



Effect of different digestates derived from anaerobic co-digestion of olive mill solid waste (omsw) and various microalgae as fertilizers for the cultivation of ryegrass

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Abstract

Aims The aim of this work was to evaluate the fertilizing effect of three anaerobic co-digestates on the growth of the herbaceous plant *Lolium rigidum*.

Methods Nine treatments, combining different nutritional solutions (organic and inorganic) and number of fertilizations (one or two) were evaluated. Organic nutritive solution: plants grown with different olive mill solid waste (OMSW) -microalgae co-digestates: 75% OMSW-25% *Raphidocelis subcapitata*, volatile solids (VS) basis (OMSW-Rs); 50% OMSW- 50% *Chlamydomonas Reinhardtii*, VS basis (OMSW-Chl); and 75% OMSW-25% *Scenedesmus*

quadricauda, VS basis (OMSW-Sq). Inorganic nutritive solution (INS): plants grown with inorganic Hoagland nutrient solution at 50%. After 60 days of experimentation, biometric and nutritional characteristics and photosynthetic activity were measured.

Results The results showed a favourable growth, development and nutritional quality of *L. rigidum* plants when digestates obtained from the anaerobic co-digestion of OMSW-microalgae are used as organic nutritional solutions as opposed to INS ones. The highest total biomass of *L. rigidum* was obtained with the treatments that involved two fertilizations. No inhibition due to excess nutrients was observed. A higher root/shoot ratio was achieved with the digestates of OMSW-Rs and OMSW-Ch as compared to that obtained with OMSW-Sq ($F = 17.23$ $p \leq 0.001$). The nitrogen shoot biomass obtained after the organic treatments with the above-mentioned co-digestates was higher than that obtained after the inorganic treatment. Net photosynthesis rates did not present differences in the co-digestates treatments, being equal or superior to the INS treatments.

Conclusions The use of the anaerobic co-digestates from OMSW-microalgae can be considered a viable and promising alternative to inorganic fertilization.

Keywords *Lolium rigidum* · Organic fertilizer · Circular economy · *Chlamydomonas reinhardtii* · *Scenedesmus quadricauda* · *Raphidocelis subcapitata*

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Introduction

The use of inorganic fertilizers has been and continues to be a very common practice among farmers. The green revolution focused on obtaining a higher crop yield, which prevailed for years in the care of the environment (Ahmed and Turchini 2021). Moreover, it is well-known that the continued abuse of inorganic fertilizers has a negative effect not only on soil as it causes a variation in pH, the deterioration of both the soil structure and the micro-fauna, but it also impacts negatively on water bodies as it causes them to eutrophy (Foley et al. 2005; Rohila et al. 2017). The harmful impact of inorganic fertilizers on farmland has, therefore, led to an increasing search for alternative sources of organic fertilization. Current environmental awareness leads us more and more to environmentally friendly practices (Zhang et al. 2018; Ülgüdür et al. 2019).

Recently, the European Commission has stated that the industrial model of “take-make-consume and eliminate” (linear economy model) is less and less efficient and threatens Europe’s competitiveness (COM/2015/0614). To counteract this problem, the establishment of policies to implement circular economy models that allow greater use of natural resources began in 2015. These models are aimed at addressing all phases of the life cycle of a product: from production, through consumption, waste management and the secondary raw materials market, with the objective of promoting and increasing the use of reprocessed nutrients and to support the development of circular economy with a more resource-effective use of nutrients. For this reason, it is necessary to return to the initial organic fertilization practices or to look for alternative organic fertilization sources, such as the use of the digestates from anaerobic digestion processes.

Anaerobic digestion (AD) has been widely studied by the scientific community in recent decades for its great potential. Basically, this process entails the degradation by microorganisms of organic matter in the absence of oxygen obtaining: i) biogas (methane up to 75–80% vol. and carbon dioxide at 20–25%) as a renewable energy and heat source, and ii) a stable and moderately hygienic organic matrix called digestate that can be used as a soil amender or fertilizer (Tambone et al. 2010; Nkoa 2014; Solé-Bundó et al. 2017; Wang et al. 2021). Therefore, not only is AD essential

for sustainable bio-waste management, contributing to a reduction in global warming, but it is strongly encouraged by international governments of countries around the world with limited fossil resources due to the reduction of energy dependency with third countries (Horizon Europe Strategic Plan 2021–2024).

Specifically, Regulation 2019/1009 lays down the rules of the market of EU fertilising products in Europe. This legislation designates 11 component material categories which include 2 for digestates (fresh crop digestate and digestate other than fresh crop digestate). Firstly, for fresh crop digestate a fertilising product may contain digestate obtained through AD of one or more plants or plant parts grown (including algae, not cyanobacteria). Secondly, for digestate other than fresh crop digestate, input materials include bio-waste collection at source, organic fertilisers and soil improvers. According to EU legislation, both living and dead organisms are allowed. In both cases, digestion additives which are needed to improve the environmental performance of the digestion process with certain specifically regulated conditions and exceptions are allowed (Regulation (EU) 2019/1009).

