

Traditional Compost and BSF-biodigested Compost in the Organic Fertilization of Ryegrass

Regina Menino^{a,*}, José N. Semedo^b, Paula Scotti-Campos^b, Amélia Castelo-Branco^a, Daniel Murta^{c,d}, Cecília Nestle^e, Vasco Fitas da Cruz^f, Olga Moreira^g

^a UEIS Sistemas Agrários e Florestais e Sanidade Vegetal-Laboratório de Solos, Plantas e Águas, Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV), Av. da República, Quinta do Marquês, 2780-159 Oeiras, Portugal

^b UEIS Biotecnologia e Recursos Genéticos, INIAV, Oeiras, Portugal

^c EntoGreen - Ingredient Odyssey, Santarém, Portugal; ^d CiiEM-Centro de investigação interdisciplinar Egas Moniz, Campus Universitário, Quinta da Granja Monte de Caparica, 2829-511 Caparica, Portugal

^e Leal & Soares, S.A., Zona Industrial Mira | Polo I – Lote 39 | 3070-337 Mira, Portugal

^f MED-Universidade de Évora

^g Unidade Estratégica de Produção e Saúde Animal, INIAV, I.P., Pólo de Investigação da Fonte Boa, Santarém, Portugal.

* Corresponding author. Email: regina.menino@iniav.pt

Abstract

The present study is a contribution to evaluate the agronomic potential of two different organic products (cattle effluents, either composted or digested by Black Soldier Fly larvae) as organic fertilizers, through the measurement of production and the photosynthetic activity of ryegrass (*Lolium multiflorum* L.), growing in a sandy soil, treated with different doses of those products. Within this aim, an experiment was conducted in a semi-controlled greenhouse with ryegrass plants cultivated in pots, with ten treatments: four different treatments of traditionally composted material, four different treatments of biodigested material, a mineral control (using the recommended rate of mineral fertilizer for ryegrass), and a zero control (without any type of fertilization). Under the experimental conditions, the results showed a significant effect of both organic composts over mineral fertilization, with a better performance of the biodigested by black soldier fly larvae. As for the informative capacity of photosynthetic activity data, in assessing the vegetative development of crops, "net assimilation rate" and "water use efficiency" proved to be the most suitable parameters.

Keywords: Biodegraded effluent, composted effluent, photosynthetic performance, ryegrass yield pot trial.

1. Introduction

The promise of the so-called "green revolution" (of the 1960s), based on the ability to create new highly productive varieties at the expense of high chemical inputs (fertilizers, phytopharmaceuticals, and fuels), was then praised for the resounding success in several countries where it was implemented, but it has been progressively revealing its perverse side, namely: in the exhaustion of fertility and in the pollution of soils and groundwater, in the reduction of water resources (by the proliferation of irrigated areas); and in climate change due to the intensive use of agricultural machinery burning fossil fuels.

In order to counteract all these inconveniences, various alternatives have been tested, either at the general level of the entire production philosophy, or at the specific level of the various factors that contribute to saving production and protecting the environment, without losing a scale of production compatible with the planet's needs.

The resurgence of organic fertilizers, which are more robust than mineral fertilizers, in improving soil fertility and reduce groundwater contamination, has been successfully tested in several situations. Among these, livestock manure, which is one of the traditional organic fertilizers discarded by the intensivist option that informed the "green revolution", is undoubtedly one of the obvious candidates.

Moreover, the progressive high concentration of intensive livestock farming in some regions, and the limited area for the disposal of the slurry produced, is a reality, causing difficult issues that need to be urgently solved. The direct disposal brings a sanitary problem, if not adequately managed, and one of the ways to overcome it is to submit the slurry to a composting process to use in crop fertilization. The organic products generated through these processes have to be used cautiously, because their composition is not standard and has to be adequate to each culture, in terms of quality and quantity. However, these organic composts are usually rich in plant nutrients, especially nitrogen (N), which is one of the most important nutrients for plant growth.

The problem with the organic "fertilizers" is that the N content is mainly in organic form and it needs to be mineralized for plant absorption. Nevertheless, this mineralization process does not always go along with the plant needs and, so, the fertilising potential of different types of composts depends, to a large extent, on the reconciliation of these processes in the relationship of the different plant genotypes with the different composts.

