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Organic fertilisation induces changes in soil nitrogen mineralisation and enzyme activities

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Abstract: In this study, we addressed the reuse of two organic waste products as fertilisers. To this end, soil fertilised with the spent mushroom substrate (SMS) or with an anaerobic digestate (DIG) was subjected to an incubation assay, and the results were compared with those from soil treated with a mineral fertiliser (MIN) and an unfertilised soil (CO). The soil was sampled after fertilisation and after 90 days of aerobic incubation. Nitrogen (N) mineralisation (NH_4^+ and NO_3^-) and oxidable carbon (OC) were determined. The impact of the treatments on the soil was evaluated by measuring the enzymatic activity of arylsulfatase (ARYL), ß-galactosidase (GAL), and urease (URE). The highest OC content was observed in the SMS treatment. After 90 days of incubation, the SMS treatment showed a lower mineral N content than the CO treatment. This finding was associated with N immobilisation. However, mineral N significantly increased ARYL activity in the DIG and MIN treatments, and URE activity was always higher at both sampling times in the SMS treatment. Initially, GAL activity was notably high in the DIG treatment but decreased after incubation, reaching similar values to those registered in the CO treatment. Organic fertilisation treatments induced different effects on soil N mineralisation, showing changes in the activity of the enzymes analysed.

Keywords: recycling; nutrients; organic residues; liquid anaerobic digestate; spent mushroom substrate of *Pleurotus*

The EU's Circular Economy Action Plan promotes the use of organic waste as fertiliser, thus allowing the recycling of nutrients in the agricultural system. Also, the Common Agricultural Policy of the EU establishes that 25% of the area will be under organic cultivation by 2030 and that there will be a 20% reduction in mineral fertiliser by this date. In this regard, the use of agricultural organic waste as fertiliser is proposed as a sustainable alternative to mineral fertiliser. In addition, organic residues contribute to the 4 × 1 000 initiative with respect to the increase in soil organic carbon (C) content (Poulton et al. 2017). This endeavour seeks to demonstrate that a small increase in organic C storage (4‰ per year) is crucial to improve soil fertility and yields and will contribute to mitigating climate change.

Agricultural production is leading to increasing amounts of organic waste, including new organic residues from agri-food industries and farms (e.g. spent mushroom substrates and digestates). In this regard, mushroom production has risen in the last decade and is forecasted to continue growing. In the context of organic waste, the volume of biogas produced in the 28 EU countries increased from 93 to 187 TWh between 2008 and 2016 (Gustafsson et al. 2021). This figure is expected to double again

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by 2030 (Kampman et al. 2017). Biogas production generates digestate as waste (180 million tons in Europe alone; Corden et al. 2019), and the separation of the raw digestate increases the quality of the liquid fraction as a fertiliser (Barduca et al. 2021).

The reuse of organic waste as fertiliser calls for prior knowledge of potential nitrogen (N) availability for crops (mineral N: NO_3^--N and NH_4^+-N). Given their composition, organic waste products derived from the agro-industrial sector are a source of organic nitrogen and nutrients for crops. However, these organic waste materials must previously undergo mine ralisation, which converts organic N to mineral N. This process is determined by soil temperature and water content, the composition of the organic residues, and soil characteristics (Cabrera et al. 2005). The mineralisation and immobilisation of N occur in the soil simultaneously.

Soil enzymes play a prominent role in nutrient availability. They transform soil organic compounds and are involved in mineralisation, nutrient immobilisation, and the biological fixation of N (Dick and Tabatabai 1993). Given the role of enzymes in these processes, their activity after fertilisation can be used as early sensitive indicators of soil nutrient changes after fertilisation application. Moreover, several authors have demonstrated the positive effect of organic fertilisers on soil enzymatic activity of soil (Kuziemska et al. 2020). Urease (URE) catalyzes the hydrolysis of urea to NH_4^+ and CO_2 and its relationship with the N cycle (Sastre and Lobo 2003). The *ß*-galactosidase (GAL) is involved in the C cycle; it serves as a marker to monitor damage to the soil microflora after the application of organic (Martínez-Iñigo et al. 2003). Arylsulfatase (ARYL) is responsible for the mineralisation of organic sulfur, and its activity is correlated with organic matter (Tabatabai and Bremmer 1970).

Here we hypothesised that the type of organic fertiliser applied to soil could condition differences in mineral N forms, oxidable C and enzymatic activities. Furthermore, we considered the effects of organic fertilisation would have implications for management practices associated with each type of organic residue.

