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Research article

High-agronomic value of selected poultry manure valorized through aerobic fermentation: The AFRODITE® process

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ABSTRACT

Chicken manure is a raw material with potential value as fertilizer. The present work documented an 8-month continuous spontaneous fermentation process, referred to as AFRODITE® (Aerobic Fermentation and Revaluation of Organic matrix to Develop and Improve The Essence of soil), where the main physical, chemical and microbiological parameters have been investigated for two years within the company FOMET S.p.A. The pelletized final product reached in both years stable value of pH (6.5–7.5), moisture (17.0–13.8%), total nitrogen (3.0–3.8%) and C/N ratio (10.46–10.66); total eubacteria population decreased (from 10 to 11 to about 7 Log CFU/g), with the selection of a thermophilic/hyperthermophilic microbiota due to the high temperatures during the process. The commercial product is microbiologically safe and rich of bacteria that were isolated and identified. The dominant species belong to the *Bacillus* genus, which includes several Plant Growth Promoting Rhizobacteria whose beneficial activities towards soil and plant are widely known. A greenhouse experiment in lettuce and eggplants showed the positive effect of two concentrations of the pelletized commercial product (2.5 and 5% w/w) in combination with a mixture of topsoil/river sand. In particular, 27% of fresh weight increase was observed in lettuce with both product amounts whereas the average production of eggplants was three times higher using 5% pelletized product compared to the untreated control. This work has highlighted that chicken manure fermentation followed by pelletization allows the obtainment of a stable product, rich in beneficial bacteria and nutritional elements, possessing growth-promoting effects on horticultural plants. The process represents a virtuous example of circular economy.

1. Introduction

Animal Manure is a valuable organic fertilizer since the dawn of time; it is rich in nutrients that contribute to improve soil fertility and crop productivity (Sadeghpour and Afshar, 2024). Nowadays, the use of manure and litter of animal origin, usually solid and/or liquid, contributes to soil fertility and sustainable agricultural practices (Carvajal-Muñoz and Carmona-Garcia, 2012; Das et al., 2017; Mengqi et al., 2021). The poultry sector is one of the largest livestock industry worldwide, generating a large amount of manure and consequently environmental pollution, including a considerable impact on soil, land, water resources and global climate change (Yang et al., 2021). Nevertheless, poultry manure is one of the main organic amendments used for soil fertility preservation to get high yields of agricultural crops (Ravindran et al., 2017). It is a mixture of organic and inorganic compounds with high nutrient levels, such as nitrogen (3–5%), potassium (1.5–3%) and phosphorus (0.9–3.5%) (Jędrczak et al., 2014); the chemical composition depends on several factors such as a breeding system, seasonality, a breed type and a production group (Dróżdż et al., 2020). It has a relatively high dry matter percentage and it is suitable for longer transports (Esperón et al., 2020). Raw poultry manure can be directly applied to agricultural soils, but the improper management and over application may cause ammonia volatilization, higher nitrogen mineralization, nutrients leaching and surface water contamination by excessive phosphorus (AWMFH, 2013). Moreover, poultry farms generally produce huge amount of manure compared to agricultural land availability (Rizzo et al., 2020). The fermented product, via biological transformation, is preferable to the fresh one since the fermentation process prevents the risk of N loss, increases soil organic matter and suppresses soil-borne pathogens due to the high temperatures reached (Darby et al., 2004; Evanylo et al., 2008; Escribano, 2016; Guo et al., 2019). It is easy to control with simple equipment requirements, it

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is applicable at different scale (on-farm, and at medium and large scale) and it contributes to odour and volume reduction (Bolan et al., 2010). Therefore, the transformation of manure into a stable product, referred to as stable poultry manure (SPM) via aerobic or anaerobic digestion, represents a valorisation of the waste product since its presence within the soil can supply high nutrient quantity and quality (Ibuot et al., 2019). Some concerns related to toxic compounds (*e.g.* antibiotics residues) still need to be considered (Andersson and Hughes, 2014), although several works evidenced that the high temperatures reached during the fermentation process can deactivate some antibiotics and reduce their amount (Diehl and LaPara, 2010; Sun et al., 2016). In particular, Ho et al. (2013) showed a significant decrease of antimicrobials in the poultry manure during 40 day-composting process, with 99% removal of administered antibiotics. Bacteria and Fungi activity is essential; the population along the process varies according to the species of animal and the litter (Dumas et al., 2011). These microorganisms are able to promote the decomposition and transformation of different organic molecules within the manure, leading to the formation of humic substances (Chilakamarry et al., 2022). Their survival is affected by process conditions such as pH, moisture, aeration and temperature. Some microorganisms recovered during the fermentation process also have decomposing abilities (e.g., white-rot fungi, *Streptomyces*, and *Bacillus* spp.) towards recalcitrant organic compounds (cellulose, lignin, grease, etc.) (Yang et al., 2017; Wang et al., 2023). *Bacillus* spp., belonging to the philum *Firmicutes*, are also known as Plant Growth Promoting Rhizobacteria (PGPR), able to improve soil fertility and plant growth through several mechanisms (Kumar et al., 2011). They play an integral role in nutrient mobilization and adsorption by accelerating the cycling of macro and micronutrients (Mader et al., 2002; Sun et al., 2015; Qiu et al., 2021; Minkina et al., 2023). In addition, they can survive hostile conditions for long time as spore forming bacteria (Voundi et al., 2016). This study aimed at investigating the whole cycle of the spontaneous fermentation and humification of poultry manure in two different seasonal conditions within a company, FOMET S.p.A., specialized in the production of SPM. FOMET S.p.A has developed a unique trademarked process of fermentation named AFRODITE® (Aerobic Fermentation and Revaluation of Organic matrix to Develop and Improve The Essence of soil). The process complies with the European legislation (CE) n° 1069/2009 and with the Italian Dlgs n° 75/2010 on fertilizer management and recently obtained the patent granting (patent n° WO2024/003703A1). The patent described an innovative fermentation process without heating control, ventilation and moisture adjustment. The present work describes the monitoring of some chemical and microbiological parameters during the fermentation process and in the pelletized commercial product. The study also focused on the isolation of microorganisms from the commercial product in order to select potential beneficial bacteria for future implementation as booster ingredients for fertilization purposes. To our knowledge, there is no evidence of isolation of beneficial bacteria from animal manure that may act as PGPR. In addition, in order to understand the valorisation outcome of the processed manure, the commercial product (PPM) was applied as fertilizer in greenhouse at different concentration with different substrates, and its effect evaluated in lettuce and eggplants. Intensive lettuce and eggplant cultivations require a large use of nutrients, thus increasing the demand for fertilizers. Testing different substrates and several PPM concentrations was mandatory for the patent processing and for exploring plant response to different soil/manure conditions in the perspective of pre-market commercialization strategy.

2. Experimental

2.1. Manure management

The stable poultry manure production plant is located in FOMET S.p.A. (San Pietro di Morubio, Verona, Italy). FOMET is a company that manufactures and distributes worldwide organic and special fertilizers and annually it manages around 100.000 t/year of manure. The production process is a continuous system of manure litters coming from different farms; the material is unloaded from the transport vehicles in the fresh manure unloading area, isolated from the rest of the industrial one. An operator uses a scraper to collect the different daily loads received, mixing the material in order to get a homogeneous heap of ~12 m high. The whole process lasts around eight months with two turning operations, after 40 and 120–140 days. The further step, after 8 months of composting, is the screening phase to remove undesirable particles and inert materials; the obtained compost is a stable product, which is ready for pelleting. There are two processing lines, composed by four and five pellet machines, respectively that reach a temperature of 70 ± 1 °C for 2 min. Both lines include a conveyor belt, which leads the pelleted product to a cooling system to stabilize the temperature of the finished product close to the environmental temperature. The pellets are then packaged in 25 and 500 kg plastic bags and storage for a month, prior quality analyses.

