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From wastes to resources: citrus hydrolates as natural biostimulants of soil microorganisms

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Introduction The hydrolates result from the industrial extraction process of the essential oils through cold pressing of the citrus peels. Today, they are generally considered a waste to be disposed of. However, as hydrolates hold some water soluble compounds (sugars, polyphenols, acids), they could be reused, as irrigation water, instead of being a high economic burden for their disposal.

The aim of this work was to evaluate the effects of citrus hydrolates applied to soil on microbial biomass, activity and microbial main groups.

Materials and methods Soil was collected from topsoil (0-10 cm) of a Citrus lemon orchard, then air-dried and sieved at 2 mm. Later, aliquots of 450 g of soil were placed in 1L plastic bottles and moistened up to 50% of the water holding capacity (WHC) by applying hydrolates or water only (control, CTR). Hydrolates (**H**) were obtained from three citrus species: Citrus sinensis **(O)**, Citrus limon **(L)** and Citrus reticulata **(T)**. The hydrolates were applied at three different doses to reach 1/3, 2/3 and 3/3 of the 50% of soil WHC. Respectively, 35, 70 and 105 mL of hydrolate were added to 450 g of air-dried soil. Distilled water was added to the control soil up to 50% of its WHC and, when necessary, to hydrolate treatments to complement the 50% of WHC. Soil aliquots (450 g) of all 10 treatments (3x3 for hydrolates, one for control) were incubated in the dark at constant temperature (23.0 ± 0.5°C) and their humidity weekly adjusted up to 50% of WHC by adding distilled water. Four replicates per treatment were run. At the same time, 20 g of soil from each above treatment were similarly incubated in 200 mL jars, sealed with rubber stoppers holding silicon septa to monitor the emission of CO_2. Microbial biomass C was determined according to the fumigation-extraction method after 1and 4 weeks of incubation. At the same time, soil fatty acid methyl esters (FAMEs) were determined and used as indicators of the main microbial groups. The CO_2 accumulated in the headspace of the jars was measured at days 2, 5, 7 during the first week, then weekly for the rest of the month.

*Table 1. Volumes of **H** and H_2O added to 1 kg of dry soil. Also amount of C-**H** added is reported.*

Treatment	C-Hydrolates	Hydrolates	H_2O
	g kg^{-1}	mL kg^{-1}	mL kg^{-1}
Ctr	0.0	0.0	235.0
L 1/3	1.9	78.3	156.7
L 2/3	3.9	156.7	78.3
L 3/3	5.8	235.0	0.0
O 1/3	5.4	78.3	156.7
O 2/3	10.9	156.7	78.3
O 3/3	16.3	235.0	0.0
T 1/3	2.2	78.3	156.7
T 2/3	4.3	156.7	78.3
T 3/3	6.5	235.0	0.0

