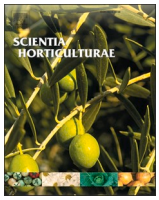
Scientia Horticulturae 337 (2024) 113545

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Research Paper

Solid fraction of digestate from olive pomace modulates abiotic and biotic processes in soil: Retention of agrochemicals and inhibition of fungal pathogens

Nicola Colatorti *, Nunzio Vito Digregorio, Salvatore Camposeo, Elisabetta Loffredo

Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, Bari 70126, Italy

ARTICLE INFO

Keywords:
Anaerobic digestion
SEM-EDX
Pesticide
Adsorption isotherm
Desorption
Fungi

ABSTRACT

A new type of solid digestate (DG) obtained exclusively from two-phase olive pomace was characterised and evaluated for physicochemical and biological properties. In slurry-type experiments, the adsorption of the fungicide boscalid and the herbicide oxyfluorfen on non-amended loam soil (SOIL) and on soil amended with DG at doses of 1 % (SOIL-DG1), 3 % (SOIL-DG3) and 6 % (SOIL-DG6) (w/w) was quantified and modelled. The DG showed a remarkable and stable capacity to adsorb the compounds over a temperature range of 5–40 °C. Based on the data of the adsorption isotherms conducted at 20 °C, the distribution coefficients of SOIL, SOIL-DG1, SOIL-DG3 and SOIL-DG6 were, respectively, 1.3, 2.2, 2.2 and 3.4 mg kg^{-1} for boscalid and 2.0, 2.1, 2.2 and 5.9 mg kg^{-1} for oxyfluorfen, which suggested a significant increase in soil retention capacity after the addition of DG, especially at the highest dose. The desorption of both compounds from all treatments, especially from SOIL-DG6, was slower than adsorption and incomplete (hysteresis coefficient < 1), thus indicating a prolonged permanence of the molecules on the soil. In lab-scale experiments, the phytopathogenic fungi *Armillaria mellea, Fusarium culmorum* and *Verticillium dahliae* were exposed to 0.02, 0.1, 0.5 and 1 % (w/w) DG alone, and 0.02 and 0.1 % DG preliminary interacted with 100 mg L^{-1} of soil humic acid (HA-DG). Fungal response was clearly influenced by the species, the treatment and the dosage adopted. In general, all treatments did not significantly modify the growth rate of *A. mellea* and *V. dahliae*, whereas all DG treatments, especially HA-DG, caused evident suppressive effects on *F. culmorum*. Based on the results of this study, it can be concluded that the addition of DG to the soil can regulate the bioavailability of agrochemicals in pore water and exert an inhibitory or irrelevant action depending on the phytopathogenic fungus.

1. Introduction

Olive pomace (OP), composed of parts of pulp, peel and stone, is the main by-product of olive pressing to obtain virgin olive oil. This material is particularly copious in olive-growing areas where olive oil represents one of the main agro-industrial products on which the local economy is based. In Italy, approximately 3 million tons of OP are produced annually, and the Apulia region contributes one third of the entire Italian production of oil olives (Valenti et al., 2017). Due to the abundance of OP and its composition, its management, especially storage, can represent a problem for the olive oil industry. Raw OP has various applications in many production sectors such as the extraction of olive pomace oil, addition to the soil as fertilizer, combustion for thermal purposes, fermentation, preparation of biostimulants, use as food, beverage and feed additive, and as an ingredient in pharmaceuticals and cosmetics (Dantas Palmeira et al., 2023; Tolisano et al., 2023). The recycling of OP is a real necessity to safeguard the environment and implement circularity in the olive oil sector.

The growing global demand for renewable energy in recent years has encouraged the use of waste biomass, such as OP, for bioenergy production (WBA, 2022). For this purpose, OP is usually mixed with agro-zootechnical wastes, such as crop residues and manure, or, less commonly, is used alone. Given its physicochemical properties and very high moisture, OP is well suited for microbiological processes. Anaerobic digestion (AD) technology consists of a conversion of organic residues into more stable material and is carried out by anaerobic bacteria and archea (Singh and Kalia, 2017; Braguglia et al., 2018). This technology represents a sustainable solution to the need for new energy

* Corresponding author.
E-mail address: elisabetta.loffredo@uniba.it (N. Colatorti).

https://doi.org/10.1016/j.scienta.2024.113545
Received 15 October 2023; Received in revised form 20 July 2024; Accepted 5 August 2024
Available online 10 August 2024

sources and the urgency of sequestering the carbon present in organic waste. In addition to producing biogas, i.e. a mixture of CH_4, CO_2, and small quantities of other gases, this process releases a large quantity of very humid (about 90–95 % moisture) and heterogeneous by-product which contains approximately 60–80 % (on dry matter) of undecomposed organic material. After a separation process, the raw anaerobic material originates a solid digestate (DG) and a clarified liquid digestate (Wang and Lee, 2021).

Although the AD technology emerged as a subject of interest in the last years of the seventeenth century (Pullen, 2015), recently, it has had a rapid development for energy needs, so that the global amount of biogas produced has tripled in the last decade (Karimi et al., 2022). In olive mill farms, the possibility of directing OP to the biogas plant represents a sustainable approach which ensures stable extra income.

During the AD process, biomass is biodegraded, homogenised and partially sanitized. The more labile organic components (sugars, lipid substances, proteins) are rapidly converted into biogas, while the more recalcitrant ones (lignocellulosic fraction) accumulate in the DG along with mineral elements that are essential for plant nutrition (Nkoa, 2014). The physicochemical properties of DG are strictly dependent on the type of ingestate and on the process parameters adopted, such as moisture, retention time and temperature in the digestor (Singh and Kalia, 2017). DG contains a mixture of undecomposed or partially decomposed substances, anaerobic microorganisms, enzymes, volatile fatty acids, microbic metabolites and inorganic particulate. After exposure to the open air and the activity of aerobic microorganisms, the formation of a humic-like fraction takes place in the DG (Provenzano et al., 2014).

In general, the DG is characterized by low content of dry matter, high levels of P, N (mainly as NH_4^+-N) and K, and pH value in the range 6.7 – 9.2 (Teglia et al., 2011). The direct application of DG to agricultural land is a common practice even if it raises concern for the very high N content which can cause ammonia or nitrogen oxides emission in the atmosphere and/or nitrate leaching into natural water. Other concerns of DG spreading on the soil is related to its content of agrochemicals and antibiotics (Wang and Lee, 2021; Brueck et al., 2023), potentially toxic elements (heavy metals) and occasional plant and animal pathogens (Peng and Pivato, 2019) that pose a serious threat to human health and ecosystem security. However, the abundance of phytonutrients is the reason of the wide use of DG as organic amendment (Möller and Müller, 2012; Caruso et al., 2018). DG spreading on the soil has been authorised by the European Commission (2019), while national or regional legislations have set limits to its application based primarily on the total N content.

The wide variability of DG characteristics is due to the considerable diversity of biomass used to feed the AD process. In the last years, this material has been mainly studied for its composition and stability (Provenzano et al., 2016). Undoubtedly, when a single type of biomass is used, such as OP only, DG properties are more stable, and this facilitates the choice of its destination and the most suitable dosage. In any case, a comprehensive DG characterization is essential to assure its sustainable and reliable use.

The addition of DG to the soil increases the content of soil organic matter, provides nutritional and edaphic benefits to plants, and reduces the demand for chemical fertilizers, which provides economic and environmental benefits (Cristina et al., 2020). Moreover, DG can regulate all the processes to which organic xenobiotics undergo in soil, such as adsorption, movement, leaching, dissipation, which in turn control their bioavailability (Loffredo et al., 2021; Dollinger et al., 2022). This is particularly important in sandy soils, where soil organic matter is scarce, and/or where plant protection products are abundantly applied. Low retention capacity of these chemicals by the soil leads to soil and water pollution.

Among widely adopted agrochemicals, there are oxyfluorfen and boscalid. Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)−4-(trifluoromethyl)benzene] is a diphenyl ether herbicide used in pre- and post-emergence to control broad-leaf weeds in several crops. The spread of oxyfluorfen in the environment is very dangerous due to its carcinogenic potential and long persistence in water and soil (EPA, 1992). The carboxamide boscalid [2-chloro-N-[2-(4-chlorophenyl)phenyl]pyridine-3-carboxamide] is a widely adopted broad-spectrum fungicide having a very long persistence in the environment (Chen and Zhang, 2010). The hydrophobic nature of these molecules (high Kow values) suggests their strong interaction with both natural and anthropogenic soil organic matter (Senesi et al., 2015).

Although DG is increasingly added to cultivated soil, and despite the well-known risks associated with this practice, its effects on soil microbiota is rather neglected (Tang et al., 2021; Karimi et al., 2022). Fungi are a very important component of soil microbiota since they ensure the maintenance of the soil carbon cycle, with consequent turnover of organic matter and continuous release of nutrients (Sanchez 2009). Furthermore, fungi can contribute to eliminate organic contaminants by means of mycodegradation (Castellana and Loffredo, 2014). However, several soilborne fungi can be very dangerous for susceptible crops and cause drastic reductions in the quantity and quality of products, resulting in huge economic loss. Fungal control is very difficult, and chemical treatments are generally considered the most effective remedy. However, the need to limit the use of chemicals has prompted researchers to investigate new eco-compatible methods for the control of phytopathogenic fungi. Studies conducted in the last two decades have shown surprising suppressiveness of pathogenic fungi by native soil organic matter, including humic acids (HA) (Loffredo et al., 2007, 2008) and fulvic acids (Moliszewska and Pisarek, 1996), and by the HA-like fraction of compost (El-Masry et al., 2002; Loffredo and Senesi, 2009), vermicompost (Szczech and Smolinska, 2001), biochar (Taskin et al., 2019) and hydrochar (Parlavecchia et al., 2021). Therefore, the appropriate use of such amendments might partially replace synthetic fungicides, with considerable benefits for the environment.

The basidiomycete *Armillaria mellea* and the ascomycetes *Fusarium culmorum* and *Verticillium dahliae* are among the most dangerous fungi for several crops. The species *A. mellea* belongs to the Physalacriaceae family and is a root pathogen mainly of woody plants (about 500 species) but also of herbaceous plants in temperate regions. This fungus is a facultative necrotrophic pathogen, being able to attack both living and dead root tissues and causing typically non-specific symptoms to the aerial part, up to the gradual or sudden plant decay (Devkota and Hammerschmidt, 2020). The mycelium of *A. mellea* can persist for a long time, even decades, in the infected dead roots left in the soil, which represent a source of primary inoculum. *F. culmorum* belongs to the Nectriaceae family and is one of the main agents of foot and root rot of cereals. It can cause significant economic loss due to yield reduction, worse grain quality, as well as kernels contamination with mycotoxins (Scherm et al., 2013). This pathogen is also very resistant, being able to survive in adverse conditions for a long time. *V. dahliae* is a cosmopolitan fungus with a notable longevity in soil where its resting propagules, called microsclerotia, persist up to 14 years in the absence of host plants (Schnathorst, 1981). *V. dahliae* infects over 400 different dicotyledonous plants, including tomato, potato, aubergine, strawberry, flax, olive trees and grapevine (Bhat and Subbarao, 1999).