The result of AD is a digestate with agronomic attributes, including organic matter content, and essential macro and micronutrients. In fact, digestates provide plant available N content (in the form of $\text{NH}_4^+\text{-N}$) and a portion of N organic fraction (10%–20%) (Möller and Müller, 2012) which enhance plant growth after fertilization (Tampio et al. 2016; Aihemaiti et al. 2020). AD also provides phosphorus which, despite being a crucial nutrient for plants, is found in soils in a very low concentration and availability (Möller and Müller 2012). Moreover, other essential nutrients such as S, Ca, Mg, and micronutrients are present, which results in a fertilizer with characteristics similar or superior to inorganic ones (Abubaker et al. 2012). However, the presence of heavy metals and pathogens must be controlled to avoid adverse effects (Bonetta et al. 2014). For this reason, regulations are very restrictive and, for example, in the aforementioned European legislation, AD procedures (temperature–time profiles) are regulated and the contents of the dry matter of 16 polycyclic aromatic hydrocarbons, of macroscopic impurities and stability criteria, in terms of oxygen uptake rate and residual biogas potential, are also limited (Regulation (EU) 2019/1009).

The quality of the final fertilizer is directly related to the original substrate that is digested since a large part of the nutrients remains in the final product (Odlare et al. 2011). Therefore, anaerobic co-digestion is considered a promising technology as it has been demonstrated that the application of co-digestates as fertilizers can effectively stimulate the growth of plants, increasing their yields. Furthermore, they can replace others of synthetic origin (Xu et al. 2021). Although co-digestion began to be studied in the late 1990s, in this decade the scientific community has been making great efforts to study this particular process as it results in greater methane yields, process stability and economic viability than single AD (Mata-Alvarez et al. 2011; Acosta et al. 2021). There are many examples of co-digestion found in literature which combine household, agricultural and industrial residues such as urban solid waste and lignocellulosic biomass (Romero et al. 2020; Arelli et al. 2021), food waste and cow manure (Xing et al. 2020), etc. and many reports have been found in the literature on the co-digestion of sewage sludge, manure and the organic fraction of municipal solid wastes (Mata-Alvarez et al. 2011). However, few of these articles address the study of the digestates produced as fertilizers. Albuquerque et al. (2012) showed that digestates derived from farm and agro-industrial residue co-digestion have a high fertilising potential (Albuquerque et al. 2012). In other study, Toumi et al. (2015) carried out an anaerobic co-digestion of dairy wastewater and cattle manure; they found that the application of the stabilised anaerobic effluent on the agricultural soil showed significant beneficial effects on forage corn and the growth of tomato plants and crops. In addition, digestate from the co-digestion of sewage sludge mixed with olive pomace or macroalgal residues was found to be more beneficial for tomato plant growth (Elalami et al. 2020).

The anaerobic digestate of microalgae is known to be used effectively as an organic fertilizer due to the presence of bio-assimilable nutrients (Solé-Bundó et al. 2017; González-González et al. 2019). However, there are very few studies on the use of anaerobic digestates from olive mill solid waste (OMSW) as fertilizers (Fernández-Rodríguez et al. 2021). To date, and to the best of our knowledge, the potential of the co-digestate from the anaerobic co-digestion mixtures of OMSW and microalgae has not been investigated as an organic fertilizer. Therefore, the objective of

this work was to evaluate the fertilizing capacity of digestates from the combination of OMSW-microalgae, whose better biogas productions were obtained in previous biochemical methane potential (BMP) experiments (Fernández-Rodríguez et al. 2019a, b), with a view to obtaining high-quality organic fertilizers capable of substituting others of synthetic origin.

Materials and methods

Anaerobic digestates

The digestates used came from different batch mesophilic anaerobic co-digestion processes treating different mixtures of OMSW and microalgae. The anaerobic sludge used as inoculum in these anaerobic assays was obtained from a full-scale upflow anaerobic sludge blanket reactor that processes wastewater derived from a brewery from Seville (Spain). The percentages of OMSW-microalgae were chosen based on previous BMP studies of methane production optimization (Fernández-Rodríguez et al. 2019a, b). The microalgae used were: *Raphidocelis subcapitata* (*R. subcapitata*), *Secenedesmus quadricauda* (*S. quadricauda*) and *Chlamydomonas reinhardtii* (*Ch. reinhardtii*). The different OMSW-microalgae mixtures used and the main characteristics of the different digestates used are shown in Table 1.

Characteristics of the studied species and experimental procedure

In order to evaluate the possible use of the digestates as organic fertilizers, the effect of these anaerobic digestates on the growth of the herbaceous plant *Lolium rigidum* was assessed. It is an annual species between 10 to 60 cm in height, well adapted to the semi-arid environment of the Mediterranean area, where it is native. This species is a self-sowing annual species and its persistence can range between 5 and 10 years, providing good quality forage with high nutritional and palatability values (high crude protein content) (Heineck et al. 2020).

