	52.0 ± 7.35 Bab

EC = electrical conductivity (dS m^{-1}); TOC = total organic carbon (%); TN = total nitrogen (%); HA= humic acids (%).

At the beginning of co-composting, butyrate esterase showed the highest activities in piles B and C ($p < 0.001$). During the composting, the butyrate esterase significantly increased the activity in pile A and C and reduced after 60 days in all piles (Figure 2). At the end of co-composting (T100), the hydrocarbon concentrations (C > 12) were 50 mg kg^{-1} in all piles, in line with the Italian limits (50 mg kg^{-1}) [44] (Dlgs. 152/2006) and the GI (A = 93%; B = 79%; C = 76%) was higher than 60% in all piles at T100 (Table 2), in accordance with Italian law [31] (D.lg. 75/2010).

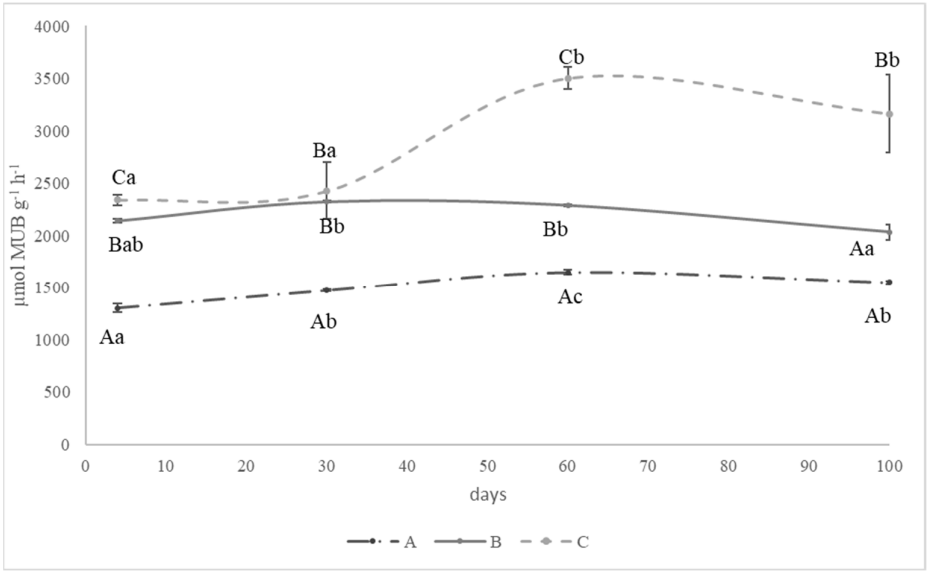

Figure 2. Time course of butyrate esterase activity during the co-composting in piles A (3S:1GW), B (1S:1GW), and C (1S:3GW). Different lowercase letters indicate statistically different values (time effect) within each co-compost. Different uppercase letters indicate statistically different values (treatment effect) within each time. Bars represent standard deviation.

In Figure 3, the enzymatic stoichiometry analyzed during co-composting and after plant cultivation is analyzed. Specifically, the dot lines in Figure 2 represent the equilibrium amongst enzyme activities (BG:NAG:AP = 1:1:1) and the variation from the equilibrium revealed the microbial nutrient limitation. At T4, co-compost A showed N-limited condition as ln(NAG)/ln(AP) was above 1 (1.05 ± 0.004), while co-compost B and C showed values for ln(NAG)/ln(AP) below 1 (0.96 ± 0.012 and 0.93 ± 0.005), respectively, resulting more P limited. The C-to N-acquiring enzyme ratio (ln(BG)/ln(NAG)) was below 1 in co-compost A (0.89 ± 0.003), confirmed the N-limited condition, while in co-composts B and C, the BG and NAG ratios were close to equilibrium (BG:NAG = 1:1) (B = 0.98 ± 0.016; C = 1.01 ± 0.011). After 60 days of co-composting, co-compost A showed the values of 1.00 ± 0.001 and 0.97 ± 0.001 for ln(BG)/ln(NAG) and ln(NAG)/ln(AP) ratios, and as a result, were P-limited. In co-compost B, the ln(BG)/ln(NAG) and ln(NAG)/ln(AP) ratios reached values of 1.02 ± 0.016 and 0.93 ± 0.013, respectively, shifting from P-limited to C- and P-limited. The co-compost C showed the following values: ln(BG)/ln(NAG) =1.05 ± 0.027 and ln(NAG)/ln(AP) = 0.92 ± 0.01, remaining in C- and P-limited conditions. At the end of the co-composting, the co-compost A resulted in a P-limited (ln(BG)/ln(NAG) = 0.97 ± 0.01; ln(NAG)/ln(AP) = 0.98 ± 0.001). Instead, the co-composts B and C were C and P limited, showing values of 1.03 ± 0.01 and 1.04 ± 0.061 in co-composts B and C, respectively, while the ln (NAG)/ln (AP) reached the following values: 0.91 ± 0.017 in B and 0.93 ± 0.043 in C.

