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Mill wastewater and olive pomace compost as amendments for rye-grass

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Abstract – A two-year experiment was carried out to study the effects of applying untreated Olive Wastewater (OWW), treated OWW and olive pomace compost as soil amendments on both rye-grass growth and soil characteristics. We analysed growth parameters (Leaf Area Index, and fresh and dry weight), leaf green colour (SPAD readings), N uptake of the rye-grass and chemical soil characteristics. The results indicate that the highest untreated OWW application increased growth parameters by 18.2% in 2001 and by 41.1% in 2002, indicating the possible use of OWW as an amendment to rye-grass. We observed a significant increase in total, extracted and humified organic carbon, and humification parameters. No accumulations of heavy metals in the soil were observed. Furthermore, the N content in OWW was used by the rye-grass for plant growth that increases N uptake, and consequently, dry matter accumulation.

olive wastewater / olive pomace compost / perennial rye-grass / controlled environment lysimeter / trace metals / organic carbon

1. INTRODUCTION

The milling process of olives produces large amounts of Olive Wastewater (OWW); if using the centrifuge method of extraction, OWW production is reduced. Worldwide production of OWW is more than 2 million tons per year and around 44% of this amount comes from the Apulia district (Southern Italy), due to its high olive oil production (20% of the global market). OWW spread on the soil could increase pollution risks because of the presence of phenolic compounds and other pollutants, especially when it is not evenly distributed on the soil and the correct doses are not applied. For this reason, Italian law [17] indicates the maximum amount of OWW ($80 \text{ m}^3 \text{ ha}^{-1}$ for the centrifuge method of extraction) that can be applied on soil for agricultural purposes. Furthermore, an OWW application could cause temporary immobilisation of soil mineral nitrogen (N), and consequently, crop yield reduction due to the plants' N deficiency [29] and lower N uptake, especially in the presence of a higher concentration of soluble carbon [23]. Moreover, OWW is characterised by its slow biological mineralisation [25], even if the phenolic compounds of OWW spread on soil are degraded with time and transformed into humic substances, as reported by [8].

On the contrary, OWW presents chemical properties (organic carbon, nitrogen, potassium and phosphorus contents) that can increase soil fertility [22, 26] and yield production,

confirming its fertilising value [2, 4]. Moreover, OWW spread on soil improves physical properties, organic matter, available P and exchangeable K content as reported by [5, 14]. Therefore, the addition of OWW and other agricultural and industrial organic materials to soil is becoming a more common agriculture practice, especially in Mediterranean conditions where the mineralisation is higher and the soil needs more organic matter. Finally, foliar applications of OWW from the centrifuge two-step olive oil mill process seem to increase yield, kernel number and grain protein content of maize [30].

In recent years, due to a policy of environmental protection, new methods regarding the possible use of OWW have been studied to improve the recycling of this material, and therefore olive pomace compost [18] and treated olive wastewater [7] were developed. In this way, untreated OWW, OWW treated with a mineral catalyser and OWW compost are being increasingly recognised as good alternatives to chemical fertilisers to sustain yield production.

Therefore, the objective of this research was: (i) to study in a controlled environment the effects of untreated OWW, treated OWW and olive pomace compost on both yield and N utilisation of rye-grass crop and on chemical soil characteristics, and (ii) to evaluate the possible use of these materials as amendments and then to extend their application on the farm scale.

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Table I. Chemical characteristics of wastewaters (mg l⁻¹) and olive pomace compost (mg kg⁻¹).