2.2. Sampling

Samples of poultry manure, subjected to the composting process, were collected in two different years (February–November 2020; September 2022–May 2023), in order to test the process reproducibility. The chemical and microbiological analyses were performed on the fresh poultry manure (FPM-2020; FPM-2023), on the stable poultry manure (SPM-2020; SPM-2023) at the end of the 8-month process, and on the pelletized poultry manure (PPM-2020; PPM-2023) one month after packaging. 100 g of FPM and SPM were taken from three positions of the manure heap, namely inner low, inner high and external. The three samples deriving from the different sections were pooled and gently mixed. PPM were sampled (100 g) from the plastic bags after one month of storage. Once collected, the samples were placed in plastic tubes for chemical and microbiological analysis, and frozen at - 80 °C for molecular analysis.

2.3. Physical and chemical analysis

During the fermentation period, the temperatures within the heap were daily monitored at representative points by means of eight probes (Deltaohm, Padova, Italy) inserted into the manure mass, removed before each turning and repositioned at the end of the procedure. Turning operations usually lasted 8–15 days. The data recording system allowed measuring, storing and sending temperature values to the acquisition system software HD35AP-S (Senseca, Padova, ITALY). All the manure samples (three replicates each sampling time) were ground in a ball mill (Mixer mill ZM200, Retsch GmbH, Haan, Germany) and sieved at 0.5 mm before analysis. The pH was measured in 1:10 mass-to-water ratio, using a polymer electrode connected to a pH-meter (pH 8+DHS stirrer XS Instruments, Carpi, MO, Italy). Moisture was determined reaching the constant weight of manure samples at 105 °C for 12 h, using an oven (UNB400 model, Memmert GmbH, Schwabach, Germany) to calculate the percentage of moisture. The formula below (1) indicate the percentage of moisture (MC %) of manure samples where TS1i, TS2i and TS1f, TS2f are the Total Solids weight of samples before (i) and after (f) the method conditions

$$MC\,\% = 100 - \frac{TS1\text{f} - tare}{TS1i} \cdot 100 \tag{1}$$

Total organic carbon (TOC) was determined according to the dichromate method (Springer and Klee, 1954). Total nitrogen (TN%) was determined after acid digestion of 0.300 g with sulfuric acid and selenium-potassium persulfate as catalyser, using a Kjeldahl automatic distiller (UDK 129 VELP Scientifica, Usmate, Monza-Brianza, Italy). The ammonium nitrogen (NH_4^+-N) was determined by means of the Kjeldhal distiller, using 20 mL of NaOH solution (33%). Briefly, the total organic nitrogen (NH_2-N) was calculated by the difference between TN and

NH_4^+-N. The ratio carbon/nitrogen (C/N) was calculated from the results of TOC and TN.

2.4. Microbiological analysis: DNA extraction and real time PCR

Total genomic DNA was extracted from FPM, SPM and PPM in both years by using the DNeasy PowerSoil Pro Kit (Qiagen, United States), according to the manufacturer's instructions. The DNA concentration and purity was evaluated measuring the ratio of absorbance at 260 and 280 nm (Infinite®200 PRO NanoQuant, Mannedorf, Switzerland). Total eubacteria were quantified via real time PCR (StepOne™ Real-Time PCR System, Applied Biosystems) according to Bozzi Cionci et al. (2022) Bozzi Cionci et al. (2022). PCR was performed in a 20 μL PCR amplification mixture, containing 10 μL of Fast SYBR® Green Master Mix (Applied Biosystems, Foster city, CA, USA), 10 μM of primers Eub338 (5′-ACT CCT ACG GGA GGC AGC AG-3′) and Eub518 (5′-ATT ACC GCG GCT GCT GG-3′), molecular grade H_2O and 2 μL DNA (5 ng/μl). The PCR protocols started with an activation step at 95 °C for 30", followed by 40 cycles at 95 °C for denaturation (3'') and 60 °C for annealing (30''). Standard curves were constructed using 16S rRNA PCR product of major isolated microorganisms from manure; data were transformed to obtain the number of microorganism as Log_{10} CFU/g manure according to the rRNA copy number (Lee et al., 2009).

2.5. Bacterial isolation and identification

Bacterial isolation and identification were performed from PPM taken both on 2020 and on 2023; after crushing, samples were processed by serial dilutions and seeded with the pouring plate method on tryptic soy agar (TSA, Oxoid Milan, Italy). Plates were incubated at 30 °C in aerobic conditions for 48–72 h. Colonies with apparent different morphologies were picked from TSA plates, re-streaked on the same medium and identified through 16S rRNA gene sequencing. First, genomic DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). 16S rRNA gene amplification was carried out with primers 8f (5′-AGAGTTTGATCCTGGCTCAG -3′) and 1520r (5′-AAGGGAGGT GATCCAGCCGCA -3′), according to Pagliarini et al. (2023a). The PCR products were purified using the NucleoSpin purification kit (Macherey-Nagel GmbH & co. KG, Germany) and then sequenced by Eurofins MWG Operon (Ebersberg, Germany). Sequence chromatograms were edited with the software program Finch TV version 1.4 and the consensus sequence built through DNAMAN software (Version 6.0, Lynnon BioSoft. Inc., San Ramon, CA, USA). Sequence assignment to species or genera was investigated by matching available 16S rRNA sequences using nucleotide BLAST (Basic Local Alignment Search Tool; http://www.ncbi.nlm.nih.gov/BLAST/).

2.6. Greenhouse pot experiments: preliminary experiment

The preliminary experiment was carried out in April 2021 in a temperate greenhouse in the Centre for Plant Nutrition (CFPN) (FOMET S.p.A, San Pietro di Morubio, Verona, Italy), by testing different substrates and different poultry manure concentrations. The experiment consisted of a randomized complete block design using crushed PPM-2020 as soil amendment at the following concentrations: 0%, 0.1%, 0.25%, 0.5%, 1%, 2.5%, 5%, 10% and 15% wt/wt., mixed with three different selected substrates: river sand (S), a commercial topsoil (T) (Huminsubstrat N.4, Klaismann Dailmann, Neuhaus Italy) and a 1:1 topsoil-river sand (M). The theses are described in Table 1. Homogenous lettuce seedlings with two leaves (*Lactuca sativa* L. cv. Gentile) were provided by Gobetti Piantine (Casaleone, VR, Italy) and transplanted to individual pots (2 L capacity) filled with the appropriate substrate and PPM%. Ten pots for each thesis, including the control with no PPM, were set up, obtaining 90 pots for each different substrate. A crop cycle length of 33 days from transplantation to harvest was adopted. Irrigation was carried out with 200 ml in each pot of groundwater every 2 days. 33 days after transplanting, all the 90 lettuce plants were collected for the foliar and root biomass determination. Plants were cut at the base and the fresh weight (FW) was determined by weighing the lettuce head immediately after cutting. The aboveground biomass was dried at 70 °C for 5 days. The difference in weight before and after drying was used to calculate the shoot dry weight of the sample (DW). Percentage of dry matter (DM%) and percentage of moisture content (MC%) were calculated according to Eq. (2) and Eq. (3):

Table 1
Preliminary greenhouse pot experiment; description of the theses and codes. S: river sand; T: topsoil; M: 1:1 topsoil-river sand.

PPM-2020	River Sand	Topsoil	Mix 1:1
0%	S1	T1	M1
0.1%	S2	T2	M2
0.25%	S3	T3	M3
0.5%	S4	T4	M4
1%	S5	T5	M5
2.5%	S6	T6	M6
5%	S7	T7	M7
10%	S8	T8	M8
15%	S9	T9	M9

$$DM\% = \frac{DW}{FW} \cdot 100 \quad (2)$$

$$MC\% = \text{FW} - \frac{DW}{FW} \cdot 100 \quad (3)$$

Moreover, measurements of root fresh weight (FW) and dry weight (DW) were also performed, as well as the corresponding dry matter and moisture content percentage (DM%; MC%).