L. rigidum seeds were sown in pots with sterile sand (9 seeds in each 400 cm³ pot). The seeds were placed in the pots in a homogeneous way, leaving space between them and burying them at a depth

Table 1 Main characteristics of the different anaerobic co-digestates used in the study. **OMSW-Rs**: digestate obtained from the anaerobic co-digestion of olive mill solid waste and *Raphidocelis subcapitata* (75%:25%, volatile solids basis); **OMSW-Chl**: digestate obtained from the anaerobic co-digestion of olive mill solid waste and *Chlamydomonas reinhardtii*

(50%:50%, volatile solids basis) and **OMSW-Sq**: digestate obtained from the anaerobic co-digestion of olive mill solid waste and *Scenedesmus quadricauda* (75%:25%, volatile solids basis). Means \pm standard errors. The performed analyses of the digestates were determined as was previously described by Fernández-Rodríguez et al. (2019a)

Parameter	OMSW- Rs	OMSW-Chl	OMSW-Sq
Carbon / Nitrogen ratio	10.6 \pm 0.4	9.9 \pm 0.8	10.40 \pm 0.5
Soluble chemical oxygen demand (g O ₂ L ⁻¹)	2.89 \pm 0.5	5.12 \pm 0.9	3.20 \pm 0.8
Chemical oxygen demand (g O ₂ L ⁻¹)	30.85 \pm 0.5	28.51 \pm 0.4	27.48 \pm 1.7
Total solids (g kg ⁻¹)	36.1 \pm 1.8	42.4 \pm 0.3	36.00 \pm 0.2
Volatile solids (g kg ⁻¹)	27.4 \pm 1.4	22.2 \pm 1.5	25.20 \pm 0.2
Soluble sugars (mg L ⁻¹)	32.31 \pm 7.3	14.83 \pm 1.7	22.21 \pm 2.5
Soluble phenols (mg L ⁻¹)	215 \pm 16	281 \pm 33	243 \pm 27

of 1–2 cm. With the aim of guaranteeing a high percentage of emergence, healthy seeds were chosen, discarding those that were broken, deteriorated or damaged. The pots were grouped onto plastic trays and placed in the greenhouse of the General Research Services of the University of Seville (CIT-IUS). The experiment was carried out at temperatures between 21°C and 25 °C, between 40 and 60% relative humidity and natural light (minimum and maximum light flux: 200 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). The pots were always hydrated with 5% Hoagland nutrient solution (Fernández-Rodríguez et al. 2021).

Then, 15 days after emergence (100% emergence), the pots were grouped into 9 treatments, combining different nutritional solutions (organic and inorganic) and number of fertigrations (one or two fertigrations). The evaluated treatments were:

i) Organic nutritive solution: plants grown with different olive mill solid waste (OMSW) -microalgae co-digestates: 75% OMSW-25% *R. subcapitata*, volatile solids (VS) basis (OMSW-Rs); 50% OMSW- 50% *Ch. Reinhardtii*, VS basis (OMSW-Chl); and 75% OMSW-25% *S. quadricauda*, VS basis (OMSW-Sq). These treatments were tested with 1 and 2 fertigrations (F1 and F2, respectively). As nutritive solution, the different liquid fractions of the OMSW-microalgae co-digestate were used obtained after centrifugation at 2000 rpm for 2 min.

ii) Inorganic nutritive solution (INS): plants grown with inorganic Hoagland nutrient solution at 50% (Hoagland and Arnon 1938). This concentration simulates a nutrient rich soil (Mancilla-Leytón et al. 2013). The selected percentage was chosen based on the average nitrogen concentration of the digestates studied (Table 2). These treatments were tested with 1 and 2 fertigrations (F1 and F2, respectively).

Table 2 Physicochemical characteristics and macronutrients solution concentration used. Hoagland nutrient solution at 50% (INS) and the different olive mill solid waste (OMSW)—microalgae co-digestates: OMSW-Rs (Rs): 75% OMSW-25% *Raphidocelis subcapitata* (volatile solids basis); OMSW-Chl

(Chl): 50% OMSW- 50% *Chlamydomonas reinhardtii* (volatile solids basis); and OMSW-Sq (Sq): 75% OMSW-25% *Scenedesmus quadricauda* (volatile solids basis). Values represent means \pm standard errors. In each row different letters show significant differences

Parameter	INS 50%	OMSW- Rs	OMSW-Chl	OMSW-Sq
Nitrogen Kjeldahl (mg L ⁻¹)	107 \pm 2 a	115 \pm 10 a	111 \pm 6 a	92 \pm 10 a
Phosphorus (mg L ⁻¹)	21 \pm 5 b	33 \pm 3 a	40 \pm 5 a	25 \pm 4 b
Potassium (mg L ⁻¹)	117 \pm 9 a	174 \pm 38 a	188 \pm 45 a	113 \pm 23 a
Magnesium (mg L ⁻¹)	17 \pm 3 a	19 \pm 4 a	18 \pm 3 a	20 \pm 4 a
pH	6.9 \pm 0.1 b	7.8 \pm 0.1 a	7.9 \pm 0.0 a	7.8 \pm 0.1 a
Electrical conductivity (dS m ⁻¹)	0.9 \pm 0.1 a	0.7 \pm 0.1 a	0.6 \pm 0.1 a	0.6 \pm 0.1 a

iii) Control treatment (control): plants grown without fertigation.

For each treatment, 5 pots were used as replicates, guaranteeing the homogeneity of the seedling size (10–12 cm). Each treatment pots were placed inside trays. In all treatments, except for the control treatment, the first fertigation was carried out 15 days after emergence, adding 20 mL of the fertilizer solution (organic or inorganic) to be evaluated. In the treatments with 2 fertigations, the second one was performed 15 days after the first. All digestate mixtures used in the study complied with the limits established by the European Nitrates Directive (EEC, 1991), the limits established as toxic by EU Directive (CEC 2003) and the limits established by Spanish Law (RD 1620/2007) for reusing water in the irrigation of pastures for animal consumption. The study treatments were reviewed twice a week. The trays were manually maintained throughout the experiment at a constant level of 3 L. The level was filled with a solution that simulated a nutrient-poor medium (5% Hoagland nutrient solution, Fernández-Rodríguez et al 2021).