The photosynthetic activity reflects the response of the plants to changes in the environment, being a sensitive physiological parameter of plant metabolism and development (Liu *et al.*, 2013) and is closely linked to N availability, since N is a major constituent of light-harvesting complexes and reaction center systems, in which chlorophyll and other

pigments are associated with proteins to form chlorophyll proteins, and there is a positive correlation between these pigments and leaf N concentration (Bredemeier and Schmidhalter, 2001).

In the present study, the effect of traditional bovine slurry compost and a compost of the same origin biodigested by Black Soldier Fly larvae (BSFL) are evaluated on ryegrass plants, through production and photosynthetic rate, between each other and with a mineral fertilization, all with equal N endowments.

2. Materials and Methods

The trial was conducted in a greenhouse, located in Oeiras (Portugal), belonging to the National Institute for Agrarian and Veterinarian Research (INIAV I.P.).

The soil used for the experiment was classified as a Gleyic Podzol (IUSS-FAO, 2006). A sample of the soil was analysed for selected physicochemical properties, according to the methods used routinely in INIAV laboratories. The results for soil surface layer (0-25 cm) analysis revealed a coarse texture (96% sand), a pH around 6, a cation exchange capacity of 3.8 cmol.kg^{-1} , poor in nutrients and with 5.1 g of organic matter per kg.

The soil collected to be used for the pot trial was air-dried, at room temperature, and sieved to pass a 2mm mesh, using a total weight of 3 Kg per pot.

The organic composts used in the experiment were analyzed for N content, by the Kjeldahl method (Bremner and Mulvaney, 1982), and the results obtained for the bovine slurry composted (designated by the C letter – for Composted) was 12.3 g N kg^{-1} , and for the Black Soldier Fly larvae (BSFL) biodigested bovine slurry (designated by the F letter – for Frass) the result was 14 g N kg^{-1} .

A greenhouse pot experiment, using ryegrass (*Lolium multiflorum* Lam.), was set up in order to evaluate the agronomic potential of a composted bovine slurry (C) and a BSFL biodigested slurry (F) of the same origin, and was carried out in cylindrical plastic pots (with 15 cm height, 12.5 cm in diameter at the bottom, and 17 cm in diameter at the top) with a surface area of 226.9 cm^2 , filled with 3 Kg of sandy soil.

During the plant growth cycle, the greenhouse temperature was kept between 18 and 25 °C, and the pots were daily watered, with deionized water, to maintain the soil moisture near to 80% of water holding capacity, estimated by weight difference. The pots were disposed in different places every day, in a randomized way, after watering, to eliminate any influence of the day light.

The experiment, in a randomized plot design, consisted of 10 treatments (1 control, 1 mineral, 4 C and 4 F, these last 8 with a complement of 10% nitro ammoniacal N) with five replicates, totalizing fifty cylindrical plastic pots. The rates of compost were calculated based on the N demand by the crop (estimated in 140 kg N per hectare), considering a mineralization rate of 50%, and a ryegrass N use efficiency of 50%. Based on these assumptions, the treatments were the following: T0, corresponding to the negative control, without fertilization; TM, with 1.2 g of mineral fertilizer per pot (equivalent to 140 kg N per hectare); TC1, TC2, TC3, and TC4, using, respectively, 83 g, 166 g, 248 g, and 331 g of compost, per pot; TF1, TF2, TF3, and TF4, using, respectively, 22.7 g, 45.4 g, 90.8 g, and 113.5 g, of biodigested compost per pot.

Before sowing, the material to be tested was mixed with soil. The seeds were surface sown in each pot, using a seed density equivalent to 40 kg per hectare.

Gas exchanges were measured in adult expanded leaves of 6 weeks old plants, prior to the first plant cut. Net photosynthesis rate (P_n), stomatal conductance (g_s) and transpiration rate (E) were determined using a portable infra-red gas analyzer (LI6400, LI-COR, Lincoln, U.S.A.), under 20-25 °C, with a light supply of $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$, as described in Semedo *et al.* (2021) Water use efficiency (WUE) was calculated as P_n/E .

After measurements of leaf exchange evaluation, the plants were harvested, at about 2 cm above the soil surface, for yield evaluation (for fresh and dry weight). After weighted, the samples of fresh material were washed with deionized water, dried at 60°C, till constant weight, and, after dry weighted, grounded for N chemical analyses, by the Kjeldahl method (Bremner and Mulvaney, 1982).