Measurements of *in vitro* growth of the fungal colony can be interpreted with various models (Dantigny et al., 2005; Tao et al., 2014). Very few investigations have been conducted on the impact of DG on soil-resident fungi (Tao et al., 2014; Santi et al., 2015; Brezáni et al., 2019). In a study by Walsh et al. (2012), DG addition to soil appeared irrelevant on fungal community, while Tao et al. (2014) demonstrated a suppressive activity of both raw and liquid DG on some phytopathogenic fungi. The limited knowledge on this topic and the conflicting results reported in the literature suggest further investigation before drawing definitive conclusions on this matter. Furthermore, most of the information available in the literature concerns DG samples obtained exclusively from livestock manure or waste of mixed plant-animal origin. As far as we know, this is the first study on the impact of DG

from OP only on soil fungi.

In soil, DG interacts chemically with native organic matter, especially HA. It is reasonable that the simultaneous presence of DG and HA in soil may influence the biotic community differently than DG alone or HA alone (Kulikova et al., 2005). This hypothesis has already been verified on *Sclerotinia sclerotiorum* treated both with allelopathic compounds or HA individually and with their combination (Loffredo and Traversa, 2014). In that study, the authors observed that the allelochemical-HA combination modified or even reversed the effects of the individual applications.

Based on the above considerations, the present study investigated: (i) the contribution of DG supply to the soil adsorption capacity of two common agrochemicals; and (ii) the effects of various doses of DG alone and in combination with a soil HA on the *in vitro* growth of three phytopathogenic fungi.

2. Materials and methods

2.1. Digestate, soil, chemicals, humic acid and fungi

The DG sample used was collected in an Apulian oil mill with adjoining biogas plant (AGROLIO S.r.l., Andria, Italy). The crude DG was released after approximately 45-day AD of two-phase olive pomace only. It had a dry matter content < 10 % (w/v); after centrifugation for solid/liquid separation, the solid DG was collected and stored in glass containers at a temperature of 4 °C.

A loam soil was sampled at 0–10 cm depth in the olive grove of the experimental station of University of Bari located at Valenzano, Southern Italy (41° 02′ N; 16° 90′ E). The soil was air-dried, sieved at particle size < 2 mm to remove the coarser fraction and thoroughly homogenized.

Boscalid (Cas number188425-85-6) and oxyfluorfen (CAS number 42874-03-3) at purity ≥ 99 % and 98 %, respectively, were purchased from Sigma Aldrich s.r.l., Milano, Italy. Molecular mass, water solubility and log Kow of boscalid are 343.2 g mol^{-1}, 4.6 mg L^{-1} at 25 °C and 2.96, while the same parameters of oxyfluorfen are, in the order, 361.7 g mol^{-1}, approximately 1 mg L^{-1} and 4.73 (PubChem Open Chemistry Database at the National Institutes of Health (NIH), 2023). Methanol (HPLC grade) solutions of boscalid and oxyfluorfen were prepared individually at a concentration of 2000 mg L^{-1}. Successively, aqueous mixtures of the two compounds were prepared by combining appropriate aliquots of each methanol solution and diluting with a mixture of double-distilled water/methanol (95:5, v/v). All other chemicals used in the experiments were of extra pure grade.

The HA sample was the standard HA isolated from a prairie soil located near Joliet, Ill, USA, belonging to the standard and reference collection of humic and fulvic acids of the International Society of Humic Substances (IHSS 2022). HA properties are: 10.2 meq g^{-1} total acidity, 8.3 and 1.9 meq g^{-1} COOH and phenolic OH contents, respectively, 581and 41 g kg^{-1}C and N contents (on dry- and ash-free basis), respectively (IHSS, 2023).

Isolates of *A. mellea* (Vahl) P. Kumm., 1871, *F. culmorum* (Wm. G. Sm.) Sacc., 1892, and *V. dahliae* Kleb., 1913 were kindly provided by the collection of prof. Antonio Ippolito of the Department of Soil, Plant and Food Sciences of the University of Bari, Bari, Italy. Fungal mycelium was cultivated on potato dextrose agar (PDA, Oxoid, 4 % w/v) in Petri dishes in the dark at 20 ± 1 °C. To maintain the characteristics of the fungal culture stable until the end of the experiments, every 20 days, a 2-mm diameter disk of the colony was transferred on fresh PDA. Fungal inoculation was performed in an AsalAir vertical 700 laminar flow hood (Cernusco sul Naviglio, Italy) using 2-mm PDA disk covered with mycelium collected from the growing margin of young colonies and placed in the center of new plates.

Table 1
Main soil characteristics.

Parameter	Value
Sand (%)	36
Silt (%)	43
Clay (%)	21
pH[a]	7.45 ± 0.03
EC[b] (dS m^{-1})	0.20 ± 0.003
Moisture (%)	4.4 ± 0.5
Organic C (g kg^{-1}.)	37.94 ± 2.34
Total N (g kg^{-1}.)	2.98 ± 0.08
Available P (mg kg^{-1}.)	2.38
C/N	12.7
K (mg kg^{-1})	101.0
CEC (cmol+ kg^{-1})	39.0 ± 3.8

[a] soil/H_2O: 1:2.5 (w/v);.
[b] soil/H_2O: 1:2 (w/v).

Table 2
Main digestate properties.

Parameter	Value
pH[a]	9.21 ± 0.03 [b]
Moisture (%)	34.20 ± 0.48
Electrical conductivity[a] (dS/m)	0.42 ± 0.02
Total volatile solids (% d.m.)	96.96 ± 0.61
Fixed total solids (% d.m.)	2.00 ± 0.23
Organic C (% d.m.)	51.03 ± 0.39
Total N (% d.m.)	1.23 ± 0.06
NH_4–N (% d.m.)	0.062
C/N ratio	41.5
Total P (% d.m.)	0.075

[a] digestate/H_2O, 1:10 w/v.
[b] SD (n = 3); d.m.: dry matter.

2.2. Soil and digestate characterization

Soil properties were determined according to the official methods of soil analysis (Gazzetta Ufficiale della Repubblica Italiana (GU), 1999). Briefly, soil moisture was measured after heating the soil at 105 °C overnight; the pH was potentiometrically measured in a soil/water suspension (1:2.5, w/v); electrical conductivity (EC) was measured at 25 °C in the water extract obtained from a soil/water suspension (1:2, w/v); organic C, total N, and available P were determined by the Walkley-Black, the Kjeldahl, and the Olsen method, respectively; cation exchange capacity (CEC) was determined by using 0.1 M $BaCl_2$ buffered to pH 8.2 with triethanolamine (2.25 %, v/v). Soil characteristics are shown in Table 1.

Proximate analysis of the DG sample, including pH, electrical conductivity (EC), moisture and ash content, was performed according to the official methods of analysis of fertilizers. The pH and EC were measured in a DG/water suspension (1:10, w/v); the ash content was measured after burning the sample in a muffle furnace at a temperature of 550 °C for 4 h Organic C was determined by the Springer-Klee method (Gazzetta Ufficiale della Repubblica Italiana (GU), 2000); total N and ammonia N were determined according to the procedures reported in European Commission (2003). Some characteristics of the air-dried digestate are referred in Table 2.

Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) analysis was performed to study DG micromorphology. For the purpose, the sample was fixed with an adhesive carbon tape, metalized with Au/Pd and analyzed using a Hitachi TM3000 scanning electron microscope (Hitachi, Tokyo, Japan) equipped with an Oxford Swift ED3000 microanalysis system. Backscattered electrons were detected, and SEM micrographs of DG were captured at both 500 × and 1800 × magnifications.

Table 3
Theoretical models used to describe sorption data.

Sorption models		
Henry	Freundlich	Langmuir
$q_e = Kd\ C_e$	$q_e = K_F\ C_e^{1/n}$	$q_e = \frac{(K_L C_e b)}{(1+ K_L C_e)}$
q_e (mg g^{-1}): equilibrium concentration of the adsorbed compound C_e (mg L^{-1}): equilibrium concentration of the compound in solution		
Kd (mg kg^{-1}): distribution coefficient	K_F (mg kg^{-1}): Freundlich adsorption constant (sorption capacity) 1/n: degree of nonlinearity between C_e and q_e n: sorption intensity	b (mg kg^{-1}): maximum adsorption capacity of the adsorbent K_L (L mg^{-1}): Langmuir constant (energy of adsorption/affinity of the solute for the adsorbent

2.3. Sorption and desorption experiments

The capacity of DG to adsorb boscalid and oxyfluorfen was evaluated in batch equilibrium experiments at temperatures of 5, 10, 20, 30 and 40 °C. For the purpose, aliquots of 20 mg of DG were interacted with 20 mL of aqueous mixtures of the compounds at the individual concentration of 2 mg L^{-1}, thus obtaining a solution/DG ratio equal to 1000. All samples were mechanically shaken at 350 *g* for 16 h to reach the steady state. Previous experiments demonstrated a rapid adsorption of the two compounds on DG, and the achievement of the equilibrium condition in about 2 h. Subsequently, the suspensions were centrifuged at 10,000 *g* for 10 min and a volume of 15 mL of supernatant solution was collected from each sample to determine the equilibrium concentration of the compounds using ultra-high performance liquid chromatography (UHPLC) analysis as reported in the next section.

Adsorption isotherms of the compounds onto unamended soil (SOIL) and soil amended with DG at 1 % (SOIL-DG1), 3 % (SOIL-DG3) and 6 % (w/w) (SOIL-DG6) were performed in batch equilibrium mode. Approximately, the dose of 1 % DG is equivalent to a soil application of 7 t ha^{-1} which corresponds to 30 kg ha^{-1} total N, considering a soil depth of 5 cm and a soil bulk density of 1.4 g cm^{-3}. Therefore, the DG doses adopted were consistent with those recommended for field applications (30–60 t ha^{-1}) (Velechovský et al., 2021). Volumes of 10 mL of aqueous mixtures of boscalid and oxyfluorfen at individual concentrations of 0.1, 0.2, 0.5, 1 and 2 mg L^{-1} were added to 2 g of each substrate in glass flasks. To reach equilibrium, samples were stirred for 24 h at 20 °C in the dark. After that, samples were centrifuged at 10,000 *g* for 10 min and the equilibrium concentration of the compounds in the supernatant solution was measured by UHPLC according to the protocol described in the next section.

Desorption of the compounds from all treatments was started immediately after adsorption using the samples added with the maximum concentration (2 mg L^{-1}) of the compounds. At each desorption step, 7 mL of supernatant solution (of 10 mL initially added) was replaced with double distilled water and the sample was stirred again for 24 h at 20 °C to reach new equilibrium. Subsequently, the sample was centrifuged, and the concentration of the compounds was determined as described previously for the adsorption experiments. All adsorption and desorption experiments were triplicated.

The concentration of the compounds on each adsorbent, q_e (mg kg^{-1}), was calculated from the equation: qe = (C_0 – Ce) V/m, where C_0 and Ce (mg L^{-1}) are the initial and the equilibrium concentration in solution, respectively, V (mL) is the solution volume and m (g) the adsorbent mass. Data obtained were statistically treated by one-way analysis of variance (ANOVA) to evaluate possible significant differences between the means of different treatments.

2.4. Chromatographic analysis and data modelling

The concentration of residual compounds in the supernatant solution was determined by UHPLC. All samples were previously filtered using 0.45 μm Millipore™ cellulose acetate filters and subsequently loaded into a WPS-3000 autosampler. A Dionex Ultimate UPLC 3000 RSLC (Waltham, MA, USA) instrument equipped with an HPG-3200 RS pump, and a TCC-3000 column compartment connected to a Supelco™ LC-18 column (150 mm × 3 mm × 3 μm) was used for analyses. Isocratic elution was adopted with water/methanol (30/70, v/v) flowing at 0.8 mL min^{-1}. A DAD-3000 RS diode array detector (Dionex, Waltham, MA, USA) set at wavelengths of 207 and 210 nm was used for boscalid and oxyfluorfen detection, respectively. The external standard method was used to quantify the compounds.