After 60 days of experimentation, the average height of the plants, biomass and photosynthetic activity were measured in order to identify the treatment with the highest positive response. Gas exchange measurements were performed on fully developed leaves ($n=10$) for each treatment (LICOR-6400XT, Inc., Neb., USA). The net photosynthesis rate (A) was measured at a CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ and at $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The temperature of the leaves was kept between 20°C and 25°C , and the relative humidity between 45 and 55%. After photosynthetic activity measurements, the plant material was identified and transferred to the laboratory, where it was dried at 80°C for 48 h. Finally, the dried biomass was weighed on a precision scale to obtain the final dry weight. The average relative growth rate (RGR) of the aerial part was calculated for each treatment using the following equation:

$$\text{RGR} = (\text{Ln}B_f - \text{Ln}B_i) * D^{-1} (\text{g g}^{-1} \text{day}^{-1}) \quad (1)$$

where, B_f is the final dry biomass; B_i is the initial dry biomass and D is duration of the experiment in days.

Macro and micro elements of the total foliar concentrations were analysed as was previously described in Fernández-Rodríguez et al. (2021).

Statistical analysis

The usual statistical tests were used to contrast normality and homoscedasticity (Kolmogorov–Smirnov Test and Levene’s Test, respectively). Data were analysed using one-way analysis of variance (ANOVA). When there were significant results for the analysis of variance, the Tukey test was used to identify the differences, two-to-two comparisons. For all statistical analyses, the IBM 25.0 SPSS statistic software for Windows (Inc., Chicago, IL, USA) was used.

Results

The nutritional differences of each OMSW-microalgae co-digestate used (mainly P, Table 2) were reflected in the results obtained for the different variables analysed. Figure 1 shows the average height of the plants obtained for each evaluated treatment. Significant differences in height were found among the different treatments ($F=6.13$; $p \leq 0.001$)—the values found after two fertigations were significantly higher (37–43 cm) than those obtained in the treatments that received only one fertigation (31–35 cm). The control treatment had the lowest height (30 cm).

The effect of OMSW-microalgae co-digestate on *L. rigidum* biometric characteristics such as total biomass, root biomass, shoot biomass and root/shoot ratio are shown in Fig. 2. The shoot biomass values were significantly higher in the treatments that received two fertigations ($4.65\text{--}5.44 \text{ t ha}^{-1}$) than those that received only one (3.45 t ha^{-1}), with significantly higher results in OMSW-Rs and OMSW-Chl co-digestates treatments ($F=34.88$; $p \leq 0.001$). The control treatment showed the lowest production (2.24 t ha^{-1}) (Fig. 2A). Regarding the RGR of the aerial part, the values were significantly higher with two fertigation than when only one was performed (mean values of $0.103 \text{ g g}^{-1} \text{d}^{-1}$ and $0.095 \text{ g g}^{-1} \text{d}^{-1}$, respectively) ($F=59.70$; $p \leq 0.001$), and no significant differences in RGR values were found among the different treatments ($p \geq 0.05$). In the control treatment, the RGR value was significantly lower ($0.086 \text{ g g}^{-1} \text{d}^{-1}$) than in the rest of the treatments.

Root biomass, the OMSW-Rs and OMSW-Chl co-digestates treatments did not present significant differences in the first fertigation with respect

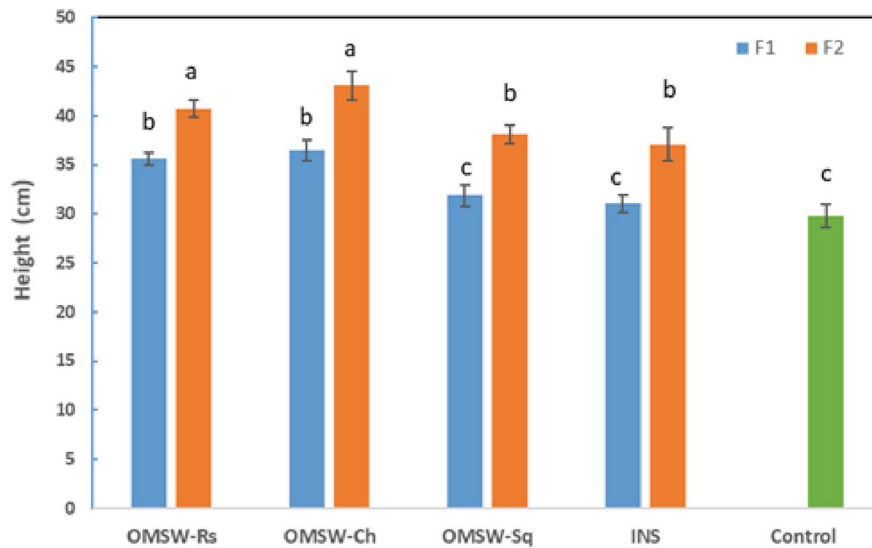


Fig. 1 Height of *Lolium rigidum* plants irrigated with different olive mill solid waste (OMSW)-microalgae co-digestates. **OMSW-Rs**: 75% OMSW-25% *Raphidocelis subcapitata* (volatile solids basis); **OMSW-Chl**: 50% OMSW- 50% *Chlamydomonas reinhardtii* (volatile solids basis); and **OMSW-Sq**: 75% OMSW-25% *Scenedesmus quadricauda* (volatile solids basis). **INS** is the control with inorganic Hoagland nutrient

solution at 50%. F1 and F2 are the number of fertigations carried out with the different liquid fractions of digestate used as nutritive solution, one or two respectively. **Control** was carried out with inorganic Hoagland nutrient solution at 5% (nutrient poor soil) without fertigations. Values represent means \pm standard errors and different letters show significant differences.