	Untreated waste water	Treated waste water	Olive pomace compost
pH	5.11	5.20	5.97
Total N	1.59	1.30	1.40
NaHCO ₃ -P	339	454	210
NH ₄ Ac-K	388	519	506
Total carbon	15.36	46.2	50.5
C/N	9.66	35.54	36.07
Phenol composts	5020	4854	.
Zn	3.75	.	40.00
Cu	1.99	.	17.2
Ni	0.05	.	0.05
Pb	1.54	.	16.2
Cr	<0.01	.	<0.01
Mn	10.38	.	11.23
Co	0.133	.	10.9
Cd	0.05	.	0.01

2. MATERIALS AND METHODS

2.1. Properties of untreated OWW, treated OWW and olive pomace compost

The untreated OWW, treated OWW and olive pomace compost characteristics are presented in Table I. The data shown (mean of 5 samples) are an average over two consecutive years, since the parameters showed no significant differences from year to year. The OWW was obtained from a two-step olive oil mill process using the centrifuge method. The OWW was also treated, immediately after its production, with a mineral catalyser (2 kg of MnO_x 100 l⁻¹) mixed with liquid material in a specific reactor of 2000 m³ in aerobic conditions for 7 weeks [6]. During the treatment the OWW was mechanically shaken and air was continually pumped into the bottom of the reactor. The compost used consisted of 82 kg 100 kg⁻¹ of the pomace, 10 kg 100 kg⁻¹ poultry manure and 8 kg 100 kg⁻¹ wheat straw, and was made in an open field [10]. Homogenisation and oxygenation were ensured through continual monitoring of its humidity and temperature and by turning over the material. The moisture ranged from between 50 and 60 °C, whereas the temperature ranged from between 50–60 °C in the thermophilic phase (40 days) and 35–45 °C in the mesophilic phase (150 days).

2.2. Site

The research was carried out in the Experimental Farm "Agostinelli" in Rutigliano (Bari – Southern Italy) (41°01' latitude, 4°39' longitude, 112 m a.s.l.), in a controlled environment (lysimeters placed in an open field) on rye-grass (*Lolium perenne* L. cv "Barvestra") during 2001–2002 (indicated as 2001) and 2002–2003 (indicated as 2002). The lysimeters presented the following characteristics: height = 128 cm, diameter = 112 cm, area = 0.985 m² and were filled with soil before the experiment.

2.3. Experimental design and measurements

The experimental design was a randomised complete block with three replications, and the following treatments were compared: untreated control (contr); OWW obtained using the centrifuge method at 80 m³ ha⁻¹ (maximum amount allowed by Italian law) equal to about 120 kg N ha⁻¹ (80ref); OWW obtained using the centrifuge method at 320 m³ ha⁻¹ (320ref); OWW treated with MnO_x catalyser at 320 m³ ha⁻¹ (320cat); 120 kg N ha⁻¹ of organic N as an olive pomace compost (120com).

The untreated OWW, treated OWW and olive pomace were applied on the 25th of February in 2001 and on the 24th of January in 2002. Rye-grass shoots were cut at 5 cm above soil level to minimise soil contamination at 25, 79, 106, 129, 169, 204, 229, 255 and 333 days after waste (waters and compost) application (indicated as DAA) in 2001 and at 47, 99, 119, 144, 166, 188, 214, 251, 272, 321, 440, 460 and 482 DAA in 2002. During the rye-grass cycles, fresh weight, dry weight (48 h at 70 °C), total N content of the plants (Fison CHN elemental analyser mod. EA 1108) and total N uptake (N content × biomass dry weight) were determined. At each sampling, Leaf Area Index (LAI) and SPAD readings (a rapid and non-destructive estimate of leaf greenness determined by a portable chlorophyll-meter) were recorded. SPAD readings were measured at mid-length on the fully expanded leaf from approximately 10 randomly selected plants for each lysimeter. Finally, nitrogen utilisation efficiency (NUE in kg kg⁻¹) as a ratio of dry weight to total N uptake was calculated, according to [11].