Sorption and desorption isotherm data were described using the linear Henry model and the non-linear empirical Freundlich and Langmuir models. The equations applied and the meaning of the various parameters are reported in Table 3. The Freundlich equation fits well when solute molecules form a multilayer on the adsorbent and this has surface heterogeneity, while the Langmuir equation is proper for solutes forming a monolayer on the adsorbent without interacting with each other and when the surface of the adsorbent is homogeneous. The Freundlich (K_F and 1/n) and Langmuir (b and K_L) parameters were calculated by the non-linear regression method using the solver add-in component of Microsoft® Excel® and a trial-and-error procedure to minimize the sum of squared residuals (SSR) between experimental and theoretical data. The accordance of the theoretical model to the experimental data was evaluated on the basis of the correlation coefficient, $r = \sqrt{\frac{\sum (q_e m - \overline{q_e})^2}{\sum (q_e m - \overline{q_e})^2 + \sum (q_e m - q_e)^2}}$, where q_em is the theoretical adsorbed concentration of the compound (mg kg^{-1}) at equilibrium, q_e is the experimental concentration (mg kg^{-1}), and $\overline{q_e}$ is the average q_e. Furthermore, the linear Henry equation was also employed to calculate the distribution coefficient, Kd, from the slope, and the organic-carbon-partition coefficient, K_{OC}, by: K_{OC} = (Kd × 100)/(% OC)), which is a measure of the quantity of solute adsorbed per unit of organic carbon of the adsorbent.

Desorption isotherm data were also theoretically evaluated using the Henry and the Freundlich equations. This allowed to calculate the desorption parameters Kd_{des}, K_{OCdes}, K_{Fdes}, and $1/n_{des}$ as described for the corresponding sorption parameters. Finally, the hysteresis coefficient, H, was calculated according to: $H = (1/n_{des})/(1/n_{ads})$. A hysteresis occurs for H value < 1, which indicates slow and incomplete desorption of the compound.

2.5. Experiments on fungi

Appropriate amounts of DG were suspended in double distilled water to obtain dosages of 0.02 % (DG0.02), 0.1 % (DG0.1), 0.5 % (DG0.5) and 1 % (DG1) (w/v). In another set of experiments, DG0.02 and DG0.1 were preliminary interacted with 100 mg L^{-1} HA (HA-DG0.02 and HA-DG0.1) under magnetic stirring for 16 h at room temperature (~ 20 ± 1 °C), in the dark.

Each suspension and double distilled water (control) was added with PDA (4 %, w/v) and autoclaved at a temperature of 121 °C for 15 min. Subsequently, a volume of 20 mL of each medium was collected under stirring and poured into a 9 cm-diameter Petri dish. After cooling and solidification at room temperature (~ 20 ± 1 °C), under axenic conditions, the fungus was inoculated as described in Section 2.1. The samples were then kept in a 60043/THTL thermostated chamber (F.lli Della Marca S.r.l., Roma, Italy) at a constant temperature of 20 °C, in the dark. During fungal growth in the plates, the colony radius (in mm) was measured until its extension was possible for all samples (colony diameter ≤ 4.4 mm), i.e., 14, 5 and 18 d for *A. mellea, F. culmorum* and *V. dahliae*, respectively. Each experiment was replicated five times.

The radial growth rate of the fungus, μ (mm h^{-1}) was calculated applying the linear model with breakpoint: r (mm) = μ (t – λ), where r is the radius, t is the sampling time (h) and λ is the lag time (h), i.e., the time required for the completion of the germination process (Dantigny

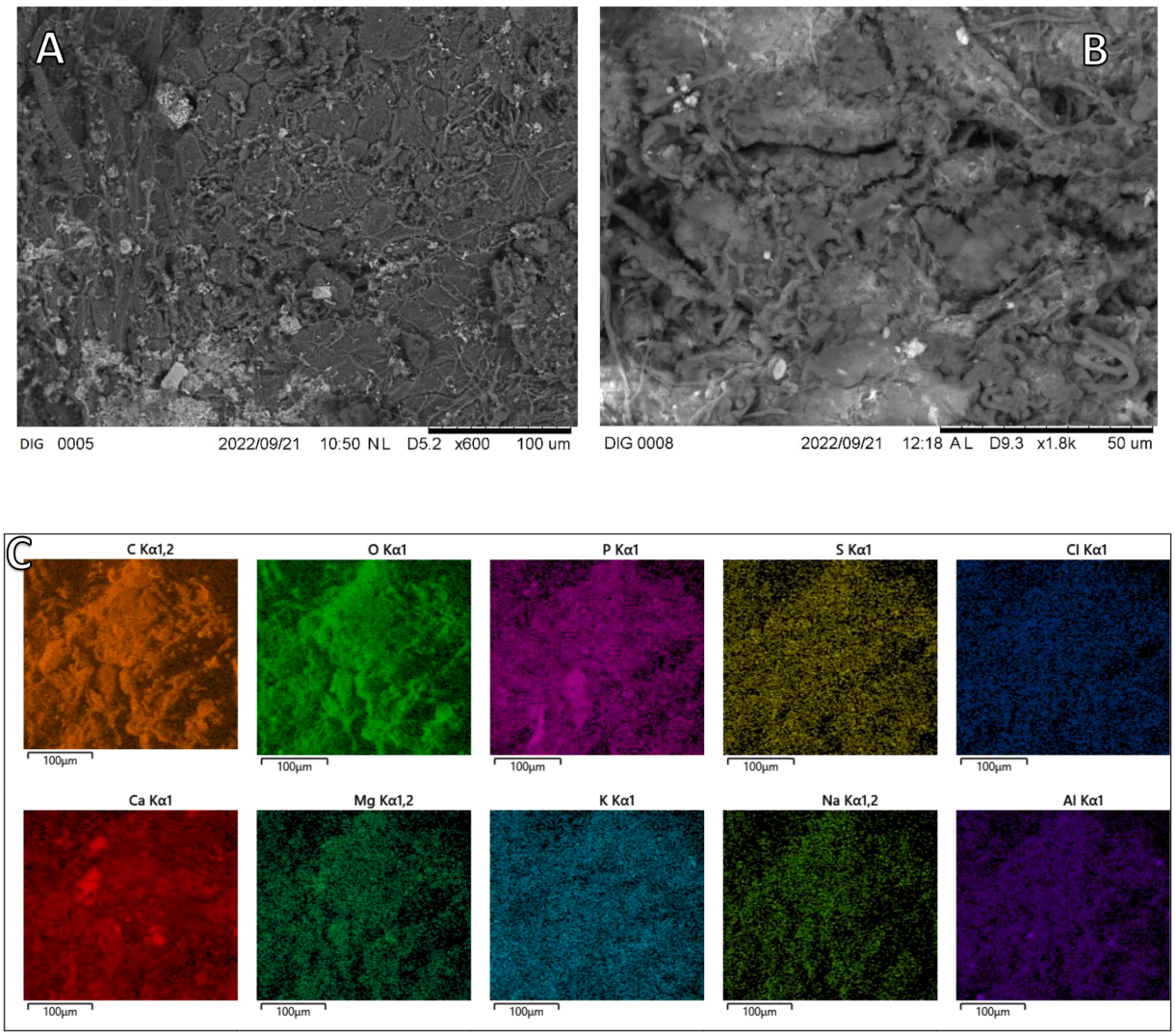


Fig. 1. Scanning electron micrographs at magnifications of 600× (A) and 1800× (B) and energy-dispersive X-ray (EDX) maps (C) of DG. Images were taken with secondary electrons.

et al., 2005). This model describes very well the growth of the colony, which is confirmed by the high values generally observed for the regression coefficient ($r \geq 0.99$). The appropriateness of this model allows to calculate the parameters μ and λ even before the complete coverage of the plate by the mycelium. In general, when fungus inoculation is carefully conducted, there is not a significant relationship between μ and λ (Dantigny et al., 2005). Using the colony diameter values (D) measured during the experiments, the absolute growth rate (AGR, mm h^{-1}) of the mycelium was calculated according to the expression: $AGR = \frac{D-2}{t}$, where the constant 2 is the inoculum diameter (mm), and t is the incubation time (h) (Tao et al., 2014). The μ values obtained for the various treatments were statistically analysed by ANOVA, and the means compared to the control by the least significant difference (LSD) test at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

3. Results and discussion

3.1. Physicochemical properties of the digestate

As is well known, DG properties are largely dependent on the type of organic waste used to feed the digester and on the duration of the AD process. The unique nature of the DG considered in this study, i.e., obtained exclusively from olive pomace, is the reason for its rather different composition compared to that of other DGs generally obtained from animal manure only or mixed plant and animal biomass (Möller and Müller, 2012). In facts, results of proximate analysis indicated that pH, ash, moisture and EC were somehow different from those averagely found for other DGs originated from mixed feedstock. In particular, the pH value (9.2) of our DG was similar to that measured for a DG obtained from 60 % maize silage and animal residues (Mukherjee et al., 2016) but higher than that found for a sewage sludge DG (Cristina et al., 2020). The high pH value measured for this DG was possibly due to a high content of alkaline and alkaline earth metals. The amount of fixed total solids (2 % on dry matter) was in the average for DG (Table 2). The EC value of 0.42 dS m^{-1} was lower than that found for a mixed feedstock DG (Loffredo et al., 2021) and a sewage sludge DG (Cristina et al., 2020). Results of ultimate analysis showed an organic C content (51 % on dry matter) higher than that usually recorded for this type of material (25–41 %) (Cesaro, 2021) and for a swine manure DG (Hung et al., 2017), but very similar to that of a mixed biomass DG (Loffredo et al., 2021). Differently, as expected, the total N content was lower than that of other DGs, reasonably attributable to its exclusive vegetal nature. Higher total N contents are reported for a swine manure DG (Hung et al., 2017) and a sewage sludge DG (Cristina et al., 2020).

Table 4
Concentration (mg kg^{-1}) of the adsorbed compounds onto DG at various temperatures.

Compound	Temperature (°C)				
	5	10	20	30	40
boscalid	1557.44 ± 25.61	1577.56 ± 16.47	1595.88 ± 11.59	1591.36 ± 22.61	1578.09 ± 18.65
oxyfluorfen	1608.11 ± 9.33	1618.52 ± 16.71	1622.91 ± 10.58	1630.15 ± 32.66	1616.45 ± 21.36

Note: data were analysed by ANOVA ($n = 3$) and no statistically significant differences were found between the means of the various treatments.

3.2. SEM analysis

Fig. 1 shows SEM images of the DG at 600 × and 1800 × magnifications. This technique allows to evaluate the surface micromorphology of a material and inquire on the allocation and distribution of pores. The lower magnification gave a general overview of the material and denoted an apparent scarce homogeneity (Fig. 1A). In facts, roughness, small cavities, furrows, and a sort of thin network, probably formed by vessels of the olive tissues, were evident. At higher magnification, surface heterogeneity was more evident. The morphological texture of the DG exhibited irregularly shaped cavities, mostly less than 10 μm, and coiled filamentary structures composed of lignocellulosic and mineral constituents (Fig. 1B). The pores generated by the cell walls and vascular tissues were not so evident perhaps due to the intense pressure exerted during the pressing of the olives. SEM micrographs of a swine manure DG showed a smooth and compact surface typical of DG obtained from animal waste (Hung et al., 2017). Diffuse porosity and large surface area are of primary importance to ensure physical and chemical interaction with organic and mineral constituents and high adsorption capacity of the material. EDX elemental analysis showed the presence on the surface of various elements, such as C, O, P and S, alkali and alkaline earth metals (Fig. 1C) that are very common in plant-based materials. The high pH and EC values obtained for this material can be reasonably attributed to the retention of alkali metals during the AD process (Cristina et al., 2020).

