Research Article

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Cattle effluents, either composted or bio-digested by Black Soldier Fly larvae, in the fertilization of ryegrass in sandy soils

Abstract

The relative agronomic performance of two organic fertilizers - effluents from intensive cattle farming, after composting either by traditional aerobic methods (compost) or by bio-digestion by larvae of the black soldier fly (*Hermetia illucens*, BSF) (frass) - in the growth of annual ryegrass (*Lolium multiflorum* L.) on a sandy soil was evaluated. For this purpose, a pot trial was conducted in a semi-controlled greenhouse. Eight treatments were defined, with four treatments using the compost and four treatments using frass (with a 10% nitroamoniacal nitrogen supplementation in all treatments), in addition to a negative control without fertilization, and a positive control with exclusively chemical nitroamoniacal fertilization. Regarding the biomass production, the BSF frass displayed a higher potential than the compost, with a significantly higher production from an N endowment equivalent to 280 kg ha^{-1} (252 kg organic N + 28 kg mineral N). While the yields obtained with compost were stable when 350 kg ha^{-1} (315 kg organic N + 35 kg mineral N) were provided, the yields obtained with BSF frass revealed a tendency to continue to progress with higher doses. This could be justified by the fact that the P_2O_5 and K_2O content of BSF frass is significantly higher than that of the compost, and these nutrients continued to be mineralized over time. Furthermore, the obtained results confirm the potentiating effect of mineral fertilizers in the maximization of biomass production, when associated with organic fertilizers in adequate proportions. In addition, it was verified that the addition of frass enhanced the water use by plants, which was significantly higher than the treatments with compost, regardless of the N endowment. It was concluded that mixing chemical and organic fertilizers positively impacts soil fertility and the sustainability of the production as a whole, by increasing productivities in a sustainable way.

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Introduction

In the pursuit of sustainable food security at the global level, in a compatible way with environmental and social security, the most appropriate solutions for agricultural production, in the countless prevailing edaphoclimatic, social and economic situations, should involve the unprejudiced weighing up of several alternative techniques, all of which are inextricably linked but often assumed to be irreconcilable. The experimental work presented here was designed to help reconcile various objectives in agricultural production on sandy soils, with a holistic approach to biomass production, namely the reduction of crop water requirements, the safe disposal of effluents from intensive livestock production, the reduced use of chemical fertilizers and the reduction of nitrate contamination of aquifers.

Sandy soils, due to their high hydraulic permeability and reduced content in mineral and organic structures, essential for the retention of mineral nutrients in their soluble formulations, are particularly prone to leaching of plant nutrients, thus constituting a factor of high risk of impoverishment of soil fertility and contamination of aquifers, particularly by nitrates.[1] Organic fertilizers, in addition to the macro and micronutrients they can make available to plants, promote the formation of soil structures with the capacity to retain water and nutrients.[2] The process of mineralization of organic nitrogen in soils, being slow and gradual,[3] may also allow for a reduction in the leaching process, with the drawback, however, of a shortage of mineral N available for the plants at the appropriate time, in particular at the start of the crops.

Considering the above, with the results presented here, we anticipate the relative potential of two organic fertilizers (one obtained by traditional composting and the other through biodigestion by *Hermetia illucens* larvae (black soldier fly - BSF), from the same effluent of intensive livestock activity) with regard to biomass production and water saving, in ryegrass cultivation on sandy soils, measured at the first cut.

Materials and methods

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

The soil used in the experiment was classified as a Gleyic Podzol.[4] After sampling the soil from the 0-25 cm layer, it was air-dried at room temperature and sieved to pass a 2mm mesh, using a total weight of 3 kg per pot (replicate). A soil sample was analyzed for selected physicochemical properties, according to the methods used routinely in INIAV laboratories. The results obtained revealed a coarse texture (96% sand), a pH around 6.0, a cation exchange capacity of 3.8 cmol kg^{-1}, low concentrations of nutrients and a value of 5.1 g of organic matter per kg of soil.

