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Promising Composts as Growing Media for the Production of Baby Leaf Lettuce in a Floating System

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Abstract: The floating system is a successful strategy for producing baby leaf vegetables. Moreover, compost from agricultural and agri-food industry wastes is an alternative to peat that can be used as a component of growing media in this cultivation system. In this study, we experimented with three composts containing tomato (*Solanum lycopersicum* L.), leek (*Allium porrum* L.), grape (*Vitis vinifera* L.), and/or olive (*Olea europaea* L.) mill cake residues, which were used as the main component (75/25 volume/volume) of three growing media (GM1, GM2 and GM3) to evaluate their effect on the growth and quality of red baby leaf lettuce (*Lactuca sativa* L.). We used a commercial peat substrate as a control treatment (100% volume) and in mixtures (25% volume) with the composts. The plants were cultivated over two growing cycles, in spring and summer, and harvested twice in each cycle when the plants had four to five leaves. We found that the percentage of seed germination was significantly higher in plants grown in peat than in those grown in compost growing media. The yield was affected by the growing media in the summer cycle, and we obtained the highest value with GM1. Furthermore, the second cut was more productive than the first one for all the growing media in both cycles. The lettuce quality was also affected by the growing media. In general, the total phenolic content and antioxidant capacity in the leaves was higher in plants grown in the compost growing media, particularly in the second cut, but the nitrate content in the leaves was greater in some of the compost treatments compared with the peat treatment. In addition, an in vitro suppressive activity study demonstrated that the interaction between different fungi and bacteria observed through metagenomics analysis could contribute to the effectiveness of the compost in controlling *Pythium irregulare*. The use of compost as a component of the growing media in the production of baby leaf vegetables in a floating system does not only favor the crop yield and product quality, but also shows suppressive effects against *P. irregulare*.

Keywords: germination; nitrate content; phenolic content; antioxidant capacity; microbial community

1. Introduction

Nowadays, there is a high demand among consumers for ready-to-eat vegetables due to a growing interest in healthy, fresh convenience foods. Demand for baby leaf vegetables has especially

increased [1]. Baby leaf vegetables come in a wide variety of textures, colors and flavors, which makes them very attractive for consumption. Lettuce is considered to be a health-beneficial food due to the high concentrations of vitamins, minerals, dietary fiber, and antioxidant compounds different lettuces contain [2]. A wide range of varieties can be used for baby leaf production.

Among the hydroponic methods used to produce baby-leaf vegetables, the floating system is a successful strategy for producing baby leaf vegetables, which consists of trays floating on a waterbed or hydroponic nutrient solution, which can be operated as a closed system [1], resulting in a more environmentally friendly crop production strategy (Nicola et al., 2016) [3]. Among other reasons for their use, floating systems make it possible to obtain clean and safe products for the processing industry and to reduce crop cycle duration with respect to soil culture [4]. In addition, some baby leaf crops can be harvested more than once, if regrowth is allowed. With this latter approach, the time to harvest is shortened, resulting in a lesser environmental impact and a reduction in the economic cost [5].

Peat is the usual substrate used to fill the holes in the trays used for growing baby leaf vegetables in floating systems [6]. Nevertheless, peat increases susceptibility to some diseases, such as damping off, which is caused by fungi or oomycetes like *Pythium* spp., which can lead to significant production losses [7]. Moreover, peat comes from peatland ecosystems, and harvesting peat despoils ecologically important peat bog areas [8]; degraded peatlands negatively and disproportionally contribute to released stored carbon and an increase in global greenhouse gas emissions, affecting the environment and CO_2 balance [9,10]. The search for organic materials that can be used as peat alternatives has become increasingly important.

Compost from agricultural and from agri-food industry waste can be an alternative to peat in soilless culture systems. Furthermore, compost can control different plant pathogens, like *Fusarium* sp. [11] and *Pythium irregulare*, and improve the yield and quality of the final product [12]. In addition, compost use is an environmentally friendly practice in light of the circular economy [13]. Depending on its composition, compost made with so-called green materials, such as pruning waste, can in exceptional cases be used directly as a standalone substrate, but it is usually used as a growing media constituent [8,14]. The main limitations to the use of composts in growing media are their physical properties, salinity, high pH, and rate of residual degradation over time [15].

Compost can also be considered an important resource for the biofertilization and bio-stimulation of crops. During composting, organic matter is decomposed and transformed by microorganisms after a polymerization process to form humic substances [16], which have a very important effect on improving soil fertility, because they are rich in mature organic matter. Besides humic substances, the hormone-like molecules secreted by microbes and nutritional elements are compost components that may play a crucial role in the bio-stimulation of plants [17]. The compost microbiome composition plays an important role in the complex relationships that occur in the rhizosphere [18]. High-throughput sequencing technologies have provided an important way to determine compost microbiome information [19], rather than the isolation and identification of microorganism species.

The objective of this study was to characterize three composts from agro-industrial wastes and evaluate their impact as a growing media component on the yield and quality of a red baby leaf lettuce crop growing in a floating system. Our hypothesis was that composts could provide a biostimulant and biofertilizing effect on baby leaf lettuce in addition to its suppressive activity against *P. irregulare*.

2. Materials and Methods

2.1. Compost Characterisation

Three types of compost produced at the University Miguel Hernandez composting site were used for the experiment. In the compost feedstocks, the following raw materials were used: vineyard pruning, tomato and leek processing by-products, and olive mill cake. Their proportion in the composts is described in Table 1. The composting process for the three composts lasted 210 days and consisted of a composting phase with a mesophilic and thermophilic phase of 166 days and a maturation phase

