1	Manuscript for submission to the
2	Bioresource Technology Report
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6	A Vermifiltration System for Low Methane Emissions and High
7	Nutrient Removal at a California Dairy
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ABSTRACT

23	Liquid storage of manure is a leading cause of methane emissions from the dairy sector and an
24	important source of air and water pollution. This study monitored the effect of vermifiltration on
25	methane emissions and water quality at a California dairy that uses an anaerobic lagoon. Methane
26	fluxes and wastewater removal rate of volatile solids, N species, salinity, major ions, and trace
27	elements were monitored for 12 months. Vermifiltration reduced methane emissions relative to an
28	anaerobic lagoon by 97-99% and removed 87% of the volatile solids, contaminants such as salts
29	and trace elements, P (83%) and N (84%) from the wastewater. Vermifiltration of dairy
30	wastewater demonstrated to be a useful tool to mitigate methane emissions, regulate excess
31	nutrients and improve water quality at dairy farms.
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34	Keywords: GHG; nutrients; liquid manure; anaerobic lagoon; nitrogen; wastewater treatment.
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39	1. Introduction

40	The livestock sector is responsible for about 14.5% of total anthropogenic greenhouse gas (GHG)
41	emissions worldwide (Gerber et al., 2013), and manure is a significant source of both agricultural
42	CH_4 and N_2O emissions (Chadwick et al., 2011). Between 1990 and 2022 in the United States
43	(US), CH4 emissions from cattle manure increased 122%, reflecting the increased use of
44	emission-intensive liquid systems over this time period (USEPA 2022). Nearly 98% of CH_4
45	emissions caused by management of manure occur during storage (Aguirre-Villegas and Larson,
46	2017; Grossi et al., 2019), an essential practice that enables farmers flexibility in the timing of
47	land applications to optimize crop production and protect environmental quality. Anaerobic
48	lagoons are the primary source of storage GHG emissions (Kaffka et al., 2016), as they provide
49	anaerobic conditions ideal for CH_4 -producing microorganisms and are also a source of N_2O and
50	NH_3 emissions. The NH_3 eventually redeposits or transforms to $\mathrm{N}_2\mathrm{O}$ or particulate matter,
51	contributing to both eutrophication and climate change (Hristov et al., 2002). The management of
52	dairy manure has a high potential for GHG emissions mitigation, making it an essential target for
53	reducing anthropogenic global warming from agriculture (Grossi et al., 2019).
54	Since the 1950s, US dairies have experienced intensification and agglomeration (Vanotti et al.,
55	2019). This has resulted in increased problems associated with the utilization and disposal of
56	animal waste, as in many areas the concentration of manure nutrients exceeds the capacity of the
57	land to receive them (Burkholder et al., 2007). The livestock sector is one of the top contributors
58	to the most serious environmental problems, including water-quality degradation, globally (FAO,
59	2006). Because of these high environmental risks, the use of livestock wastewater stored in
60	anaerobic lagoons is often subject to regulations, and off-farm manure export requirements are
61	increasing (Vanotti et al., 2019).

Manure nutrients can be recovered and used for crop production using solid-liquid separation,
where manure nutrients are removed and/or treated with a variety of technologies to generate
value-added products (Gollehon et al., 2016; Vanotti et al., 2019). These technologies vary in
operational costs, use of additives, complexity, energy input, and production of sludge requiring
disposal.

67 As animal production has intensified, offensive odors are increasingly a concern (Stowell et al., 2015). Also, livestock water use can represent a large proportion of total agricultural water use in 68 69 areas with intensive dairy farming (Le Riche et al., 2017). The reuse of dairy wastewater provides 70 a potential means for farmers to reduce the demand for high-quality water (Pimentel et al., 2004). Vermifiltration offers the opportunity to reduce the dairy GHG emissions (both N_2O and CH_4), 71 remove organics and excess nutrients from wastewater, increase flexibility in water use, avoid 72 odors, and recover the manure nutrients in the treated wastewater and vermicompost. A 73 vermifilter serves simultaneously as a solid-liquid separator, a treatment system for wastewater 74 75 and separated solids, and a nutrient recovery technology. The practice consists of spreading wastewater over a filtering system containing earthworms (Arora and Saraswat, 2021). The 76 method uses the joint action of earthworms and microorganisms to aerobically treat the 77 78 wastewater. Although microorganisms biochemically degrade the organic waste, the earthworms aerate and fragment the substrate and modify its physical and chemical characteristics, promoting 79 80 microbial activity and decomposition (Manyuchi and Phiri, 2013). 81 Vermifiltration can be used to treat wastewater containing high organic matter from variable sources, including livestock liquid manures (Samal et al., 2018; Singh et al., 2021). The 82

performance of a vermifiltration system is affected by the earthworm loads (Wang et al., 2015),

hydraulic loading rates (Singh et al., 2019), filter materials used (Adugna et al., 2019), and

conditions affecting the survival of the earthworms, such as toxicity, humidity, temperature, and 85 pH (Sinha et al., 2010). Other characteristics reported in the literature are low technology and 86 87 power requirements to operate (Sinha et al., 2010), lack of odor during treatment (Arora and Saraswat, 2021), the ability to remove solids, excess nutrients, and contaminants, including 88 pathogens, from wastewater (Arora and Saraswat, 2021), and allowing on-farm recycling of waste 89 90 and water. The technique doesn't produce sludge (Yand et al., 2008) but vermicompost, which has beneficial effects on soils and crops. It is a source of plant macro-and micronutrients (Hussain 91 92 and Abassi, 2018), increases soil microbial biomass and diversity (Saha et al., 2022), enhances 93 soil health (Lazcano and Domínguez, 2011; Hussain and Abbasi, 2018), and has the potential to sequester carbon. 94

Industrial-scale dairy vermifiltration systems in the US range in size from 45 m² to 29,000 m² and
treat wastewater from up to 6,000 dairy cows and 2,840,000 L of wastewater per day (BioFiltro
personal communication).