to the INS ($p \geq 0.05$; Fig. 2B) where a lower root biomass was obtained when *L. rigidum* herbaceous plants were treated with the co-digestate from the OMSW-Sq (which does not present significant differences with respect to the control treatment; $p \geq 0.05$). However, after the second fertigation, the root biomass in the three co-digestates did not present significant differences ($p \geq 0.05$), whereas it varied with respect to the INS treatment (Fig. 2B). With these results, a higher root/shoot ratio is clearly achieved in OMSW-Rs and OMSW-Chl co-digestates than in the OMSW-Sq co-digestate ($F = 17.23$; $p \leq 0.001$, Fig. 2C).

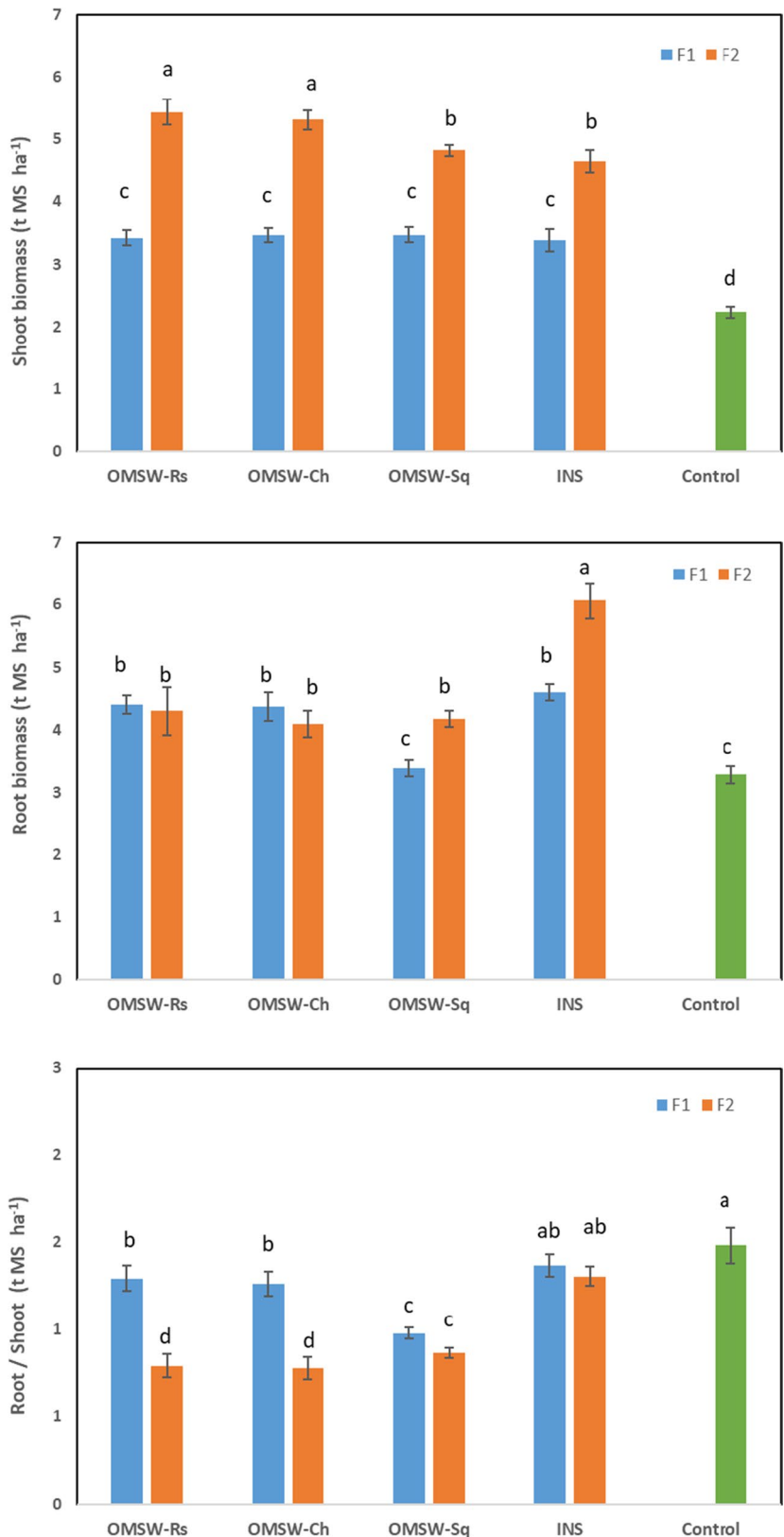
Turning to photosynthetic activity, net photosynthesis rates were not significantly different during the first fertigation when seedlings were fertilized with the anaerobic organic nutritional solution (OMSW-Rs, OMSW-Chl or OMSW-Sq) with respect to the INS or control treatments ($p \geq 0.05$; Fig. 3). However, in the second fertigation, net photosynthesis rates significantly improved when OMSW-Chl and OMSW-Sq co-digestates were added ($F = 12.92$; $p \leq 0.001$, Fig. 3).