of 44 days. The temperatures reached were >60 °C. The physical properties of the compost and peat (bulk density, total pore space, and total water holding capacity) were measured as described by Bustamante et al. [20] pH and electrical conductivity (EC) were measured in a water-soluble extract 1:10 (*w*/*v*) using a conductivity/pH meter (Crison). The total organic carbon (TOC) and total nitrogen (N) were measured using a LECO TruSpec C/N Elemental Analyzer. P, K, Ca, Mg, B, Fe, Mn, Mo, and Zn were determined by inductively coupled plasma-mass spectrophotometry (ICP-MS PQExCell, VG-Thermo Elemental, Winsford, Cheshire, UK), after $HNO_3/HClO_4$ high pressure digestion. Organic N, nitrate, and ammonium N were determined following the McKenzie and Young [21] method. Available phosphorus was extracted with ammonium citrate pH 7 and it colorimetrically determined on the extracts according to Watanabe and Olsen [22]. Available K was extracted with ammonium acetate pH 7 and later filtered through whatman 0.22 mm^2; it was determined by inductively coupled plasma-mass spectrophotometry, as the rest of the above-measured elements. All the analyses were performed in triplicate. Available humic acids were measured according to Sanchez-Monedero et al. [23]. For the biological characteristics, the bacterial and fungal colony forming units (CFUs) were counted after plating different tenfold serial dilutions of water extract from the composts/peat in Trypto-casein Soy Agar (TSA) plus cycloheximide (100 mg mL^{-1}) and potato dextrose agar (PDA) plus streptomycin (50 mg mL^{-1}), respectively. The Petri plates were incubated at 28 °C, and a standard plate count (SPC) was performed to determine the number of colonies of bacteria and fungi grown on the respective media after 7 and 5 days, respectively. The CFUs were counted and the values were multiplied by the dilution factor and expressed in log CFU g^{-1} of dry compost. Finally, dehydrogenase activity (DHA) was determined according to García et al. [24]

Table 1. Composition of composts in percentage of dry matter.

Composts.		**C1**	**C2**	**C3**
Feedstocks (% dry matter)	Vineyard wastes	54	42	41
	Tomato wastes	46	25	21
	Leek wastes	-	-	8
	Olive mill cake	-	33	30

C1, C2, and C3 represent the composts used.

The main physical, chemical, and biological characteristics of the composts are shown in Table 2.

Table 2. Main physical, chemical, and biological characteristics of the composts used.

	Peat	**C1**	**C2**	**C3**	
			Physical characteristics		
BD (g cm^{-3})	0.38 ± 0.01 b	0.19 ± 0.1 a	0.20 ± 0.1 a	0.20 ± 0.1 a	***
TPS (Vol %)	75.12 ± 0.10	88.4 ± 0.1	87.6 ± 0.2	87.9 ± 0.1	n.s.
AC (Vol %)	20.45 ± 1.23 a	20.6 ± 0.4 a	32.7 ± 0.3 b	34.6 ± 0.7 b	***
WHC (Vol %)	547 ± 11.25 a	678 ± 4.5 b	548 ± 4.9 a	533 ± 6.5 a	***
			Chemical characteristics		
pH	5.6 ± 0.03 a	8.41 ± 0.02 b	8.59 ± 0.03 b	8.84 ± 0.08 b	***
EC (dS m^{-1})	1.2 ± 0.01 a	5.44 ± 0.01 d	3.65 ± 0.04 b	4.75 ± 0.01 c	***
C/N	49.6 ± 0.3	10.9 ± 0.1	13.2 ± 0.5	12.2 ± 0.3	
TOC (g kg^{-1})	466 ± 0.2 b	378 ± 1 a	433 ± 1 b	404 ± 3 b	***
HA (g kg^{-1})	252.1 ± 9.0 c	44.2 ± 2.0 a	70.9 ± 1.0 b	70.5 ± 0.3 b	***
Total N (g kg^{-1})	9.4 ± 0.3 a	36.5 ± 0.5 b	35.2 ± 0.6 b	31.3 ± 0.4 b	***
Organic N (g kg^{-1})	9.3 ± 0.1 a	35.1 ± 0.5 b	34.1 ± 0.5 b	29.8 ± 0.5 b	***
Nitric N (g kg^{-1})	0.10 ± 0.03 a	0.81 ± 0.07 b	0.51 ± 0.09 b	1.1 ± 0.09 b	***
Ammonium N (g kg^{-1})	<0.01 a	0.61 ± 0.02 b	0.6 ± 0.03 b	0.5 ± 0.02 b	***

Table 2. *Cont.*

	Peat	C1	C2	C3	
			Chemical characteristics		
Total P (P_2O_5, g kg^{-1})	4.5 ± 0.2 a	21.4 ± 7.8 b	12.9 ± 5.6 b	15.1 ± 7.7 b	***
Available P (P_2O_5, g kg^{-1})	4.1 ± 0.1 a	19.6 ± 0.7 b	11.7 ± 0.8 b	14.4 ± 0.6 b	***
Total K (K_2O, g kg^{-1})	3.2 ± 0.3 a	28.4 ± 1.8 b	27.4 ± 3.8 b	32.3 ± 2.8 b	***
Available K (K_2O, g kg^{-1})	2.8 ± 0.3 a	25.4 ± 3.7 b	23.9 ± 2.8 b	29.6 ± 3.0 b	***
Ca (g kg^{-1})	18.1 ± 3.0 a	40.0 ± 3.0 b	22.5 ± 2.2 a	28.3 ± 0.41 ab	***
Mg (g kg^{-1})	1.8 ± 0.8 a	6.4 ± 0.1 b	4.0 ± 0.3 b	4.4 ± 0.8 b	***
B (mg kg^{-1})	0.3 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	n.s.
Cu (mg kg^{-1})	5.5 ± 1.1 a	21.6 ± 1.2 b	19.3 ± 1.5 b	20.1 ± 1.1 b	***
Fe (g kg^{-1})	1.2 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	n.s.
Mn (mg kg^{-1})	70.8 ± 5.7 a	120.1 ± 2.7 b	82.4 ± 5.7 ab	98.6 ± 5.6 ab	***
Mo (mg kg^{-1})	1.5 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	1.0 ± 0.3	n.s.
Zn (mg kg^{-1})	14.3 ± 1.1 a	46.4 ± 1.3 b	28.9 ± 1.8 b	28.0 ± 1.9 b	***
			Biological characteristics		
Total fungi (log(10) CFUs g^{-1})	4.88 ± 0.26 a	4.51 ± 0.02 a	5.40 ± 0.29 b	5.18 ± 0.04 b	***
Total Bacteria (log(10) CFUs g^{-1})	7.23 ± 0.08 a	8.60 ± 0.02 b	9.19 ± 0.04 c	9.41 ± 0.01 c	***
DHA (μmol INTF g^{1-} h^{-1})	3.7 ± 0.1 a	16.43 ± 0. 59 b	20.49 ± 0.89 b	17.35 ± 0.15 b	***

Values are the mean ± SD (n = 3). Asterisk indicates significances at *** $p < 0.001$; n.s: non-significant. Different letters indicate significant differences. C1, C2, and C3 represent the compost used. BD: Bulk density; TPS: total pore space; AC: air capacity; WHC: water holding capacity; EC: electrical conductivity; TOC: total organic carbon; HA: humic acids; CFUs: colony formed units; DHA: dehydrogenase activity; INTF: p-iodonitrotetrazolium formazan.