Very little is known about GHG emissions from vermifiltration systems. Only a very limited 98 number of studies are available (Luth et al., 2011; Lai et al., 2018). Quantification of the annual 99 CH₄ emissions from vermifilters is needed to help establish the technique as a recognized tool to 100 101 mitigate agricultural GHG emissions and can spur the process by allowing dairy farmers to participate in the carbon market. In addition, most vermifiltration studies have focused on the 102 efficiency of removing organics and nutrients from wastewater and have consisted of small-scale 103 104 laboratory experiments and short-term observations (Singh et al., 2019; Wang et al., 2015). This study monitored a commercially available vermifiltration system (BIDA system, BioFiltro) for 105 one year, operating on a typical Central Valley California dairy farm with an anaerobic lagoon. 106 The study focused on quantifying the CH_4 emissions of a vermifiltration system treating dairy 107

108	wastewater. The study also aimed to address vermifiltration effects on the wastewater nutrient
109	contents. Further research is needed to assess the vermifiltration GHG life cycle, including GHGs
110	emitted for building and operating the vermifilter and the potential GHG sequestration from land
111	application of vermicompost.
112	Quantification of CH4 emissions from manure management for national and regional GHG
113	inventories as well as carbon market methodologies are based on IPCC equations which include a
114	treatment-specific parameter denoted as methane conversion factor (MCF; IPCC, 2006). The
115	factor allows estimation of CH4 emissions from the different manure management systems
116	without monitoring CH ₄ fluxes annually.
117	The study objectives were to 1) quantify CH ₄ emissions of a dairy vermifilter and compare
118	vermifilter and anaerobic lagoon CH ₄ emissions; 2) determine the methane conversion factor; and
119	3) assess the effects of vermifiltration on dairy wastewater constituents such as organic solids,
120	nutrients, trace elements, and EC.
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123	2. Materials and Methods
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124	The study was conducted on a commercial dairy (Fanelli Dairy) located in Hilmar, in the
125	California Central Valley, that housed 800 milking cows and 700 replacements. The farm had
126	1500 animals, a typical herd size for the Central Valley of California, which hosts 90% of the
127	dairy cows in the State (CDRF, 2020). Manure was flushed from the barn floors and stored in an

anaerobic lagoon. The vermifilter was built in 2015 for a pilot project studying vermifiltration

effects on dairy N dynamics and GHG emissions. The pilot vermifilter was approximately 10% of
an estimated full-size plant for the farm and treated circa 2,500 tons of manure and 15,000-45,000
L of dairy wastewater per day. The hydrologic rate of raw influent was regulated by recirculating
wastewater in order to maintain total suspended solids concentration below 10,000 mg L⁻¹.

Milking cows were housed in free-stall barns and replacements in open lots. The free-stall barns and the feeding areas of the open lots were flushed three times daily for 10 minutes using recycled wastewater from an anaerobic lagoon built with a holding capacity of ~5.7 million L and a surface area of 10,800 m². Flushing water from the barns flowed through a vibrating screen primary separator and then to the anaerobic lagoon. The separated solids were air-dried and used for bedding. Water from the lagoon was used for crop irrigation or recycled for flushing (as described in Lai et al., 2018).

The vertical flow vermifiltration system treated wastewater collected after the first separator 140 141 (Figure 1). The vermifiltration system included a second rotary separator that removed manure fibers with diameters larger than 0.8 mm to prevent clogging of the sprinkler system used to apply 142 the wastewater on the vermifilter bed. The resulting influent water was then directed into a 143 holding tank. Every 30 minutes, influent was applied for 2 minutes to the vermifilter surface by 144 the sprinkler system. The applied influent percolated through the vermifilter to the underlying 145 drainage space and drained under gravity in about 4 hours. Treated water was then used for 146 flushing. 147

The analysis assumed flushing collected 100% of the VS excreted by milking cows housed in free-stalls, where the cows spent all of their time, and 30% of the replacement excreted VS in open lots, where manure was collected exclusively from regularly flushed feeding areas. The

screen separator VS removal was 17% (CARB, 2019; Pain, 1978). The daily production rates of 7.6 kg VS per milking cow and 3.4 kg VS per replacement and the maximum methane producing capacity for the specific type of animal manure (B_0) of 0.24 m³ CH₄ kg VS⁻¹ are values currently used in the GHG US inventory for California (USEPA, 2022). A cow population of 895 animals, obtained by weighting the VS contribution of milking and replacement cows, was used when determining emissions rates (or other metrics) per animal.

157 The vermifilter consisted of a concrete rectangular enclosure $(49 \times 11 \times 1.5 \text{ m})$ inhabited by

158 worms (*Eisenia fetida*) within the top 30 cm of the 0.5 m layer of woodchips. A 30-cm deep space

at the bottom of the vermifilter bed collected drainage and provided aeration through 20

160 peripheral PVC exhaust pipes (15 cm diameter) that allowed air exchange (passively) with

ambient air (Fig 1A, B). Monthly tilling of the vermifilter surface layer increased aeration in the

162 woodchips and avoided ponding of water. The handheld tiller required less than three hours and

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163 was pulled by a winch powered by a car battery.
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164 Figure 1. Overview of the vermifiltration wastewater management system at the Fanelli diary. A) Schematic 165 diagram of the vermifilter. B) The vermifilter bed with vents, irrigation lines, and CH₄ fluxes measurement 166 collars C) The manure treatment process at the Fanelli Dairy. Water flushed from the free-stall barn was 167 stored in the anaerobic lagoon (An Lagoon). The lagoon water was recycled as flush water or to irrigate 168 crops. The wastewater (INF) passed through a secondary separator to remove sand and large manure fiber before it was applied over the top of the vermifilter. The effluent wastewater (EFF) was recycled as flush 169 170 water. The yellow symbols show sampling locations for water quality and the orange cylinders for flux measurements. The shaded boxes follow the pathway of the volatile solids (VS) and nitrogen (N) produced 171 172 by one typical California cow over one year, assuming all water was used for crop irrigation.