Table 3 shows the concentration of the main macro and micro nutrients of the *L. rigidum* plants obtained in this study. In all treatments, nitrogen contents was higher in organic nutritional solutions than in inorganic ones; better results were attained with the anaerobic OMSW-Rs and OMSW-Chl co-digestate treatments. As with nitrogen, after the second fertigation the phosphorus values in the plant were higher; it can be observed that plants treated with OMSW-Chl co-digestate have a significantly higher phosphorus content (0.41%), a value which is higher than those obtained with the other two co-digestates (0.12–0.19%) (Table 3). The heavy metal content (Cu, Zn, Cd) found in the herbaceous plant after the anaerobic co-digestate treatment was low and are shown in Table 4.

Discussion

The results found in the present study show a favourable growth and development of *L. rigidum* plants

Fig. 2 Shoot biomass (A), root biomass (B) and root/shoot ratio (C) of *Lolium rigidum* plants irrigated with different olive mill solid waste (OMSW)—microalgae co-digestates. **OMSW-Rs**: 75% OMSW-25% *Raphidocelis subcapitata* (volatile solids basis); **OMSW-Chl**: 50% OMSW- 50% *Chlamydomonas reinhardtii* (volatile solids basis); and **OMSW-Sq**: 75% OMSW-25% *Scenedesmus quadricauda* (volatile solids basis). **INS** is the control with inorganic Hoagland nutrient solution at 50%. F1 and F2 are the number of fertigations carried out with the different liquid fractions of digestate used as nutritive solution, one or two respectively. **Control** was carried out with inorganic Hoagland nutrient solution at 5% (nutrient poor soil) without fertigations. Values represent means \pm standard errors and different letters show significant differences



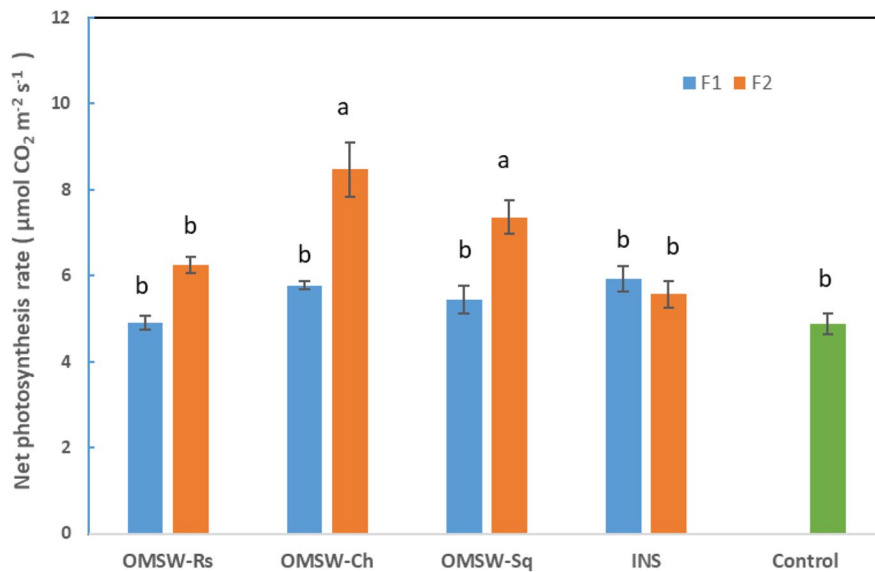


Fig. 3 Net photosynthesis rates of *Lolium rigidum* var. *Wimmera* plants irrigated with different olive mill solid waste (OMSW)—microalgae co-digestates. **OMSW-Rs**: 75% OMSW-25% *Raphidocelis subcapitata* (volatile solids basis); **OMSW-Chl**: 50% OMSW- 50% *Chlamydomonas reinhardtii* (volatile solids basis); and **OMSW-Sq**: 75% OMSW-25% *Scenedesmus quadricauda* (volatile solids basis). **INS** is the control with inorganic Hoagland nutrient solution at 50% and

Control was carried out without fertilization. F1 and F2 are the number of fertigation carried out with the different liquid fractions of digestate used as nutritive solution, one or two respectively. **Control** was carried out with inorganic Hoagland nutrient solution at 5% (nutrient poor soil) and without fertigation. Values represent means \pm standard errors and different letters show significant differences

Table 3 Concentration of macro and micronutrients in aerial vegetation of *Lolium rigidum* plants irrigated with different olive mill solid waste (OMSW)—microalgae co-digestates. **OMSW-Rs (Rs)**: 75% OMSW-25% *Raphidocelis subcapitata* (volatile solids basis); **OMSW-Chl (Chl)**: 50% OMSW-50% *Chlamydomonas reinhardtii* (volatile solids basis); and **OMSW-Sq (Sq)**: 75% OMSW-25% *Scenedesmus quadricauda* (volatile solids basis). **INS** is the control with inorganic