At the beginning of the cropping cycles (T0) and at the end of 2001 (T1) and 2002 (T2), soil samples (at 50 cm depth) from each lysimeter were taken, air dried, ground to pass through a 2-mm sieve and then analysed. The following selected soil characteristics were determined: nitrate, extracted with KCl 1N and then analysed using the cadmium reduction method which produces a red dye through a diazotisation reduction; exchangeable ammonium, extracted with KCl 1N and then analysed using Nessler's reagent [21]; soil total N by the Kjeldhal digestion and distillation method; available P (NaHCO₃-P) by the Olsen method and exchangeable cations by the Thomas method [21]. Total organic carbon (TOC) was determined according to the Springer and Klee method [21]; extracted total organic (TEC) and humified organic carbon [C(HA+FA)] were determined by [27]; degree of humification (DH), the humification rate (HR), carbon organic extract and not humified (NH) and the humification index (HI) were calculated according to [9]; total content of trace metals was determined by hydrochloric and nitric acid and measured by atomic absorption spectrometry [21]. Furthermore, at T0 the particle-size analysis was determined using the standard pipet method [16] and the soil presented the following values: sand = 13.88%, silt = 29.62% and clay = 56.50%.

2.4. Statistical analysis

Statistical analysis was carried out using the SAS procedures [24]. The effect of the treatment was evaluated considering the years as a random effect and waste application as a fixed effect, while the differences between the means were evaluated using the Least Significant Difference (LSD) and Duncan's Multiple

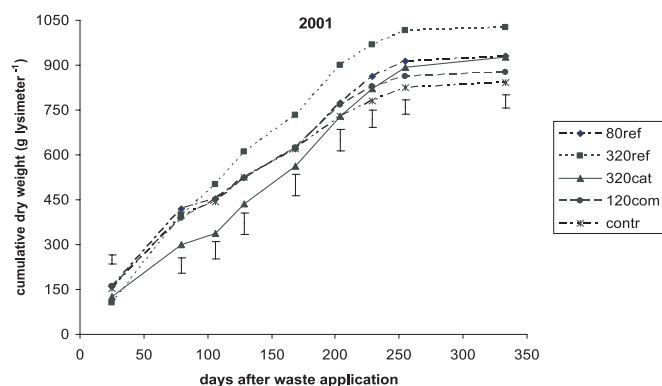


Figure 1. Cumulative dry weight of the rye-grass during the 2001 growing cycle. Bars represent the LSD at $P \leq 0.05$ for mean value comparison at each sampling data.

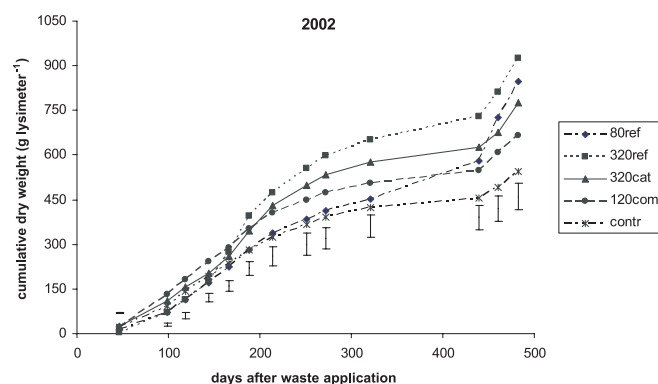


Figure 2. Cumulative dry weight of the rye-grass during the 2002 growing cycle. Bars represent the LSD at $P \leq 0.05$ for mean value comparison at each sampling data.

Range Test (DMRT). Finally, Pearson correlation coefficients were used to relate yield performance, N uptake and growth parameters. The full analysis of variance, which involved the principal factors and interactions, was significant for the two-year experiment, hence the data presentation in the tables being divided into 2001 and 2002.

3. RESULTS AND DISCUSSION

3.1. Effects of untreated OWW, treated OWW and olive pomace composts on rye-grass growth

In Figures 1 and 2 the cumulative dry weight of the rye-grass during the cropping cycles in both 2001 and 2002 are presented. OWW and compost applications influenced dry weight production, which was higher than the control, mainly in the last part of both cycles.