174 **2.1 Methane emissions**

175 CH₄ emissions were measured from the vermifilter and the anaerobic lagoon at the Fanelli Dairy
176 using the chamber technique and a dynamic closed measurement system (Pavelka et al., 2018).
177 Fluxes from 16 locations on the vermifilter and 12 locations on the lagoon were measured
178 monthly from December 2019 to November 2020.

179

180 2.1.1 Gas Flux Measurement System

The portable Trace Gas Analyzer used Optical Feedback-Cavity Enhanced Absorption 181 182 Spectroscopy (Li-7810, LI-COR) to measure CH₄ and CO₂ concentrations once per second in the volume enclosed by a chamber positioned on the media surface. The instrument has a 183 measurement range 0 to 100 ppm and precision of 0.60 ppb at 2 ppm with 1 second averaging. A 184 5 L min⁻¹ pump circulated the air in a closed loop between the chamber and the analyzer, and 185 fluxes were calculated from the changes in CH₄ concentrations over time (Parkin and Venterea, 186 2010). Flux calculation was limited to the initial linear increase in CH₄ concentration. Before 187 positioning the chamber, the CH₄ concentration inside the chamber was allowed to equilibrate 188 with ambient concentration to ensure that the analyzer chamber and tubing were free of CH₄ from 189 previous measurements. Measurement on each of the 28 measured locations lasted less than 5 190 minutes. The order of the measurement changed during each site visit, and fluxes were measured 191 mid-morning in less than 4 hours to reduce the effects of daily temperature fluctuations. 192 The chamber was built using non-emitting CH₄ materials (PVC and HDPE) and included a vent to 193 avoid pressure effects (Pavelka et al., 2018). The ratio of surface to volume of the chamber was 194

determined by the need to avoid a rapid CH₄ build up, which lead to an insufficient number of

readings before exceeding the analyzer measurement range. The size of the lagoon chamber was
limited by the need for floating the chamber and positioning it without disturbance using a 6 m
pole on the lagoon surface.

199 The vermifilter chamber had a diameter of 31 cm and a volume of 39 or 54 L. The lagoon 200 chamber had a diameter of 25 cm and a volume of 49 L. The chambers were tested for leaks 201 before deployment in the field following guidelines in Pavelka et al. (2018), and leaks were less 202 than 0.006 μ mol CH₄ m⁻² s⁻¹.

203

204 **2.1.2 Vermifilter measurements**

205 In the vermifilter, sources of CH₄ emissions were the vermifilter bed and potentially the underlying drainage and aeration space. Therefore, CH₄ fluxes at 12 locations on the vermifilter 206 bed and four vents connected to the underlying space were monitored (Figure 1B). On the 207 208 vermifilter bed, measurements were located on three equidistant transects and in areas of varying moisture content and distances from sprinkler heads and walls. During measurements, the 209 chamber was fastened to a PVC collar (17 cm high, 30 cm diameter, permanently inserted 10 cm 210 into the woodchip layer (Figure 1). A tight seal was obtained by a locking mechanism pressing 211 the chamber on a rubber gasket attached to the top of the collar. The collars didn't interfere with 212 the spraying of the wastewater or the periodic, hand-operated tilling. The collars ensured repeated 213 measurements at the same locations and prevented disturbance to the media surface and below-214 surface air exchange during measurement. On each date, measurements were repeated on each of 215 216 the vermifilter locations two times and three times when increase in CH₄ concentration over time was irregular. Repeated measurements were averaged for each location. At each monitoring 217

location, temperature at a depth of 15 cm was measured. To measure CH₄ emissions from the
vents, the chamber sampling area was reduced to the size of the vent (15 cm diameter) by placing
a 5-cm thick foam layer on the bottom of the chamber. The modified chamber was pushed onto
the vents to form a tight seal.

The vermifilter CH₄ emissions are equal to the sum of the CH₄ emissions from the vermifilter bed 222 and the vents. These were calculated by scaling up measured vermifilter bed and vent flux 223 densities (μ mol CH₄ m⁻² s⁻¹) for the corresponding surface area. CH₄ fluxes for a full-size 224 vermifilter, defined as the size required to treat the entire Fanelli Dairy animal population and that 225 would eliminate the need for long-term lagoon storage, were also calculated. The 4,630 m² full-226 size was determined using the ratio between cow population and size of a full-size vermifilter 227 currently operating in Washington State, US (circa 5 m² per cow, BioFiltro personal 228 229 communication).

230

231 **2.1.3** Anaerobic lagoon measurements

For the 12 measurement locations on the lagoon, a floating chamber was attached to a 6 m pole 232 and was lowered onto the lagoon surface, about 5 m from the lagoon edge. The chamber opening 233 was sealed to and floated upon a 1 x 1 m, 5-cm thick foam board. This created an air-tight seal on 234 the lagoon surface. It was not possible to replicate measurements in the same location because 235 lifting the chamber at the end of the measurement cycle disturbed the lagoon surface and would 236 have likely affected gas exchanges. The lagoon water temperature was measured at each sampling 237 238 event. Measurement locations were at varying distances from the lagoon inlet and outlet and included areas of open water and areas covered by a scum layer. Lagoon CH₄ emissions were 239

calculated by multiplying the mean fluxes densities (μ mol CH₄ m⁻²s⁻¹) by the total surface area of the lagoon (10,800 m²).