2.7. Greenhouse and tunnel experiments on lettuce and eggplants

The experiments on lettuce and eggplants were carried out in June 2023 by selecting the best two theses from the preliminary test. The selected substrate was the 1:1 topsoil/river sand. The randomised block design was set up as above; for lettuce, 2 L pots (ten for each thesis) were filled with the selected substrate mixed with 2.5% and 5% of crushed PPM-2023 (L1 and L2, respectively). Control pots (L-CTR) were filled with only the selected substrate. Lettuce seedlings were transplanted and growth was monitored for 33 days; totally, 30 plants were set up. 200 ml of tap water every three days was used to water the plants. Destructive lettuce analysis was performed as described above in section 2.6. The agronomic experiment with eggplant (*Solanum melanogena* cv. Dalia) was performed in tunnels in a randomized complete block design organized in three lines on mulching soil with 150 cm distance among each other. Plants were equipped with natural horizontal wires with 50 cm distance within the lines. Theses were set up in 14 L pots (8 pots each thesis) and consisted of a control (E-CTR: 1:1 topsoil/sand) and the two theses, E1 and E2, consisting of 1:1 topsoil/sand with addition of crushed PPM-2023 at 2.5% and 5%, respectively. Seedlings eggplants (15 cm height; 4–5 leaves) were purchased from Gobetti Piantine (Verona, Italy) and transplanted on the pots. Plants were micro-irrigated (one drippers 2 L h^{-1} per pot) and received the same water volume (440 L·pot^{-1}). Fruits were collected during the growing season (once a week) and weighted to obtain the average production of the whole cycle. After 120 days (end cycle), the epigeal biomass of all 24 plants was collected to register the fresh foliar (FW).

2.8. Statistical analysis

Statistical analyses on chemical, microbiological and pot experiments were done by using R software (www.r-project.org); a one-way analysis of the variance was conducted with a significance threshold set at $p \leq 0.05$. The HSD Tukey test was employed to determine homogeneous groups.

3. Results and discussion

3.1. Physical and chemical analysis

Fig. 1 showed the temperature monitored along the fermentation process. Temperature increased rapidly from 15.1/18.7 °C to 62.3/88.8 °C after 9 days from the manure delivery, both in 2020 and 2023. Then, within the 40th day, the detected average temperature was 83.7 °C in 2020 and 93.2 °C in 2023. This difference was due to the different seasonal period where the process took place in 2020, during spring/summer and in 2023 during autumn/winter. The different external temperature at the beginning of the process (Fig. 1) may have affected the temperature increase within the heap. From the first turning (at 41st day) until the second turning (135th day), the temperature registered an average value of 78.6 ± 8.4 °C and 69.9 ± 5.5 °C in 2020 and 2023, respectively. After the second turning, the values were above 70 °C, both in 2020 and in 2023. Except for the first 8–9 days, temperatures always exceeded 50 °C for 8 months. Temperature is an essential parameter and it is strictly dependent from the microbial metabolic activities along the different degradation steps, as reported in the literature (Tiquia et al., 1996). This is a spontaneous process without heating control and the temperature increase was exclusively generated by microbial metabolism. The mesophilic phase, which refers to temperatures up to approximately 40 °C (Miller, 1996), occurred within the first 8–9 days. Then a thermophilic and hyperthermophilic phase from 50 up to 96 °C was observed for all the process, thus improving the decomposition rate of the organic matter (Mengqi et al., 2021). The thermophilic and hyperthermophilic microorganisms gradually overcame the mesophilic population and completed the decomposition of organic matter, thus reaching the safety standards. Despite the process could be stopped after the first turning operation (120 days), the company moved the pile in another building to continue the fermentation period until the 240th day. The pH (Table 2) significantly decreased in both years (FPM-2020: 7.91 ± 0.26; SPM-2020:7.12 ± 0.36; FPM-2023: 7.46 ± 0.25 SPM-2023: 6.57 ± 0.20) due to chemical transformation and biological activities (Eklind and Kirchmann, 2000). pH is strongly influenced by the raw initial material, as well as the ventilation and temperature conditions (Bernal et al., 2009; Maheshwari et al., 2014) and it is an important indicator of manure maturity as it influences the bioavailability of nutrients for plants (Kong et al., 2024). Usually it represents an indicative parameter for observing the whole process. Nevertheless, pH value between 6.7 and 8.5 is the optimal range to achieve the maximum degradation rate (Bernal et al., 2009). Physical variables such as moisture content (MC%) on FPM were 34.46 ± 2.95% and 30.01 ± 3.25% in 2020 and 2023, respectively, in agreement with Higgins et al. (2021). The initial MC% is an essential factor affecting the aerobic fermentation and, as reported by Dunlop et al. (2015), these value are suitable for microbial metabolic activity. SPM values were 25.10 ± 3.50% in 2020 and 20.13 ± 3.11% in 2023. This almost 10% significant reduction was found in the two different processes; this is obviously associated to the temperature increase during the 8 months-stock period, which causes water evaporation of the compost heap (Quiroga et al., 2010). The MC% further decreased significantly after the pelleting phase with values of 17.00 ± 3.15% and 13.80 ± 2.30%, due to subsequent water losses because of the high temperature during the pellet production. Total nitrogen percentage (TN%) showed a slight increase during 2020, from 2.70 ± 0.10% (FPM-2020) to 2.90 ± 0.10% (SPM-2020); then it significantly increased, after the pelleting phase (PPM-2020: 3.20 ± 0.1%) (Table 2). These values are in agreement with those reported by different authors in the fresh manure (Gao et al., 2010; Qiu et al., 2021). NH_4^+-N had an opposite behaviour and decreased significantly from 0.93 ± 0.06% (FPM-2020) to 0.43 ± 0.1% (SPM-2020); it remained stable after pellet production (PPM-2020: 0.50 ± 0.10%). Regarding organic nitrogen (NH_2-N), the analyses evidenced a significant increase from 1.77 ± 0.11% (FPM-2020) to 2.43 ± 0.12% (SPM-2020), to reach a value of 2.73 ± 0.15% in the PPM. In 2023, a similar trend was observed (Table 2); TN% increased significantly from 2.70 ± 0.10 to 3.10 ± 0.05 and remained stable after pelleting (3.0 ± 0.20%). A stable value of NH_4^+-N was maintained, from 1.06 ± 0.06 (FPM-2023) to 0.93 ± 0.05 (SPM-2023); it decreased significantly in PPM-2023 (0.43 ± 0.15%). Finally, NH_2-N significantly increased from 1.71 ± 0.10% (FPM-2023) to 2.19 ± 0.11% (SPM-2023) and reached a value of 2.60 ± 0.10% (PPM-2023). Overall, data showed the nitrogen loss via ammonia emission in both years, thus evidencing the critical role played by nitrogenous compounds during fermentation and humification. However, the increase of NH_2-N also demonstrated that nitrogen was partially recovered along the process as humic substances, thanks to microorganism's activity that are responsible for the nitrogen preservation (Mengqi et al., 2021). Microorganisms play a crucial role in the conversion of ammonia to nitric nitrogen, in particular under high temperature, thus allowing nitrogen incorporation into the humic matter and the consequent nitrogen storage in the soil organic matter (Qiu et al., 2021). The C/N ratio (Tale 2) was evaluated after the heap formation and was 7.77 ± 1.43 (FPM-2020) and 10.24 ± 1.04 (FPM-2023); a slight increase was then observed after the fermentation period reaching value of 9.60 ± 1.42 and 10.33 ± 1.15 in SPM-2020 and

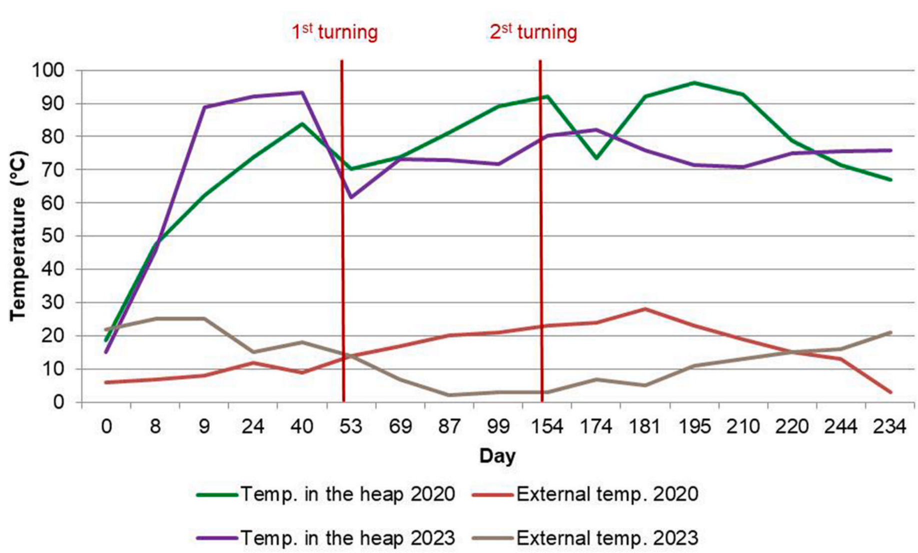

Fig. 1. Temperature monitoring along the fermentation process in 2020 and 2022–2023.