2.2. Compost Microbial Community

Total DNA was extracted from 500-mg compost samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), following the modification described by Taskin et al. [25] For bacteria, the V4 region of bacterial 16S rDNA was amplified using the barcoded primers 515F and 806R [26]. For fungi, the ITS2 region was amplified with the primer pair gITS7/ITS4 [27]. Each sample was amplified in triplicate as described previously by Žifčáková et al. [28] Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and the DNA concentration was measured by Qubit (Thermo Fisher, Waltham, MA, USA). A TruSeq PCR-Free kit was used for library preparation. Sequencing of the bacterial and fungal communities was performed on Illumina MiSeq, and the sequences were generated with the MiSeq Reagent Kit v2 on a paired-end mode with sizes of 251 base pairs (Institute of Microbiology of the CAS, Prague, Czech Republic).

2.3. In Vitro Suppressiveness against Pythium Irregulare

Pytium irregulare isolate, originally recovered from over-used floating trays where baby-leaf lettuces were grown, was selected from the pathogen culture collection of CEBAS-CSIC. The pathogenicity of the isolate was tested every three months, by passing it through baby-leaf plants and re-isolating it again to assure that pathogenicity was not lost due over culture in petri dishes. The mycelial growth of *P. irregulare* was estimated on potato dextrose agar plates (PDA). An 8-mm agar disk of *P. irregulare* was placed on the edge of one side of the plate, and 0.5 mL of dilution 10^{-4} of each compost and peat water extract was spread over the PDA surface on the other side of the plate. As a control, 0.5 mL of sterile water was spread on a PDA surface. Three replicates were performed per treatment. The plates were incubated in the dark at 28 °C, and the radial growth of the pathogen was measured every 24 h for 7 days. Growth inhibition was expressed by Mycelia Growth Inhibition, MGI (%) [29].

MGI% = ((RGcontrol − RGcompost)/RGcontrol) × 100;
RGcontrol = radial growth of pathogen in control plates;
RGcompost = radial growth of pathogen in plates with compost.

2.4. Experimental Conditions

The experiments were conducted at the 'Tomás Ferro' Experimental Agro Food Station of the Technical University of Cartagena (UPCT; lat. 37_410 N; long. 0_570 W). A cultivar of red baby leaf lettuce (*Lactuca sativa* L., cv. 'Ligier') from Rijk Zwaan, De Lier, the Netherlands, was cultivated in a floating system in an unheated greenhouse covered with thermal polyethylene. In the greenhouse, the light conditions during the experiments were an average daily light integral (DLI) of 14.07 mol m^{-2} d^{-1}; the minimum, maximum, and average air temperatures, in the spring cycle were 8.10 °C, 39.10 °C, and 19.41 °C, respectively; in the summer cycle, the average DLI was 15.48 mol m^{-2} d^{-1} and the minimum, maximum, and average air temperatures were 14.02 °C, 44.15 °C and 28.69 °C, respectively. Two crop cycles were carried out with sowings on 29 March 2019 (spring) and 14 June 2019 (summer).

Seeds were sown in 60 × 40-cm styrofloat trays [30] filled with the three compost-growing media (GM1, GM2, GM3) composed using each compost (C1, C2, C3) mixed with commercial peat (75:25, *v*/*v*). A commercial peat 315 (Blond/black 60/40 Turbas y Coco Mar Menor S.L.) was used as a control. The main chemical characteristics of the peat were as follows: pH 5.6; EC 1 dS m^{-1}; total C 466.8 g kg^{-1}; total N 9.4 g kg^{-1}; total P 0.3 g kg^{-1}; and total K 0.9 g kg^{-1}. After sowing, the trays were placed in a climatic chamber at 18 °C and 90% relative humidity and left in the dark for 48 h to improve germination. After seedling emergence, the trays were transferred to flotation beds (1.35 × 1.25 × 0.2 m). Each level of treatment (peat, GM1, GM2, and GM3) was carried out in beds randomly located at three places inside the greenhouse described above, in both growing seasons; each bed had three floating trays of 60 cm × 41 cm. The trays were floating on tap water with an EC of 1.1 dS m^{-1} and pH 7.8. Aeration was provided using a blow pump connected to a perforated pipe trellis positioned at the bottom of each flotation bed.

A week after sowing, the lettuce plants were thinned, and 10 plants were left per cell (2000 plants m^{-2}). At the same time, the tap water in the beds was replaced with the nutrient solution [31]. The nutrient solution was adjusted to EC 2.5 dS m^{-1} and pH 5.8. The EC and temperature of the nutrient solution were monitored throughout the growing cycles using Campbell CS547 sensors (Campbell Scientific In. Logan, UT) with an average of 2.76 dS m^{-1} and 19.53 °C in spring and 2.76 dS m^{-1} and 29.66 °C in summer, respectively. The oxygen concentrations were monitored using Campbell CS512 sensors located in each flotation bed with an average of 7.09 mg L^{-1} and 6.97 mg L^{-1} in the spring and summer cycles, respectively.

Harvesting was carried out twice per cycle at the same phenological stage for both cycles, when the plants had four to five leaves. The plants were harvested on April 25 (1st cut) and May 6 (2nd cut) in the spring cycle and on July 5 (1st cut) and July 12 (2nd cut) in the summer cycle. For each growing media, 90 plants from three cells fissure were randomly chosen from each tray for harvest; they were then stored at −80 °C for analysis.