242

243 2.1.4 Methane Emission Calculation

The CH₄ emissions were calculated as 1) flux densities, i.e., the CH₄ flux per unit area of lagoon
and vermifilter (in µmol CH₄ m⁻² s⁻¹), and 2) to account for the different footprints of the
vermifilter (4,630 m²) and lagoon (10,800 m²), as total CH₄ fluxes for a full-size vermifilter and
lagoon. The full-size vermifilter was the size required to treat the dairy's entire animal population.
The total surface area of the lagoon was determined using satellite imagery. To calculate GHG
emissions in CO₂ equivalent (CO₂eq), the GWP of 25 for CH₄ was used, following the IPCC
Fourth Assessment Report (2007).

The vermifilter emission reduction was calculated for each measurement event as the difference between lagoon and vermifilter CH_4 emission, divided by the lagoon CH_4 emissions. The monthly emission reductions values were averaged to estimate the mean effect (± standard error) of the vermifilter on the dairy lagoon CH_4 emissions.

Daily CH₄ emissions were estimated by linearly interpolating data between measurement dates.
Daily values were summed to calculate monthly and annual CH₄ emissions from vermifilter and
lagoon. Uncertainty in the annual CH₄ emissions for the lagoon and the vermifilter was estimated
as the standard error of the mean of the 12 annual sums obtained by linearly interpolating fluxes
for each of the 12 measurement locations.

260 The CH₄ emissions of the solids separated by the vermifiltration separator (CH₄f) were calculated
261 using IPCC quantification guidelines (IPCC, 2006) as:

$$CH_4 f = VS_{yr} \cdot B_0 \cdot 0.66 \cdot MCF \cdot MS$$
(1)

where VS_{yr} is the annual VS after primary separation, B_0 is 0.24, 0.66 is the density of CH₄ at 25°C (kg CH₄·m⁻³ CH₄), MS is the fraction of livestock manure handled by the secondary separator and was 0.1 (BioFiltro personal communication). The MCF of 0.01 is the IPCC value for composting manure in passive windrow. The CH₄ emissions of these separated solids were added to the CH₄ emissions measured on the vermifilter to quantify the CH₄ emissions of the vermifiltration system.

269 The IPCC guidelines (IPCC, 2006) base the calculation of CH₄ emissions from manure

270 management on treatment specific MCF parameter values. The MCF quantifies the percentage of

271 VS that each management system converts to CH₄ compared to a maximum methane-producing

272 capacity for the specific type of animal manure (B_0) in a particular climate. A MCF for

vermifiltration is currently not available. The Fanelli Dairy emission data were used to determinethe vermifiltration system MCF for climatic conditions of the study site (average air temperature

of 16 °C) by applying the method described in Mangino et al. (2001):

276
$$MCF = \frac{Annual Methane Emission}{Bo x Annual Volatile Solids Production}$$
(2)

Where the *Annual Methane Emission* is the sum of the CH₄ emissions of the full-size vermifilter and the vermifiltration separator; B_o was 0.24 m³ CH₄·(kg VS⁻¹); and the *Annual Volatile Solids Production* was the VS produced annually by the dairy cow population, excluding the 17% VS retained by the solid-liquid separator. The MCF for the vermifilter was based on monthly monitoring. Monthly CH₄ emissions was the timescale used by Mangino et al. (2001) to determine the anaerobic lagoon MCF for the US and adhered to the IPCC recommendation for the determination of MCFs to include the effects of seasonal changes in VS, temperature, and VS retention time.

285

286 **2.2. Water Quality**

287 The quality of the wastewater effluent determined the residual capacity of the treated wastewater to produce GHG emissions and the amount of macro and microelements provided to crops from 288 land application. To determine the effect of the vermifilter on water quality, the vermifilter 289 influent and effluent were sampled monthly from March 2019 to March 2020. Grab samples were 290 291 kept refrigerated after collection and delivered in less than 24 hours to an accredited laboratory 292 for testing (BSK Associates, Fresno, CA). Data were assessed to ensure that laboratory quality assurance/control measures (duplicates, matrix spikes, matrix spike duplicates, and blanks) were 293 within the prescribed limits. Samples were analyzed for solids (total solids, total dissolved solids, 294 295 total suspended solids, total volatile solids, total volatile suspended solids), N species (ammonia, nitrates, nitrites, total nitrogen, total Kjeldahl nitrogen), dissolved and total organic carbon, other 296 297 nutrients (calcium, magnesium, potassium, chloride, sulfate, phosphorous, sodium), trace elements (boron, cadmium, chromium, copper, iron, lead, manganese, nickel, zinc) pH and 298 electrical conductivity. Frequency of the analysis varied from monthly to seasonal. Frequency of 299 sampling and analysis methods for each constituent are listed in Table 2. 300 Constituent removal rates were calculated monthly as the ratio of the difference between influent 301

- 302 and effluent concentrations divided by the influent concentration. Mean removal rates were
- 303 calculated as the averages (±standard error) of all available values. We quantified the N recovered

over one year by comparing the annual N produced by the cows with the sum of the N containedin the wastewater and in the vermicompost.