In addition to the physico-chemical analysis required for legal certification in force, to authorise its use on agricultural land, the two organic fertilizers used in the experiment - produced from the same slurry resulting from intensive cattle farming - were analyzed for N content, by the Kjeldahl method,[5] and the results obtained for the composted (designated by the **C** letter – for **C**omposted) was 12.3 g N kg^{-1}, and for the biodigested (designated by the **F** letter – for BSF **F**rass,) the result was 14g N kg^{-1}. The P_2O_5 and K_2O contents of these fertilizers, measured according to legal standards (EN 13650:2001), were, respectively, 0.60% and 1.01% in compost, and 3.35% and 1.91% in BSF frass.

A greenhouse pot experiment, using annual ryegrass (*Lolium multiflorum* Lam.), was set up in order to evaluate the agronomic potential of **C** and **F**, 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 227 cm^2, filled with 3 kg of sandy soil. During the plant growth cycle, the greenhouse temperature was kept between 18 and 25 ºC, 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, disposed in a randomized plot design, consisted of 10 treatments with five replicates. The control treatment (T0), did not benefit from fertilizers. The N allocations for the remaining treatments were calculated on the basis of the average used for ryegrass crops (estimated at 140 kg N ha^{-1}), considering a mineralization rate of 50% and an N use efficiency of 50%. The N endowment, referred as kg ha^{-1}, in the treatment with mineral fertilization (TM), was 140, and for the mixed fertilizations (90% N organic, 10% N mineral) were, respectively: 70 (TC1 and TF1) and 140 (TC2 and TF2) for low N treatments, and 280 (TC3 and TF3) and 350 (TC4 and TF4) for high N treatments.

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 ha^{-1}. The amount of irrigation water delivered to each treatment repetition was recorded daily.

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 analyser (LI6400, LI-COR, Lincoln, U.S.A.), under 20-25 ºC, with a light supply of 700 µmol m^{-2} s^{-1}, as described in Semedo et al.[6]

After measuring the leaf gas exchange, the plants were harvested (first cut), at about 2 cm above the soil surface, for yield evaluation (fresh and dry weight). After weighting, the samples of fresh material were washed with deionized water, dried at 60ºC, until constant weight and then grounded for N chemical analyses by the Kjeldahl method.[5]

The data were analyzed by means of a one-way ANOVA, considering a significance level of 5%, which was applied to test for differences between treatments in respect to weight, N tissues concentration, and gas exchange parameters. The Tukey HSD test for comparison of means was performed (for a 95% confidence level). Results were statistically analyzed by 'Statistica 9.0, Analytical Software'.

Results and discussion

The results obtained for ryegrass yield are presented in Table 1. It was verified that the highest yield (both for fresh and dry weight) was obtained in treatment TF4 by a significant difference compared to all other treatments, and the second highest yield was observed for treatments TC4 and TF3 by significant margin compared to the other treatments.

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 lowercase letter do not differ significantly ($p \leq 0.05$), as assessed by the Tukey test.

As expected, the lowest production was recorded for treatment T0, by a significant difference compared to the others. Also, for the TM treatment, a lower yield than the organic treatments would be expected, since it is devoid of the remaining macronutrients, once its role in the trial was only to gauge the N content in plant tissues, compared to the mixed treatments. However, this only occurred for the mixed treatments with lower N endowment (TC1 and TF1 with 63 kg ha^{-1} of organic N + 7 kg ha^{-1} of mineral N) and, even in this case, only by a significant margin for the production of fresh weight of biomass; for the other mixed treatments biomass production was always significantly higher.

The use of composts and other organic amendments, such as frass, may generate differential responses in relation to crops' growth, due to many variables. Frass is usually considered a biologically unstable fertilizer when used fresh (immediately after the biodigestion process), as is the case of this study, releasing nutrients at a slow rate.[7] Nevertheless, the growth of ryegrass followed similar patterns between frass and compost blended together with a mineral fertilizer (Table 1), demonstrating that either the nutrients of frass were made available for plants in the experimental period, or the initial provision of nutrients was a result of the mineral fertilization. This hypothesis should be tested in a further study.