2.5. Germination

To calculate the germination percentage, we used nine trays, i.e., three trays per growing media. Twenty baby leaf lettuce seeds were sown in each fissure on the tray, with 154 fissures per replication. After two days in a germination chamber at 18 °C and 90% relative humidity, the trays were transferred to flotation beds randomly placed on three stainless steel beds located in the greenhouse described above with tap water for five days (7 days after sowing (das)), with temperature conditions of 10.17 °C, 31.39 °C, and 17.63 °C as minimum, maximum, and average air temperature, respectively. Then, the percentage of seed germination with respect to the total seeds sown was calculated.

2.6. Plant Analysis at Harvesting

At harvesting time, the following parameters were analyzed in both cycles: biomass production (yield), calculated as g of fresh mass $plant^{-1}$; nitrate content in leaves and in the nutrient solution; and the total phenolic content and antioxidant capacity in the leaves.

The nitrate content was determined by ion chromatography following Lara et al. [32]

The total phenolic content (TPC) was determined by the Folin-Ciocalteu colorimetric method, as previously described by Singleton and Rossi [33], with modifications previously reported by Martínez-Hernández et al. [34]

The total antioxidant capacity (TAC) was determined as described by Klug et al. [35], using three different approaches: via the free radical scavenging capacity with 2,2–diphenyl–1–picrylhydrazil (DPPH) [36]; the ferric-reducing antioxidant power (FRAP) [37]; and 2 2′–azino–bis (3–ethylbenzothiazoline– 6–sulfonic acid) (ABTS) [38]. The DPPH method was conducted by measuring the decrease in absorbance at 515 nm for 30 min. The TAC extract (21 µL) was mixed with a volume (194 µL) of DPPH solution (≈ 0.8 mM and adjusted to Abs_{515} = 1.1 ± 0.02) and allowed to react for 30 min. The ABTS method was conducted by measuring the absorbance increase at 734 nm for 30 min. A volume (200 µL) of ABTS solution (14 mM $ABTS^{+}$ and 4.9 mM $K_2S_2O_8$ by 1:1 (*v*/*v*)) was added to each extract sample (11 µL) and allowed to react for 30 min. The FRAP method was conducted by measuring the increase in absorbance at 593 nm for 60 min. The freshly made FRAP solution (prepared at a ratio of 10:1:1 (*v*/*v*/*v*) using sodium acetate buffer, pH 3.6; 10 mM TPTZ solution in 40 mM HCl; and 20 mM $FeCl_3$, respectively, and preincubated at 37 °C for 2 h) was added (198 µL) to the extract (6 µL) and allowed to react for 60 min. All TAC reactions were conducted at room temperature in darkness, and absorbance was measured using the same microplate reader that was used for TPC. TAC was expressed as mg of Trolox equivalent per 100 g DW of lettuce leaves, as the mean of three replicates per each treatment and cut.

2.7. Statistical Analysis

Data were analyzed using Statgraphics Plus. To determine the compost characteristics, we performed an analysis of variance of measured parameters (one-way ANOVA). For the greenhouse experiment, we performed an analysis of variance of the measured parameters (two-way ANOVA), in which the growing media (peat, GM1, GM2, GM3) and time of cutting (1st cut and 2nd cut) were included for each crop cycle. When the interaction between factors was significant, ANOVA was carried out for each factor independently.

The amplicon sequencing data were processed using the SEED 2 program [39,40]. Pair-end reads were merged using fastq-join [41], and whole amplicons were processed. Chimeric sequences were detected using Usearch 7.0.1090 [42] and removed. Non-chimeric sequences were clustered to 97% similarity using UPARSE implemented within Usearch [43]. Consensus sequences were constructed for each cluster, and the closest hits at the genus level were identified using BLASTn against the GenBank databases for both bacteria and fungi [44]. Sequences identified as non-bacterial or non-fungal were excluded from subsequent analyses.

3. Results

3.1. Compost Characterisation

The three composts showed a similar BD (ca. 0.20 g cm^{-3}), but it was significantly lower than in peat (Table 2). There were no significant differences between treatments for TPS. Composts C2 and C3 showed a significantly higher AC (more than 30 vol %) than C1 and peat (ca. 20 vol %). However, the same two composts and peat showed a significantly lower WHC than C1 678 mL L^{-1}. The three composts showed a basic pH higher than 8.0, significantly higher to peat pH (5.6). The EC values of the three composts were significantly higher than peat, with compost C1 showing the highest EC values, followed by C3 and C2 (Table 2). Composts C2 and C3 showed significantly higher TOC than compost C1 and peat (Table 2). HA was significantly higher in peat with respect to the composts, C2 and C3 also being significantly higher than C1. Composts showed significantly higher values in total and available N, P, and K. In general, peat had lower values with respect to composts for Ca, Mg, Cu, Mn, and Zn. Finally, there were no significant differences between treatments for Mo, Fe, and B.

The fungal and bacterial content (CFUs) of composts C2 and C3 was significantly higher than in C1 and peat (Table 2). The total bacteria content (CFUs) and DHA activity of composts was significantly higher than in peat, CFUs values in C2 and C3 being significantly higher than in C1. Nevertheless, no significant differences were observed between the three composts in terms of DHA activity (Table 2).

3.2. Compost Microbial Community

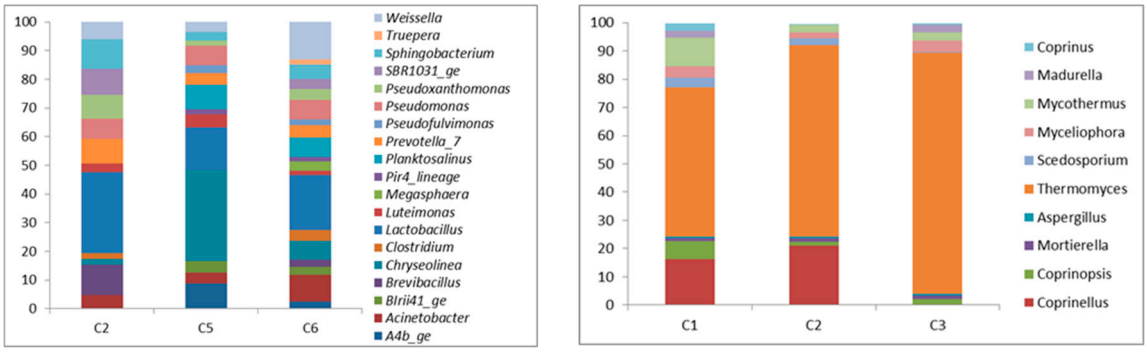
The Shannon and Simpson diversity index for bacteria did not show significant differences among composts, while for fungi, the compost C2 showed the highest diversity indexes followed by C3 and C1 (Table 3). The coverage value was estimated to be >99% and did not significantly differ between composts.