Hoagland nutrient solution at 50%. F1 and F2 are the number of fertigation carried out with the different liquid fractions of digestate used as nutritive solution, one or two respectively. **Control** was carried out with inorganic Hoagland nutrient solution at 5% (nutrient poor soil) and without fertigation. Values represent means \pm standard errors and different letters show significant differences

	N Kjeldahl (%)	Protein (%)	P (%)	Ca (%)	Mg (%)	Na (%)	K (%)	Fe (mg kg ⁻¹)
Rs F1	1.22 \pm 0.01d	7.63 \pm 0.06d	0.14 \pm 0.01e	0.23 \pm 0.00d	0.28 \pm 0.01c	1.31 \pm 0.01b	2.41 \pm 0.01b	102.68 \pm 0.01c
Rs F2	2.40 \pm 0.01b	14.98 \pm 0.13b	0.17 \pm 0.00d	0.43 \pm 0.01c	0.21 \pm 0.02d	1.95 \pm 0.01a	2.49 \pm 0.02b	118.47 \pm 0.01b
Chl F1	1.11 \pm 0.01d	6.94 \pm 0.06d	0.36 \pm 0.01b	0.24 \pm 0.00d	0.28 \pm 0.02c	1.02 \pm 0.02c	2.96 \pm 0.02a	101.52 \pm 0.23c
Chl F2	2.54 \pm 0.00a	15.90 \pm 0.04a	0.41 \pm 0.02a	0.46 \pm 0.00c	0.21 \pm 0.01d	1.94 \pm 0.00a	2.89 \pm 0.02a	128.98 \pm 0.04a
Sq F1	0.94 \pm 0.02d	5.85 \pm 0.25d	0.12 \pm 0.00e	0.64 \pm 0.00b	0.36 \pm 0.00b	0.91 \pm 0.02d	1.30 \pm 0.01 g	80.65 \pm 0.00d
Sq F2	1.70 \pm 0.01c	10.65 \pm 0.10c	0.19 \pm 0.01c	0.62 \pm 0.00b	0.38 \pm 0.01a	1.26 \pm 0.01b	1.67 \pm 0.02e	115.25 \pm 0.01b
INS F1	0.93 \pm 0.02d	5.84 \pm 0.26d	0.13 \pm 0.00e	0.69 \pm 0.02a	0.38 \pm 0.01a	0.44 \pm 0.01f	1.55 \pm 0.02f	67.63 \pm 0.02e
INS F2	1.16 \pm 0.01d	7.25 \pm 0.12d	0.16 \pm 0.00d	0.67 \pm 0.01a	0.39 \pm 0.00a	0.50 \pm 0.01e	1.85 \pm 0.02d	73.40 \pm 0.00e
Control	0.81 \pm 0.02d	4.81 \pm 0.20d	0.10 \pm 0.01e	0.29 \pm 0.02d	0.18 \pm 0.01d	0.45 \pm 0.01f	1.40 \pm 0.01f	64.25 \pm 0.02e

when digestate obtained from the anaerobic co-digestion of OMSW- microalgae is used as an organic nutritional solution as opposed to inorganic ones. The

important presence of bio-assimilable nutrients in the digestate, mainly N and P (Reuland et al. 2021) causes a better performance in vegetative growth with

Table 4 Concentration of some heavy metals in aerial vegetation of *Lolium rigidum* plants irrigated with different olive mill solid waste (OMSW)—microalgae co-digestates. OMSW-Rs (**Rs**): 75% OMSW-25% *Raphidocelis subcapitata* (volatile solids basis); OMSW-Chl (**Chl**): 50% OMSW- 50% *Chlamydomonas reinhardtii* (volatile solids basis); and OMSW-Sq (**Sq**): 75% OMSW-25% *Scenedesmus quadricauda* (volatile solids basis). **INS** is the control with inorganic Hoagland nutrient solution at 50%. F1 and F2 are the number of fertigation carried out with the different liquid fractions of digestate used as nutritive solution, one or two respectively. **Control** was carried out with inorganic Hoagland nutrient solution at 5% (nutrient poor soil) and without fertigations. Values represent means \pm standard errors and different letters show significant differences

	Cu	Zn	Cd
	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)
Rs F1	9.59 \pm 0.02b	29.25 \pm 0.39c	0.02 \pm 0.01f
Rs F2	7.15 \pm 0.01e	25.39 \pm 0.00d	0.04 \pm 0.01d
Chl F1	9.91 \pm 0.04a	33.53 \pm 0.05a	0.10 \pm 0.01a
Chl F2	8.27 \pm 0.04d	29.22 \pm 0.00c	0.08 \pm 0.01b
Sq F1	9.76 \pm 0.05a	31.79 \pm 0.24b	0.07 \pm 0.00c
Sq F2	9.21 \pm 0.03c	33.47 \pm 0.03a	0.03 \pm 0.00e

more robust and stronger plants, which are adaptable to environmental and nutritional changes (Coaguila et al. 2019).