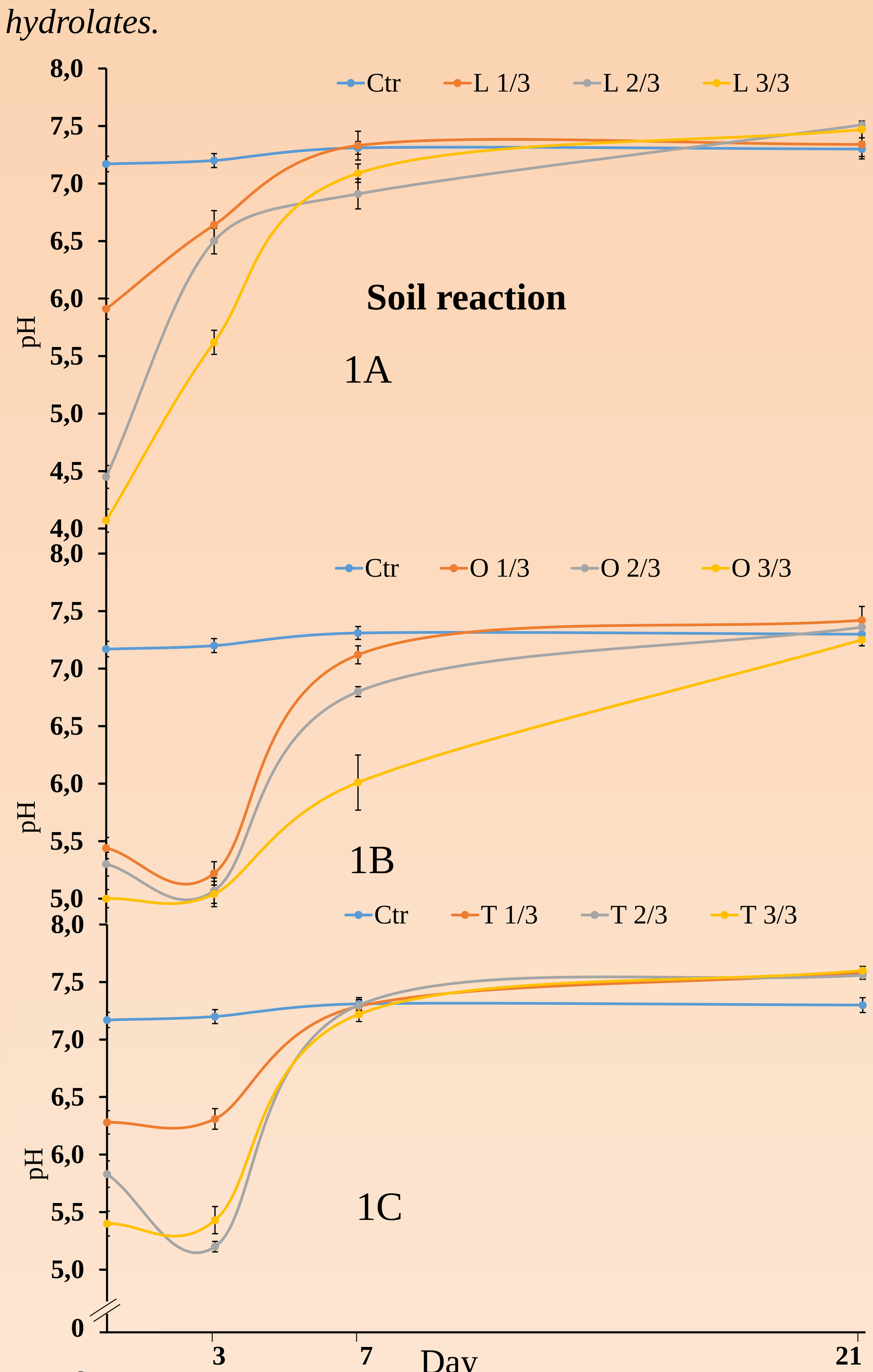
Results

Table 2. Chemical composition of hydrolates (H) added to soil.

H	Density	Total N Kjeldahl	Ashes	Dry Matter 105°C	°Brix Index	Total soluble Mono-saccharides	Total Carbo-hydrates	Total Fibers	pH	Total Acidity
	g cm^{-3}	%	%	%		%	%	%		%
Lemon	1.01	0.28	2.6	5.0	5.5	1.1	1.4	0.9	2.4	0.3
Orange	1.04	0.54	1.4	12.4	10.3	3.1	3.6	1.1	3.1	0.1
Tangerine	1.01	0.16	2.2	5.0	6.8	2.0	2.9	0.3	2.9	0.1