Table 3. The Shannon and Simpson diversity index for compost bacteria and fungi.

Compost		C1	C2	C3	
	Diversity Index				
Bacteria	Shannon (H)	7.88 ± 0.14	7.80 ± 0.11	7.92 ± 0.04	n.s.
	Simpson (Ds)	0.99 ± 0.01	0.98 ± 0.01	0.99 ± 0.01	n.s.
Fungi	Shannon (H)	1.92 ± 0.07 a	2.60 ± 0.10 b	2.09 ± 0.14 a	***
	Simpson (Ds)	0.49 ± 0.03 a	0.73 ± 0.01 c	0.60 ± 0.03 b	***

Values are the mean ± SD (n = 3). Asterisk indicates significances at *** $p < 0.001$; n.s: non-significant. Different letters indicate significant differences.

The dominant bacteria and fungi genera are shown in Figure 1. We identified different bacterial genera belonging to phyla Proteobateria (*Pseudomonas*, *Pseudoxanthomonas*, *Pseudofulvimonas*, *Luteimonas* or *Acinetobacter*); Bacteroidete (*Sphingobacterium*, *Prevotella* or *Chryseolina*); Firmicutes (*Weisella*, *Lactobacillus*, *Megasphaera*, *Clostridium* or *Brevibacillus*); and Thermus (*Truepera*). As for fungi, we recognized different genera belonging to Ascomycota (*Aspergillus*, *Thermomyces*, *Myceliophthora*, *Mycothermus*, *Madurella* and *Scedeosporium*); Basicomycota (*Coprinellus*, *Coprinopsis* and *Coprinus*); and Mucoromycota (*Mortierella*).



(**A**) (**B**)

Figure 1. Relative abundance of different genera of bacteria (**A**) and fungi (**B**) in the three composts.

3.3. Suppressiveness: Composts with Added Value

In vitro, the three composts used in this study showed a higher percentage of mycelium growth inhibition (MGI) of *P. irregular* in comparison with peat, where no inhibition was observed. Compost C1 showed the highest MGI (100%), followed by C2 (73%), C3 (65%), and peat (49%) (F = 22.44, p = 0.001).

3.4. Compost as a Component of Growing Media in Floating Systems

3.4.1. Percentage of Germination and Yield

The percentage of seed germination was significantly higher ($F = 10.37$, $p = 0.0001$) in plants grown in peat, at 94%, compared with those grown in compost growing media (GM) (82–84%).

The yield, on the other hand, was only affected by the growing media in the summer cycle (Table 4). The highest yield was observed in GM1, reaching more than 3 g/plant of the total yield (adding the two cuts), which means an increase of about 23% with respect to that obtained in peat (Table 4). Comparing the different cuts, the second cut was more productive than the first in both cycles, independent of the growing media.

Table 4. Yield and nitrate content in baby leaf red lettuce grown on different growing media (peat, GM1, GM2, GM3) in a floating system.

	Spring		Summer	
	Yield (g Plant^{-1})	**Nitrate Content (mg kg^{-1} FW)**	**Yield (g Plant^{-1})**	**Nitrate Content (mg kg^{-1} FW)**
Substrate (A)				
Peat	1.95 ± 0.15	1618.6 ± 24.6 a	1.28 ± 0.08 a	1968.2 ± 39.4 a
GM1	1.91 ± 0.17	1806.7 ± 27.3 b	1.55 ± 0.08 b	1955.9 ± 58.6 a
GM2	1.94 ± 0.18	1848.6 ± 47.3 b	1.31 ± 0.06 a	2035.1 ± 32.0 b
GM3	2.14 ± 0.22	1833.6 ± 80.6 b	1.40 ± 0.09 a, b	2000.4 ± 22.2 a, b
Cut (B)				
1st cut	1.34 ± 0.04 a	1654.5 ± 21.0 a	1.26 ± 0.04 a	1855.8 ± 18.4 a
2nd cut	2.63 ± 0.09 b	1899.2 ± 40.3 b	1.51 ± 0.07 b	2124.0 ± 16.4 b
A × B				
Peat × 1st cut	1.48 ± 0.05	1584.0 ± 46.7 a	1.06 ± 0.04	1835.6 ± 34.7 b
Peat × 2nd cut	2.42 ± 0.19	1653.3 ± 9.4 a, b	1.49 ± 0.12	2100.7 ± 31.8 d, e
GM1 × 1st cut	1.24 ± 0.07	1757.0 ± 20.2 b, c	1.37 ± 0.03	1722.3 ± 25.0 a
GM1 × 2nd cut	2.57 ± 0.09	1856.3 ± 46.2 c	1.76 ± 0.13	2189.6 ± 18.5 f
GM2 × 1st cut	1.27 ± 0.09	1699.8 ± 34.5 a, b	1.32 ± 0.08	1906.8 ± 12.2 c
GM2 × 2nd cut	2.61 ± 0.13	1997.5 ± 52.6 d	1.35 ± 0.05	2163.4 ± 9.0 e, f
GM3 × 1st cut	1.36 ± 0.10	1577.3 ± 31.9 a	1.33 ± 0.07	1958.6 ± 5.5 c
GM3 × 2nd cut	2.91 ± 0.24	2090.0 ± 99.7 d	1.46 ± 0.16	2042.1 ± 40.4 d
Analysis of variance				
Substrate (A)	n.s.	***	*	*
Cut (B)	***	***	**	***
A × B	n.s.	***	n.s.	***

Asterisks indicate significances at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s.: non-significant. Different letters indicate significant differences. FW: fresh weight.