306

307 **2.3. Vermicompost Analysis**

The vermicompost is the product of the action of the worms and microbes on the organic matter 308 removed from wastewater and the wood chips. Vermicompost is typically removed after an 18-309 month period, during which no chips are added. The mass of vermicompost produced was 310 quantified from the volume of material in the vermifilter at extraction and its bulk density. In 2021 311 312 the Fanelli Dairy vermicompost was analyzed by Prof. W. Horwath's research group at UC Davis. Samples were dried at 45 °C, for 48 h. A subsample was acidified with 3M HCl to prevent N loss 313 and dried at 45 °C for 48 h. Samples were ground to <0.25 mm using a ball mill. After, 10 mg of 314 315 each sample was analyzed for total C and N by dry combustion (AOAC Method 972.43). Wet bulk density was determined for a 10 L composite sample. A subsample was dried at 105 °C for 48 h to 316 determine moisture content by mass difference. 317

318

319

3. Results and Discussion

320 **3.1. Methane fluxes**

The effects of the vermifilter on the dairy wastewater CH_4 emissions were evaluated using 1) the comparison of CH_4 emissions from the vermifilter with the lagoon CH_4 emissions and 2) the efficacy of the vermifilter to remove VS from the wastewater.

324	Methane emissions from the vermifilter were substantially lower than emissions from the lagoon
325	throughout the year (Figure 2, Table 1). Over a year, the vermifilter emitted 97% less CH ₄ than
326	the lagoon over the same unit area and 99% less CH_4 at the full-size scale (p < 0.01). The
327	vermifilter reduction of the lagoon CH ₄ flux density ranged between 89% and 100% (Figure 2).
328	Even extrapolating the vermifilter maximum measured CH ₄ flux rate of 6.4 μ mol CH ₄ m ⁻² s ⁻¹ over
329	the year resulted in vermifilter CH4 emissions 94% lower than the lagoon CH4 emissions.
330	Methane emissions from the vermifilter increased steadily between December 2019 and May
331	2020, and from June 2020 declined gradually through November 2020 (Figure 2).
332	Air temperature didn't explain any of the observed variation in CH ₄ emissions ($r^2 < 0.1$), and soil
333	(or water) temperature explained only 17% of the vermifilter (p <0.001) and 28% of the lagoon
334	seasonal variations in CH ₄ fluxes ($p < 0.001$). The temperature in the vermifilter bed varied by
335	only 13 °C over the year (Figure 2), in part due to the consistent wastewater application. The
336	vermifilter homogeneous design and consistent operation, the limited variations in humidity and
337	temperature, and the weak relationship with temperature support the reliability of the monthly
338	flux monitoring. Even with a low temporal resolution, the monitoring provided the first
339	quantification of the annual CH4 emissions of an industrial-scale dairy vermifilter and its MCF.
340	Furthermore, the methods to determine CH ₄ emissions in this study were similar to those reported
341	in the literature. Of the 17 studies included in a review of all available publications on CH ₄
342	emissions measurements from liquid manure storages (Leytem et al., 2017), only four monitored
343	uncovered lagoons and provided annual CH4 emissions. The reported annual estimates were based
344	on gas measurements made monthly or seasonally for 1-3 days.



Figure 2. A: Comparison of the vermifilter and the anaerobic lagoon CH₄ emissions measured monthly between December 2019 and November 2020. Columns represent the average of 12 locations (\pm standard errors). The red line represents the reduction in CH₄ emissions (%) of the vermifilter compared to the anaerobic lagoon. B: Seasonal trends of CH₄ flux densities (µmol CH₄ m⁻²s⁻¹) from a) the vermifilter and b) the anaerobic lagoon in a California Dairy from December 2019 to November 2020. Symbols are the average of 12 locations (\pm standard errors). c) Average air and vermifilter bed temperatures (at 15 cm).

A weak relationship between CH₄ fluxes and temperature for an anaerobic lagoon was also 353 reported by Safley and Westerman (1992) and Leytem et al. (2017). In the Leytem et al. (2017) 354 355 study, CH₄ emissions had a stronger relationship with wind and lagoon physicochemical properties such as total solids, chemical oxygen demand, and VS than temperature. 356 The water level in the lagoon was constant until June 2020, followed by a gradual decrease until 357 November 2020 due to the use of lagoon water for irrigation. The VS availability in the lagoon 358 359 decreased with water levels and because of the increased consumption of VS due to the high temperatures as described by Mangino et al. (2001). The decreased VS availability offset the 360 effect of the increased temperature and resulted in lower CH₄ emissions. Because the vermifilter 361 received the lagoon water recycled for flushing, the vermifilter received less VS during the 362

summer. Thus, the decreasing CH₄ emissions from the vermifilter during summer could in part be
due to the decreasing lagoon VS content.

The vermifilter was tilled monthly to increase porosity and aeration and thus eliminate conditions
generating CH₄ emissions. Anoxic conditions built up gradually after each tilling event.
Therefore, the length of the interval between tilling and measurements could also explain part of

the observed temporal variability in the vermifilter CH₄ fluxes.

Estimated annual emissions of CH₄ from the lagoon were 253,854 kg CH₄ compared to 2,970
kg CH₄ from the vermifilter and the additional 308 kg CH₄ from the solids separated by the
separator in the vermifiltration system. Even though CH₄ emissions from the solids separated by
the second separator were not directly measured in the study, their contribution to the total
vermifilter emissions was minimal (10%).

- In one year, the full-size vermifilter system could reduce CH₄ emissions by 6,264 t CO₂eq (Table
- 1). The results are consistent with the low vermifiltration CH₄ emissions reported by Luth et al.
- 376 (2011) and Lai et al. (2018).

- **Table 1: Emissions of CH4 from manure management systems (MMS) at the Fanelli Dairy.**
- 378 Monthly CH₄ emissions are calculated by linearly interpolating the fluxes between
- 379 consecutive sampling dates. CH₄ emissions of the lagoon are compared to emissions from a

380 vermifilter of the size required to treat all VS produced in the dairy.