Table 2
Physical and chemical parameters analysed in 2020 and 2022–2023 in the chicken manure along the fermentation process. MC: moisture content; TN: total nitrogen; NH_4^+-N: ammonium nitrogen; NH_2-N: organic nitrogen; C/N: carbon/nitrogen ratio.

Parameter	Year 2020				Year 2023			
	FPM	SPM	PPM	*s*	FPM	SPM	PPM	*s*
pH	7.91 ± 0.26^a	7.12 ± 0.36^b	6.7 ± 0.26^c	*	7.46 ± 0.25^a	6.57 ± 0.20^b	6.56 ± 0.15 ^{b}	**
MC (%)	34.46 ± 2.95^a	25.10 ± 3.50^b	17.0 ± 3.15^c	***	30.01 ± 3.25^a	20.13 ± 3.11^b	13.80 ± 2.30^b	**
TN (%)	2.70 ± 0.10^a	2.90 ± 0.10^a	3.20 ± 0.10^b	**	2.70 ± 0.10^b	3.10 ± 0.05^a	3.00 ± 0.20^{ab}	**
NH_4^+-N (%)	0.93 ± 0.06^a	0.43 ± 0.12^b	0.50 ± 0.10^b	**	1.06 ± 0.06^a	0.93 ± 0.05^a	0.43 ± 0.10^b	***
NH_2-N (%)	1.77 ± 0.11^b	2.43 ± 0.12^a	2.73 ± 0.15^a	***	1.71 ± 0.10^b	2.19 ± 0.11^a	2.60 ± 0.10^a	***
C/N	7.77 ± 1.43^a	9.60 ± 1.42^a	10.66 ± 0.78^b	*	10.24 ± 1.04	10.33 ± 1.15	10.46 ± 0.54	*ns*

([a,b,c]) different letters indicate significant difference between the manure samples FPM (fresh poultry manure), SPM (stable poultry manure) and PPM (pelletized poultry manure). HSD Tukey's test; ns,*, ** and ***: effect not significant or significant at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$.

SPM-2023, respectively. It remained stable (10.66 ± 0.78 and 10.46 ± 0.54) after pelleting and after 30 days of batch storage. Usually C/N is the main indicator of compost quality and maturity; authors frequently reported a C/N ratio of 30–35:1 as more suitable for the initial stage of process (Michel et al., 1996), but this average value may be lower in poultry manure that is richer in nitrogen (Cáceres et al., 2018). The analysed heaps may also have undergone a pre-fermentation process, with a consequent reduction of the C/N before its storage into the facilities since manure derives from big/medium farms that release the litter at the end of the production cycle and collection may take one week. C/N remains a critical factor affecting the process; as reported by Gao et al. (2010), co-composting materials (sawdust, cattle/pig manure, spent mushrooms) are always taken into consideration in order to adjust the C/N ratio and optimize the degradation process. However, Cáceres et al. (2018) indicated that a manure with a lower C/N ratio, such as chicken or swine manure, is able to a greater nitrogen mineralization compared to manures with higher C/N ratios like cow manure.

3.2. Microbiological analysis and microorganism isolation

3.2.1. Real time PCR

Real time PCR on the manure before and after the fermentation process is described in Fig. 2. The results are expressed as average ± sd of three independent determinations associated to the three samplings (inner low, inner high and external). FPM-2020 and FPM-2023 exhibited a bacterial load of 10.17 ± 0.39 Log_{10} CFU/g and 11.18 ± 0.85 Log_{10} CFU/g, respectively. These values are in agreement with different authors (Fries et al., 2005; Omeira et al., 2006) who evaluated the microbiota of different poultry litters and the count ranked from 9 to 12 Log_{10} CFU/g. Counts decreased of about 2 log after the aerobic degradation process, with values of 8.90 ± 0.28 Log_{10} CFU/g and 9.08 ± 0.35 Log_{10} CFU/g. The resulting decrease is related to the increased temperature due to the aerobic metabolism that reached values above 70 °C for several months, thus leading to microbial selection, pathogens death and non-spore formers mesophilic bacteria. Finally, the enumeration in the finished product further decreased to 6.81 ± 0.10 Log_{10} CFU/g and 6.78 ± 0.21 Log_{10} CFU/g, respectively in PPM-2020 and PPM-2023. The intrinsic bacterial load recovered in the pellets was probably associated to the thermophilic and hyperthermophilic population (see section below) and heat-resistant species that survived along the 8 months process that were gradually selected by the increasing temperatures within the heap (Vithanage et al., 2016; Mengqi et al., 2021).

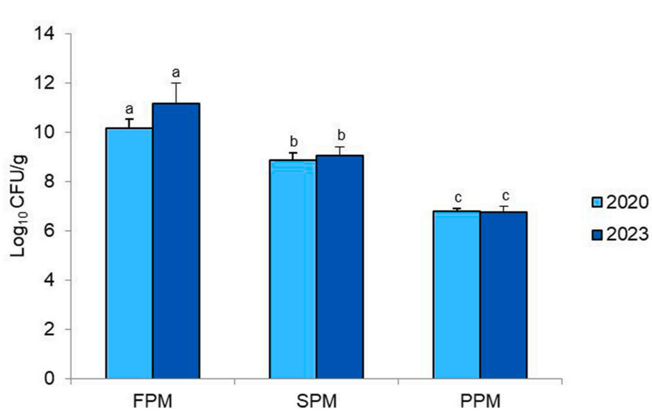

Fig. 2. Real Time PCR; quantitative measurements (Log10 CFU/g) of total eubacteria in 2020 and 2022–2023 in fresh poultry manure (FPM), stable poultry manure (SPM) and pelletized poultry manure (PPM).

3.2.2. Bacterial isolation and identification in fermented manure

The isolation procedure in PPM-2020 and PPM-2023 allowed the picking up of 25 and 20 bacterial colonies, respectively (Tables 3 and 4). This number is not representative of the whole microbial community of the matrix, but the objective of this work was to characterize the prevalent autochthonous strains surviving the pelleting process with potential plant growth promoting activities. The most representative isolated species, 39 strains out of 43, were ascribed to the genus *Bacillus and Oceanobacillus* able to survive the high temperatures during fermentation and pelleting thanks to the endospore formation (Cho and Chung, 2020). This high capability of surviving has also been reported in other studies; the work of Pagliarini et al. (2023a) evidenced the isolation of

Table 3
Best match identification phylotypes of purified PCR products deriving from the 16S rRNA amplification of the isolated strains in PPM-2020.

ID Strain (2020)	Closest match	% similarity	Accession Number
ST_5_1	*Bacillus aryabhattai* B8W22	100	ON754386
ST_5_8	*Bacillus subtilis* strain soilG2B	99.93	PP537171
ST_5_2	*Bacillus subtilis* JCM 1465	99	ON754387
ST_5_9	*Bacillus subtilis* JCM 1465	99.95	PP537172
ST_5_3	*Bacillus licheniformis* DSM 13	100	ON754388
ST_5_10	*Bacillus licheniformis* TB212	99.93	PP537173
ST_5_11	*Bacillus licheniformis* TB212	99.86	PP537174
ST_5_12	*Bacillus licheniformis* TB212	99.86	PP537175
ST_5_13	*Bacillus licheniformis* TB212	99.83	PP537176
ST_5_4	*Bacillus stratosphericus* 41KF2a	100	ON754389
ST_5_15	*Pseudomonas psychrotolerans* C36	99.36	PP537178
ST_5_5	*Pseudomonas psychrotolerans* C36	99.36	ON754390
ST_5_6	*Microbacterium oleivorans* BAS69	99.93	ON754391
ST_5_14	*Bacillus piscis* 16MFT21	100	PP537177
ST_5_7	*Bacillus haynesii* NRRL B-41327	99.96	ON754392
ST_7_1	*Bacillus methylotrophicus* IHB B 7249	99.85	ON755161
ST_7_2	*Bacillus licheniformis* TT2	100	ON755162
ST_7_8	*Bacillus licheniformis* PB3	99.72	PP537180
ST_7_3	*Bacillus subtilis* soilG2	100	ON755163
ST_7_4	*Pantoea* sp. CWB600	99.71	ON755164
ST_7_7	*Bacillus subtilis* ML215	99.79	PP537179
ST_7_5	*Bacillus paralicheniformis* FA6	99.98	ON755165
ST_7_6	*Bacillus glycinifermentans* KBN06P03352	99.93	ON755166

Table 4
Best match identification phylotypes of purified PCR products deriving from the 16S rRNA amplification of the isolated strains in PPM-2023.