The use of OMSW-microalgae co-digestate as organic nutritional solution affected *L. rigidum* biometric characteristics such as total biomass, root biomass, shoot biomass and root / shoot ratio. This can be explained as a normal plant response to higher nutrient supply, where shoot growth is enhanced and increased in contrast to root growth, which results in a decrease in root/shoot ratio. OMSW-Rs and OMSW-Chl co-digestate contained higher values of essential and limiting macro elements for the growth of the crop. The N content was slightly higher as well as the P content (Table 2). The production of *L. rigidum* can be highly variable, especially in dry land, since it basically depends on the water regime. Under optimal conditions, Peralta and Royuela (2020) found productions of 9 t ha⁻¹ of dry matter, a somewhat higher value than those obtained in the present work. Tampere and Viiralt (2014) found a total yield in grasslands with *L. perennial* of 6.44 t ha⁻¹ of dry matter when they used digestate from cattle slurry as a fertilizer, values very similar to those achieved in the present research.

The root/shoot ratio is proportional to the nutrient supply (which is stronger with N, less so with P and, usually shows no variations with other nutrients, except Mg) with a greater relationship at low nutrient supply (Lynch et al. 2012). Nitrogen is essential in the production of proteins and enzymes as it controls every one of the metabolic processes in plants (Islam et al. 2010). In the same way, phosphorus is also a very important element for vegetables since it is present in adenylates, nucleic acids and phospholipids (Möller and Müller 2012). That plants treated with OMSW-Chl co-digestate have a significantly higher phosphorus content (0.41%), which is similar to the excellent value (0.44%) described in the Spanish Foundation for the Development of Animal Nutrition (FEDNA).

The results showed an excellent performance in the production of photosynthetic plant material, with no nutritional limitation of the different fertilizers for the functioning of the photosystem (Fig. 3). This improvement in photosynthetic capacity, i.e., the maximum rate of carbon assimilation by leaves (without limitation of light saturation and adequate growth conditions), is directly related to the leaf content of certain macronutrients involved in the photosynthetic function (i.e. N and Mg). Most of the nitrogen in a plant leaf is spent on chloroplasts and photosynthesis (limiting growth) and, therefore, the role of nitrogen in photosynthesis is crucial because it controls the nitrogen-photosynthesis relationship achieving an increase in culture production without adding more nitrogen through fertilization (Chapin et al. 1987; Xu et al. 2021). Magnesium also plays an important role in the functions of photosynthesis—it is key in RuBP modulation carboxylase (Sun et al. 2018) and, therefore, the increase reported in some treatments could have contributed to the increase in the net assimilation rate. In all study treatments, magnesium values were higher than those described by FEDNA (0.18%) (Table 3).

Likewise, significant differences in height were found among the different treatments (Fig. 1). Although height is not an indicative variable of plant production, it is of great interest in mowing processes and in systems where animals are fed directly “by teeth” in the field. This suitable height has very positive implications for cattle grazing since it facilitates access to livestock and is considered good pasture. In fact, numerous

authors have demonstrated the positive correlation between height and forage consumption per bite dimensions in ryegrass (Gilliland et al. 2002; Smit et al. 2005). With this in mind, Laca et al. (1992) concluded that this higher intake occurs in tall pastures with low density compared to pastures with less height and density, while maintaining the same biomass. In addition, nitrogen availability for animals is sourced mainly from forage proteins, which are required by animals for milk and meat production (Boland et al. 2013). Small ruminants prefer plants with high nitrogen content over other nutrients (Thomas et al. 2010; Mancilla-Leytón et al. 2013), with a similar occurrence with the sodium content (to maintain osmotic pressure), which acts as an attraction for cattle (Watson et al., 2018). The results of the present study show that *L. rigidum* plants grown with organic nutrient solution (OMSW- microalgae co-digestates) presented higher protein values and sodium content than those grown with inorganic ones (Table 3), and this is also closely linked to the quality of the pasture. On the other hand, certain macro-minerals such as calcium and phosphorus are essential for the good development and functioning of livestock. Both elements are important in dairy animals and are often supplemented, along with magnesium, in the feed of milking cattle (Kronqvist et al. 2011). In most cases, the concentration of these elements in *L. rigidum* plants grown with digestates was equal to or higher than those grown in inorganic nutrient solution (Table 3). Their high contents are mainly related to good milk production in dairy animals and good growth in cattle (Xu et al. 2021). Furthermore, one of the key parameters to ensure the quality of the forage is the concentration of heavy metals after fertilization to prevent damage to human health through the food chain (Xu et al. 2021). In the present study, very low in heavy metals contents was present (Table 4); all the values were below the limits established as toxic by EU Directive (CEC 2003). The low levels of these heavy metals observed in the plants are indicative of the scarce contamination and low metal contents of the input substrates that were used for anaerobic co-digestion as was demonstrated in a previous study (Fernández-Rodríguez et al. 2021).

Conclusions

The results show that the use of the anaerobic digestates obtained from the co-digestion of OMSW-microalgae, as an organic nutritional solution for the forage species *L. rigidum* cultivation, can be considered a viable and promising alternative to inorganic fertilization. The results of the anaerobic co-digestate fertilization improved not only the growth and development of the *L. rigidum* plants compared to the inorganic fertilizer, but also the quality of the nutritional forage obtained. These differences may be due to the greater presence of bio-assimilable essential nutrients in the AD digestate (mainly P). To date, AD digestate is an underutilized by-product that can contribute to a more sustainable and environmentally friendly agriculture. This study represents the first step in the right direction but requires further studies in order to assess its suitability for other forage species.

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Declarations

Conflicts of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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