In this sense, without the occurrence of leaching, the TM treatment determined a much higher N content in plant tissue than that found for

TF4, in an inverse relation to that recorded for biomass production, despite the fact that the latter benefited from 2.5 times more N (mineral plus organic). On the other hand, and as expected, the pattern of N contents in the harvested plants, verified for the progressive N endowments, in both mixed treatments tested, showed an identical trend with each other and with the pattern registered for the N content in the plant tissues. This can be understood as clear evidence that in order to maximise biomass yields, there is a need for a greater proportion of mineral N (Timsina, 2018), in addition to other macro nutrients that, in the present study, were in deficit in the compost, and only approached the recommended amounts for ryegrass cultivation in treatment TF4.

Compared to the results obtained by Menino et al.[8] 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 provided for the start of the ryegrass cultivation, as it was hypothesised in the referred work.

The 2nd degree polynomial curves of biomass production (dry weight) as a function of N dosage is shown in Figure 1, which was measured for both mixed fertilizers. For the fertilizer based on BSF frass, a potential for further increase in biomass production is indicated, starting from the maximum N application tested. This conclusion is further suggested by the difference, between TM and TF4, of the N content in the leaf tissues, in counter-cycle with the positive correlation of the yield as a function of the leaf content of this macronutrient. Therefore, it is reasonable to admit the need for a complement of mineral N, in the mixed fertilization with BSF frass, greater than that used in the present study.

Conversely, regarding the compost fertilizer, a potential reduction in the biomass production is apparent beyond the N application equivalent to 280 kg ha^{-1} (Figure 1). Such a decrease, however, is not consistent with the statistically significant difference between this N application level and that of 350 kg ha^{-1}, as previously pointed out, suggesting that there should not be a decrease but rather a stabilization in the biomass production.

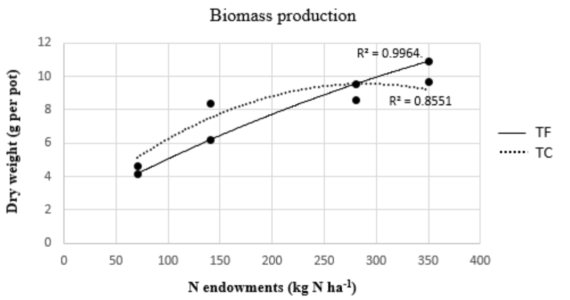

Figure 1 Second degree polynomial curves for biomass production as a function of N endowments in the mixed fertilizers.

The results obtained in the physiological monitoring of the trial are presented in Table 2. The pattern of the values recorded for P_n over the progressive allocations of N for the mixed fertilizers is consistent with that observed for biomass production, confirming their informative value with respect to the vegetative state of the plants.[9] HoweverHowever, here, it was possible to observe that the higherhighest net assimilation rate was recorded for the TF4 treatment, relative to TF3 and TC4, although by a wide margin, did not reveal statistical significance,. n ervertheless Nevertheless, with a statistically significant difference compared to the remaining treatments was observed. These results are within the range of values referred by Concenço et al.[10] for ryegrass crops.

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 Pn/E, for ryegrass grown under different fertilization treatments

Treatment	P_n (mmol m^{-2}s^{-1})	gs (mmol m^{-2}s^{-1})	E (mmol m^{-2}s^{-1})	WUE [mmol (CO_2) mol^{-1} (H_2O)]
T0	3.0 f	106 b	2.3 c	1.3 c
TM	10.3 bc	332 a	5.6 a	1.8 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	2.6 ab
TF1	5.9 e	194 b	3.3 bc	1.8 bc
TF2	8.2 d	318 a	4.4 ab	1.9 bc
TF3	11.1 ab	312 a	4.6 ab	2.4 ab
TF4	12.5 a	291 a	4.1 ab	3.0 a

The results presented by Jia et al.[11] for the physiological characteristics of ryegrass, indicating Pn values ranging between 10.8 and 16.5 mol m^{-2}s^{-1}, respectively at low and high atmospheric CO_2, are only compatible with those obtained, in the present trial, for the treatments TM, TC4, TF3 and TF4, which are within those values. Given the systematically increasing pattern of results for both organic fertilizers, we can therefore assume that, here as well, the N endowments tested are still below the optimum.