3.3. Sorption and desorption of the agrochemicals on digestate and soil

The quantities of each compound adsorbed at equilibrium on the mass unit of DG at various temperatures are reported in Table 4. The sorption study conducted at temperatures between 5 and 40 °C allowed the evaluation of the retention efficiency of the compounds by the DG in different application conditions, such as different seasons, different latitudes and different depths. Statistical treatment (ANOVA) of the data showed that the quantities of each compound adsorbed at equilibrium at the five temperatures considered were not significantly different ($P \leq 0.05$) (Table 4). As far as we know, there is no information in the literature on this matter, therefore a comparison between our results and those of other authors is not possible. The only work reporting the effects of temperature on the adsorption of boscalid only was conducted using zeolites as adsorbent; the authors concluded that the temperature had no significant influence on the sorption process (Silvestrini et al., 2015). At the various temperatures, the quantities of boscalid and those of oxyfluorfen adsorbed on the DG were almost identical, the absorbed oxyfluorfen being (on average) only 2 % higher than the adsorbed boscalid (Table 4). Considering the very low water solubility of both compounds (few mg per L at 25 °C), these results were expected. The DG used in this study showed a sorption capacity of boscalid that was approximately three times higher than that of a DG produced from mixed feedstock (Loffredo et al., 2021) and much higher than that of a soil/30 % DG biomixture (Mukherjee et al., 2016). Although, as expected, the quantity of boscalid adsorbed on the DG was lower than that adsorbed on other organic matrices richer in carbon, such as biochar (Mukherjee et al., 2016), it is relevant for the purpose of preventing the transfer of the compound into natural waters with consequent contamination. Due to their good capacity to adsorb oxyfluorfen and other pesticides, local organic wastes from olive oil production were employed in the preparation of biobed systems (Delgado-Moreno et al., 2017).

Adsorption isotherms were performed to describe quantitatively the interaction between the soil and the two compounds at a fixed temperature. The experiments were carried out on both untreated soil and soil treated with 1, 3 and 6 % DG (w/w). By interpreting adsorption data with different models, we calculated the sorption parameters, such as the adsorption constant and the maximum adsorption, and obtained information on the mechanism of interaction between the DG and the molecules, including the type of DG surface and the allocation of the compounds on it. The adsorption data were described with the Freundlich, Langmuir and Henry equations. The Freundlich model is the most appropriate one for reversible adsorption on substrates that have heterogeneous surfaces and when the solute molecules form a multilayer on the surface of the substrate. The Langmuir equation describes very well the adsorption on homogeneous surfaces and when the solute forms a monolayer on the adsorbent, being irrelevant the interactions among the adsorbed molecules. The Henry equation was used to calculate the distribution coefficient, Kd_{ads}, which expresses the sorption efficiency of a substrate. The adsorption parameters (Kd_{ads}, KF_{ads} and $1/n_{ads}$) and the coefficient K_{OCads} are reported in Table 5. To estimate the congruence between the experimental data and the theoretical model, both the correlation coefficient (r) and the sum of squared residuals (SSR) were considered.

Based on r and SSR values, the adsorption of both compounds on all soil treatments was well described by the Freundlich model, although

Table 5
Sorption parameters of the compounds on different soil treatments.

Compound	Theoretical models											
	HENRY				FREUNDLICH				LANGMUIR			
	SSR	r	Kd_{ads} mg kg^{-1}	K_{OCads} mg kg^{-1}	SSR	r	K^a_{Fads} mg kg^{-1}	$1/n_{ads}$	SSR	r	b^a mg kg^{-1}	K^b_L L mg^{-1}
	SOIL											
Boscalid	0.06	0.995	1.29	34.04	0.01	0.999	1.21	1.25	0.06	0.988	458.42	0.003
Oxyfluorfen	0.07	0.997	2.03	53.69	0.07	0.993	2.04	0.99	0.07	0.993	34.38	0.064
	SOIL-DG1											
Boscalid	0.19	0.992	2.17	50.77	0.03	0.996	2.23	0.78	0.02	0.998	7.53	0.439
Oxyfluorfen	0.18	0.992	2.12	49.62	0.15	0.986	2.09	1.11	0.19	0.982	3115.11	0.001
	SOIL-DG3											
Boscalid	0.04	0.998	2.24	42.99	0.03	0.998	2.22	1.07	0.04	0.996	1048.81	0.002
Oxyfluorfen	0.15	0.994	2.21	42.50	0.02	0.998	2.08	1.24	0.15	0.986	4273.03	0.001
	SOIL-DG6											
Boscalid	0.04	0.999	3.45	52.11	0.03	0.999	3.45	0.95	0.04	0.998	67.73	0.054
Oxyfluorfen	0.34	0.996	5.93	89.39	0.32	0.991	6.00	1.04	0.34	0.990	240.19	0.025

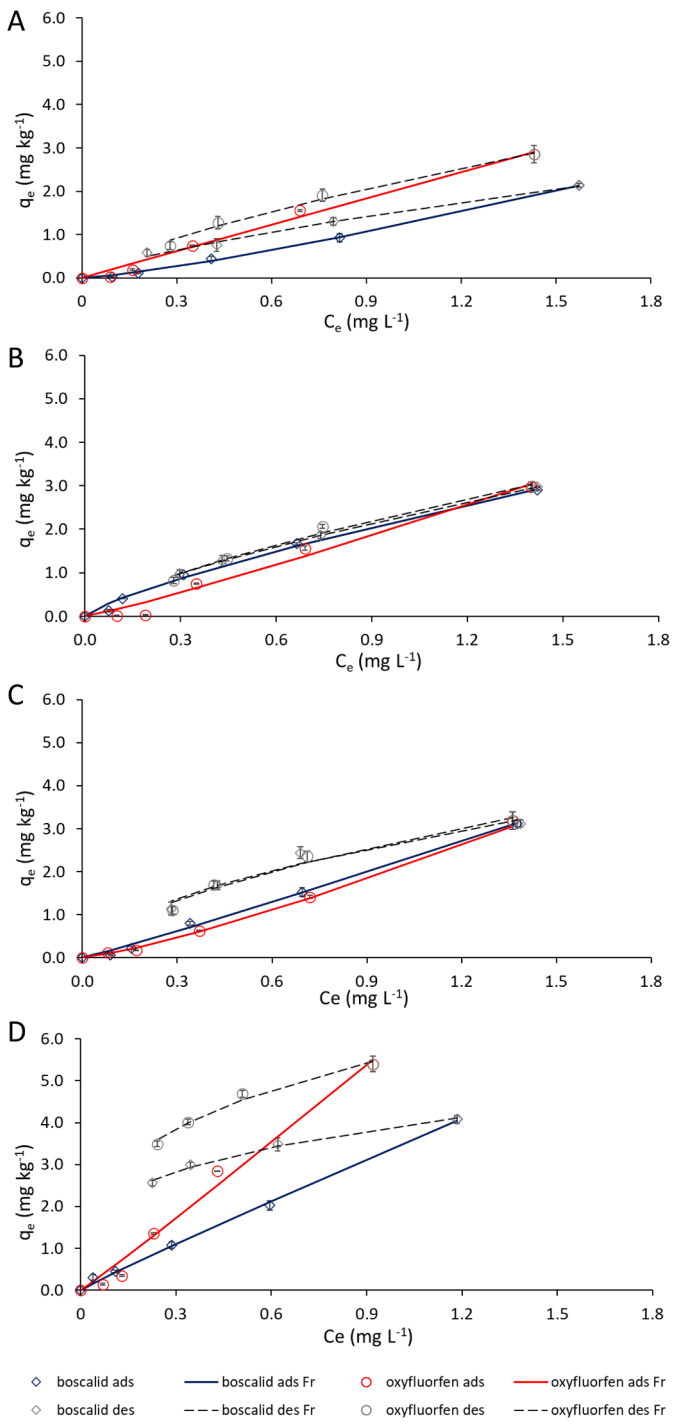


Fig. 2. Adsorption and desorption isotherms of the compounds on unamended soil (A) and soil amended with DG at 1 (B), 3 (C) and 6 % (D). The points indicate experimental data, while solid and dashed lines are plots of the Freundlich model for adsorption and desorption, respectively. The vertical bar on each point is the standard error ($n = 3$).

high r values were also obtained applying the other equations, especially the Henry model (Table 5). Therefore, it was evident that both unamended soil and DG/soil mixtures had heterogeneous surfaces on which the adsorbed compounds formed multiple layers.

Native and exogenous soil organic matter exert a remarkable influence on the dynamic of organic xenobiotics in soil, interacting through various mechanisms and influencing the adsorption process both quantitatively and qualitatively (Senesi et al., 2015). Chemical and physical adsorption may occur simultaneously, although, based on the physicochemical properties of the interacting species, one mechanism generally prevails on the other. Chemical and physical bonding of different strength, such as hydrogen, ionic, covalent bonding, electron donor-acceptor interaction, ligand exchange, van der Waals forces and others, are responsible of the intensity and duration of adsorption (Senesi et al., 2015). The presence of a multitude of functional groups, including carboxylic and phenolic OH, alcoholic OH, ketonic C=O, amine groups and others, on DG surface controls the retention and release of organic compounds. Unprotonated, non-ionizable and non-polar or low-polar molecules, such as boscalid and oxyfluorfen, may be linked to soil organic matter through non-specific hydrophobic interaction or partitioning processes between water and hydrophobic sites of DG (Senesi et al., 2015). Of course, experimental conditions, including pH and ionic strength, can affect both retention and release of the compounds.

Considering the values of the sorption parameters, Kd_{ads}, KF_{ads} and K_{OCads}, it was evident that the addition of the DG, especially at the highest dose, increased the overall retention of both compounds on the soil,. The KF_{ads} value of boscalid on SOIL-DG1, SOIL-DG3 and SOIL-DG6 were, respectively, 1.8, 1.8 and 2.8 times higher than that on SOIL, while that of oxyfluorfen on SOIL nearly tripled when 6 % DG was added (Table 5). Furthermore, the sorption parameters were higher for oxyfluorfen than for boscalid on SOIL and SOIL-DG6 treatments and were similar in the other treatments (Table 5). The maximum K_{OCads} values of both compounds were observed in the treatment with the highest DG dose (SOIL-DG6) (Table 5).

Little information is present in the literature on the extent and modeling of the adsorption of boscalid and oxyfluorfen on soil. Vallee et al. (2014) studied the adsorption of boscalid on two different soils and reported KF_{ads} values of 4.7 and 8.5 μg g^{-1}. Pérez-Lucas et al. (2021) added composted sheep manure in an agricultural soil and observed a noticeable increase in boscalid adsorption which was attributed to the increase in organic matter. In a very recent study, Bhatt et al. (2023) investigated the adsorption kinetics of boscalid on different soils and reported equilibrium adsorptions between 12.1 and 14.3 μg g^{-1}, the latter value for the soil with the highest organic matter content. The Kd values calculated by Hall et al. (2015) for oxyfluorfen adsorption on three Hawaiian soils ranged from 166 to 351 μg g^{-1}. In a study on oxyfluorfen adsorption on different soils, Wu et al. (2019), found KF_{ads} values ranging from 62 to 116 μg g^{-1}. Even though the DG used in this study had a greater adsorbent capacity of the compounds than other DGs, the overall adsorbent capacity of the treated soil was lower than that reported in the above-cited studies; this is reasonably due to the lower clay content and, above all, the lower organic matter content of the soil used here. A significant increase (2.6 times, from 28 to 72 μg g^{-1}) in oxyfluorfen adsorption on a sandy clay loam soil was observed after the addition of olive-oil mill waste (Calderón et al., 2015).