In particular, in 2001 the dry weight in 320ref was significantly higher during the entire cycle, whereas the 80ref and 320cat were different only at the end of the rye-grass cycle (255 and 333 DAA). Furthermore, in the first year the application of the highest dose of untreated OWW shows a constant increase in dry weight compared with the other treatments and the control, confirming that OWW application could be used as an amendment to sustain yield production [1, 28]. The 320cat presented an initial limited increase in dry weight, probably due to different molecular weights of humic-like substances obtained using catalysts in respect to those already present in the soil [6]. Furthermore, the catalysed treatment may improve biological activity, and consequently, some OWW nutrients could be utilised by microorganisms, and therefore be temporarily subtracted from the plant's root apparatus.

In 2002 the difference in cumulative dry weight between the OWW and compost with control was higher than the 2001 experimental trial, possibly due to residual effects of previous waste applications. Furthermore, the data of cumulative dry weight of the tested treatments in 2002 confirmed the results obtained in the 2001 experiment, indicating that the application of 320 m³ of OWW had positive effects on rye-grass and presented the highest increase compared with other treatments.

3.2. Effects of untreated OWW, treated OWW and olive pomace composts on rye-grass yield and N utilisation

In Table II the fresh weight, dry weight, LAI and N uptake (sum of 9 cuttings), SPAD readings and NUE (mean of 9 cuttings) of rye-grass in 2001 are presented. The 320ref presented the highest performance for dry weight (1027 g lysimeter⁻¹), LAI (11.12) and SPAD readings (31.86), indicating a possible use as an amendment. This large amount of OWW could reduce the use of mineral fertilisers in plant growth and development. Similar results were found by [12] and [28], which indicated a total substitution of mineral fertiliser with OWW application, whereas [7] suggested that OWW could only integrate mineral fertilisation. Furthermore, the 80ref treatment (80 m³ ha⁻¹ of OWW, which is the maximum amount allowed by Italian law) presented growth parameters (fresh, dry weight and LAI) and SPAD readings higher than the values observed in control lysimeters, indicating that poliphenols content in the 80 m³ ha⁻¹ wastewater (Tab. I), although reducing the soil bacteria's biological activity [1], did not influence its productive performance. In 2001 no significant difference between treatments was found for N uptake and NUE. This latter parameter was low in the tested treatments (about 30–38 kg kg⁻¹ in both years) in comparison with other research [11], and if NUE is poor then more

Table II. Fresh weight, dry weight, LAI and N uptake (sum of 9 cuttings), and SPAD and NUE (mean of 9 cuttings) of rye-grass cropped in a controlled environment in 2001.

	80ref	320ref	320cat	120com	contr
Fresh weight (g lysimeter ⁻¹)	4109.9	4480.3	4094.6	3853.8	3599.5
Dry weight (g lysimeter ⁻¹)	929.5ab	1027.3a	926.2ab	877.0ab	840.4b
LAI	10.12ab	11.12a	10.11ab	9.49ab	8.79b
SPAD readings	31.91a	31.86a	31.67a	29.42b	29.24b
N uptake (kg ha ⁻¹)	276.4	303.3	303.2	266.9	264.2
NUE (kg kg ⁻¹)	34.05	34.14	30.57	33.19	32.06

Values in a row followed by different letters are significantly different according to DMRT at $P \leq 0.05$.

Table III. Fresh weight, dry weight, LAI and N uptake (sum of 13 cuttings), and SPAD and NUE (mean of 13 cuttings) of rye-grass cropped in a controlled environment in 2002.