After plant cultivation, the following values were detected in co-compost A: ln (BG)/ln (NAG) = 1.01 ± 0.03 and ln (NAG)/ln (AP) = 0.84 ± 0.02 in *Viburnum* trial and ln (BG)/ln (NAG) = 1.02 ± 0.01 and ln (NAG)/ln (AP) = 0.88 ± 0.05 in *Photinia* trial (Figure 2). The co-compost B showed the following enzymatic ratios: ln (BG)/ln (NAG) = 1.03 ± 0.06 and 1.13 ± 0.02 as well as ln (NAG)/ln (AP) = 0.85 ± 0.01 and 0.81 ± 0.01 in *Viburnum* and *Photinia* trials, respectively. The co-compost C showed values of 1.07 ± 0.03 and 0.86 ± 0.02 for ln (BG)/ln (NAG) and ln (NAG)/ln (AP), respectively, in *Viburnum* trial. In the *Photinia* trial, ln (BG)/ln (NAG) and ln (NAG)/ln (AP) acquired values of 1.16 ± 0.02 and 0.84 ± 0.03, respectively.

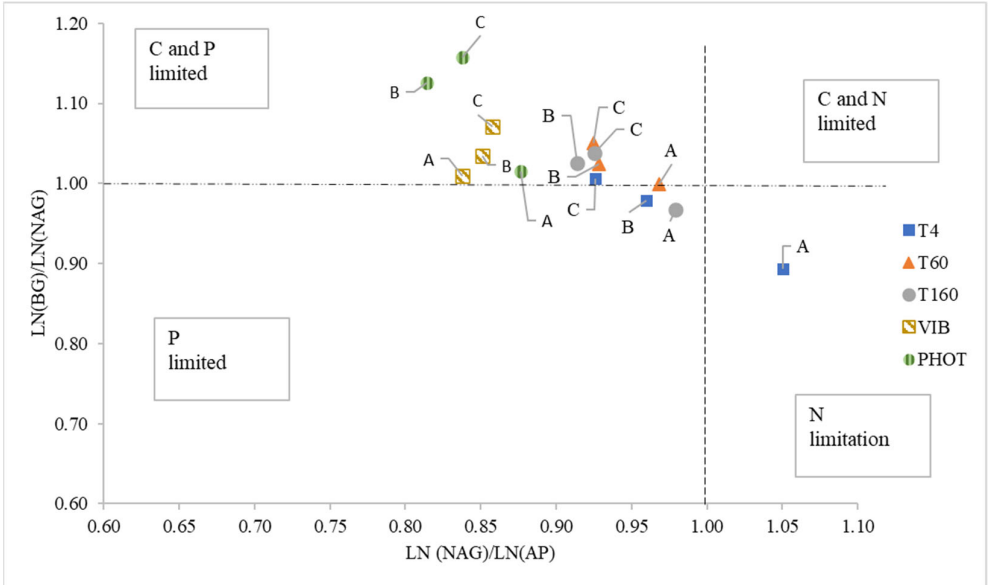

Figure 3. Ecoenzymatic stoichiometry of the co-composts (A = 3S:1GW; B = 1S:1GW; C = 1S:3GW) after 4 (T4), 60 (T60), and 100 (T100) days of co-composting and after the plant cultivation. The dot

lines represent the equilibrium amongst enzymatic activity ratios. BG = β-glucosidase; AP = acid phosphatase; NAG = *N*-acetylglutamate synthase. VIB = *Viburnum tinus*; PHOT = *Photinia* and *phaseri*. Dotted lines represent the equilibrium between enzyme activities (lnBG:lnNAG:lnAP 1:1:1).

3.2. Microbial Biomass, Respiration, and Abundance

Microbial biomass differed amongst piles at T0, with a higher value in pile B, increasing during co-composting, especially in pile C (Figure 4A,B). At T100, the highest microbial biomass was observed in pile C (Figure 4B). The microbial respiration generally decreased during the co-composting and at T100, the piles showed significant differences. The pile C had a higher respiration than B, while pile A showed middle values (Figure 4C,D). The qPCR results showed that the abundance of the bacterial and fungal communities differed in pile B compared to A and C at T0 (Figure 5A,B). At T10, Pile C had the highest abundance of bacteria and fungi than pile A (Figure 5B).

