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Final Report

The effect of vermifiltration on gaseous emissions from dairy wastewater

Submitted to: Sustainable Conservation

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- 1 **Abbreviations:** BOT, bottom of vermifilter; EFF, effluent; GHG, greenhouse gas; INF, influent;
- 2 LAG, lagoon water; N, nitrogen; TOP, top of vermifilter; VOC, volatile organic compound
- 3

1 Core ideas

- 2 1. Vermifiltration decreases emission of ammonia from dairy wastewater by 90.2%.
- 3 2. Vermifiltration slightly increased N₂O, CO₂, CH₄, and EtOH emission from wastewater.
- 4 3. The vermifilter is not a significant source of GHG or noxious emissions.

5

1 **1 ABSTRACT**

2 Dairy lagoon water contains high concentrations of nitrogen (N), giving it the potential to
3 pollute groundwater and the atmosphere. To reduce N loading of an anaerobic lagoon at a
4 commercial dairy, a pilot project vermifilter was installed, which used earthworms embedded in
5 woodchips to enhance removal of solids and contaminants. However, it was unclear whether the
6 removal of N occurred at the expense of increasing nitrogenous gases, greenhouse gases
7 (GHGs), volatile organic compounds (VOCs), and criteria pollutants from lagoon water treated
8 with this new technology. Thus, emissions were measured from untreated dairy lagoon water
9 (LAG), as well as from the vermifilter's influent (INF), effluent (EFF), the top (TOP), and
10 bottom (BOT) of the filter to assess filter performance. Ammonia (NH₃) and ethanol (EtOH)
11 were measured using an Innova 1412 analyzer, while nitrous oxide (N₂O), carbon dioxide (CO₂),
12 methane (CH₄), and hydrogen sulfide (H₂S) were measured using Thermo analyzers. Gases were
13 measured using flux chambers for LAG, INF, and EFF, a triangular shaped wind tunnel for TOP,
14 and from an exhaust pipe for BOT. Results suggested the vermifilter versus the untreated lagoon
15 water reduced NH₃ emission by 90.2% without substantially increasing emission of N₂O, CO₂,
16 CH₄, and EtOH from other sampling locations of the vermifilter system. Although this study
17 must be replicated across other dairy operations to verify these results, these preliminary findings
18 suggest vermifiltration technology is a potential solution for N removal particularly in regions
19 like the San Joaquin Valley of California, where dairy air- and water quality issues are most
20 sensitive.

1 **2 INTRODUCTION**

2 In Central California, increasingly intensive dairy production produces more waste than
3 dairy farmers can apply to cropland at agronomical rates. Combined with the shallow water
4 basin, the large amount of waste produced threatens ground water - and air quality, soil quality,
5 and downstream ecosystems. To mitigate nutrient loading in dairy wastewater, a vermifilter was
6 built at a commercial dairy in Central California to treat lagoon water before it was applied to
7 cropland or recycled as flush water to the freestall barns. Vermifiltration is a wastewater
8 treatment system composed of a bed of organic media, such as woodchips or sawdust, seeded
9 with earthworms that bio-oxidizes applied waste, outputting effluent that is lower in nutrients
10 and vermicompost and earthworms that can be sold for profit. Previous studies have reported
11 high nutrient removal under optimal wet:dry ratios and earthworm loading rates, reaching
12 removal efficiencies up to 77.9% for NH₃-N, and 66.6% for total N (TN) (Wang et al., 2014b,
13 Wang et al., 2013). Because this technology is a relatively low-cost, low-maintenance
14 wastewater system, vermifiltration has been adopted recently to treat domestic wastewater in
15 developing countries (Xing et al., 2015, Xing et al., 2016), industrial waste (Lin et al., 2013), and
16 livestock waste (Li et al., 2008, Luth et al., 2011, Wang et al., 2014a). Similar to these studies,
17 preliminary chemical analysis of the influent and effluent by the manufacturer (BioFiltro)
18 reported high nitrogen (N) removal efficiency (>90%) from the wastewater.

19 Although these results were promising, it was unclear how the vermifilter affected
20 gaseous emissions, namely ammonia (NH₃), nitrous oxide (N₂O), carbon dioxide (CO₂), methane
21 (CH₄), ethanol (EtOH), and hydrogen sulfide (H₂S). NH₃ and H₂S are nuisance gases and
22 respiratory irritants, whereas CO₂, CH₄, and N₂O are greenhouse gases (GHGs) with global
23 warming potentials (GWPs) of 1, 28, and 298, respectively (IPCC, 2013). Specifically, the

1 removal of N from wastewater via enhanced N-cycling in the vermifilter had the potential to
2 increase production of N_2O , a byproduct of incomplete denitrification (Figure 3). Similarly, it
3 was imperative that nutrients removed from the wastewater were not converted into EtOH or
4 other volatile organic compounds (VOCs), a group of smog precursors regulated in the present
5 air shed.