The results presented by Jia et al.[11] for the physiological characteristics of ryegrass, indicating Pn values ranging between 10.8 and 16.5 mol m$^{-2\,b}$s^{-1}, respectively at low and high atmospheric CO_2, are only compatible with those obtained in the present trial, for the treatments TM, TC4, TF3 and TF4, which are within those values. Given the systematically increasing pattern of results for both organic fertilizers, we can therefore assume that, here as well, the N endowments tested were still below the optimum.

Means in the same column with the same lowercase letter do not differ significantly (p≤0.05), as assessed by the Tukey test.

Regarding g_s and E, the recorded values were higher in the treatment with exclusive mineral fertilization, although only statistically significant in comparison with both organic treatments at their lower nitrogen endowments. In this trial, these parameters did not prove to be particularly relevant for analysing the relative behaviour of the different treatments.

In Table 3 are presented the values for average volume of irrigation water used, considering that it was estimated to reach 80% of field capacity, and for dry weight of biomass produced per volume of irrigation water.

Means in the same column with the same lowercase letter do not differ significantly (p≤0.05), as assessed by the Tukey test.

In the present table, as verified for the WUE values (Table 1) for the mixed fertilization treatments, the biomass productions were always higher for the BSF frass treatment, at any of the N fertilization levels, although by statistically non-significant margins. These results reveal that apparently, BSF frass is more efficient in increasing the water holding capacity of soils in comparison to exclusive mineral fertilization or even with the combined application of a manure-derived compost.[12,13]

Table 3 Mean values for Average Water Volume (AWV) and for weight of biomass per volume of irrigation water

Treatment	AWV (litres per pot)	Dry weight/AWV [g (ml cm^{-3})$^{-1}$]
T0	2.3 d	0.7 f
TM	3.8 c	1.4 cd
TC1	3.9 c	1.1 e
TC2	4.7 b	1.3 d
TC3	5.9 a	1.6 bc
TC4	6.0 a	1.8 ab
TF1	3.7 c	1.3 de
TF2	4.9 b	1.7 ab
TF3	4.9 b	1.8 ab
TF4	5.1 b	1.9 a

Conclusion

In the context of the safe disposal of effluents from intensive cattle production, and with a view to a preliminary assessment of the relative potential on sandy soils, of two organic fertilizers, obtained by composting and by biodigestion by BSF from the same slurry, the present study allows for the following conclusions to be drawn after the first cut: i) regarding the **production of biomass**, BSF frass had a higher potential in comparison to the compost, with a significantly higher production from an N endowment equivalent to 280 kg ha^{-1} (252 organic N + 28 mineral N). Furthermore, while the yields obtained with compost showed a stabilisation from N endowments greater than 350 kg ha^{-1} (315 organic N, 35 mineral N), the yields obtained with BSF frass show a tendency to continue to progress; ii) regarding **water saving**, as the biomass production per unit of water required is higher, also the most favourable results were registered for the BSF frass treatments, at all levels of N endowment, although by a non-significant margin. Moreover, the N content of BSF frass being higher than that of compost, the amount of organic fertiliser required for each treatment is lower in the treatments with BSF frass. In synthesis, the results recorded in the present study, confirm the greater fertilising potentiality of BSF frass, in comparison with compost, along with a higher economy of water. Nevertheless, the results verified here confirm the potentiating effect of mineral fertilizers in the maximization of biomass productions, when associated with organic fertilizers in the adequate proportion for the sustainability (or even increase) of soil fertility.

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Conflicts of interest

The author declares there is no conflict of interest.

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