	80ref	320ref	320cat	120com	contr
Fresh weight (g lysimeter ⁻¹)	4019.7ab	4643.3a	3619.0ac	3004.8bc	2571.6c
Dry weight (g lysimeter ⁻¹)	848.1ab	926.0a	776.1ab	664.3ab	545.0b
LAI	10.55ab	11.98a	9.33ac	7.96bc	6.47c
SPAD readings	38.72a	38.37a	38.03a	38.22a	35.90b
N uptake (kg ha ⁻¹)	222.7ab	248.7a	197.3ab	172.4ab	144.2b
NUE (kg kg ⁻¹)	38.01	37.71	39.36	38.63	37.80

Values in a row followed by different letters are significantly different according to DMRT at $P \leq 0.05$.

N available in the soil/crop system will be susceptible to loss [15].

In 2002, 120com presented a significant decrease (Tab. III) in dry weight (sum of 13 cuttings) that can probably be attributed to the temporary immobilisation of the N induced by both a high C/N ratio (Tab. I) and the organic N distributed (120 kg ha⁻¹). In fact, different researches [13, 29] indicate that compost can enhance soil organic matter and crop nutrient supply, but compost with a high C/N ratio can temporarily deplete N reserves in the soil for plant utilisation [29], requiring more N to ensure crop growth. Also in 2002, the 320ref presented the highest performance for the measured parameters, whereas the intermediate response of the OWW application for 320cat and 80ref and the lowest for the control treatment were found. Significant difference in N uptake (248.7 and 144.2 kg ha⁻¹, for 320ref and contr, respectively) was found in the second year of the experiment due to the cumulative effects of the absence of mineral fertilisation.

Table IV shows correlation coefficients between the different parameters measured. The LAI and N uptake were positively and highly correlated with fresh and dry weight, whereas a significant negative correlation between dry weight and NUE was found, probably because the NUE parameter, which represents the ability of the plant to translate the N uptake into yield, decreased as N applied increased [11, 20]. Although with different absolute values, SPAD readings were correlated with N uptake and dry weight, and therefore could be used as a practical indicator for OWW and compost level applications. Higher significant correlation between SPAD readings and yield production was found in other research with olive pomace compost application [19]. Furthermore, the SPAD readings could indicate the temporary reduction of leaf greenness that can be used to modulate OWW in more sensitive plants.

3.3. Effects of OWW, treated OWW and olive pomace composts on soil characteristics

Tables V and VI show the soil characteristics by years and treatments, respectively. Soil N-NO₃ (7.26, and 1.59 mg kg⁻¹

Table IV. Correlation coefficients between the different parameters measured.

	Fresh weight	Dry weight	LAI	SPAD readings	N uptake	NUE
Fresh weight	1.0000	0.9645***	0.9902***	-0.1652 n.s.	0.8604***	-0.3718*
Dry weight		1.0000	0.9302***	0.3551**	0.9400***	-0.5013***
LAI			1.0000	-0.0575 n.s.	0.8032***	-0.2823 n.s.
SPAD readings				1.0000	0.5573***	0.7883***
N uptake					1.0000	-0.7605***
NUE						1.0000

*, **, *** = Significant at the $P < 0.05$, 0.01 and 0.001 levels, respectively. n.s. = not significant.

Table V. Soil characteristics at the beginning (T0) and end of the first year (T1) and at the end of the experiment (T2).

	T0	T1	T2
Nitrate (mg kg ⁻¹)	7.26a	3.58b	1.59c
Ammonium (mg kg ⁻¹)	6.34a	7.44a	3.74b
Total N (%)	0.124	0.129	0.141
NaHCO ₃ -P (mg kg ⁻¹)	17.59	18.79	20.79
NH ₄ Ac-K (mg kg ⁻¹)	398.7b	511.1a	540.3a
Na (mg kg ⁻¹)	356.7b	262.9b	626.5a
Mg (mg kg ⁻¹)	699.5b	780.9b	1017.1a
Ca (mg kg ⁻¹)	4977.3	5234.0	4863.5
TOC (g kg ⁻¹)	13.01b	13.32b	15.54a
TEC (g kg ⁻¹)	5.06ab	4.39b	6.29a
HA+FA (g kg ⁻¹)	1.33b	1.20b	2.16a
DH (%)	26.48b	27.36b	33.45a
HR (%)	10.29b	9.16b	14.10a
NH	3.73ab	3.19b	4.12a
HI	2.97	2.76	2.34
Zn (mg kg ⁻¹)	73.96a	73.59a	66.64b
Cu (mg kg ⁻¹)	31.97	33.97	32.84
Ni (mg kg ⁻¹)	54.99	53.48	49.45
Pb (mg kg ⁻¹)	44.71	41.13	44.37
Cr (mg kg ⁻¹)	46.30	46.75	48.85
Mn (mg kg ⁻¹)	29.70	32.26	32.51
Co (mg kg ⁻¹)	79.44	81.45	81.92
Cd (mg kg ⁻¹)	0.45	0.63	0.65