The data were analyzed statistically using a one-way AOV with a significance level of $P < 0.05$, which was applied to test for differences between treatments in respect to weight, N tissues concentration, and gas exchange parameters. Tukey HSD test for mean comparison was performed (for a 95% confidence level). Results were statistically analyzed by Statistix 9.0, Analytical Software.

3. Results and Discussion

The results obtained for ryegrass production and N concentration in leaf tissues are presented in Table 1. From the data, and as registered both for fresh and dry weight, it is possible to observe that the higher yield was obtained in the treatment TF4, by a significant difference compared to all the other treatments and the second highest yield was

observed for treatments TC4 and TF3, by a significant margin over the remaining treatments, which means that the biodegraded was more efficient than the traditional compost and the biological fertilisers gave a higher yield than the mineral fertiliser treatment. In fact, the TM treatment only led to a higher yield than that recorded for organic fertilizers for treatments TC1 and TF1, which proves that the allocation to these treatments is insufficient.

Table 1. Mean values (n=5) for ryegrass production, expressed as fresh and dry weight (g) per pot, and total N concentration in plant tissues, in each treatment

Treatment	Fresh weight (g)	Dry weight (g)	Total N (g kg ⁻¹)
T0	9 f	1.7 g	10.2 g
TM	42 d	5.3 de	34.2 a
TC1	28 e	4.7 ef	11.1 fg
TC2	61 c	8.4 c	15.7 de
TC3	64 c	8.6 bc	16.2 de
TC4	76 b	9.7 b	17.6 cd
TF1	26 e	4.2 f	12.5 f
TF2	41 d	6.2 d	14.8 e
TF3	76 b	9.5 b	19.0 bc
TF4	89 a	10.9 a	20.5 b

Means in the same column with the same small letter do not differ significantly ($p \leq 0.05$), as assessed by the Tukey test

As expected for a sandy soil, the lowest production was recorded for treatment T0, by a significant difference compared to the others.

Compared to the results obtained by Menino *et al.* (2021), also working in similar conditions (but in a different soil, with another organic compost and no mineral fertilizer complement) the yield values obtained in the present experiment are much higher, maybe due to the higher rates of the composts but undoubtedly also due to the complementary mineral fertilizer for the start of the ryegrass crop, as it was hypothesised in the referred work.

With regard to the concentration of N in the plant tissue, the highest values, and significantly different from the other treatments, were recorded for TM, since the directly assimilable formulation of N is here much more adapted to the needs of the plant. On the other hand, again as expected for a sandy soil, the lowest N concentration was recorded for treatment T0, by a significant difference compared to the others. The remaining data for N concentration followed the same pattern recorded for the production of biomass.

The results obtained for the gas exchange measurements are presented in Table 2, and suggest the following comments:

Table 2. Mean values (n=9) for net assimilation rate (P_n), stomatal conductance (g_s), transpiration (E), and water use efficiency (WUE), calculated as P_n/E , for ryegrass grown under different fertilization treatments

Treatment	P_n ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	g_s ($\text{mmol m}^{-2}\text{s}^{-1}$)	E ($\text{mmol m}^{-2}\text{s}^{-1}$)	WUE [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}(\text{H}_2\text{O})$]
T0	3.0 f	106 b	2.3 c	1.3 c
TM	10.3 bc	332 a	5.6 a	1.9 bc
TC1	5.4 e	196 b	3.5 bc	1.5 bc
TC2	6.4 de	331 a	4.7 ab	1.4 c
TC3	8.3 cd	239 ab	4.2 ab	2.0 bc
TC4	10.4 abc	244 ab	4.0 abc	2.8 ab
TF1	5.9 e	194 b	3.3 bc	1.8 bc
TF2	8.2 d	318 a	4.4 ab	2.1 bc
TF3	11.1 ab	312 a	4.6 ab	2.9 ab
TF4	12.5 a	291 a	4.1 ab	4.0 a

Means in the same column with the same small letter do not differ significantly ($p \leq 0.05$), as assessed by the Tukey test

The pattern of the values recorded for P_n and WUE is consistent with that observed for biomass production, confirming their informative value with respect to the vegetative state of the plants. However here, the higher net assimilation rate recorded for the TF4 treatment, relative to TF3 and TC4, although by a wide margin did not reveal statistical significance. On the other hand, regarding g_s and E, the values registered do not suggest relevant conclusions regarding their relationship with the production.