Date		Vermifilter	Lagoon
		(kg CH ₄ month ⁻¹)	(kg CH ₄ month ⁻¹)
Year	Month		
2019	December	38	20,981
	January	114	17,999
	February	236	21,723
	March	422	30,141
	April	948	30,769
2020	May	708	26,850
2020	June	195	32,578
	July	163	25,766
	August	57	12,628
	September	7	13,724
	October	21	8,763
	November	61	11,931
Annual CH4 emissions (kg CH4 yr ⁻¹)		Vermifilter 2,970 (\pm 631) + Solids from vermifiltration separator 308 (\pm 154) = Total vermifilter system 3,278 (\pm 649)	253,854 (±35,423)
Potential manure CH ₄ emissions (kg CH ₄ yr ⁻¹)		327,951	
MCF		1%	77%
Emission per animal: (kg CH ₄ yr ⁻¹ cow ⁻¹ yr ⁻¹)		3.7*	284
	$(t CO_2 eq yr^{-1} cow^{-1} yr^{-1})$	0.1*	7.1
Emission per unit-area of MMS (kg CH ₄ m ⁻²)		0.7*	23.5

* Including emissions from the vermifiltration separator;

The lower vermifilter CH₄ emissions compared to the lagoon were due both to a lower emission rate per unit area (CH₄ flux density) and the smaller surface area of the vermifilter, as the full-size vermifilter was 43% of the lagoon. The vermifilter system emitted annually 3.66 kg CH₄ yr⁻¹ per cows (or 0.1 t CO₂eq cow⁻¹yr⁻¹), and 0.7 kg CH₄ m⁻² yr⁻¹ per unit area of vermifilter, compared to 284 kg CH₄ cow⁻¹ yr⁻¹ (7.1 tCO₂eq cow⁻¹yr⁻¹) and 23.5 Kg CH₄ m⁻²yr⁻¹ of the lagoon (Table 1).

The large size of the lagoon and the inability to reach the lagoon center increased uncertainty in the estimate of the lagoon CH₄ emissions (Figure 2b). However, this was not the case for the vermifilter CH₄ emissions. Also, the lagoon CH₄ flux rates measured in this study are comparable with the emissions rate of 20 kg CH₄ m⁻²yr⁻¹ reported by Owen and Silver (2014) for dairy anaerobic lagoons. They are also within the range of 0.4-37 kg CH₄ m⁻²yr⁻¹ (12-1030 kg CH₄ ha⁻¹ ¹day⁻¹) summarized by Leytem et al. (2017) and also reported by Kupper et al. (2020).

CH₄ fluxes for the same vermifilter were previously measured by Lai et al. (2018). This study 394 also observed low CH₄ emissions from the vermifilter, but the authors reported CH₄ emission 395 rates from the vermifilter higher than from the lagoon (0.8 compared to 0.4 kg CH₄ d⁻¹ per 50,000 396 L of daily treated wastewater, respectively). The emission rates reported in the study did not 397 account for the size of the lagoon. Scaling up the lagoon emissions from the sampled volume to 398 its total volume would increase the reported lagoon CH₄ emissions well above the vermifilter 399 emission. In fact, the vermifilter CH₄ emission rates measured using a triangular sampling tunnel 400 401 covering a section of the surface of the vermifilter during July by Lai et al. (2018) were lower than the 1.9 kg $CH_4 d^{-1}$ measured in the month of July in this study. 402

403 The vents contributed minimally to the total vermifilter CH₄ fluxes. The 20 vents were connected 404 to an air volume similar in size to the vermifilter bed. The low vent CH₄ (on average 0.12 ± 0.1 μ mol CH₄ m⁻² s⁻¹) and high CO₂ (on average 521 ± 175 µmol CO₂ m⁻² s⁻¹) emission rates provided evidence that aerobic conditions predominated at depth in the vermifilter. Because the maximum contribution of the vents to the vermifilter CH₄ emissions was 0.3% (data not shown), they were excluded from the annual CH₄ flux estimation.

Additional research is needed to improve understanding of how vermifilter design, animal type,
climate, and system performance affect emissions of CH₄.

The VS left in treated wastewater determines its capacity to produce further CH₄ emissions. On average, the vermifilter system removed 87% of the VS (Table 2) from the wastewater. Combined with the VS removed by the first separator (17%), only 11% of the total VS produced were present in the vermifilter effluent (Figure 1). The VS reduction was continuous during the year (Figure 3) and ranged from 77% to 96%. Even if all treated water was stored in the existing lagoon under current management, CH₄ emissions would be 87% lower than without vermifiltration.

The influent VS content was variable. Similar fluctuations observed by Wilkie et al. (2004) were 418 explained by the way in which the manure particulates moved through the system and not by 419 420 changes in wastewater characteristics. Fluctuations were also measured by Miito et al. (2021) in a Washington State dairy deploying the same vermifilter. The authors sampled total solids and total 421 suspended solids every two weeks between July and December. The study found no difference in 422 solid content during warmer months compared to winter months. These results suggest 423 seasonality has little effect on dairy wastewater quality and that the monthly sampling sufficiently 424 425 accounted for the existing temporal variation.