3.4.2. Nitrate Content in Leaves and in the Nutrient Solution

Regarding the nitrate content in leaves, the two-way ANOVA indicated a significant interaction between the growing media and cut in both cycles (Table 4). In the spring cycle, the highest nitrate content values were obtained in the 2nd cut for GM2 and GM3. In the summer, the nitrate content was greater in the 2nd cut than in the 1st cut in all growing media, and the highest values were obtained with GM1 and GM2 in the 2nd cut.

During the spring cycle, in the 1st cut, the nitrate content measured in the nutrient solution was higher in plants grown in compost growing media than in plants grown in peat (Figure 2). In the 2nd cut, the nitrate concentrations decreased slightly for every compost growing media but not for peat, which remained constant, thus equalizing the values of all treatments by the end of the cycle. In the summer cycle, the lowest concentrations of nitrate in the nutrient solution in the 1st cut were found for GM1. In the 2nd cut, the nitrate concentration increased for every growing media, while GM1 maintained the lowest value.

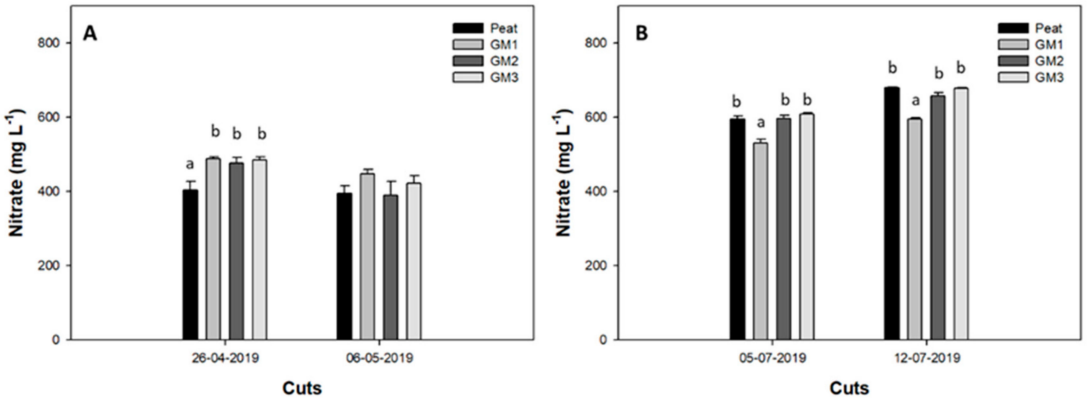


Figure 2. Nitrate content in the nutrient solution for both cuts in the spring (**A**) and summer (**B**) cycles using different growing media (peat, GM1, GM2, GM3). Values are the mean ± SD (n = 9).

3.4.3. Total Phenolic and Antioxidant Capacity in Red Baby Leaf Lettuce Leaves and Roots

Regarding the total phenolic content, there was an interaction between growing media and cuts in both cycles (Table 5). The plants showed a similar phenolic content pattern in leaves when they were cultivated in the different growing media, although those with compost showed higher values, particularly in the 2nd cut. In general, the lowest values were found in the 1st cut in plants grown with peat. GM3 stood out for the high phenolic content values found in leaves in the 2nd cut in both cycles. The total phenolic content was always higher in summer than in spring.

Table 5. Total phenolic content in the leaves of baby leaf red lettuce grown on different growing media (peat, GM1, GM2, GM3) and harvested twice (1st and 2nd cut), cultivated in spring and summer cycles in a floating system.

Total Phenolic Content (mg GA 100 g^{-1} DW)		
	Spring	**Summer**
Substrate (A)		
Peat	2467.8 ± 225.6 a	2638.8 ± 177.5 a
GM1	2741.8 ± 226.9 a, b	2932.7 ± 156.2 a, b
GM2	2838.3 ± 94.4 a, b	3046.8 ± 216.5 b, c
GM3	2976.6 ± 192.2 b	3268.4 ± 24.1 c
Cut (B)		
1st cut	2461.0 ± 99.2 a	2577.0 ± 68.0 a
2nd cut	3051.2 ± 119.7 b	3366.4 ± 114.4 b
A × B		
Peat × 1st cut	2023.5 ± 133.4 a	2304.9 ± 121.4 a
Peat × 2nd cut	2912.2 ± 198.3 b, c	2972.8 ± 176.4 b, c
GM1 × 1st cut	2488.5 ± 144.5 a, b	2624.2 ± 128.1 a, b
GM1 × 2nd cut	2995.1 ± 415.1 b, c	3241.1 ± 102.0 c, d
GM2 × 1st cut	2641.7 ± 75.6 a, b, c	2615.0 ± 72.5 a, b
GM2 × 2nd cut	3035.0 ± 12.2 b, c	3478.6 ± 206.8 d, e
GM3 × 1st cut	2690.5 ± 182.8 b, c	2763.9 ± 94.8 b
GM3 × 2nd cut	3262.7 ± 263.5 c	3772.9 ± 171.9 e
Analysis of variance		
Substrate (A)	*	**
Cut (B)	***	***
A × B	*	***

Asterisks indicate significances at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s.: non-significant. Different letters indicate significant differences. GA: gallic acid. DW: dry weight.

The antioxidant capacity, measured by the ABTS and FRAP methods, highlighted a similar pattern to that found in the total phenolic content for both cycles, with higher values in the compost growing media, particularly in the 2nd cut and in the leaves of plants grown with GM3 (Table 6). However, there were some exceptions: we did not find significant differences between the growing media in terms of antioxidant capacity measured in leaves using either the ABTS method in the spring cycle or the FRAP method in the summer.

Table 6. Total antioxidant capacity (FRAP and ABTS methods) in leaves of baby leaf red lettuce grown on different substrates (peat, GM1, GM2, GM3) and harvested twice (1st and 2nd cut), cultivated in spring and summer cycles in a floating system.