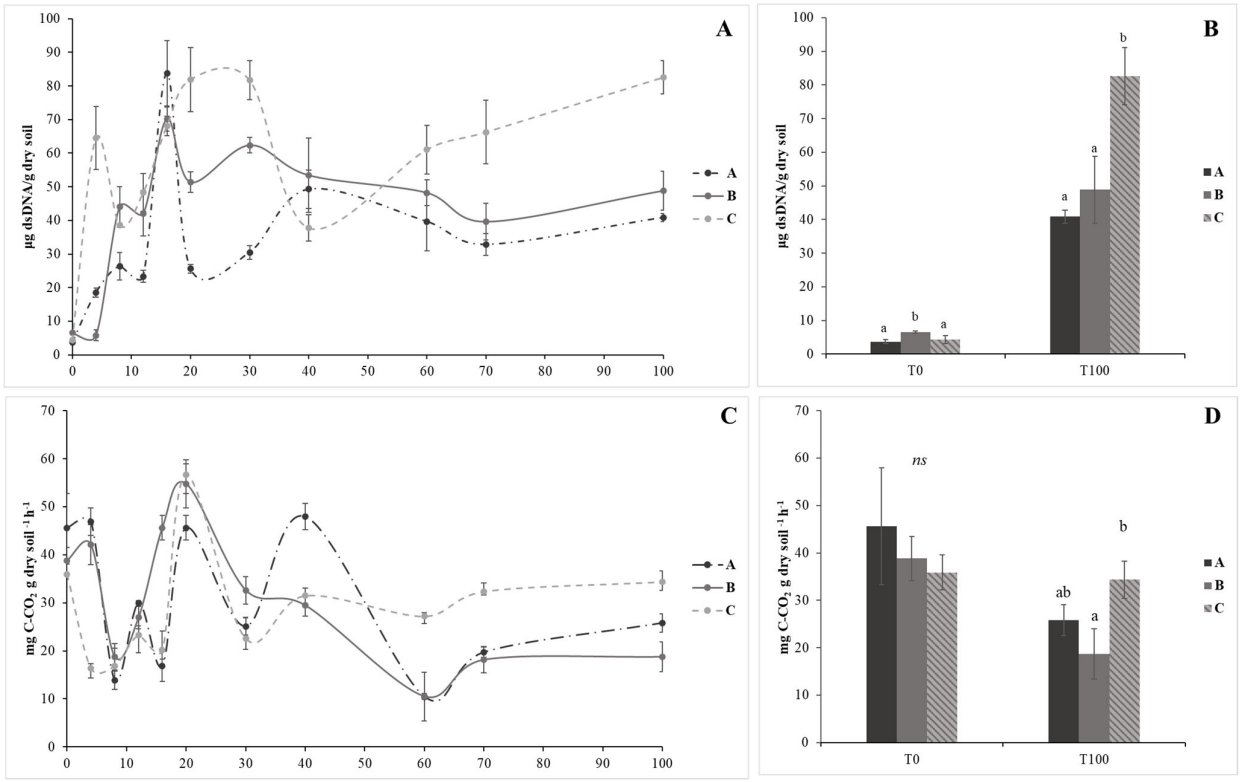

Figure 4. Microbial biomass (**A**,**B**) and respiration (**C**,**D**) (±standard deviation) in the three piles during the composting process. Compost pile A (3S:1GW), B (1S:1GW), and C (1S:3GW). Different lowercase letters represent significant differences for $P < 0.05$. ns = not significant.