In this regard, we studied how the application of two organic residues (spent fungal substrate or the liquid fraction of an aerobic digestate) contributes to soil functionality. To this end, we analysed the availability of mineral N forms (NO_3^- and NH_4^+) and OC and the activities of soil enzymes (ARYL, GAL, and URE).

MATERIAL AND METHODS

Three representative subsamples from the surface layer (0–30 cm) of soil from agricultural plots located in Alcalá de Henares (Madrid, Spain) were used (Table 1). The soil is classified as silty clay loam (USDA, 5% sand, 31% clay, and 64% silt).

All visible fragments, including roots and stones, were removed. The dried bulk soil was thoroughly mixed to ensure uniformity and was then air-dried and passed through a 2-mm sieve.

The organic residues used were spent mushroom (*Pleurotus* spp.*)* substrate (SMS) and liquid anaerobic digestate (DIG). The SMS was obtained from a commercial facility after 3–4 fructifications in a commercial facility. The DIG was supplied by a commercial anaerobic digestion plant that uses tobacco and cereal with fattening pig slurry after filtration (750-µm).

Three representative subsamples of each organic residue were used to determine their main chemical and microbiological characteristics (Table 2).

The doses of the organic treatments were calculated based on the rate of N allowed by regulations in non-vulnerable areas (210 kg N/ha year; Council Directive 91/676/EEC). The fresh weight of the organic residues was applied at rates of 86.4 t/ha for SMS and 37.8 t/ha for DIG.

Table 1. Chemical characteristics of the topsoil

Parameter	Mean
pH (1:2.5)	8.17 ± 0.05
Electrical conductivity (EC; 1:5; S/m)	0.011 ± 0.001
Dry matter (DM; %, 105 °C)	99.7 ± 0.01
Organic carbon (% DM, Walkey-Black)	0.82 ± 0.05
Total N (% DM, Kjeldahl)	0.09 ± 0.01
C/N	9.1
Available phosphorus (mg P/kg DM, Olsen)	29 ± 1.0
Calcium carbonate (%; Bernard calcimeter)	3.2 ± 0.1
Exchange cations (mmol/kg DM, ammonium acetate pH = 7)	
Ca^{2+}	172.7 ± 7.6
Mg^{2+}	16.2 ± 0.8
Na^+	1.0 ± 0.1
K^+	8.7 ± 0.8
Cation exchange capacity (CEC): CEC = Σ (Ca^{2+} + Mg^{2+} + Na^+ + K^+)	198.5

DM – dry matter. pH and EC ratio soil:distilled water. ± standard deviation

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To establish the N mineralisation from the native soil OC, a control (CO) treatment without fertilisation was included. In addition, a treatment using mineral fertiliser (MIN) at a rate of 210 kg N/ha from ammonium nitrate (27%) was conducted to allow a comparison of the efficiency of the organic fertilisers (SMS and DIG). The N efficiency was expressed as the percentage ratio between the mineral N content of the organic treatments and the MIN treatment, previously subtracting the mineral N content of the CO treatment.

An aerobic incubation assay was carried out in an incubator chamber with controlled conditions in darkness at 25 °C maintaining soil moisture at 60% of field capacity, without leaching for 90 days (Stanford and Smith 1972). Briefly, 80 g of soil was placed in 100 mL plastic pots. Fertilisers were applied with the amount of water needed to adjust soil moisture and were mixed with the soil. Each container had seven holes (5 mm diameter) in the lid, thereby minimising moisture loss while allowing oxygen exchange. Sample moisture content was maintained by spraying distilled water, the amount of which was adjusted regularly (twice or three time weekly) by weighing the containers. Destructive sampling was performed by removing soil samples in triplicate for each treatment at each soil sampling.