	Total Antioxidant Capacity (mg Trolox Equivalents 100 g^{-1} DW)			
	Spring		**Summer**	
	ABTS	**FRAP**	**ABTS**	**FRAP**
Substrate (A)				
Peat	2552.4 ± 76.0	6444.5 ± 183.4 a	2593.0 ± 114.6 a	7223.6 ± 98.7
GM1	2477.0 ± 93.9	6717.3 ± 196.9 a, b	2654.5 ± 61.4 a	7807.5 ± 258.6
GM2	2755.9 ± 76.9	70004.7 ± 224.9 b, c	2990.6 ± 226.9 b	7518.7 ± 281.2
GM3	2574.5 ± 133.3	7290.6 ± 197.3 c	2906.4 ± 246.5 b	7661.5 ± 392.2
Cut (B)				
1st cut	2461.4 ± 49.4 a	6557.0 ± 108.6 a	2456.8 ± 49.9 a	7158.3 ± 129.8 a
2nd cut	2717.5 ± 71.8 b	7171.5 ± 161.2 b	3115.4 ± 110.4 b	7947.4 ± 183.5 b
A × B				
Peat × 1st cut	2448.2 ± 103.9 a	6221.2 ± 97.0 a	2423.4 ± 133.9 a	7224.9 ± 202.2 a, b
Peat × 2nd cut	2656.5 ± 85.2 a, b, c	6667.7 ± 330.1 a, b	2762.7 ± 137.7 b	7222.8 ± 88.6 a, b
GM1 × 1st cut	2432.9 ± 107.9 a	6449.6 ± 245.8 a, b	2545.9 ± 82.5 a, b	7361.2 ± 335.2 a, b
GM1 × 2nd cut	2521.1 ± 247.0 a, b	6985.0 ± 248.5 b, c	2763.0 ± 15.7 b	8253.7 ± 151.2 c
GM2 × 1st cut	2635.12 ± 82.72 a, b, c	6518.8 ± 102.4 a, b	2493.3 ± 114.5 a, b	6999.64 ± 36.10 a
GM2 × 2nd cut	2872.74 ± 92.97 c	7490.6 ± 79.2 c	3484.8 ± 12.7 c	8037.8 ± 353.1 b, c
GM3 × 1st cut	2329.41 ± 6.17 a	7038.5 ± 20.3 b, c	2361.6 ± 84.1 a	7047.2 ± 417.5 a
GM3 × 2nd cut	2819.57 ± 169.57 b, c	7542.7 ± 367.6 c	3451.1 ± 10.8 c	8275.8 ± 466.0 c
Analysis of variance				
Substrate (A)	n.s.	**	**	n.s.
Cut (B)	**	**	***	**
A × B	*	*	***	*

Asterisks indicate significances at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s.: non-significant. Different letters indicate significant differences. DW: dry weight.

4. Discussion

To date, there have been few studies that have investigated the ideal growing media for a floating system. Among them, Cros et al. [4] demonstrated that a peat-based floating cultivation system can be considered the most suitable growing medium to grow purslane, because of its ideal physical and chemical characteristics. Nicola et al. [43] also recommended a peat-based horticultural medium for baby leaf vegetables grown in floating system. However, in recent years, there have been increasing environmental and ecological concerns about the use of peat as a growing medium because its harvest is jeopardizing endangered wetland ecosystems worldwide [45]. Furthermore, increasing demand and rising costs for peat as growing media in horticulture have led to a search for high-quality and low-cost substrates as an alternative. Compost may have physical, chemical, and biological properties that can contribute to partial peat reduction in growing media formulations [46]. In the case of a floating system, the three assayed composts showed physical properties similar to peat [47], although C1 showed a significantly higher WHC than peat, which could bring higher moisture and cause some negative aspects on plant growth [40]. Yet this issue was easily overcome given the type of trays used in this study, which contain a low volume of substrate per hole, and the system of cultivation (floating trays), where substrates obtain the water that they need and the roots mostly grow into

the nutrient solution [30]. This makes the compost physical properties in this cultivation system a non-limiting factor for plant growth.

The C/N ratio of the three composts was less than 25, which indicates that the composts can be considered matured [48]. By using unmatured composts and/or unstable organic materials with a high C/N ratio, such as wood fibers, as the plant material degrades, N-immobilization occurs. This is accompanied by a decrease in soil volume (shrinkage), pore space, and air content [49–51]. Both pH and EC have an essential influence on seedling quality and plant growth. The three composts used in this study showed a basic pH ranging from 8.4 to 8.8; these values are higher than those recommended for growing media [52]. By mixing the composts with 25% peat, however, the pH was reduced for all growing media used (to 7.7, 7.8 and 7.9, for GM1, GM2 and GM3, respectively). As a result, we obtained a good germination rate and good seedling growth. These results are in accordance with those of Morales et al. [53]. With respect to EC, an EC $\leq$ 3.5 dS m^{-1} is considered to be the limit for seedling growth in a growing medium [54]. Moreover, an EC > 4 dS m^{-1} has been reported to inhibit seed germination [55]. In our study, the growing media presented a percentage of seed germination: ca. >82%, a level of germination that can be considered standard within the range normally found for this species.

Even if C1 and C3 exceeded the abovementioned EC limit, the peat used in the mixtures served as a thinner and reduced the salt concentration of the growing media to EC 3.3, 1.8, and 2.6 dS m^{-1} for GM1, GM2, and GM3, respectively. Moreover, the high water holding capacity of C1 may also have positively influenced seed germination in GM1, because water retention is a decisive factor to this process [56]. Nevertheless, other factors could influence seed germination, due to the complexity of the mechanisms involved in it.

Furthermore, the growing media did not have any adverse effects on plant growth. In fact, the compost growing media promoted plant growth to a greater extent than peat, reaching higher yields. This beneficial effect of compost on yield could be due to the availability of nutrients and the production of auxin-like components from humic substances (Table 2). According to Trevisan et al. [57], compost acts as a reservoir for nutrients, ensuring their slow release to plant roots [58]. Moreover, some microorganisms found in our compost have been described as plant growth promoters (PGP). According to Castellano-Hinojosa et al. [59], strains of *Pseudoxanthomonas* promote plant growth via the production of ACC deaminase and siderophore and the solubilization of phosphate. In addition, Kuan et al. [60] found that inoculating maize with N2-fixing PGP strains belonging to genera *Acinetobacter* significantly increased the total N content and dry biomass of plants.