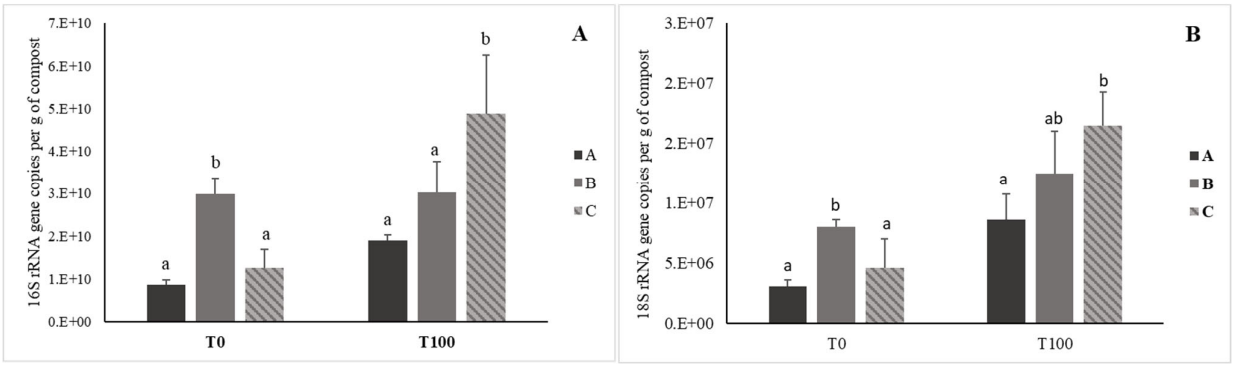

Figure 5. The 16S (bacterial, **A**) and 18S (fungi, **B**) rRNA genes copy numbers (±standard deviation) in the three piles during the composting process. Compost pile A (3S:1GW), B (1S:1GW), C (1S:3GW)**.** Different lowercase letters represent significant differences for $P < 0.05$.

3.3. Plant Trial

The results of the cultivation test with S-GW-based growing media showed different responses between the *Photinia* and *Viburnum* plants. All the growth data (total height, above and below ground dry matter, leaf area) for *Photinia* resulted in significantly higher control plants, while S/R and DW/FW ratios showed no differences (Table 3). Instead, the *Viburnum* plants did not show any effect due to the tested growing media: the differences in all the growth data were not significant among the different substrates (Table 4).

Table 3. Average (±standard deviation) growth measures in *Photinia x fraseri* plants (n = 4), growing on 3S:1GW (A), 1S:1GW (B), and 1S:3GW (C)-based growing media. Different letters indicate statistically different values amongst substrates, according to the one-way ANOVA followed by HSD Tukey's test ($p \leq 0.05$). ns = not significant.

		Dry Weight (kg)					
Substrate	**Height (cm)**	**Aboveground**	**Belowground**	**Total**	**Shoot/ Root**	**DW/ FW**	**Leaf Area (m^2)**
Control	128 ± 2.5 a	0.46 ± 0.05 a	0.14 ± 0.02 a	0.60 ± 0.03 a	3.31 ± 0.78 ns	0.53 ± 0.01 ns	0.92 ± 0.02 a
A	104 ± 7.0 b	0.31 ± 0.03 b	0.08 ± 0.01 b	0.39 ± 0.02 b	3.57 ± 0.49 ns	0.55 ± 0.02 ns	0.61 ± 0.07 b
B	113 ± 9.5 ab	0.34 ± 0.03 b	0.11 ± 0.01 ab	0.45 ± 0.02 b	3.24 ± 0.60 ns	0.53 ± 0.02 ns	0.66 ± 0.10 b
C	110 ± 4.0 b	0.33 ± 0.04 b	0.09 ± 0.02 b	0.42 ± 0.05 b	3.60 ± 0.30 ns	0.55 ± 0.01 ns	0.76 ± 0.08 ab

DW = dry weight; FW = fresh weight.

Table 4. Average (±standard deviation) growth measures in *Viburnum tinus* plants (n = 4), growing on 3S:1GW (A), 1S:1GW (B), and 1S:3GW (C)-based growing media. Different letters indicate statistically different values amongst substrates, according to one-way ANOVA followed by HSD Tukey's test ($p \leq 0.05$). ns = not significant.

		Dry Weight (kg)					
Substrate	**Height (cm)**	**Aboveground**	**Belowground**	**Total**	**Shoot/ Root**	**DW/ FW**	**Leaf Area (m^2)**
Control	45.3 ± 5.77 ns	0.25 ± 0.01 ns	0.05 ± 0.02 ns	0.30 ± 0.03 ns	5.13 ± 1.58 ns	0.42 ± 0.03 ns	0.63 ± 0.08 ns
A	48.7 ± 5.77 ns	0.23 ± 0.05 ns	0.07 ± 0.01 ns	0.30 ± 0.07 ns	3.25 ± 0.35 ns	0.43 ± 0.01 ns	0.61 ± 0.17 ns
B	46.7 ± 8.15 ns	0.22 ± 0.05 ns	0.06 ± 0.01 ns	0.28 ± 0.05 ns	3.94 ± 1.03 ns	0.43 ± 0.03 ns	0.56 ± 0.11 ns
C	44.7 ± 2.08 ns	0.20 ± 0.04 ns	0.05 ± 0.01 ns	0.25 ± 0.05 ns	3.58 ± 0.13 ns	0.44 ± 0.02 ns	0.54 ± 0.05 ns