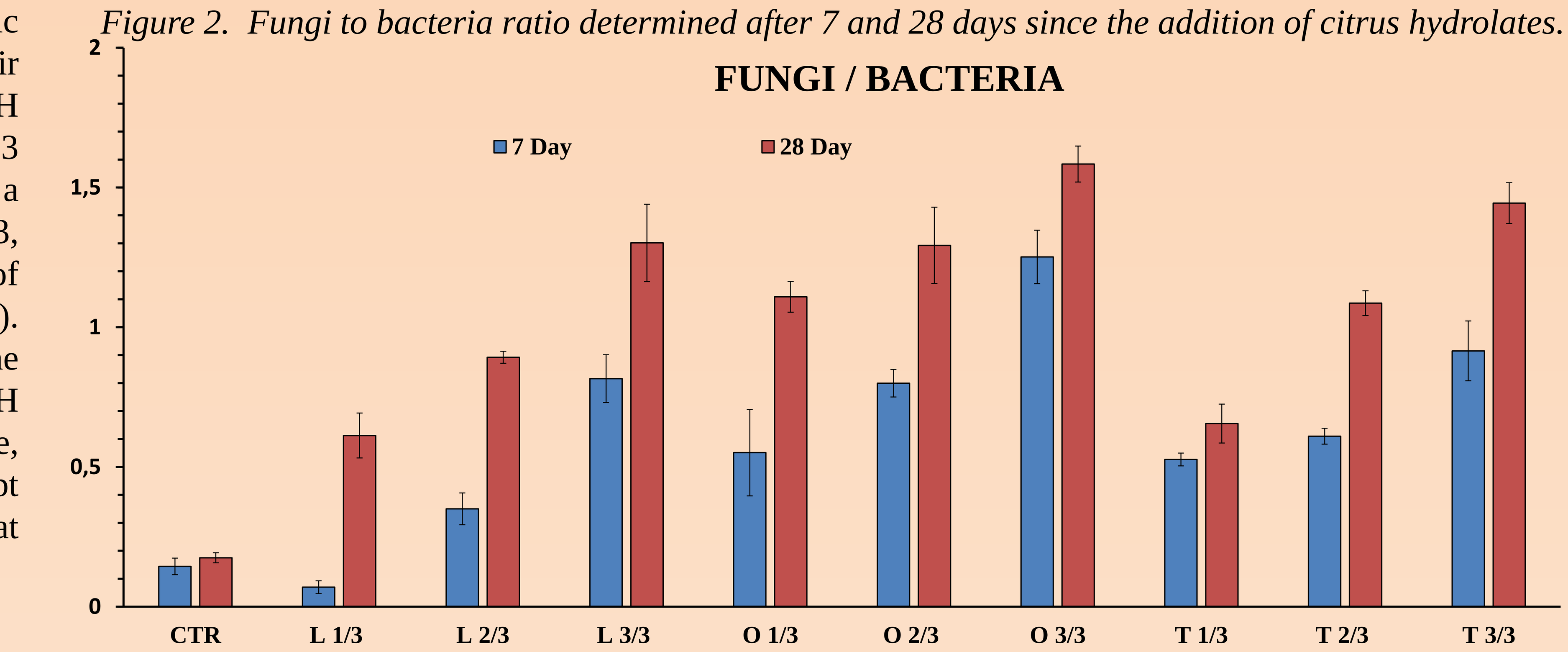
The citrus hydrolates (H) used had similar chemical characteristics, with orange H having the highest dry matter, °Brix index, total soluble monosaccharaides and carbohydrates, while lemon H having the lowest pH .

Figure 1. Soil reaction during the first 21 days following the application of lemon (1A), orange (1B) and tangerine (1C) hydrolates.