Table 2. Chemical and microbiological characteristics of organic residues

Parameter	SMS	DIG
pH (1:2.5)	8.14	8.62
Electrical conductivity (1:2.5; S/m)	0.28	0.88
Dry matter (DM %)	22.7	6.2
Oxidable carbon (% DM)	42.3	24.4
Total N (% DM)	1.1	8.9
C/N	38.5	2.7
Organic N (% DM)	0.94	2.63
NH_4^+-N (% DM)	0.13	6.29
NO_3^--N (% DM)	nd	nd
Ureic N (% DM)	nd	nd
Total P (% DM)	nd	1.21
Soluble P (% DM)	nd	1.13
Available cations (% DM)		
K^+	0.89	0.91
Na^+	nd	nd
Ca^{2+}	0.77	0.58
Mg^{2+}	0.13	0.13
Microbiological parameters		
Salmonella spp. (CFU/25g soil)	nd	nd
Escherichia coli (MPN)	> 110	As

pH and EC ratio soil:distilled water. DM – dry matter. Organic N: calculated by the difference between total N and N in inorganic forms (sum of NH_4^+-N and NO_3^--N). nd – not detectable; CFU – colony-forming unit; MPN – most probable number; As – absence; SMS – spent mushroom substrate; DIG – anaerobic digestate

Soil sampling was carried out after the application and mixing of the treatments with the soil (0 days) and after 90 days of incubation under controlled conditions. Inorganic N (NO_3^--N and NH_4^+-N) was measured in 2 mol/L KCl (ISO 14256-2, 2005; Maynard et al. 2006). The oxidable C (OC) was measured in both soil samplings following the method described by Walkley and Black (1934).

The activity of ARYL, GAL and URE was determined using the standard conditions of each specific protocol with the appropriate substrate, following ISO 20130, 2018.

All results (N fractions, OC and enzymatic activities) are expressed as dry matter (DM) (at 105 °C). The experimental design included three blocks, the treatments being randomly distributed in each.

Statistical analysis was carried out by multiple ANOVA (Duncan's multiple range test at the 95% probability level) to determine significant differences between treatments (SAS Institute 1999–2001).

RESULTS AND DISCUSSION

Notable differences in salinity, DM, OC, C/N, and nutrient content were observed between SMS and DIG treatments (Table 2). The presence of de *Escherichia coli* in the SMS was associated with an isolated cross-contamination incident during storage.

Initially, regarding the mineral N fraction, the NH_4^+-N content was significantly higher in the DIG and MIN treatments compared to the CO and SMS treatments, which showed values < 1.5 mg NH_4^+-N/kg dry soil (Figure 1).

The supply of NH_4^+-N to the soil was higher in the DIG and MIN treatments, reaching 70.7% of total N in the DIG treatment (~148 kg NH_4^+-N/ha, Table 2) and 50% in the MIN treatment (~105 kg NH_4^+-N/ha). This proportion remained in the soil, which showed values of 38 and 29 mg NH_4^+-N/kg dry soil, respectively, regardless of the organic or mineral fertiliser source used.

Given the immediacy of fertiliser, the application and soil water content potential NH_3 losses were

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minimised. After 90 days of incubation, the NH_4^+-N content was similar in all treatments, achieving < 5 mg NH_4^+-N/kg dry soil.

This decrease in NH_4^+-N corresponds to an increase in NO_3^--N in the DIG and MIN treatments associated with the nitrification of NH_4^+-N and/or the potential mineralisation of the organic N provided by the DIG treatment (Figure 1). However, in the SMS treatment, a decrease in the NO_3^--N fraction was observed after 90 days, the values being even lower than those in the CO treatment. This decrease was associated with soil N immobilisation, which was probably caused by the low N supply provided by the SMS treatment, which showed a high C/N ratio (Table 2). Therefore, SMS may limit N uptake by the plant because of the use of soil N reserves (NO_3^--N) by microorganisms (Corbeels et al. 1999). This notion would explain the lower NO_3^--N content in the SMS compared to the CO treatment caused by the so-called "nitrogen starvation" effect. Nitrogen immobilisation usually occurs in field conditions when cereal stubble organic residues rich in OC are applied to the soil (Mary et al. 1996). This process is transient until OC mineralisation occurs and mineral N is released into the soil. In non-crop conditions or when crops have a low N demand, this immobilisation prevents NO_3^- loss by leaching.

In our experiment, soil N immobilisation continued until 90 days of SMS treatment in aerobic conditions. These results are consistent with those obtained by Wang et al. (2021), who detected significantly lower mineral N in the soil after treatment with maize residue (C/N:28) for 175 days compared to the control (without fertilisation). This microbial N immobilisation (microbial N mining) could be associated with increased microbial activity in treatment with maize residues compared to control and mineral fertiliser treatments during the process.

Regarding the efficiency of SMS and DIG after 90 days of incubation compared to the MIN treatment, SMS cannot be considered a source of N for crops, as it immobilises the N available in the soil in a mineral form. Consequently, it would be necessary to fertilise with mineral N to compensate for this immobilisation. The SMS can be supplemented with other organic residues, thus conferring new properties to the mixture, such as lower salt and higher OC content (Paredes et al. 2016), or it could be used solely as an amendment to improve soil fertility (Ngan et al. 2021) while achieving N immobilisation (NO_3^-) in soil.