ID Strain (2023)	Closest match	% similarity	Accession Number
ST5_23_1	*Bacillus stratosphericus* 41KF2a	99.93	OQ933393
ST5_23_2	*Bacillus licheniformis* ATCC 14580	99.93	OQ933394
ST5_23_3	*Oceanobacillus caeni* S-11	99.85	OQ933395
ST5_23_5	*Oceanobacillus caeni* S-11	99.64	OQ933397
ST7_23_2	*Oceanobacillus caeni* S-11	99.64	OQ933384
ST7_23_9	*Oceanobacillus caeni* S-11	99.64	OQ933391
ST7_23_10	*Oceanobacillus caeni* S-11	99.64	OQ933392
ST5_23_4	*Bacillus toyonensis* BCT-7112	99.78	OQ933396
ST5_23_6	*Bacillus proteolyticus* MCCC 1A00365	99.85	OQ933398
ST5_23_8	*Bacillus proteolyticus* MCCC 1A00365	99.85	OQ933400
ST7_23_1	*Bacillus proteolyticus* MCCC 1A00365	99.95	OQ933383
ST5_23_9	*Bacillus licheniformis* BCRC 11702	99.93	OQ933401
ST7_23_8	*Bacillus licheniformis* BCRC 11702	99.95	OQ933390
ST5_23_10	*Bacillus licheniformis* DSM 13	99.50	OQ933402
ST7_23_7	*Bacillus licheniformis* DSM 13	99.85	OQ933389
ST7_23_3	*Bacillus niameyensis* SIT3	99.83	OQ933385
ST7_23_4	*Bacillus velezensis* CBMB205	99.92	OQ933386
ST7_23_5	*Bacillus pervagus* 8-4-E12	99.48	OQ933387
ST7_23_6	*Bacillus amyloliquefaciens* NBRC 15535	99.93	OQ933388

several *Bacillus* species from coffee waste after the high temperature roasting process of coffee beans. The two isolation sources slightly differed in terms of recovered species (e.g. *Oceanobacillus* derived only from from PPM-2023), probably because of the different manure origin. It is widely known that rhizospheric *Bacillus* strains play important functions in plant nutrition, interacting with plant root and physiology (Pagliarini et al., 2023b). In particular, we isolated 15 species whose PGP activities have been described in different crops by several authors. The best well-known species, such as *B. subtilis, B. amyloliquefaciens, B. licheniformis and B. paralicheniformis* are known to produce phytohormones, antimicrobials, siderophores, to solubilize phosphorous and other minerals and, through enzyme activities, they are actively involved in the degradation of the organic matter. *Bacillus velezensis* strains have also been isolated; Chebotar et al. (2022) reported increasing yield and quality of two strawberry varieties by including a strain of B. *velezensis* alone and in combination with nitrogen fertilizers in field experiments. Wang et al. (2023) studied the succession of microbial communities in a 60 days fermentation experiment, by mixing different proportions of chicken and pig manure; results evidenced that *Firmicutes* was the most represented phylum. Among the less-studied species (*B. aryabhattai*, *B. methylotrophicus*, *B. glycinefermentas etc.*) some evidences of plant growth promoting activity have already been reported (Madhaiyan et al., 2010; Ramesh et al., 2014; Yadav et al., 2022; Mun et al., 2024). In addition, a few isolates of *M. oleivorans, P. psychrotolerans and Pantoea* sp were also identified, although these species are not spore formers and probably they survived as well to the high temperatures. Some reported studies further evidenced the important role of such species as PGP bacteria (Hahm et al., 2017; Kubi et al., 2021; Patel et al., 2021). It could be, therefore, hypothized that these autochthonous PGPR, together with the stable manure mineral content, can increase the quality of the stable poultry manure enhancing, when administrated to soil, the soil fertility and the plant growth stimulation. As reported by Yildirim et al. (2011) the inoculation of PGPR, in addition to manure, led to a significant increase of mineral content and yield in broccoli plants. PGPR represent, therefore, a sort of "biological bridge" between organic matrix nutrients and the root adsorption activity. Thanks to biochemical activities such as N_2 fixation, solubilisation of inorganic phosphate and mineralization of organic phosphate and/or other nutrients, rhizospheric PGPR would create a positive association with the root surface, thus increasing nutrient adsorption and, as consequence, productivity. This is one of the possible mechanism; however, it is extremely challenging due to the wide complexity of soil-bacteria-roots interaction. In future studies, once the PGP activities of the isolated microorganisms will be confirmed by *in vitro* and dedicated pot studies, it will be possible to enrich the pelletized manure with a known concentration of the best microbial strains. A rich body of literature suggests that the enrichment of animal manure with PGPR microorganisms stimulate root and plant development and mineral solubilisation, once added to manure as organic fertilizer in crop management (Billah and Bano, 2015).

3.3. *Greenhouse: preliminary experiments*

This preliminary test on lettuce plants was aimed at evaluating the effects of the three different substrates (river sand, topsoil and the 1:1 mixture) in combination with increasing percentages of PPM (0%, 0.1%, 0.25%, 0.5%, 1%, 2.5%, 5%, 10% and 15% wt/wt) (Table 1). This step was necessary to select the most suitable substrate, in combination with at least two PPM concentrations for a further test implementation. Lettuce (*Lactuca sativa* L.) is one of the most cultivated vegetables in the Mediterranean countries, including Italy (Still, 2007). This vegetable has a short growing cycle and is well suited for fertilization tests and short-term phenotypic results (Foteinis and Chatzisymeon, 2016). Harvest was carried out 33 days after the transplant to determine foliar and root yield. In particular, the following parameters were considered: fresh and dry leaf and root biomass (FW and DW), percentage of relative humidity (MC %) and total dry matter (DM %) (Figs. 3 and 4). The control theses (S1, T1, and M1) evidenced the effect of the different substrates without manure addition, in particularly for what concern the foliar biomass. The FW registered the following average values: 6.67 ± 0.99 g·plant^{-1} (S1), 76.36 ± 5.80 g·plant^{-1} (T1), and 56.14 ± 4.73 g·plant^{-1} (M1), respectively (Fig. 3a). The results evidenced the topsoil and the mixture topsoil/river sand as the best substrates for biomass development, whereas the river sand alone did not sustain foliar growth. Sandy soil has two main problems for crop development: the nutrient deficiency and water repellency. However, a test was mandatory in order to evaluate a potential successful interaction with the stable manure. The fresh weight was significantly higher in S5 (16.69 ± 3.90 g·plant^{-1}), S6 (39.77 ± 5.47 g·plant^{-1}), S7 (57.05 ± 12.06 g·plant^{-1}) and S8 (20.45 ± 8.23 g·plant^{-1}), compared to S1. The DW in foliar biomass (Fig. 3b) showed significantly higher values in S5 (1.46 ± 0.31 g·plant^{-1}), S6 (2.54 ± 0.31 g·plant^{-1}) and S7 (3.33 ± 0.64 g·plant^{-1}) compared to S1 (1.01 ± 0.25 g·plant^{-1}). MC% progressively increased with the higher manure concentrations (Fig. 3c), although not significantly, while a significant decrease was observed for DM% (Fig. 3d), from S3 to S8. S9 did not reach the end of the cycle; all the lettuce plants died. Probably, the high nutrient content displayed a phytotoxic effect on the growth. The topsoil control thesis (T1) showed an average foliar biomass of 76.36 ± 5.80 g·plant^{-1}, a reasonable value for a biomass index of a lettuce plant grown in greenhouse (Fig. 3a). T9 did not complete the growth cycle, since all the plants died. From T2 to T4, a constant, but not significant, increase was observed. T5 showed a slight FW decrease, although not significant (83.05 ± 17.73 g·plant^{-1}). T6 (2.5% PPM-2020) was the best foliar FW, significantly higher compared to T1, with an average value of 99.55 ± 17.57 g·plant^{-1}. T8 (10% PPM-2020) was tenfold lower than all thesis, thus confirming that the excessive nutrient concentration in manure may result detrimental for the growth (4 out of 10 plants died before the end of the cycle). The DW showed the highest value in the control thesis (T1), with a decrease as the manure percentage increased (Fig. 3b). Indeed, manure application led to an increasing of water retention into the soil and probably a higher water adsorption, although values are significant only in T5, T6 and T7. MC% (Fig. 3c) did not evidence significant differences (except for T8);