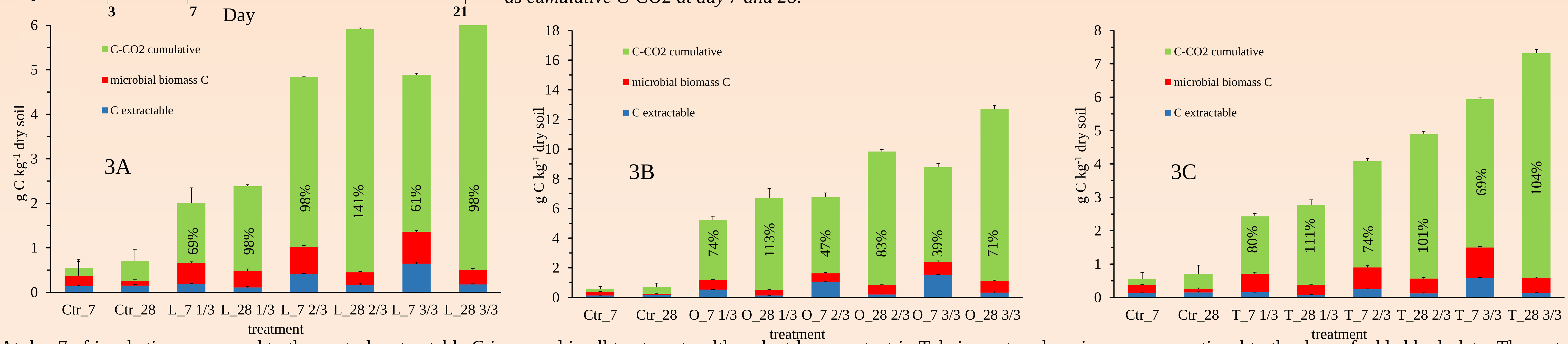
Due to the H acidic reaction, following their application, the soil pH rapidly decreased from 7.3 to a minimum of 4 and a maximum of 6.3, depending on the type of hydrolates (Figure 1). However, 7 days after the application, soil pH recovered its initial value, regardless of H type, except for O 3/3 treatment that recovered at day 21.

Figure 2. Fungi to bacteria ratio determined after 7 and 28 days since the addition of citrus hydrolates.



The fungi to bacteria ratio at both days 7 and 28 was always higher in soil added with hydrolates compared to the control (Figure 2). Furthermore, within each H treatment and regardless of incubation day, it increased by increasing the dose of hydrolate applied. During the incubation, the F/B ratio increased in all treatments due to more a reduction of bacteria than an increase of fungi. Probably, such trend of the two main microbial groups is to be ascribed to the drastic decrease of pH occurring during the first 7 days and inhibiting bacteria while favoring fungi.

Figure 3. Extractable and microbial biomass C, and cumulative C-CO2 mineralized after 7 and 28 days since the application of lemon (3A), orange (3B) and tangerine (3C) hydrolates. Number within each histogram indicates the percentage of the added H-C mineralized as cumulative C-CO2 at day 7 and 28.



At day 7 of incubation, compared to the control, extractable C increased in all treatments, although at lesser extent in T, being yet such an increase proportional to the dose of added hydrolate. Then, at day 28, extractable C drastically decreased, compared to day 7, from 42 to 81%, being the decrease % generally proportional to H dose and following the order O>L>T. Microbial biomass C, at day 7 of incubation in Ctr, L and T treatments was generally twice than at day 28, regardless of concentration, while in O treatment only at 1/3 dose and no difference at 2/3 and 3/3 doses. The trend of C mineralization was rather complex as it depended on H both type and concentration, but likely also on their interaction. For example, in O and T treatments, at both days 7 and 28 of incubation the % of added H-C mineralized as cumulative C-CO2 decreased with increasing H concentration, as expected, except for T at day 28 and 3/3 H, which slightly increased compared to 2/3 H. On the contrary, with L treatment the highest % of added H-C mineralized as cumulative C-CO2 occurred with the intermediate H concentration (2/3) at both days 7 and 28. Remarkably, after only 7 days all the C held in the added H was virtually mineralized (98%), while at day 28 a large priming positive effect occurred (141%). Similar, but smaller in extent, positive priming effects occurred, still at day 28, for O and T treatments with the lowest H concentration (1/3, 113% and 111%, respectively). However, as the H-C added for O treatment was more than 2.5 times higher than for L and T treatments, the two latter comparable between them, the lower observed % of added H-C mineralized to CO2 with O treatment were only apparent and did not indicate a lower microbial stimulating efficiency. Therefore, the observed positive priming effect likely occurred when the C held in the added H was exhausted and microbial biomass was constrained to resort to native soil organic matter.

Conclusions

1. **Citrus hydrolates were sources of readily available carbon to microbial assimilation;**
2. **Citrus hydrolates stimulated both microbial C immobilization and mineralization;**
3. **The stimulation effect depended on type and concentration of hydrolates;**
4. **For the same amount of H-C added, the efficiency of stimulation seemed to follow the order O>T>L;**
5. **A positive priming effect can occur following the application of hydrolates.**