The desorption data and the plot of the Freundlich model of both compounds on all treatments are shown in Fig. 2, while the desorption parameters obtained by fitting the experimental data in the Henry and Freundlich models are referred in Table 6. The release of boscalid and oxyfluorfen from each treatment occurred differently than adsorption. After three desorption steps, approximately 73, 74, 64 and 37 % of the initially adsorbed boscalid, and 74, 72, 65 and 35 % of adsorbed oxyfluorfen were desorbed from SOIL, SOIL-DG1, SOIL-DG3 and SOIL-DG6, respectively (Fig. 2). For both molecules, all KF_{des} values were higher and all $1/n_{des}$ values were lower than the corresponding adsorption parameters (Table 5), which denoted a poor reversibility, i.e., a hysteretic behavior. The capacity of the soil to release the compounds in the diluted solution followed the order SOIL-DG6 < SOIL-DG3 < SOIL-DG1 < SOIL. This last sequence is exactly the reversal of that seen for the adsorbent capacity of the treatments. The hysteresis phenomenon is dependent on the type of binding between the solute and the adsorbent and hinders the complete release of the compound (Senesi et al., 2015). Both the native organic matter of the soil, such as humic substances, and

Table 6
Desorption parameters of the compounds from different soil treatments.

Compound	Theoretical models								
	HENRY				FREUNDLICH				H
	SSR	r	Kd_{des} mg kg $^{-1}$	K_{OCdes} mg kg $^{-1}$	SSR	r	K^{a}_{Fdes} mg kg $^{-1}$	$1/n_{ads}$	—
SOIL									
Boscalid	0.14	0.990	1.45	38.33	0.01	0.996	1.54	0.71	0.57
Oxyfluorfen	0.27	0.990	2.18	57.42	0.03	0.994	2.23	0.72	0.73
SOIL-DG1									
Boscalid	0.39	0.987	2.27	53.13	0.10	0.979	2.32	0.71	0.91
Oxyfluorfen	0.30	0.990	2.34	54.69	0.04	0.998	2.38	0.72	0.65
SOIL-DG3									
Boscalid	1.13	0.971	2.65	50.80	0.11	0.973	2.68	0.56	0.52
Oxyfluorfen	0.88	0.978	2.69	51.62	0.07	0.985	2.71	0.59	0.48
SOIL-DG6									
Boscalid	6.46	0.924	4.39	66.24	0.01	0.997	3.92	0.27	0.28
Oxyfluorfen	8.04	0.948	7.47	112.62	0.04	0.991	6.61	0.31	0.30

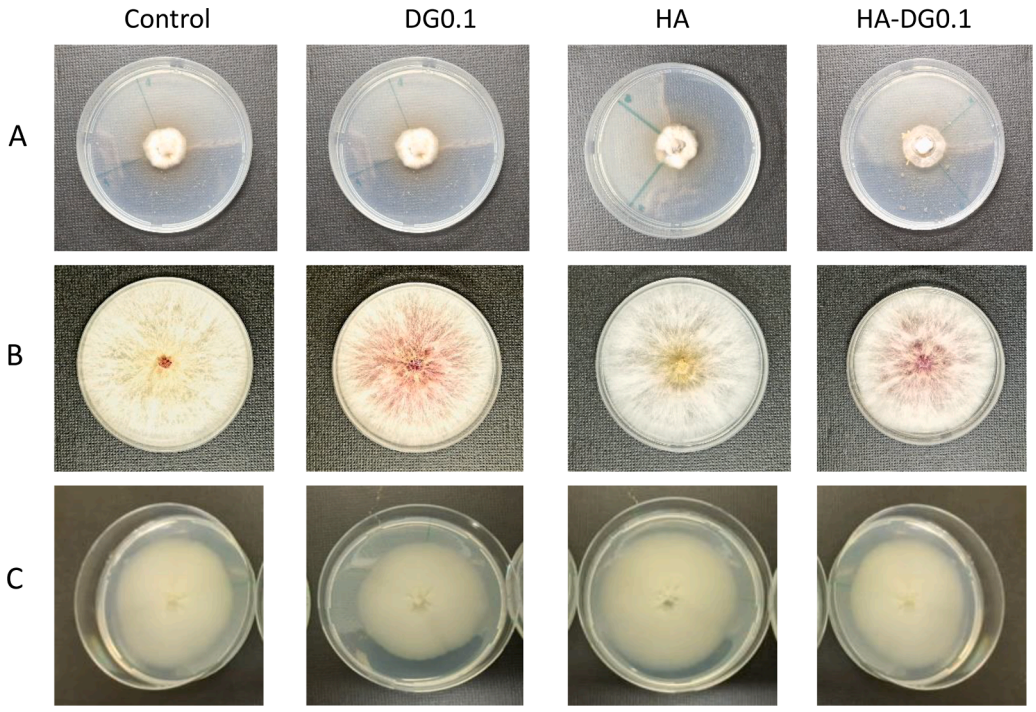


Fig. 3. Fungal colony of *A. mellea* at 288 h from inoculation (A), *F. culmorum* at 120 h from inoculation (B) and *V. dahliae* at 350 h from inoculation (C).

that introduced with amendments, such as DG, generally form both weak and strong bonds with organic xenobiotics; the weak bonds favor the rapid release of the compounds while the strong bonds cause sorption hysteresis. Recently, Bonnar et al. (2023) studied the adsorption of oxyfluorfen in rice soils and reported that the maximum release of the adsorbed compound was 27 %, which indicated a significant sorption hysteresis in all soils.

3.4. Fungal response to digestate treatment

In recent years, the agricultural practice of spreading DG directly into the soil has gained more and more relevance. Despite this, information on the impact of this material on soil-resident fungi is very scarce. It is very important to understand the possible benefits of DG on the biological fertility of the soil as well as the risks related to the promotion of microorganisms harmful to plants. Fig. 3 shows the appearance of fungal colonies on PDA substrate not treated (control) and treated with DG only and HA-DG interaction products. To evaluate the *in vitro* growth of fungi and estimate the mycelium growth rate, different models have been proposed (Dantigny et al., 2005; Tao et al., 2014). The linear model with breakpoint suggested by Dantigny et al. (2005) is generally very accurate to describe the growth data of filamentous fungi on PDA. In facts, fitting the growth in this model, frequently, the values of the regression coefficient are very close to the unit, which ensures accurate calculation of the growth parameters, such as radial growth rate, μ, and lag time, λ (Dantigny et al., 2005). The latter parameter represents the time between fungal inoculation and the beginning of hyphal elongation. Another valid parameter for estimating the mycelium extension over time is the absolute growth rate (AGR), which is considered the best parameter to ascertain stimulating or inhibitory effects of specific substances or external conditions on fungi (Tao et al., 2014).

3.4.1. Armillaria mellea

The radial extension of the mycelium was measured at regular intervals up to 14 d after inoculation. The experiments were ended before the fungus reached the edge of the plate because, after 14 d, the colonies of some samples started generating fungal rhizomorphs randomly. In the

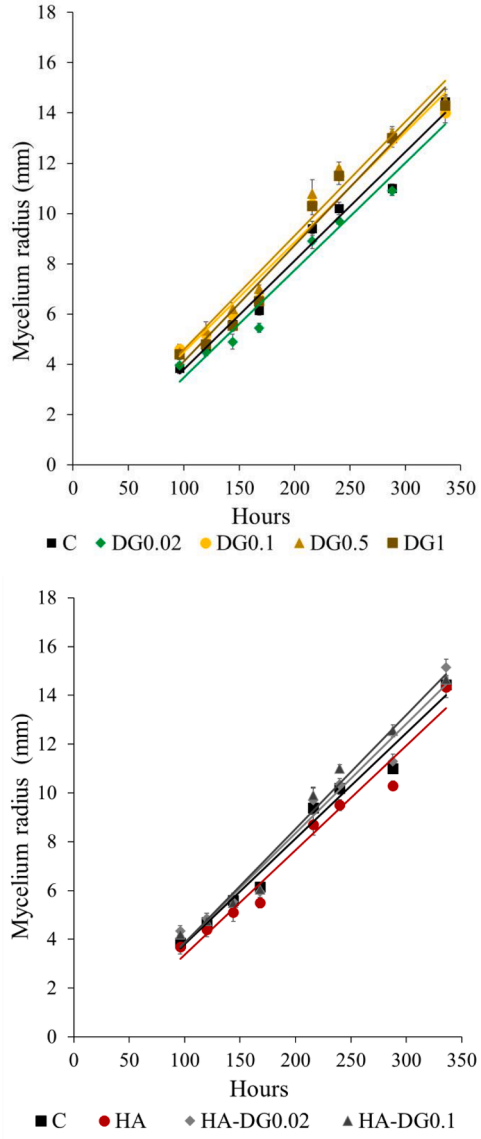


Fig. 4. Effects of DG alone (above) and DG interacted with HA (below) at various doses on the radial extent of *A. mellea* as a function of time. The graph shows the experimental points with standard errors ($n = 5$) and linear plots.

experimental conditions adopted, *A. mellea* showed the slowest growth among the fungi considered in this study. The data collected in the various samplings highlighted that none of the treatments substantially changed the growth trend of the fungus compared to the control. This occurred for both non-interacted DG and HA-interacted DG and is clearly illustrated in Fig. 4 where the linear fits to the experimental data appear nearly parallel to each other and to the control.

Linear regressions of growth data showed r values ranging from 0.983 to 0.990, while statistical analysis of data (ANOVA) indicated that all treatments were not statistically different from the control (Table 7). On the other hand, a great variability of the lag time (λ) was observed. Compared to the control, HA caused the longest latency time (~ 20 h) which was almost twice that of the control, indicating a marked delay of the growth initiation induced by this substrate (Table 7). Since there were no changes in growth rate or other evident effects of DG alone or HA-interacted DG in the *in vitro* experiments, it can be hypothesized that the addition of DG to the soil may not significantly affect the pathogenic activity of this fungus. Compared to the control, only slight differences in AGR were observed for all treatments (Fig. 5), which confirmed the irrelevant impact of any DG treatment on *A. mellea*.

Table 7
Growth parameters of the fungi calculated from the linear model.