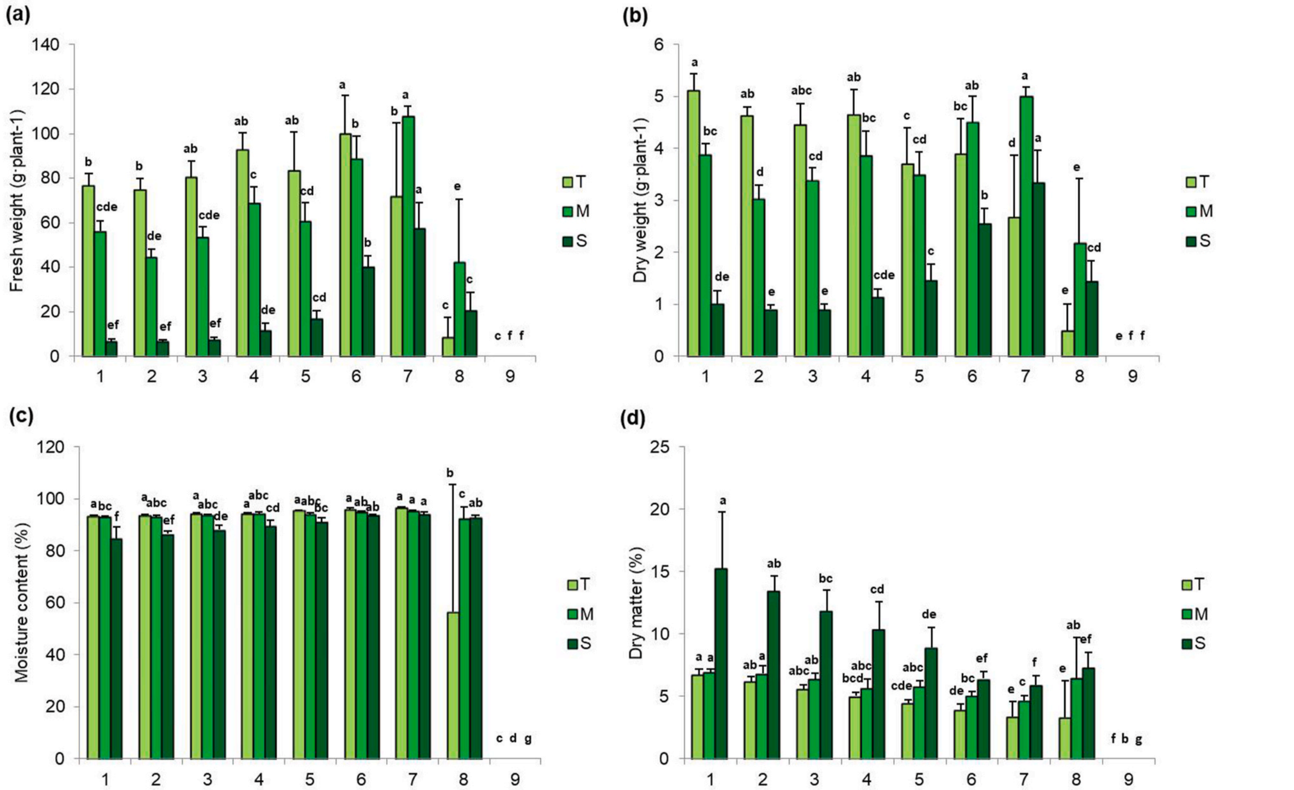

Fig. 3. Mean values (±SD) of fresh weight (a), dry weight (b), moisture content % (c) and dry matter % of foliar lettuce plants with increasing concentration of pelletized poultry manure (PPM) and three substrates (T: topsoil; M: 1:1 topsoil-river sand; S: river sand). PPM concentrations: 1: 0%, 2: 0.1%; 3: 0.25%; 4: 0.5%; 5: 1%; 6: 2.5%; 7: 5%; 8: 10%; 9: 15%. Different letters within each column indicate significant differences according to Tukey post-hoc test ($p \leq 0.05$).

DW% (Fig. 3d) decreased significantly from T4 to T8 compared to T1. The whole experiment showed that the combination topsoil/manure could lead to a relevant increase of productivity; however, T5, T6 and T7 showed a high standard deviation as a consequence of the spreading of the data weight over a wider range of values, thus leading to high variability and uncertainty. The combination of topsoil and sand evidenced the most interesting results (Fig. 3). The control thesis M1, without stable manure, had a foliar biomass average of 56.14 ± 4.73 g·plant^{-1} (Fig. 3a). Again, M9 did not reach the end cycle and all the plants died. The foliar FW progressively increased as in the topsoil thesis: 44.40 ± 3.82 g·plant^{-1} (M2), 53.23 ± 4.76 g·plant^{-1} (M3), and 68.78 ± 7.26 g·plant^{-1} (M4). The increasing FW is, therefore, associated to the increasing of manure percentage and to nutrient availability, although values were not significant compared to M1. Contrarily, M5, as T5, showed a decrease in foliar FW. Both thesis with the two substrate (T and M) and with the same manure concentration (1%) were subjected to a growth stop. M6 and M7 displayed the highest value (88.62 ± 10.40 g·plant^{-1} and 107.39 ± 4.91 g·plant^{-1}) with a significant FW increase compared to M1 (Fig. 3a); FW values of M8 thesis (42.02 ± 28.60 g·plant^{-1}) confirmed the phytotoxic effect of 10% manure concentration, as already observed in T8. Concerning the DW, the interesting output is the significant DW increase in M6 and M7 (4.49 ± 0.51 g·plant^{-1}, 4.99 ± 0.19 g·plant^{-1}) compared to M1 (3.87 ± 0.22 g·plant^{-1}) (Fig. 3b). These values evidenced that the right association of substrate and manure concentration could improve both foliar biomass, nutrient and water adsorption. These data differed from the thesis with only topsoil where the DW progressively decreased and the MC% significantly increased with the amount of PPM addition. This is in agreement with the observation that the addition of stable manure led to an improvement of soil structure due to the formation of stable aggregates, thus increasing soil porosity; consequently, the soil hydraulic conductivity and the water adsorption increase. Soil with the right proportion of organic matter had good physical properties and the ability to absorb water up to several times its dry weight, and had a high porosity (Yunanto et al., 2022). Concerning root analysis (Fig. 4), a significant FW increase was observed in S4 (12.16 ± 2.51 g·plant^{-1}), S5 (12.68 ± 2.32 g·plant^{-1}), S6 (15.47 ± 2.66 g·plant^{-1}) and S7 (11.79 ± 4.70 g·plant^{-1}) compared to S1 (6.08 ± 0.56 g·plant^{-1}) (Fig. 4a). No differences were observed in the root DW (Fig. 4b). Overall, the theses S were discarded, since the foliar biomass after 33 days did not reach a reasonable commercial productivity amount and the analysed parameters were not agronomically attractive. The root FW in the topsoil assay did not show relevant information; most of the thesis showed a significant decrease, except for T2 (20.52 ± 3.01 g·plant^{-1}) and T3 (17.49 ± 3.19 g·plant^{-1}) that were significantly higher compared to T1 (13.37 ± 1.46 g·plant^{-1}). The same trend was observed in the root DW (Fig. 4b). At root level, the thesis with the mixture topsoil/river sand (except M8 and M9) did not show significant differences compared to M1. In general, root weight was stable in all thesis without relevant differences; literature on manure application in lettuce is scarce, although some positive effects have been shown (Vella and Sacco, 2022).