426

427 The determination of the MCF coefficient can facilitate the ability of the dairy vermifiltration practice to access the carbon market and other incentive programs aiming to reduce agricultural 428 GHG emissions. The methane conversion factor (MCF) for the vermifiltration system determined 429 during this study was 1%, the same value suggested by the IPCC guidelines for composting for 430 similar climatic conditions (IPCC, 2006). The vermifilter system MCF was much lower than the 431 lagoon MCF of 77% (Table 1). The IPCC suggests a MCF of 75% for an anaerobic lagoon in the 432 region, consistent with our measured values. The higher estimated lagoon MCF relative to the 433 IPCC value suggests that the vermifilter CH₄ emissions reduction was not due to an 434 435 underestimation of the lagoon CH₄ emissions.

436

438Table 2: Average concentration of key water quality constituents in influent and effluent

439 samples and percent reduction in concentration for the vermifiltration system at the Fanelli

- 440 Dairy. Data are averages of monthly or seasonal values between March 2019 and March
- 441 **2020.**

Constituent	Units	Method	Average concentration				Reduct ion	Range	N
			Influen t	SE	Effluen t	SE	%	%	
NITROGEN									
Ammonia (NH ₃ +NH ₄ ⁺ as N)	mg l ⁻¹	EPA 350.1	494	25	13	6	97%	87-100	13
Nitrate (as N)	mg 1-1	EPA 300	ND		54	12			13
Total Kjeldahl Nitrogen	mg l ⁻¹	EPA 351.2	810	80	74	15	92%	88-100	13
Total N	mg l ⁻¹	CALC	810	80	134	17	84%	72-100	13
SOLIDS									
Total Solids	mg l ⁻¹	SM 2540B	19,258	1737	4,250	454	79%	72-95	9
Total Dissolved Solids	mg l ⁻¹	SM 2540C	5,333	410	3,300	316	42%	12-64	10
Total Suspended Solids	mg l ⁻¹	SM 2540D	13,969	1966	666	174	95%	84-96	13
Total Volatile Solids	mg l ⁻¹	SM 2540E	14,127	1344	1,798	240	87%	82-96	13
Total Volatile Suspended Solids	mg 1 ⁻¹	SM 2540E	11,392	1716	525	155	95%	85-98	10
CARBON									
Dissolved Organic Carbon	mg l ⁻¹	SM 5310C	373	126	127	24	55%	17-85	4
Total Organic Carbon	mg l ⁻¹	SM 5310C	640	160	163	28	68%	33-86	4
Conductivity	µScm	SM 2510B	8,700	241	4,518	345	48%	30-72	11
рН		SM 4500-H+ B	7.8	0.08	8.4	0.04	Incr.* 8%	Incr.*1- 15	12
Calcium	mg l ⁻¹	EPA 200.7	530	56	84	4	83%	79-89	4
Magnesium	mg l ⁻¹	EPA 200.7	320	39	89	15	70%	56-89	4
Potassium	mg l ⁻¹	EPA 200.7	2,324	977	1,679	673	26%	82-91	11
Chloride	mg l ⁻¹	EPA 300.0	435	99	358	75	12%	27-37	4
Sulfate	mg l ⁻¹	EPA 300.0	ND		62	24			
Phosphorous	mg l ⁻¹	EPA 365.4	233	19	39	7	84%	83-91	8
Sodium	mg l ⁻¹	EPA 200.7	295	10	223	25	25%	9-37	4
Boron	μg l ⁻¹	EPA 200.8	1,875	250	395	75	76%	57-9	4
Cadmium	μg l ⁻¹	EPA 200.8 DRC	1.1	0.1	ND		100%		4
Chromium	μg l ⁻¹	EPA 200.8 DRC	39	9	1	1	97%	90-100	4
Copper	μg l ⁻¹	EPA 200.8 DRC	770	147	100	18	86%	75-92	4
Iron	μg l ⁻¹	EPA 200.8 DRC	22,750	5422	913	97	95%	92-98	4
Lead	μg l ⁻¹	EPA 200.8 DRC	18	7	1	0	94%	82-99	4
Manganese	μg l ⁻¹	EPA 200.8 DRC	4,550	712	468	53	89%	83-92	4

Nickel	μg l ⁻¹	EPA 200.8 DRC	94	16	19	3	78%	63-89	4
Zinc	μg 1 ⁻¹	EPA 200.8 DRC	3,750	746	446	107	91%	85-94	4

• Measured increase (Incr.)



30000 -VS INF а 25000 -O-VS EFF 20000 VS (mg I⁻¹) 15000 10000 5000 0 🛦 Ammonia INF b 1400 N (mg l⁻¹) Nitrate INF 1100 • Total N INF 800 500 200 -100 1400 С –**∆**– Ammonia EFF N (mg l⁻¹) - Nitrate EFF 1100o... Total N EFF 800 500 200

444

-100

F-19

A-19

J-19

J-19

S-19

Figure 3. concentration of a) volatile solids (VS); ammonia, nitrate, and total nitrogen in the dairy
wastewater b) before (INF) and c) after (EFF) the vermifiltration system. Concentrations were
measured monthly from March 2019 to March 2020.