Treatment	μ (mm h^{-1})	λ (h)	r
Armillaria mellea			
Control	0.043 ± 0.001[a]	11.64 ± 2.53	0.990
DG0.02	0.043 ± 0.001	18.60 ± 2.41	0.987
DG0.1	0.043 ± 0.001	16.81 ± 2.33	0.983
DG0.5	0.046 ± 0.002	15.03 ± 4.22	0.986
DG1	0.046 ± 0.002	11.70 ± 2.14	0.985
HA	0.043 ± 0.002	20.03 ± 3.21	0.983
HA-DG0.02	0.045 ± 0.000	14.65 ± 5.89	0.983
HA-DG0.1	0.047 ± 0.000	17.24 ± 2.47	0.990
Fusarium culmorum			
Control	0.379 ± 0.002	18.25 ± 0.90	0.997
DG0.02	0.370 ± 0.005	18.59 ± 0.62	0.991
DG0.1	0.354 ± 0.010*	18.36 ± 1.33	0.990
DG0.5	0.347 ± 0.004**	19.08 ± 0.41	0.992
DIG1	0.358 ± 0.003*	18.46 ± 0.43	0.992
HA	0.412 ± 0.002**	16.70 ± 0.60	0.996
HA-DG0.02	0.356 ± 0.011*	16.98 ± 0.99	0.995
HA-DG0.1	0.313 ± 0.012***	15.13 ± 0.84	0.993
Verticillium dahliae			
Control	0.091 ± 0.001	54.27 ± 2.10	0.997
DG0.02	0.091 ± 0.001	11.48 ± 1.06	0.998
DG0.1	0.092 ± 0.002	42.17 ± 7.46	0.993
DG0.5	0.088 ± 0.002	52.63 ± 1.72	0.989
DG1	0.092 ± 0.002	64.94 ± 3.17	0.989
HA	0.080 ± 0.005	19.78 ± 6.52	0.995
HA-DG0.02	0.082 ± 0.009	40.03 ± 10.72	0.997
HA-DG0.1	0.080 ± 0.005	8.17 ± 1.55	0.997

μ: radial growth rate; λ: lag time;.
[a] Standard error ($n = 5$);.
* $P \leq 0.05$,.
** $P \leq 0.01$,.
*** $P \leq 0.001$ according to the least significant difference (LSD) test. No significant difference between each treatment and the control was found for *A. mellea* and *V. dahliae*.

To date, this is the first report concerning the impact of anaerobic DG on the growth of *A. mellea* on solid substrate, therefore a comparison between our results and those of other studies is not possible. Musatti et al. (2017) found that *A. mellea* was not able to grow in submerged conditions using DG as the only carbon and energy source. Applying different soil amendments on *Armillaria* spp., Otieno et al. (2003) found that coffee pulp slightly reduced the vitality of the fungus *A. gallica*, while Malecka et al. (2015) found that Scots pine sawdust, composted bark, and woody debris caused no relevant effects on *A. ostoyae*.

3.4.2. *Fusarium culmorum*

In the experimental conditions adopted, the growth of *F. culmorum* was very rapid and the mycelium reached the edge of the plate in just 5 days (Fig. 3). Linear regression of the mycelium growth data of any treatment showed their excellent alignment, and the correlation coefficients ranged from 0.990 to 0.997 ($n = 5$) (Table 7). Unlike what observed for *A. mellea*, this fungus responded significantly differently to the various treatments. This is evident by observing the linear plot of the mycelium growth data (Fig. 6). The treatment lines, except for DG0.02, showed different slopes from that of the control (Fig. 6). In general, DG addition slowed down the mycelial extension, which graphically corresponded to lines with a lower slope than the control.

With the exclusion of DG0.02 treatment, which was statistically equal to the control, and HA treatment, which showed the highest μ value, all other μ values were significantly or highly significantly lower than the control (Table 7). In this study, HA alone appeared to stimulate hyphal elongation, compared to the control (Table 7). However, when HA was present in combination with the DG, a clear inhibition was observed even at the lowest DG dose; the suppressive action reached its maximum expression in HA-DG0.1 combination (Table 6).

Previous studies demonstrated that the main fraction of stable soil organic matter, namely HA, could control plant diseases caused by

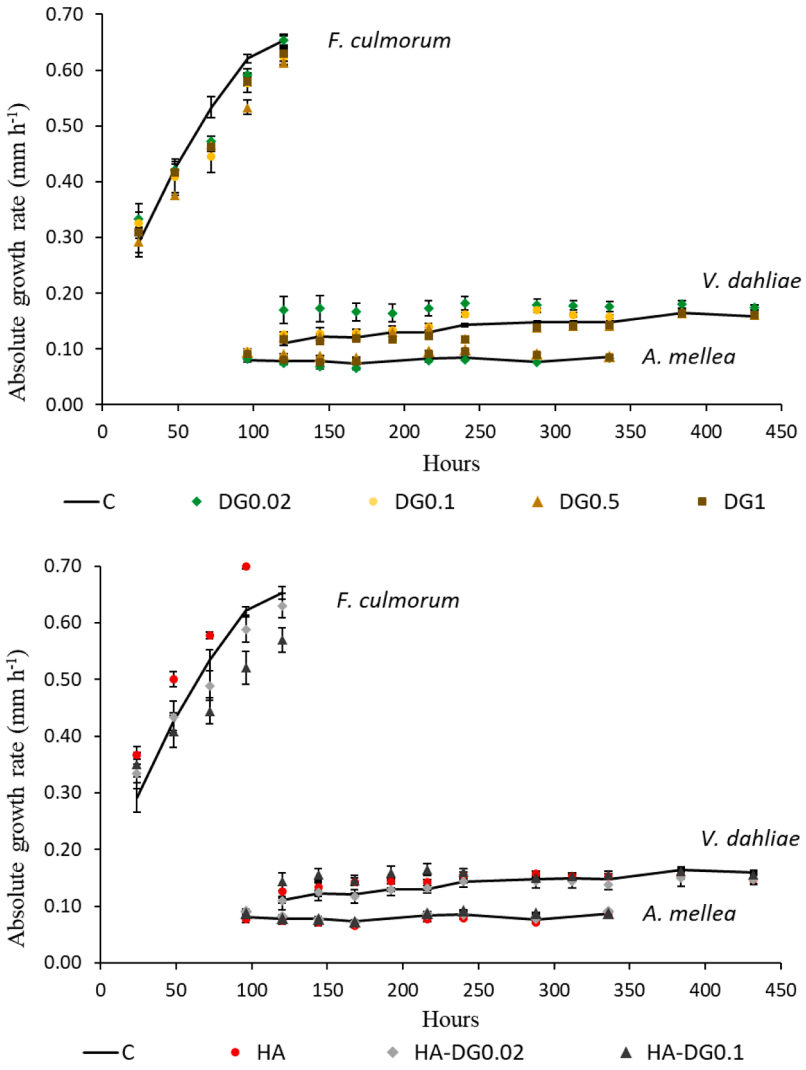


Fig. 5. Absolute growth rate of the fungal colonies.

fungal phytopathogens, especially when abundantly present in the rhizosphere (Pascual et al., 2002; Loffredo et al., 2007, 2008, 2009). However, the activity of HA depends not only on its physicochemical, structural and functional properties amd on the environmental conditions but also on the fungal species considered. Furthermore, there is previous evidence on changing, or even reversing, the activity of bioactive compounds on soil-resident fungi after interaction with HAs (Loffredo and Traversa, 2014). The high adsorption capacity of HA towards both hydrophilic and, especially, hydrophobic compounds (Senesi et al., 2015) might explain the change in HA activity observed for this fungus. Slowing down the growth of the fungus is very important in soils where the fungus lives and attacks the vegetation as the margin of time gained allows the plants to grow and strengthen, thus avoiding, at least in part, the fungal attack.

Only slight differences in the latency time of this fungus were observed between the various treatments and the control (Table 7). Although HA-DG0.1 produced the strongest inhibition on *F. culmorum*, it also showed the shortest latency time (Table 7). This result agrees with the lack of correlation between growth rate and lag time reported by Dantigny et al. (2005). Finally, AGR data (Fig. 5) confirmed that all DG and HA-DG treatments reduced fungal growth, compared to the control.

Unfortunately, there are no previous laboratory or field studies on the influence of DG on the growth of *F. culmorum*, while some investigations concern other *Fusarium* spp. Previous studies demonstrated suppressive effects on the fungus *F. oxysporum* by various organic amendments, including DG (Loffredo and Senesi, 2009; Pane et al., 2014; Tao et al., 2014). An antifungal activity of a cow slurry DG on *F. solani* was found by Vitti et al. (2021). In a recent field study, a sewage sludge DG showed suppressive effectiveness on the Fusarium vascular wilt disease caused by *F. oxysporum* f. sp. *lycopersici* (De Corato et al., 2023).

3.4.3. *Verticillium dahliae*

Compared to the control, no morphological changes were observed for *V. dahliae* mycelium during the 18-day growth in the presence of any DG or HA-DG treatment at any concentration (Fig. 3).

Radial mycelial growth on PDA only (control) and PDA supplemented with DG and/or HA as well as linear plots of the experimental data are depicted in Fig. 7. Linear regression of the experimental data showed r values ranging from 0.989 to 0.998 (Table 7). Similarly to what was observed for *A. mellea*, also in the case of this fungus, none of the doses of DG applied significantly modified the mycelial growth, compared to the control. Graphically, the interpolation of the fungal growth data in the various treatments appeared as a series of lines almost parallel to each other and parallel to the control (Fig. 7).

As previously reported for *A. mellea*, only the latency time was quite different in the various treatments, being minimum in HA-DG0.1 and maximum in DG1 (Table 7). However, under field conditions, the latency time does not appear to significantly influence the growth and pathogenicity of the fungus (Dantigny et al., 2005). These results are in

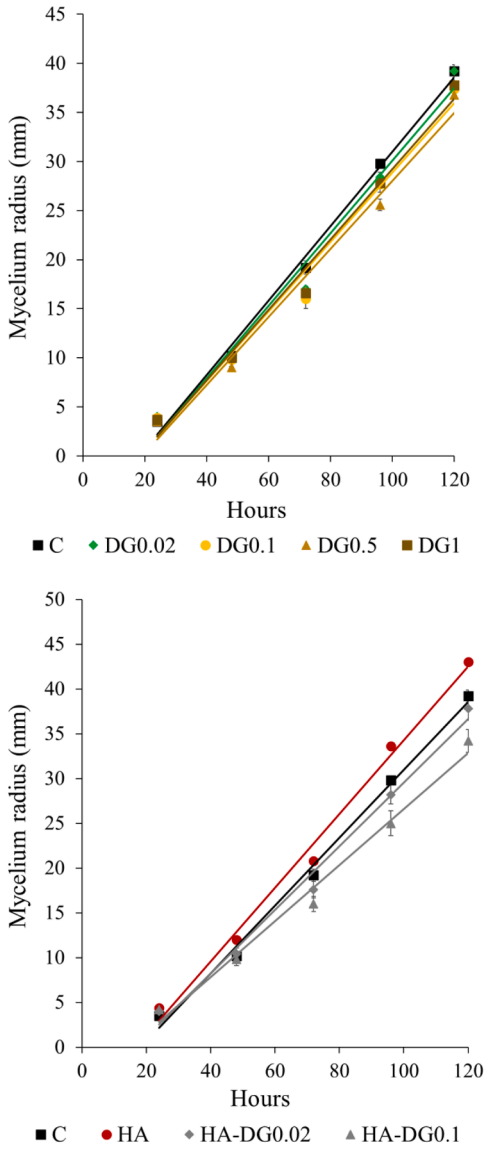


Fig. 6. Effects of DG alone (above) and DG interacted with HA (below) at various doses on the radial extent of *F. culmorum* as a function of time. The graph shows the experimental points with standard errors (n = 5) and linear plots.