3.4. Greenhouse: experiment on lettuce and eggplant

The mixture topsoil/river sand was selected for the second greenhouse assay with lettuce and eggplants with the addition of 2.5% and 5% PPM, since these concentrations significantly increased the lettuce FW in the previous assay. Results on above biomass of lettuce are shown in Fig. 5a–d; the FW of L-CTR (54.36 ± 5.98 g·plant^{-1}) was significantly lower compared to L1 (75.57 ± 5.05 g·plant^{-1}) and L2 (75.75 ± 6.59 g·plant^{-1}), thus confirming that the addition of 2.5% and 5% stable manure influenced the biomass growth by increasing the FW of above 27%. This agrees with the preliminary assay; the control thesis and the thesis with 2.5% manure addition displayed the same foliar fresh weight

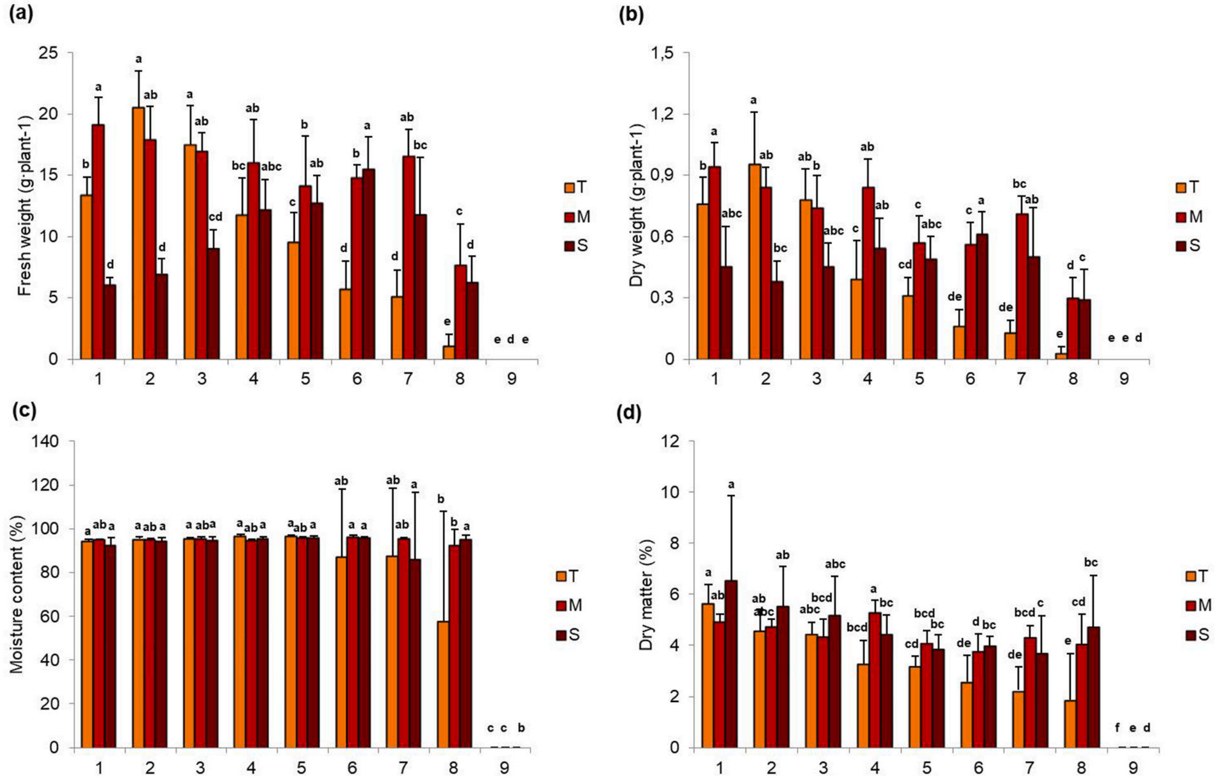

Fig. 4. Mean values (±SD) of fresh weight (a), dry weight (b), moisture content % (c) and dry matter % of root lettuce plants with increasing concentration of pelletized poultry manure (PPM) and three substrates (T: topsoil; M: 1:1 topsoil-river sand; S: river sand). PPM concentrations: 1: 0%, 2: 0.1%; 3: 0.25%; 4: 0.5%; 5: 1%; 6: 2.5%; 7: 5%; 8: 10%; 9: 15%. Different letters within each column indicate significant differences according to Tukey post-hoc test ($p \leq 0.05$).

observed in the preliminary test; the thesis with 5% of manure addition is slightly different, probably because the greenhouse tests have been performed in a different seasonal period and environmental temperature could have influenced the final outputs. Overall, all studies investigating the addition of different kinds of animal manure led to an increase of foliar biomass due to higher organic matter content and nutrients (Castro et al., 2009; Pizarro et al., 2019). The DW did not differ among theses (Fig. 5b), whereas the MC% of L-CTR (91.99 ± 0.55%) was significantly lower compared to L1 and L2 (93.72 ± 0.51% and 93.58 ± 0.43 %) (Fig. 5c); as in the preliminary assay the higher MC% could be related to a better soil structure and ability to retain water because of manure addition. The DM% had an opposite behaviour (Fig. 5d), as in the preliminary experiments with higher significant values in the control thesis compared to L1 and L2. Differently, the root biomass did not evidence any significant difference among the theses, neither FW and DW, nor MC and DW (data not shown). This result agreed with Chowdhury et al. (2019), who found that the organic amendment did not have any effect on root increase and development. On the contrary, the addition of stable organic matter and rich-available nutrients had a positive consequence in root weight and development in a study performed on lettuce by Solaiman et al. (2019). However, little information is available; plant root growth is frequently associated to soil physical properties such as soil porosity, aggregation and density (Zhao et al., 2019), and to the rhizospheric microbiota that also contribute to root development through phyto-hormones synthesis. Substantially, no differences have been observed in the present study; this result confirmed the behaviour observed in the preliminary experiment. The 2.5% and 5% PPM addition to the mix topsoil/river sand have also been tested on eggplants, which has a crop cycle of about four months. Eggplant (*Solanum melanogena* L.) is a very common crop in the Mediterranean area as well, consumed all over the world and China is the biggest producer (Luthria and Mukhopadhyay, 2006). All plants completed the production cycle and had vigorous vegetative growth, particularly the treated plants. Results confirmed that both concentrations improved the FW and the average production, (Fig. 6a). It was likely a dose-dependent effect; FW was significantly higher in both E1 and E2 (286.25 ± 70.09 g·plant^{-1}; 400.38 ± 59.43 g·plant^{-1}) compared to E-CTR (120.13 ± 23.22 g·plant^{-1}). For what concern the average production (Fig. 6b), the observed results evidenced a dose-dependent effect of manure addition but only E2 average production was significant compared to E-CTR (2409 ± 1182.27 g vs 811.62 ± 341.56 g), whereas the increase in E1 was not significant (1293.62 ± 426.64 g). No dedicated literature is available regarding poultry manure and eggplant; but, similarly, Demir et al. (2010) showed the successful addition of increasing concentrations of poultry manure (from 1% to 4%) in tomatoes. Sahin et al. (2014) reported that pepper plants were significantly affected in fruit production by 2% upon poultry manure addition. The obtained results confirmed the importance to obtain organic fertilizer from animal waste after a deep characterization of the whole fermentation process. This supports the European strategy that moves towards the reduction of chemical fertilizers that entail a progressive soil impoverishment and a considerable energy/environment costs.