The time that the plants needed to reach the adequate phenological stage for the first cut was longer (27 and 21 days in spring and summer cycle, respectively) than the time from the first to the second cut (11 and 7 days in spring and summer cycle, respectively). As Jasper et al. [61] demonstrated recently in rocket plants, the growth rate prior to the first cut is slower than the subsequent regrowth rate due to the initial plant establishment. In addition, the second cut was more productive than the first in every growing media. Awan and Ahmad [62] and Suzuki et al. [63] also found that spinach foliage weight at the second harvest was greater than the weight at the first harvest, suggesting that new roots, which developed vigorously during the regrowth period, had a positive effect on the absorption of water and nutrients by the plants [63].

The three composts used in our study all showed suppressive activity against *P. irregulare*, which was not observed in the peat [64,65]. Hoitink et al. [66] pointed out a direct relationship between compost microbial activity and the suppression of *Pythium* and *Phytophthora* root rots. In our study, compost C1 showed the highest suppressiveness, which may be related to the fact that the primed plants displayed a faster and stronger activation of the various cellular defenses [64], it could also be due to microbial antagonism, nutrient competition, parasitism, and antibiosis [65]. Dehydrogenase activity, a potential indicator of general microbial activity [11], cannot be considered as an indicator for determining compost suppressiveness in this study, since no significant differences for this parameter were observed among the composts tested. Several studies have shown that compost microbial

composition primarily depends on the microbial competition for nutrients in different types of feedstock, which deeply influence compost recolonization during curing time [67,68]. The composts investigated here were recolonized by different microbial communities. Among them, we found microorganisms belonging to specific beneficial groups, such as *Aspergillus*, *Pseudomonas*, and *Morteriella* sp. These microorganisms are effective against different pathogens [69,70] and can induce systemic resistance in plants [71]. *Brevibacillus*, which has also been found to produce bioactive compounds against pathogens [72], showed higher relative abundance than other beneficial microorganisms in C1. Interaction between different beneficial microorganisms in the composts studied could contribute to their effectiveness in controlling *P. irregulare.*

According to Nicola et al. [1], baby leaf vegetables are a significant source of nitrates, so the nitrate content is an important quality characteristic to consider. Our data reveal that the nitrate concentrations did not exceed the maximum level allowed by the EU for this type of lettuce and cultivation system, although the use of compost did increase the amount of nitrates in comparison with peat. In both cycles, the nitrate content in the 2nd cut was higher than in the first. This fact could be linked to changes in the nutrient solution, due to both a gradual release of nitrate from the compost growing media and the evaporation of water from the floating beds, particularly in summer. Moreover, the nitrogen mineralization rate from organic substrates is higher in summer than in spring, due to the higher temperatures [73]. The influence of enriched nitrates in the nutrient solution could overcome the effect produced by the higher LDI in the summer cycle on nitrate reductase activity, which would increase the conversion rate of nitrate to amino acids, reducing nitrate levels in the leaves [74].

Plants grown in compost growing media showed a higher total phenolic content levels than plants grown in peat. The compost feedstocks used (tomato, leek, vineyard, and olive mill cake residues) are rich in compounds with the capacity to activate an oxidative process in plants [75]. As suggested by Santos et al. [76], these kinds of compounds induce the stimulatory effects of secondary metabolites on different parts of lettuce grown in different agro-industrial composts. The season also influenced the accumulation of phenolic compounds in the lettuce leaves: the total phenolics were higher in summer. This agrees with the results of Marín et al. [77], who found a positive correlation between the total phenolic content and temperatures. Besides, the root zone temperature has been found to influence the production of plant metabolites in several plants [78–80]. Temperature increases in the root zone in leafy vegetables can lead to alterations in the production of some secondary metabolites in greenhouse cultivation, phenolic compounds being the most pronounced secondary metabolites [81,82]. Furthermore, the higher phenolic compound levels after the 2nd cut could be linked to the increase in some phenolic metabolism enzymes due to the signals that spread from the injured tissue to the adjacent non-injured tissue after wounding, as observed by Salveit [83], who reported a 6 to 12-fold increase in PAL activity within 24 h after cutting in Batavia lettuce.

In our study, antioxidant capacity, measured by ABTS or FRAP methods, had a positive correlation with the total phenolic content in the leaves. The correlation coefficients were $r = 0.67$ and $r = 0.86$ for the first and second cuts in spring and $r = 0.91$ and $r = 0.85$ in summer, respectively. Santos et al. [76] found similar results in lettuce using agro-industrial composts as substrates. Moreover, we found a higher antioxidant capacity after the second cut. This agrees with the results of Kang and Saltveit [84], who demonstrated that the antioxidant capacity of lettuce leaf tissue increases after wounding. The baby leaf lettuce in our study showed the highest antioxidant capacity and total phenolic content in GM2 and GM3 growing media. This is most probably due to the original presence of olive mill cake, given that Chrysargyris et al. [85] reported an increase in antioxidant enzyme metabolism in marigold and petunia grown in soilless media, using up to 30% olive mill cake in place of peat. In general, these findings suggest that compost amendments can help add value to lettuce by increasing its antioxidant activity to a greater extent than other organic resources such as peat [76].

5. Conclusions

Composts from different raw materials like vineyard wastes, tomato wastes, leek wastes, and olive mill cake can be an alternative to peat as a central component of the growing media in the production of baby leaf vegetables in a floating system. They not only increase crop yields due to their biofertilizer activity, but also boost the final quality of the product with a higher total phenolic content and antioxidant capacity. Moreover, composts were able to control the effect of *P. irregulare* due to their suppressive effect of bacterial–fungal interactions. However, the percentage of seed germination was higher in plants grown in peat than in those grown in compost growing media and the nitrate content in the leaves was greater in some of the compost treatments than in the peat treatment. Further studies are needed on the standardization of feedstocks origin, composting and stabilization processes in order to obtain standard growing media for this cultivation system.

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