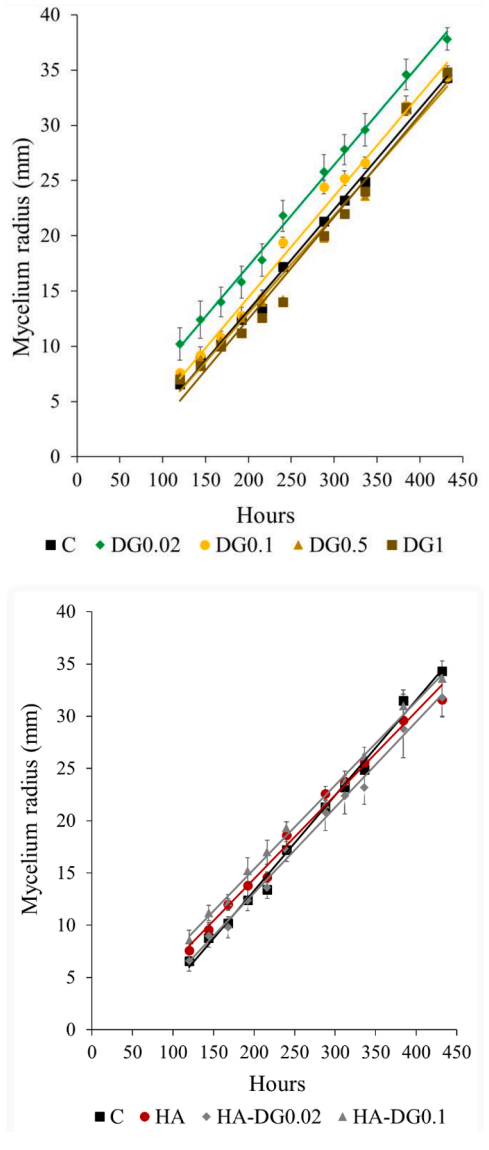


Fig. 7. Effects of DG alone (above) and DG interacted with HA (below) at various doses on the radial extent of *V. dahliae* as a function of time. The graph shows the experimental points with standard errors (n = 5) and linear plots.

agreement with the results of previous studies conducted both *in vivo* and *in vitro* by Pane et al. (2014) who found that a sterilized liquid extract from animal waste DG did not show significant inhibition of *V. dahliae*. Differently, in field trials, a liquid DG from mixed feedstock showed a noticeable reduction (50 %) in the *V. dahliae* density (Wei et al., 2016). To the best of our knowledge, no further information on this matter is available in the literature.

4. Conclusions

The anaerobic digestion of OP, a waste released in large quantities in geographical areas such as Apulia region, provides renewable energy (biogas or biomethane) and releases a byproduct, namely solid DG, which appears to improve the chemical and biological fertility of soil. This study demonstrated that a new DG produced exclusively from two-phase OP has a noticeable adsorbent capacity of the fungicide boscalid and the herbicide oxyfluorfen and, when incorporated into the soil, contributes significantly to the increase in the overall adsorbent capacity of the soil. This action is of extreme importance as it limits the transfer of agrochemicals into natural surface and ground waters and reduces their absorption by cultivated plants with consequent lower risk of entry into the human and animal food chain. The results obtained also demonstrated that the sorption capacity of the DG remains unchanged over a wide range of temperatures, guaranteeing its effectiveness in various environmental and geographical conditions. The examination of the effects of DG on some important fungi that devastate agricultural crops highlighted suppressive effects on *F. culmorum*, effects that were even more intense after the interaction of DG with the humic fraction of the soil, which is what happens in real field conditions. Further studies could help to understand the biochemical mechanisms underlying this phenomenon and which possible DG modification could enhance its suppressiveness on phytopathogenic fungi. Inhibitory effects were not observed on *A. mellea* and *V. dahliae* whose growth appeared unaltered at normal field dosage of the DG. However, considering the benefits of DG on soil fertility and the need to dispose of this byproduct, even the irrelevant activity observed on the latter fungi can be considered favourably. Ultimately, the production and use of DG from OP appears to be a sustainable and recommendable strategy in both the energy and agricultural sectors.

Funding

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

CRediT authorship contribution statement

Nicola Colatorti: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. **Nunzio Vito Digregorio:** Data curation, Formal analysis, Investigation. **Salvatore Camposeo:** Conceptualization, Investigation, Resources. **Elisabetta Loffredo:** Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing 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 paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank AGROLIO S.r.l. (Andria, Italy), for providing the digestate sample used in this study and for logistic support. We are grateful to prof. Antonio Ippolito of University of Bari for providing the fungal isolates. The authors are also grateful to the reviewers for their valuable comments and suggestions.

References

Bhat, R.G., Subbarao, K.V., 1999. Host range specificity in *Verticillium dahliae*. Phytopathology 89, 1218–1225. https://doi.org/10.1094/PHYTO.1999.89.12.1218.

Bhatt, D., Srivastava, A., Srivastava, P.C., 2023. An insight into the sorption kinetics of boscalid onto soils: effect of general soil properties. Chemosphere 325. https://doi.org/10.1016/j.chemosphere.2023.138274.

Bonnar, D.J., Eichler, J.P., Parikh, S.J., Blandino, A., Lybrand, R.A., Morris, M.L., et al., 2023. Soil-water partitioning behavior of oxyfluorfen under California rice field conditions. ACS Agric. Sci. Technol. 3, 1169–1176. https://doi.org/10.1021/acsagscitech.3c00341.

Braguglia, C.M., Gallipoli, A., Gianico, A., Pagliaccia, P., 2018. Anaerobic bioconversion of food waste into energy: a critical review. Bioresour. Technol. 248, 37–56. https://doi.org/10.1016/j.biortech.2017.06.145.

Brezáni, A., Svobodová, K., Jablonský, I., Tlustoš, P., 2019. Cultivation of medicinal mushrooms on spruce sawdust fermented with a liquid digestate from biogas stations. Int. J. Med. Mushrooms 21, 215–223. https://doi.org/10.1615/IntJMedMushrooms.2019030022.

Brueck, C.L., Nason, S.L., Multra, M.G., Prasse, C., 2023. Assessing the fate of antibiotics and agrochemicals during anaerobic digestion of animal manure. Sci. Total Environ. 856, 159156 https://doi.org/10.1016/j.scitotenv.2022.159156.

Calderón, M.J., Real, M., Cabrera, A., Koskinen, W.C., Cornejo, J., Hermosín, M.C., 2015. Influence of olive oil mill waste amendment on fate of oxyfluorfen in southern Spain soils. Clean Soil Air Water 43, 1107–1113. https://doi.org/10.1002/clen.201400560.

Caruso, C., Maucieri, C., Barco, A., Barbera, A.C., Borin, M., 2018. Effects of mycorrhizal inoculation and digestate fertilisation on triticale biomass production using fungicide-coated seeds. Ir. J. Agric. Food Res. 57, 42–51. https://doi.org/10.1515/ijafr-2018-0005.

Castellana, G., Loffredo, E., 2014. Simultaneous removal of endocrine disruptors from a wastewater using white rot fungi and various adsorbents. Water Air Soil Pollut. 225, 1872. https://doi.org/10.1007/s11270-014-1872-6.

Cesaro, A., 2021. The valorization of the anaerobic digestate from the organic fractions of municipal solid waste: challenges and perspectives. J. Environ. Manag. 280, 111742 https://doi.org/10.1016/j.jenvman.2020.111742.

Chen, L., Zhang, S., 2010. Dissipation and residues of boscalid in strawberries and soils. Bull. Environ. Contam. Toxicol. 84, 301–304. https://doi.org/10.1007/s00128-010-9934-y.

Cristina, G., Camelin, E., Tommasi, T., Fino, D., Pugliese, M., 2020. Anaerobic digestates from sewage sludge used as fertilizer on a poor alkaline sandy soil and on a peat substrate: effects on tomato plants growth and on soil properties. J. Environ. Manag. 269, 110767 https://doi.org/10.1016/j.jenvman.2020.110767.

Dantas Palmeira, J., Araújo, D., C. Mota, C., Alves, Rita C., P. P. Oliveira, M.B., Ferreira, H.M.N., 2023. Fermentation as a strategy to valorize olive pomace, a by-product of the olive oil industry. Fermantation 9, 442. https://doi.org/10.3390/fermentation9050442.

Dantigny, P., Guilmart, A., Bensoussan, M., 2005. Basis of predictive mycology. Int. J. Food Microbiol. 100, 187–196. https://doi.org/10.1016/j.ijfoodmicro.2004.10.013.

De Corato, U., Cancellara, F.A., Lacolla, G., Caranfa, D., Cucci, G., 2023. Short-term soil amendment by sewage sludge anaerobic digestate in a tomato monoculture suppresses Fusarium vascular wilt disease by changing the taxonomic characteristics of soil microbiota. Appl. Soil Ecol. 189, 104915 https://doi.org/10.1016/j.apsoil.2023.104915, 1.

Delgado-Moreno, L., Nogales, R., Romero, E., 2017. Wastes from the olive oil production in sustainable bioremediation systems to prevent pesticides water contamination. Int. J. Environ. Sci. Technol. 14, 2471–2484. https://doi.org/10.1007/s13762-017-1335-x.

Devkota, P., Hammerschmidt, R., 2020. The infection process of *Armillaria mellea* and *Armillaria solidipes*. Physiol. Mol. Plant Pathol. 112, 101543 https://doi.org/10.1016/j.pmpp.2020.101543.

Dollinger, J., Bourdat-Deschamps, M., Pot, V., Serre, V., Bernet, N., Deslarue, G., et al., 2022. Leaching and degradation of S-metolachlor in undisturbed soil cores amended with organic wastes. Environ. Sci. Pollut. Res. 29, 20098–20111. https://doi.org/10.1007/s11356-021-17204-z.

El-Masry, M.H., Khalil, A.I., Hassouna, M.S., Ibrahim, H.A.H., 2002. *In situ* and *in vitro* suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World J. Microbiol. Biotechnol. 18, 551–558. https://doi.org/10.1023/A:1016302729218.

EPA, 1992. Pesticide Tolerance for Oxyfluorfen. USEPA, Washington, USA (Fed Regist 57-22202) (accessed on 24 May 2023).

European Commission (EC) 2003. Regolamento CE, N. 2003/2003, 13 October 2003 relativo ai concimi. https://eur-lex.europa.eu/legal-content/IT/TXT/PDF/?uri=CELEX:02003R2003-20141215&from=BG (accessed on 24 May 2023).

European Commission (EC) 2019. https://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32019R1691 (accessed on 24 May 2023).

Gazzetta Ufficiale della Repubblica Italiana (GU). DM 13-09-1999. Metodi ufficiali di analisi chimica del suolo. https://www.gazzettaufficiale.it/eli/gu/1999/10/21/248/so/185/sg/pdf (accessed 12 May 2023).

Gazzetta Ufficiale della Repubblica Italiana (GU). DM 21-12-2000. Metodi ufficiali di analisi per i fertilizzanti. https://www.gazzettaufficiale.it/eli/id/2001/01/26/21/sg/pdf (accessed 12 May 2023).

Hall, K.E., Ray, C., Ki, S.J., Spokas, K.A., Koskinen, W.C., 2015. Pesticide sorption and leaching potential on three Hawaiian soils. J. Environ. Manag. 159, 227–234. https://doi.org/10.1016/j.jenvman.2015.04.046.

Hung, C.Y., Tsai, W.T., Chen, J.W., Lin, Y.Q., Chang, Y.M., 2017. Characterization of biochar prepared from biogas digestate. Waste Manag. 66, 53–60. https://doi.org/10.1016/j.wasman.2017.04.034.

Karimi, B., Sadet-Bourgeteau, S., Cannavacciuolo, M., Chauvin, C., Flamin, C., et al., 2022. Impact of biogas digestates on soil microbiota in agriculture: a review. Environ. Chem. Lett. 20, 3265–3288. https://doi.org/10.1007/s10311-022-01451-8.

Kulikova, N.A., Stepanova, E.V., Koroleva, O.V., 2005. Mitigating activity of humic substances: direct Influence on Biota. Use of Humic Substances to Remediate Polluted Environments: from Theory to Practice. Springer, The Netherlands, pp. 285–309. https://doi.org/10.1007/1-4020-3252-8_14. Perminova I.V. et al.,.