The DIG treatment supplied N in the form of NH_4^+-N, which was mineralised to NO_3^--N during the incubation. At the end of the incubation, the efficiency of the DIG treatment with respect to MIN reached 61% in relation to the total N supplied calculated by the ratio between 38.4 and 62.5 mg mineral N/kg dry soil in DIG (68.6 mg mineral N/kg dry soil, Figure 1) and MIN (92.7 mg mineral N/kg dry soil, Figure 1), respectively, after subtracting the mineral N value corresponding to the CO treatment (30.2 mg

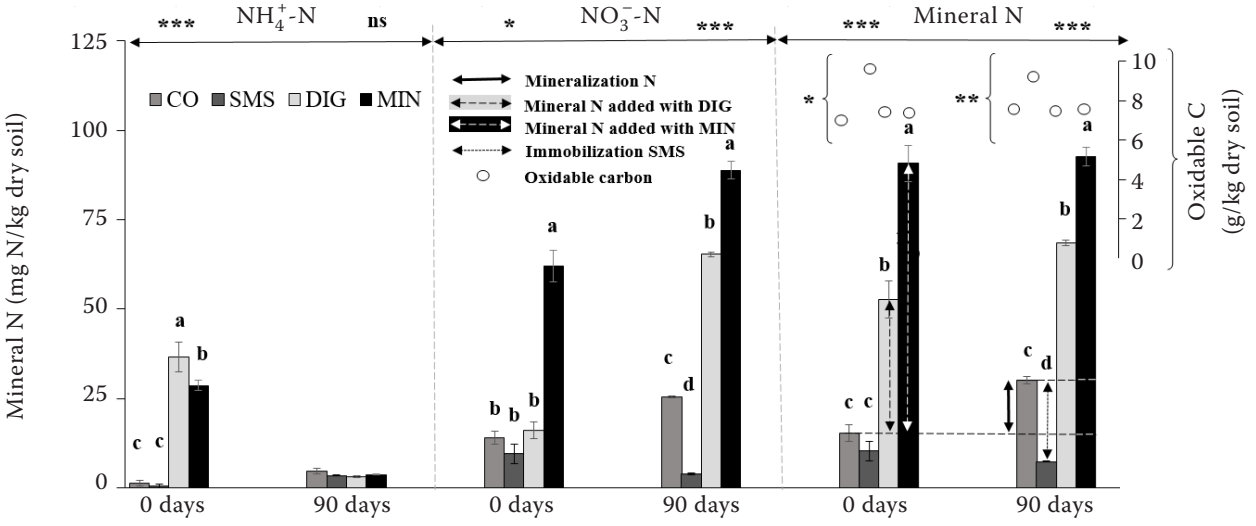

Figure 1. Forms of mineral nitrogen (NH_4^+-N and NO_3^--N) in the soil 0 and 90 days after aerobic incubation. Different letters between treatments in each form of N and sampling time indicate significant differences $P < 0.05$; Duncan's test. *$0.05 \leq P < 0.01$; ***$0.001 \leq P < 0.0001$; ns – not significant ($P > 0.05$); CO – control; SMS – spent mushroom substrate; DIG – anaerobic digestate; MIN – mineral fertiliser

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mineral N/kg dry soil, Figure 1). The mineral N content supplied by the DIG treatment was similar to values reported by Tambone et al. (2017) in an aerobic incubation assay.

It should be noted that the entire dose of N in the MIN treatment was applied as mineral N (NH_4^+-N and NO_3^--N), whereas in the DIG treatment, only 70.7% of total N was NH_4^+-N.

When comparing mineral N at the end of the incubation attributable to that associated with the NH_4^+-N applied in the DIG treatment, we estimate 38 kg mineral-N/kg dry soil, after subtracting the mineral N in the soil (30 kg mineral-N/kg dry soil) with a contribution of 148 kg NH_4^+-N/ha, whereas the MIN treatment, is estimated to provide 62 kg mineral-N/kg dry soil, after subtracting the mineral N in the soil, half of which attributable to the NH_4^+-N contributed, 31 kg mineral-N/kg dry soil with a supply of 105 kg NH_4^+-N/ha. Then, in the MIN treatment, the supply of 3.39 kg NH_4^+-N/ha allowed us to obtain 1 kg mineral-N/kg dry soil, while in the DIG, 3.85 NH_4^+-N/ha was necessary to obtain 1 kg mineral-N/kg dry soil. These observations indicate that the DIG treatment has an efficiency of 86% based on the NH_4^+-N applied with respect to the MIN treatment.