The results from a work developed by Jia *et al.* (2010), on physiological characteristics of ryegrass, found values of

P_n varying between 10.8 and 16.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$, respectively with low and high atmospheric CO_2 , which are higher than the values obtained in the present experiment, except for the treatments TM, TC4, TF3 and TF4, that were within the low values obtained in that experiment. Given the systematically increasing pattern of results for both organic fertilizers, we can therefore assume that the maximum amounts tested were sub-optimal.

4. Conclusions

The main hypothesis that informed the present study is confirmed in the results that show, in a statistically demonstrated manner, the better performance, in ryegrass production, of cattle slurry biodigested by the black soldier fly larvae, in comparison with traditional compost with the same raw material (both with a complement of readily available N). Both organic fertilizers gave a higher yield than the mineral fertilizer treatment, very clearly, due to the addition of directly available N, which again proved to be paramount to accelerate initial plant growth under any organic fertilization.

As for the informative capacity of photosynthetic activity data, in assessing the vegetative development of crops, "net assimilation rate" and "water use efficiency" proved to be suitable parameters. As a matter of fact, significant effect of both organic composts was also perceived through overall photosynthetic activity of ryegrass plants and biomass production, showing the potential of these organic products as fertilizers.

The pattern of the values recorded for P_n and WUE is consistent with that observed for biomass production, confirming their informative value with respect to the vegetative state of the plants.

With regard to the concentration of N in the plant tissue, the highest values, and significantly different from the other treatments, were recorded for TM, which suggests the possibility that the additional 10% N added to the organic fertilizer may have been insufficient.

Given the systematically increasing pattern of results for both organic fertilizers, we can therefore assume that the maximum amounts tested were sub-optimal.

Acknowledgements

This work is co-financed by the FEADER and the Portuguese government through PDR2020 under the project EFLUENTES (PDR2020-101-031831).

References

- Bredemeier, C., and U. Schmidhalter, 2001. Laser-induced chlorophyll fluorescence to determine the nitrogen status of plants. In *Plant nutrition - Food security and sustainability of agro-ecosystems*. Dordrecht. The Netherlands: Kluwer Academic Publishers. Eds., W.J. Horst *et al.* 92, 726-727. https://doi.org/10.1007/0-306-47624-X_352
- Bremner, J.M., and C.S. Mulvaney, 1982. Nitrogen-Total. In *Methods of soil analysis. Part 2. Chemical and microbiological properties*. 2nd ed. (Agronomy 9). American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin. Eds., Page A.L., R.H. Miller and D.R. Keeney. 595-624.
- Conceição, G., E.A. Ferreira, A.A. Silva, F.A. Ferreira, L. Galon, M.R. Reis, L. d'Antonino, L. Vargas, L.V.B.D. Silva, 2008. Fotossíntese de biótipos de azevém sob condição de competição. *Planta Daninha*. 26 (3), 595-600. <https://doi.org/10.1590/S0100-83582008000300015>
- IUSS-FAO Working Group WRB, (2006): World reference base for soil resources 2006 - A framework for international classification, correlation and communication, Rome, Food and Agriculture Organization of the United Nations. [10013/epic.43321.d001](https://doi.org/10.1013/epic.43321.d001)
- Jia, Y., S. Tang, R. Wang, X. Ju, Y. Ding, S. Tu, D.L. Smith, 2010. Effects of elevated CO_2 on growth. Photosynthesis, elemental composition, antioxidant level, and phytochelatin concentration in *Lolium multiflorum* and *Lolium perenne* under Cd stress. *Journal of Hazardous Materials*. 180 (1-3), 384-394. <https://doi.org/10.1016/j.jhazmat.2010.04.043>.
- Liu, X., Y.Fan, J.Long, R. Wei, R. Kjelgren, C. Gong, J. Zhao, 2013. Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of *Robinia pseudoacacia* seedlings. *Journal of Environmental Sciences*, 25 (3), 585–595. doi:10.1016/s1001-0742(12)60081-3
- Menino R., F. Felizes, M.A. Castelo-Branco, P. Fareleira, O. Moreira, R. Nunes, D.Murta, 2021. Agricultural value of Black Soldier Fly larvae frass as organic fertilizer on ryegrass. *Heliyon*, Jan 2, 7 (1), e05855. doi: 10.1016/j.heliyon.2020.e05855. PMID: 33426352, PMCID: PMC7785954.