IHSS Natural organic matter research 2022. https://humic-substances.org/(accessed on 10 June 2023).

Loffredo, E., Berloco, M., Casulli, F., Senesi, N., 2007. *In vitro* assessment of the inhibition of humic substances on the growth of two strains of *Fusarium oxysporum*. Biol. Fertil. Soils 43, 759–769. https://doi.org/10.1007/s00374-006-0160-z.

Loffredo, E., Berloco, M., Senesi, N., 2008. The role of humic fractions from soil and compost in controlling the growth *in vitro* of phytopathogenic and antagonistic soil-borne fungi. Ecotoxicol. Environ. Saf. 69, 350–357. https://doi.org/10.1016/j.ecoenv.2007.11.005.

Loffredo, E., Carnimeo, C., Silletti, R., Summo, C., 2021. Use of the solid by-product of anaerobic digestion of biomass to remove anthropogenic organic pollutants with endocrine disruptive activity. Processes 9, 2018. https://doi.org/10.3390/pr9112018.

Loffredo, E., Senesi, N., 2009. *In vitro* and *in vivo* assessment of the potential of compost and its humic acid fraction to protect ornamental plants from soil-borne pathogenic fungi. Sci. Hortic. 122, 432–439. https://doi.org/10.1016/j.scienta.2009.05.030.

Loffredo, E., Traversa, A., 2014. Soil and compost humic fractions regulate the response of *Sclerotinia sclerotiorum* to exogenously added allelochemical compounds. Biol. Fertil. Soils 50, 1281–1290. https://doi.org/10.1007/s00374-014-0944-5.

Malecka, M., Kwaśna, H., Szewczyk, W., 2015. Fungal communities in barren forest soil after amendment with different wood substrates and their possible effects on trees, pathogens, insects and nematodes. J. Plant Prot. Res. 55, 301–311. https://doi.org/10.1515/jppr-2015-0042.

Moliszewska, E., Pisarek, I., 1996. Influence of humic substances on the growth of two phytopathogenic soil fungi. Environ. Int. 22, 579–584. https://doi.org/10.1016/0160-4120(96)00048-7.
Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12, 242–257. https://doi.org/10.1002/elsc.201100085.
Mukherjee, S., Weihermüller, L., Tappe, W., Hofmann, D., Köppchen, S., Laabs, V., et al., 2016. Sorption-desorption behaviour of bentazone, boscalid and pyrimethanil in biochar and digestate based soil mixtures for biopurification systems. Sci. Total Environ. 559, 63–73. https://doi.org/10.1016/j.scitotenv.2016.03.145.
Musatti, A., Ficara, E., Mapelli, C., Sambusiti, C., Rollini, M., 2017. Use of solid digestate for lignocellulolytic enzymes production through submerged fungal fermentation. J. Environ. Manag. 199, 1–6. https://doi.org/10.1016/j.jenvman.2017.05.022.
Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron. Sustain. Dev. 34, 473–492. https://doi.org/10.1007/s13593-013-0196-z.
Otieno, W., Jeger, M., Termorshuizen, A., 2003. Effect of infesting soil with *Trichoderma harzianum* and amendment with coffee pulp on survival of *Armillaria*. Biol. Control 26, 293–301. https://doi.org/10.1016/S1049-9644(02)00168-8.
Pane, C., Celano, G., Zaccardelli, M., 2014. Metabolic patterns of bacterial communities in aerobic compost teas associated with potential biocontrol of soilborne plant diseases. Phytopathol. Mediterr. 53, 277–286. https://doi.org/10.14601/Phytopathol_Mediterr-13363.
Parlavecchia, M., Gattullo, R., Perri, G., Loffredo, E., 2021. Modulatory effects of biochar, hydrochar and vermicompost on the growth of horticultural plants and phytopathogenic fungi. Acta Hortic. 1311, 541–548. https://doi.org/10.17660/2021.1311.69.
Pascual, J.A., Garcia, C., Hernandez, T., Lerma, S., Lynch, J.M., 2002. Effectiveness of municipal waste compost and its humic fraction in suppressing *Pythium ultimum*. Microb. Ecol. 44, 59–68. https://doi.org/10.1007/s00248-001-0040-x.
Peng, W., Pivato, A., 2019. Sustainable management of digestate from the organic fraction of municipal solid waste and food waste under the concepts of back to earth alternatives and circular economy. Waste BioMass Valorization 10, 465–481. https://doi.org/10.1007/s12649-017-0071-2.
Pérez-Lucas, G., El Aatik, A., Vela, N., Fenoll, J., Navarro, S., 2021. Exogenous organic matter as strategy to reduce pesticide leaching through the soil. Arch. Agron. Soil Sci., 67, 934–945. 10.1080/03650340.2020.1768531.
Provenzano, M.R., Cavallo, O., Malerba, A.D., Di Maria, F., Cucina, M., Massaccesi, L., Gigliotti, G., 2016. Co-treatment of fruit and vegetable waste in sludge digesters: chemical and spectroscopic investigation by fluorescence and Fourier transform infrared spectroscopy. Waste Manag. 50, 283–289. https://doi.org/10.1016/j.wasman.2016.02.026.
Provenzano, M.R., Malerba, A.D., Pezzolla, D., Gigliotti, G., 2014. Chemical and spectroscopic characterization of organic matter during the anaerobic digestion and successive composting of pig slurry. Waste Manag. 34, 653–660. https://doi.org/10.1016/j.wasman.2013.12.001.
PubChem Open Chemistry Database at the National Institutes of Health (NIH), U.S. National Library of Medicine. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/8814 (accessed on 20 June 2023).
Pullen, T., 2015. Anaerobic Digestion-Making Biogas-Making Energy. Routledge, New York, NY. https://doi.org/10.4324/9781315770772.
Sanchez, C., 2009. Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol. Adv. 27, 185–194. https://doi.org/10.1016/j.biotechadv.2008.11.001.
Salvestrini, S., Vanore, P., Iovino, P., Leone, V., Capasso, S., 2015. Adsorption of simazine and boscalid onto acid-activated natural clinoptilolite. Environ. Eng. Manag. J. 14, 1705–1712. https://doi.org/10.30638/eemj.2015.181.
Santi, G., Muzzini, V.G., Galli, E., Proietti, S., Moscatello, S., Battistelli, A., 2015. Mycelial growth and enzymatic activities of white-rot fungi on anaerobic digestates from industrial biogas plants. Environ. Eng. Manag. J. 14, 1713–1719. https://doi.org/10.30638/eemj.2015.182.
Scherm, B., Balmas, V., Spanu, F., Pani, G., Delogu, G., Pasquali, M., Migheli, Q., 2013. *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. Mol. Plant Pathol. 14, 323–341. https://doi.org/10.1111/mpp.12011.
Schnathorst, W.C., 1981. Life cycle and epidemiology of *Verticillium*. Fungal Wilt Diseases of Plants. Academic Press, New York, pp. 81–111. Mace, M.E., Bell, A.A., Beckman, C.H. (eds).
Senesi, N., Loffredo, E., D'Orazio, V., Brunetti, G., et al., 2015. Adsorption of pesticides by humic acids from organic amendments and soils. In: Clapp, C.E., et al. (Eds.), Humic Substances and Chemical Contaminants, Soil Science Society of America Journal, pp. 129–153. 10.2136/2001.humicsubstances.c8. Madison, WI, USA.
Singh, L., Kalia, V.C., 2017. Waste Biomass Management—A Holistic Approach. Springer International Publishing AG, Cham, Switzerland.
Szczech, M., Smolinska, U., 2001. Comparison of suppressiveness of vermicomposts produced from animal manures and sewage sludge against phytophthora *Nicotianae breda* de haan var. nicotianae. J. Phytopathol. 149, 77–82. https://doi.org/10.1046/j.1439-0434.2001.00586.x.
Tang, Y., Luo, L., Carswell, A., Misselbrook, T., Shen, J., Han, J., 2021. Changes in soil organic carbon status and microbial community structure following biogas slurry application in a wheat-rice rotation. Sci. Total Environ. 757, 143786 https://doi.org/10.1016/j.scitotenv.2020.143786.
Tao, X., Shang, B., Dong, H., Chen, Y., Xin, H., 2014. Effects of digestate from swine manure digester on *in vitro* growth of crop fungal pathogens: a laboratory study. Trans. ASABE 57, 1803–1810. https://doi.org/10.13031/trans.57.10622.
Taskin, E., Perri, G., Loffredo, E., 2019. Impact of type and dose of biochar and hydrochar on growth response of phytopathogenic and antagonistic soil resident fungi. Fresenius Environ. Bull. 28, 9070–9076.
Teglia, C., Tremier, A., Martel, J.L., 2011. Characterization of solid digestates: part 1, review of existing indicators to assess solid digestates agricultural use. Waste BioMass Valorization 2, 43–58. https://doi.org/10.1007/s12649-010-9051-5.
Tolisano, C., Luzi, F., Regni, L., Proietti, P., Puglia, D., Gigliotti, G., et al., 2023. A way to valorize pomace from olive oil production: lignin nanoparticles to biostimulate maize plants. Environ. Technol. Innov. 31, 103216 https://doi.org/10.1016/j.eti.2023.103216.
Valenti, F., Arcidiacono, C., Chinnici, G., Cascone, G., Porto, S.M.C., 2017. Quantification of olive pomace availability for biogas production by using a GIS-based model. Biofuels Bioprod. Biorefin. 11, 784–797. https://doi.org/10.1002/bbb.1784.
Velechovský, J., Malík, M., Kaplan, L., Tlustoš, P., 2021. Application of individual digestate forms for the improvement of hemp production. Agriculture 11. https://doi.org/10.3390/agriculture11111137.
Vallée, R., Dousset, S., Billet, D., Benoit, M., 2014. Sorption of selected pesticides on soils, sediment and straw from a constructed agricultural drainage ditch or pond. Environ. Sci. Pollut. Res. 21, 4895–4905.
Vitti, A., Elshafie, H.S., Logozzo, G., Marzario, S., Scopa, A., Camele, I., Nuzzaci, M., 2021. Physico-chemical characterization and biological activities of a digestate and a more stabilized digestate-derived compost from agro-waste. Plants 10, 386. https://doi.org/10.3390/plants10020386.
Walsh, J.J., Rousk, J., Edwards-Jones, G., Jones, D.L., Williams, A.P., 2012. Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils. Biol. Fertil. Soils 48, 889–897. https://doi.org/10.1007/s00374-012-0681-6.
Wang, W., Lee, D.J., 2021. Valorization of anaerobic digestion digestate: a prospect review. Bioresour. Technol. 323, 124626 https://doi.org/10.1016/j.biortech.2020.124626.
Wei, F., Passey, T., Xu, X., 2016. Effects of individual and combined use of biofumigation-derived products on the viability of *Verticillium dahliae* microsclerotia in soil. Crop Prot. 79, 170–176. https://doi.org/10.1016/j.cropro.2015.09.008.
World Bioenergy Association (WBA). Annual report 2022. https://www.worldbioenergy.org/uploads/230510%20Annual%20Report%202022%20Public.pdf (accessed on 29 May 2023).
Wu, C., Liu, X., Wu, X., Dong, F., Xu, J., Zheng, Y., 2019. Sorption, degradation and bioavailability of oxyfluorfen in biochar-amended soils. Sci. Total Environ. 658, 87–94. https://doi.org/10.1016/j.scitotenv.2018.12.059.

Further reading

U.S. National Library of Medicine. PubChem open chemistry database at the National Institutes of Health (NIH). Available online: https://pubchem.ncbi.nlm.nih.gov (accessed on 10 May 2023).