N-19

D-19

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M-20

F-20

3.2 Nutrient removal and recovery

449 The effect of the vermifilter on water quality not only determines the residual capacity of treated wastewater to emit GHG gases and pollutants, but also to provide nutrients to crops when land 450 451 applied. During 2019-2020, vermifiltration reduced wastewater NH₃ concentrations by 97% 452 $(\pm 5\%)$ and total N by 84% $(\pm 8\%)$ (Figure 3, Table 2). High rates of N and NH₃ reduction by vermifiltration were reported in several studies (Adugna et al., 2019; Dey Chowdhury and Buhnia 453 454 2021). Our results were consistent with the Lai et al. (2018) study on the Fanelli Dairy vermifilter 455 N dynamics. The vermifilter removed most of the N from the wastewater, and this was 456 transformed into benign N_2 gas through denitrification. The study measured minimal N_2O emissions (0.14 kg N₂O d⁻¹) during vermifiltration. Volatilization of NH₃ from the vermifilter was 457 458 0.1 kg NH₃ d⁻¹and was 90% lower than from the lagoon. The vermifiltration reduction of the N load in dairy wastewater reduces the potential losses to the 459 460 atmosphere, surface, and groundwater. When regulations limit the maximum load of N to apply with irrigation to land, vermifiltration results in the reduction in the amount of land required by a 461 farmer to dispose of the dairy wastewater. Also, the improved quality of the treated wastewater 462 relative to the lagoon increases the options for recycling treated water and can result in the 463 reduction of the farm's demand for high-quality water. 464 Vermifiltration can still provide N for crops in both the treated water and vermicompost (Table 465 2). Water treated by a full-size vermifilter (460,000 L day⁻¹) at the Fanelli Dairy would provide 466 annually 22 t N, 50% of which is plant available ammonia and nitrate (Table 2). Considering a 467 generic N fertilization rate of 150 Kg N per hectare, the treated wastewater would provide N 468 469 fertilization to 147 ha.

470 Vermicompost produced at the Fanelli Dairy after circa 18 months of use had 1.4% N content,

471 42% C, and a bulk density of 190 kg_{dw} m⁻³. Thus, a full-size vermifilter at the Fanelli Dairy would

472 produce 563 t of vermicompost (wet weight and 60% humidity), with 148 t of C (165 kg C cow⁻¹)

473 and 5 t of N (6 kg N cow⁻¹) that can be applied to soils.

474 The life cycle of the N produced annually from one cow was followed until the dairy wastewater stored in the anaerobic lagoon was used for irrigation (Figure 1c). In addition to the data resulting 475 476 from this study, N production and loss rates estimated regionally by Pettigrove and Eagle (2009) were used. Of the 153 kg N produced annually by one typical cow in the region, on average 31 477 478 kg N are lost during storage in anaerobic lagoons. The 20% loss included the N removed by the first separator, as N is minimally affected by separators because soluble nutrients and salts 479 predominantly remain in the liquid system (Harter et al., 2007). The researchers reported a typical 480 481 28% loss (34 kg N) after land application. This leaves 88 kg N for use by the crop. In contrast, the use of the vermifilter resulted in 25 kg N remaining in the treated wastewater and an additional 6 482 kg N in the vermicompost. Losses of N as emissions of N₂O and NH₃ during vermifiltration 483 measured by Lai et al. (2018) were minimal (<1 kg N). Thus, the vermifilter recovered in both the 484 treated water and vermicompost 20% of the initial N that can be applied to crops. This was lower 485 486 than the 60% of initial N provided by applying lagoon water. However, this can help mitigate the excess nutrients associated with intensive dairies operation. Also, losses from soils after land 487 application of vermifiltration-treated wastewater are unknown but likely reduced compared to the 488 489 lagoon because of the lower amount applied, higher microbial activity able to cycle and store nutrients (Saha et al., 2022), and low initial concentrations of NH₃. The difference between N 490 excreted, N₂O emitted during vermifiltration, and left in the effluent/vermicompost was emitted 491 as N₂. This loss may represent a missed opportunity to recover nutrients that are a valuable 492

493	resource. A cost-benefit analysis can determine the most appropriate strategy for a dairy.
494	However, the analysis should assess not only cost and feasibility of the nutrient-recovering
495	technologies but also their effects on GHG emissions and air and water quality.
496	The vermifilter removed additional constituents from the dairy manure wastewater. Phosphorous
497	was reduced by 84% (\pm 8%). Total dissolved solids and electrical conductivity decreased by 42%
498	($\pm 14\%$) and 48% ($\pm 11\%$), respectively. There were also reductions from the wastewater in most
499	major ions and all trace elements (Table 2). Only sulfates and nitrates increased compared to pre-
500	treatment conditions, and concentrations in the effluent were low (Table 2).
501	Among other available studies Miito et al. (2021) and demonstrated the efficacy of vermifiltration
502	as a technically viable alternative for on-site dairy wastewater treatment. A 68% wastewater
503	reduction in total suspended solids (TSS), 81% reduction in total nitrogen, 48% reduction in
504	phosphorus were reported on a Washington State dairy using a similar vermifiltration system
505	(Miito et al., 2021). These authors reported higher reduction efficacy of the vermifilter at higher
506	temperatures and higher influent concentrations. This can in part explain our study's higher TSS
507	and phosphorus wastewater reduction rates (95% and 84%, respectively). At the California dairy,
508	the annual average air temperature was circa 10 °C higher, and the influent phosphorus
509	concentrations were higher than at the Washington dairy (190-290 mg L ⁻¹ compared to 54-127
510	mg L ⁻¹).

4. Conclusions

513	Vermifiltration of dairy wastewater caused minimal CH ₄ emissions of 0.7 Kg CH ₄ m ⁻² yr ⁻¹ or
514	3.7 kg CH ₄ m ⁻² yr ⁻¹ cow ⁻¹ and greatly reduced the CH ₄ emissions of an anaerobic lagoon. The
515	emissions were only 1% of the CH ₄ emissions potentially produced by the liquid manure.
516	Vermifiltration significantly decreased the wastewater nutrient load, increasing opportunities to
517	recycle wastewater. Thus, vermifiltration can be a useful tool to mitigate agriculture CH4
518	emissions and manage excess nutrients. Further research is needed to assess factors controlling
519	GHG fluxes, GHG life cycle of vermifiltration, and the potential for carbon sequestration from
520	land application of vermicompost and treated water.
521	
522	5. Acknowledgment
523	The study was funded by the vermifiltration wastewater treatment company BioFiltro.

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