4. Discussion

Although the suitability of GW-S co-composting has been already demonstrated, in previous works, restrictions had been observed for the agricultural application of co-composts, especially those with a high S amount [14,17,18] (Mattei et al. 2016; Peruzzi et al. 2020; Macci et al. 2023). The biological assessment (microbial biomass and abundance, respiration rate, eco-enzyme stoichiometry, plant cultivation) allowed for the extensive investigation of the dynamic of S-GW composting and the stability of final composts to better valorize their application for agricultural purposes.

The initial chemical properties and the monitoring of co-composting confirmed the suitability of the S and GW to be co-composted, as previously seen in Macci et al. [17] (2023). In fact, the pH was in an optimal range (5.5–8) for the microbial development and activities [1] (Azim et al. 2018). The increase in microbial biomass and respiration rate during co-composting suggests the optimal conditions for biological activities and the absence of possible osmotic stress, despite the initial high EC in all piles [45–47] (Bremer and Krämer, 2019; Yan et al., 2012; Sanchez 2023). The relative amount of TOC and TN was an important indicator of nutrient contents and organic matter degradation fate [48] (Onwosi

et al., 2017). The three piles showed a lower initial TOC/TN than the recommended values (25–30), suggesting a fast organic matter degradation process and the possible ammonia volatilization [1,48,49] (Jurado et al., 2014; Onwosi et al., 2017; Azim 2018). However, the feasibility of composting at lower TOC/TN ratios had been previously demonstrated [50] (Kumar et al. 2010), including for S-GW co-composting [17] (Macci et al. 2023). During the co-composting, the TOC reduction is expected due to the conversion of organic carbon into CO_2 by microbial metabolism [1] (Azim 2018). However, such a decrease could be limited in co-compost with high S amount due to the low content of less degradable carbon sources in the S [17] (Macci et al. 2023). In fact, the TOC significantly reduced only in pile C, while in the other piles, the TOC reduction was not statistically significant. However, the trend of the respiration rate better highlighted that the organic degradation occurred in all piles: in fact, after the first phase of the reduction in respiration rates, probably due to the microbial acclimatation to experimental conditions [51] (Nikaeen et al. 2015), the microbial respiration rate increased in all piles. The microbial respiration is positively correlated to the presence of the labile fraction of organic matter, stimulating the biological activities and the organic matter degradation [47,51] (Nikaeen et al. 2015; Sanchez 2023). Also, the reduction in TN occurred in pile C and it could be related to the release of ammonia, which could have phytotoxic effects [1] (Azim et al. 2018). However, the germination test highlighted the absence of toxic effects in pile C, as well as in others, with values above 50%, thus the final co-compost resulted suitable for plant growth [18] (Peruzzi et al. 2020). This was also enforced by the metal concentrations below the legislation limits for the use of the co-compost as growing media [31] (D.lgs. 75/2010).

The eco-enzyme stoichiometry revealed the differences in microbial energy and nutrient limitation amongst piles. At the beginning of the process, in pile A, the microorganisms were N-limited as a result, showing an unbalance in the enzyme activities towards the N acquisition. This is also confirmed by the lower TN detected in A than in the other co-composts due to the high S content [14,17] (Mattei et al. 2016; Macci et al. 2023). The co-compost B in the starting phase showed P-limited conditions, in association with the higher abundance of bacteria and fungi than the other piles. The increasing percentage of GW in the co-compost (pile B) increased the presence of readily available compounds that could stimulate the microbial community, as the GW acted as an energy source increasing the microbial P demand [14,17,52] (Elser et al. 2003; Mattei et al. 2017; Macci et al. 2023). However, the high GW content (pile C) also led to microbial C and P limitations, probably due to the high amount of recalcitrant polymer (e.g., lignin) that reduce the accessibility to cellulose and hemicellulose. Because the lignin degradation is species-specific [4] (Nemet et al. 2021), the microbial abundance and growth reduced in pile C, thus the abundance of bacteria and fungi resulted similar to A. During the co-composting, changes in microbial limitation were observed, reflecting differences in the co-compost stability. A reduction in the nitrogen limitation occurred in pile A, as confirmed by the balance between BG and NAG activities. Instead, the unbalance towards the P-related enzyme production could be related to the increase in microbial biomass and respiration during the process, and thus, the microbial requirement for P to sustain the microbial growth [52] (Elser et al. 2003). The P-limited condition in pile A remained at the end of the process; however, the enzymatic ratio close to the equilibrium (BG:NAG:AP = 1:1:1), coupled with the reduction in microbial respiration (i.e., biological activity), conferred high stability as growing media upon co-compost A [25,47] (Barret et al. 2016; Sanchez 2023). Pile B shifted from P-limited to C- and P-limited, and thus, the microbial needs for C-acquisition increased and remained until the end of the process. Pile C did not change the microbial energy and nutrient (C and P) limitations, but at the end of the co-composting showed the higher bacteria and fungi abundance as well as microbial biomass and respiration. This suggested the persistence of biodegradable organic matter in pile C, which led to a higher respiration rate and affected the microbial community, resulting in co-composts that were less stable than others [47,53] (Gomez et al. 2006; Sanchez 2023).