Values in a row followed by different letters are significantly different according to DMRT at $P \leq 0.05$.

Table VI. Soil characteristics of the N treatments in the first year (T1) and at the end of the experiment (T2).

	T1					T2				
	80ref	320ref	320cat	120com	contr	80ref	320ref	320cat	120com	contr
Nitrate (mg kg ⁻¹)	5.24	2.38	4.85	2.71	2.74	1.34	1.66	1.67	1.55	1.70
Ammonium (mg kg ⁻¹)	8.94	7.68	8.95	6.31	5.32	3.06	4.72	3.91	3.86	3.16
Total N (%)	0.130	0.140	0.122	0.124	0.127	0.123	0.157	0.147	0.153	0.127
NaHCO ₃ -P (mg kg ⁻¹)	19.13ab	23.14a	19.13ab	15.91b	16.65b	13.67	13.32	18.17	12.08	11.33
NH ₄ Ac-K (mg kg ⁻¹)	452.7ab	690.7a	738.0a	339.3b	334.7b	510.0c	808.7a	678.7b	374.7d	329.3d
Na (mg kg ⁻¹)	230.7a	253.3ab	260.7ab	270.0ab	300.0a	647.3	678.7	600.7	622.0	584.0
Mg (mg kg ⁻¹)	804.0	770.7	831.3	769.3	729.3	960.0	1070.0	1021.3	1002.7	1031.3
Ca (mg kg ⁻¹)	4838.0	5296.0	5345.3	5497.3	5193.3	5281.3a	5036.7a	5012.7a	4297.3b	4689.3ab
TOC (g kg ⁻¹)	14.23	13.28	13.17	14.23	11.69	15.14	17.10	14.09	17.30	14.05
TEC (g kg ⁻¹)	4.82ab	4.89a	4.39ab	3.92b	3.93b	5.50b	7.00a	6.59ab	6.56ab	5.79b
HA+FA (g kg ⁻¹)	1.21	1.44	1.09	1.26	1.01	1.78	1.97	2.47	2.33	2.27
DH (%)	25.13	29.12	24.99	31.86	25.72	29.01	27.02	37.12	35.54	38.56
HR (%)	8.64	10.85	8.34	9.14	8.83	11.32	11.23	17.88	13.64	16.44
NH	3.61a	3.46a	3.30ab	2.66b	2.91ab	3.71ab	5.04a	4.12ab	4.23ab	3.51b
HI	3.02	2.47	3.05	2.35	2.94	3.55	2.78	1.81	1.84	1.70
Zn (mg kg ⁻¹)	75.87	73.90	77.90	66.67	72.47	70.13	72.00	63.10	63.00	64.97
Cu (mg kg ⁻¹)	33.50	32.87	34.37	33.53	35.60	31.73	32.80	34.53	32.40	32.73
Ni (mg kg ⁻¹)	57.23	52.20	49.68	58.03	50.25	47.56	51.50	46.43	47.57	54.17
Pb (mg kg ⁻¹)	41.83	41.40	43.13	39.03	40.23	42.30	46.10	46.83	43.90	42.70
Cr (mg kg ⁻¹)	47.38	47.01	48.09	46.05	45.20	50.73	47.01	48.38	49.00	49.18
Mn (mg kg ⁻¹)	31.83	32.42	32.80	32.53	31.74	32.65	32.80	33.20	32.33	31.56
Co (mg kg ⁻¹)	81.23	81.85	84.13	78.60	81.47	79.28	82.59	84.81	82.00	80.97
Cd (mg kg ⁻¹)	0.59	0.63	0.64	0.61	0.48	0.57	0.64	0.65	0.62	0.50