3.5. Scalability and future implications

AFRODITE® complies with the European legislation (CE) n° 1069/2009 and with the Italian Dlgs n° 75/2010 for the safe use of fertilizers of animal origin. Beside chemical analysis, the manure is periodically subjected to pathogen detection that assure quality standards for the distribution and commercialization. The fertilizer sector is fluctuating because of the trend of raw material prices. This is a promising sector for the development of cost effective and low environmental-impact products, made by recovering matrices deriving from different animal wastes. The obtained patent will guarantee FOMET S.p.A. to stand out in the fertilizer market, where the increasing energy costs, get more and more difficult the recovering and transforming of certain types of

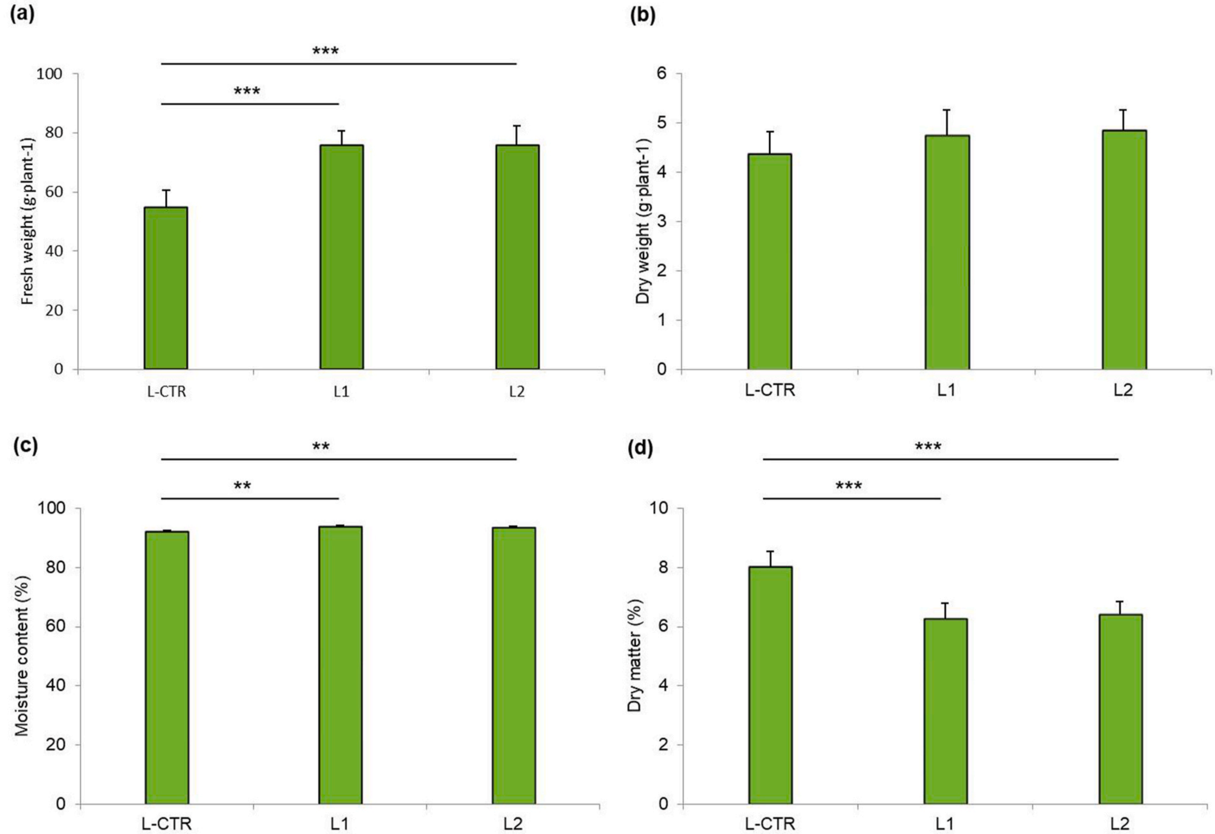

Fig. 5. Mean values (±SD) of fresh weight (a), dry weight (b), moisture content % (c) and dry matter % of foliar lettuce plants. L-CTR: control plants; L1: 2.5% PPM; L2: 5% PPM. ** and ***: significant at $p \leq 0.01$ or $p \leq 0.001$.

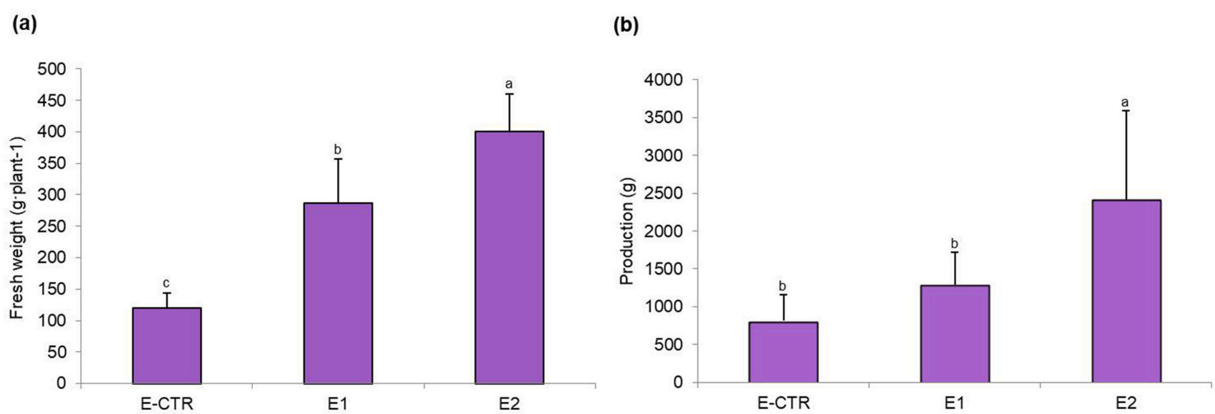

Fig. 6. Mean values (±SD) of foliar fresh weight (a), fruits productivity (b) of eggplants. E-CTR: control plants; E1: 2.5% PPM; E2: 5% PPM. Different letters within each column indicate significant differences according to Tukey post-hoc test ($p \leq 0.05$).

organic raw materials. FOMET S.p.A. supplies an organic fertilizer whose process transformation is well characterized, starting from the raw materials. The application of such amendments is suitable for all soil preparation; it can be used in conventional, integrated and organic farming. In particular, it is excellent for background fertilization, pre-sowing and pre-transplanting in the horticulture, cereal, and flower sector. It is estimated that will cover approximately 20% of the demand since the lack of organic substance in the soil and the reduction in soil fertility are issues that transversally involve farmers at both national and European level. The demand for Made in Italy products with low environmental impact, rich in organic substance and beneficial bacteria, is also increasing from non-EU customers.

4. Conclusions

AFRODITE® is a spontaneous fermentation process bringing to a chemical and microbiological stable manure of poultry origin. The right association of substrate (topsoil and river sand) with 2.5% and 5% PPM positively affected lettuce and eggplant productivity, increasing the amount of nutrient availability. Lower and higher PPM concentrations did not show the same effect. Moreover, PPM evidenced the presence of putative PGPR, mainly belonging to the *Bacillus* genus, which may play additional activities for soil fertility and plant nutrition, once released into the rhizosphere. In the next future, PPM fertilization potential value will be tested on further crops, with different characteristics and growth cycles, even at field level. PGP features (mineral solubilisation, phytohormones synthesis, siderophores production etc.) of isolated microorganisms will be evaluated *in vitro* assays in order to select target strains for enrichment strategy of the commercial product.

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CRediT authorship contribution statement

Elia Pagliarini: Investigation, Formal analysis, Data curation. **Francesca Gaggìa:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Michele Rossin:** Writing – review & editing, Supervision, Methodology. **Clizia Franceschi:** Writing – review & editing, Supervision, Methodology. **Diana Di Gioia:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Elia Pagliarini reports a relationship with FOMET S.p.A that includes: funding grants. Elia Pagliarini has patent #WO2024/003703A1 issued to assignee. Michele Rossin has patent #WO2024/003703A1 issued to assignee. Clizia Franceschi has patent #WO2024/003703A1 issued to assignee. Dr. Elia Pagliarini was the recipient of a grant to achieve his Master Thesis in Agricultural Science. If there are other authors, they 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.

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