The incubation time did not affect the OC value in any treatments. This parameter was significantly increased only in the SMS (Figure 1; 9.1–9.6 g/kg dry soil). The application of SMS as an amendment would imply an increase of between 2.2‰ and 2.7‰ of OC to the soil.

The immobilisation of soil N in the microbial biomass by the addition of SMS may contribute to soil N preservation by preventing NO_3^- leaching. Moreover, SMS could contribute to the storage of ~ 9–11 t of OC per hectare per year.

The ARYL activity showed similar values in the DIG and MIN treatments compared to CO, although the SMS treatment had the highest initial values of this enzyme. After 90 days of incubation, the differences in ARYL activity between the SMS treatment and the others increased (Table 3). Given that the activity of this enzyme is used as an indirect indicator of fungi presence because these organisms contain sulfate ester, the substrate of ARYL (Bandick et al. 1999), it may explain the greater ARYL activity in the SMS treatment compared to the others. Furthermore, the increase in OM and ARYL activity in this treatment is correlated with soil organic matter (Tabatabai and Bremmer 1970).

The GAL activity increased in the treatments that supplied OC, namely SMS and DIG (Table 3). The increase was associated with the OC supplied by the treatments, which provided substrate and energy to microbial biomass and, as a result, increased the activity of this enzyme (Sekeran et al. 2018).

The low URE activity observed in the DIG and MIN treatments (similar to the CO treatment, Table 3) can be explained by the presence of inorganic N forms, which makes the synthesis of this enzyme unnecessary in these cases. This effect is consistent with the results of Dick et al. (1998), who reported that the addition of products with NH_4^+ suppresses URE activity. This observation would explain why URE activity was initially higher in the SMS treatment and why it remained higher than the other treatments after 90 days of incubation. These elevated URE values would be associated with N immobilisation during the incubation, thus keeping the soil inorganic N low.

The enzymatic activities of ARYL, GAL, and URE in soil treated with organic treatments showed values similar to or higher than those obtained in the CO and MIN treatments, and no negative effects were observed.

Table 3. Enzyme activity (nmol PNP/min g dry soil) of arylsulfatase (ARYL), ß-galactosidase (GAL) and urease (URE) at 0 and 90 days after aerobic incubation

Treatment	0 days			90 days		
	ARYL	GAL	URE	ARYL	GAL	URE
CO	15.9 ± 0.9^b	17.3 ± 1.5^c	3.2 ± 1.6^b	13.7 ± 1.5^b	12.7 ± 1.0^b	6.0 ± 0.5^b
SMS	18.5 ± 1.4^a	20.2 ± 3.3^b	15.8 ± 3.9^a	22.7 ± 1.7^a	23.1 ± 1.3^a	9.8 ± 0.9^a
DIG	15.3 ± 1.0^b	71.3 ± 6.1^a	3.2 ± 1.5^b	11.8 ± 0.8^c	11.0 ± 0.6^c	6.4 ± 0.2^b
MIN	13.0 ± 1.1^b	16.0 ± 1.1^c	1.6 ± 0.7^b	–	–	–
Significance	***	***	***	***	***	***

Different letters between treatments in each column indicate significant differences $P < 0.05$; Duncan's test. ***$0.001 \leq P < 0.0001$. There are no data on the MIN treatment at 90 days due to a lack of soil samples; CO – control; SMS – spent mushroom substrate; DIG – anaerobic digestate; MIN – mineral fertiliser

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Due to the differences in soil N mineralisation depending on the residues applied, prior knowledge of this parameter is relevant to ensure efficient fertilisation, as well as the sustainable recycling of these materials. The DIG treatment had an efficiency of 86% with respect to the MIN treatment, which allows its substitution in relation to NH_4^+ applied. In contrast, SMS provides soil with a C source to the soil. The immobilisation of mineral N in soil when this residue is applied could prevent NO_3^- leaching in soils with a high NO_3^- content. None of the organic residues had a negative effect on the enzymatic activities studied. The recycling of these waste products as fertilisers or soil amendments provides a sustainable alternative within the framework of circular economy policies.

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