Values in a row followed by different letters are significantly different according to DMRT at $P \leq 0.05$.

for T0 and T2, respectively) and exchangeable N-NH₄ contents (6.34 and 3.74 mg kg⁻¹), significantly decreased from the beginning to the end of the experiment (Tab. V) due to N uptake, but no difference between treatments was found (Tab. VI). Therefore, the N content in OWW and pomace compost was used by the plants for growth and development and the OWW application did not modify the mechanisms of the uptake [25], N immobilisation in the soil [26], or accumulation of exchangeable ammonium due to bacteria inhibition [3].

In accordance with other research results [26, 32] a significant increase in exchangeable K (NH₄Ac-K) was found at T2 (398.7, and 540.3 mg kg⁻¹ for T0 and T2, respectively) confirming that OWW and pomace compost could be used as an alternative K supply. The humification parameters (TOC, TEC and [C(HA+FA)]) and humification indices (DH and HR) significantly increased at the end of the experiment (Tab. V), indicating the positive effects of OWW waste application on the organic carbon content in the soil. This result may reflect the application of phenolic substances in the OWW (Tab. I) applied on the soil [8]. In general, the findings observed from the soil pointed out the great importance of OWW and pomace compost

application as soil amendments, because of the semiarid conditions in Southern Italy, characterised by a high mineralisation rate, the improvement of soil properties is more important than fertiliser application. Furthermore, no significant accumulations throughout the years and amongst the treatments (Tabs. V and VI, respectively) were found for the total content of heavy metals, and the values recorded were lower to average values recorded in Italian soil [31]. Finally, nitrate and ammonium content in the soil presented no significant differences between treatments (Tab. VI). Therefore, the N content in OWW waste was used by rye-grass for plant growth, increasing total N uptake (Tab. III), and consequently, the dry weight accumulation at the end of the experiment (Tab. III) due to a high correlation between N uptake and dry weight content (Tab. IV).

4. CONCLUSIONS

The results of this research show the importance of OWW and olive pomace compost application as a soil amendment in Southern Italy, which is characterised by its high temperatures

in the summer, and consequently, soil water deficit that reduces the transport of nutrients from the soil to the plants. In this environment the amendment functions of a fertiliser could be more important than the presence of nutrients, due to a high mineralisation rate and the consequent depletion of organic matter in the soil. Furthermore, the data confirm the possibility of applying OWW and olive compost as an organic fertiliser with increased rye-grass growth (fresh and dry weight, and LAI) in comparison with unfertilised treatment. In fact, the highest OWW application (320ref) increased dry weight by 18.2% and 41.1% for 2001 and 2002, respectively.

Since SPAD readings indicate the temporary reduction of leaf greenness, they could be used as a practical indicator for scheduling the OWW and olive pomace compost applications on plants.

The treatments that exceeded the maximum amount permitted by Italian law ($80 \text{ m}^3 \text{ ha}^{-1}$) did not reduce the yield and growth of rye-grass, and therefore on this crop a higher volume can be applied without risks. Furthermore, the OWW spread on soil did not show any potential risks for the soil and agro-system and the nutrients present in the OWW waste were used for plant growth. Therefore, recycling organic material obtained from this agro-industrial process could represent a valid alternative to incineration, and consequently, reduce the greenhouse effects by organic carbon sequestration.

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