As seen by Nicese et al. [21] (2024), the plant trial revealed a species-specific plant response to S-GW co-compost. *Viburnum tinus* similarly grew in all the co-composts and in control substrates, and thus, the co-compost did not affect plant elongation. Similar results had been observed by Nin et al. [54] (2022) and Vannucchi et al. [24] (2022) for cherry laurel and *V.tinus*, growing on S-based and S-GW compost-based growing media, respectively. In contrast to Nicese et al. [21] (2024), and Mattei et al. [20] (2017) results, in this study *Photinia* appeared more sensitive to S-GW co-composts than *V. tinus*, showing values in plant growth parameters (plant height and aboveground) lower than in the control substrate. Instead, no differences were found among the S-GW co-composts, suggesting that the co-compost suitable was, as a result, suitable, and independently so from the S and GW ratio, while the plant performance depended upon the plant sensibility to substrate substitution. After plant cultivation, the co-compost A maintained a BG and NAG ratio close to equilibrium (lnBG:lnNAG = 1:1), while an unbalance towards the production of P-acquiring enzyme occurred (decrease in NAG/AP). Instead, the other co-composts (B and C) showed higher unbalances than A. This suggested the possible reutilization of co-compost A for other vegetative cycles, e.g., mixing with other substrates to reduce the P limitation, improving the sustainability in crop production [55] (Diara et al. 2012).

5. Conclusions

The biological assessment better investigated the dynamic of S-GW composting and the stability of S-GW composts. Although the initial TOC/TN was below the recommended values and the TOC reduction was limited during the co-composting, the increase in the microbial respiration and the following reduction during the co-composting highlighted that the organic matter degradation occurred in all the co-compost piles. Microbial abundance reflected the S-GW proportion in the co-compost piles. The high GW content in the pile led to the persistence of biodegradable compounds at the end of the process, increasing the abundance of bacteria and fungi. At the end of the process, the eco-enzyme stoichiometry revealed a greater stability for the co-compost with a higher proportion of dredged sediment (3S:1GW), as the enzymatic activity ratios grew closer to equilibrium as a result (BG:NAG:AP = 1:1:1). The stability was also maintained after the plant cultivation, making the 3S:1GW co-compost suitable for more than one vegetation cycle. The plant trial also confirmed the suitability of all tested S-GW co-composts to plant cultivation, even though the plant sensitivity to the substrate affected the plant performance. These results contributed to better valorization, the application of the S-GW co-compost in agriculture, especially in the prospective of law legislation updating.

Author Contributions: Conceptualization, G.P., P.A., F.P.N., G.M., and C.M.; methodology, F.V., E.P., S.D., L.A. and S.I.P.; software, F.V. and S.I.P., validation, F.V., E.P., S.D., D.M., G.M., and C.M., formal analysis, F.V., S.I.P., and P.A; investigation, F.V., E.P., S.D., D.M., L.A., F.P.N., G.M., and C.M.; resources, G.P., F.P.N., G.M., and C.M.; data curation, F.V., E.P., S.D., D.M., and C.M.; writing—original draft preparation, F.V. and C.M.; writing—review and editing, F.V., E.P., S.D., L.A., F.P.N., G.M. and C.M.; visualization, F.V. and S.I.P.; supervision, C.M.; project administration, C.M.; funding acquisition, C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by AGRISED (LIFE17 ENV/IT/269) "Use of dredged sediments for creating innovative growing media and technosols for plant nursery and soil rehabilitation" and by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUPB83C22002930006, Project title "National Biodiversity Future Center—NBFC".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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