6 Although earthworms in vermifilters do not directly emit significant amounts of NH_3 ,
7 CO_2 , CH_4 , and H_2S , the physical and chemical modifications they impose upon the media impact
8 the emission of these gases. As for N_2O , current literature debates whether earthworms increase
9 or decrease emissions of this GHG. Drake et al. (2007) hypothesized that the earthworm gut
10 favored incomplete denitrification to N_2O production over complete denitrification to dinitrogen
11 gas (N_2) due to restriction in moisture and nutrient availability at the posterior of the gut.
12 Experimentally, Lubbers et al. (2013) found that earthworms increased emission of N_2O and CO_2
13 from soil by 42% and 33%, respectively. Contrarily, in a pilot study examining the effects of
14 vermifiltration on pig slurry, Li et al. (2008) observed minor increases in N_2O emissions and a
15 50% reduction in NH_3 emission on a whole wastewater system basis. Furthermore, Li et al.
16 (2008) attributed these observations to increased rates of nitrification and complete
17 denitrification to N_2 in the vermifilter. Similarly, Luth et al. (2011) examined the effect of
18 application rate of pig slurry on NH_3 and GHG emissions and found that under the optimal
19 application rate, vermifiltration reduced NH_3 , N_2O , and CO_2 emissions, and acted as a CH_4 sink
20 compared with equivalent filters without earthworms. To explain these conflicting results for
21 N_2O emission, Luth et al. (2011) proposed that there was a threshold of N input above which the
22 presence of earthworms would decrease N_2O emission; however, the dosage dependence of N_2O
23 emission on N input has yet to be explicitly tested.

1 Little research has examined VOC emissions from dairy production and waste
2 management (Shaw et al., 2007), let alone the specific effect of vermifiltration on VOC emission
3 from dairy wastewater. Although Bhattacharya et al. (2016) showed that the presence of
4 earthworms in composting cattle manure decreased VOC emission, they did not measure EtOH
5 emission. To our knowledge, no literature has directly assessed the effect of vermifiltration on
6 H₂S, although Sinha et al. (2008) alluded to the potential of vermifiltration to reduce H₂S
7 emissions.

8 The majority of previous research in vermifiltration focused on optimizing vermifilter
9 performance as measured through nutrient removal efficiency (Liu et al., 2012, Wang et al.,
10 2014b, Wang et al., 2011, Wang et al., 2013) and elucidating the microbial communities
11 responsible for these effects (Li et al., 2013, Liu et al., 2012, Wang et al., 2014b, Wang et al.,
12 2016, Wang et al., 2011); however, very little is known about the effect of vermifiltration on
13 emissions. Additionally, the bulk of these studies involved domestic wastewater; few studies
14 examined vermifiltration of livestock wastewater. To date, the only study using cattle manure as
15 substrate focused on vermicomposting, which involved actively mixing and turning over the
16 media (Bhattacharya et al., 2016), as opposed to vermifiltration, in which the media is
17 undisturbed during operation. Thus, because the present study concerns the only vermifilter built
18 at a commercial dairy in Central California, it is descriptive by nature. The objective of this study
19 was to assess the effectiveness of the vermifilter on the reduction of GHGs, VOCs, and criteria
20 pollutants from treated lagoon water. Based on past findings (Li et al., 2008, Luth et al., 2011),
21 we hypothesized that the vermifilter enhanced nitrification and complete denitrification to reduce
22 NH₃ emission without significantly increasing N₂O emission.

1 3 MATERIALS AND METHODS

2 3.1 Experimental site

3 The present study was conducted at a commercial dairy with approximately 760 milking
4 cows and 1,300 head total in the California San Joaquin Valley, CA. The first sampling period
5 occurred during the summer in July 2015, while the second sampling period occurred during the
6 winter in December 2016. The dairy that hosted the vermifilter, used a conventional wastewater
7 management system, in which the manure in the freestall barns was flushed daily 3 times for 6
8 min to remove manure from the barn floor. Flushwater was stored in an uncovered anaerobic
9 lagoon with a ~5.7 million L holding capacity. A portion of this water was recycled through the
10 freestall barns during the next flushing period but eventually, all water was applied to
11 surrounding cropland as fertilizer (FIG. 1**Error! Reference source not found.**). In contrast, the
12 vermifilter wastewater management system treated the lagoon water before it was recycled back
13 to the freestall barns as flushwater (Fig. 1**Error! Reference source not found.**). During the July
14 2015 sampling, the vermifilter system was composed of a solids separator followed by
15 application to the vermifilter. However, during the December 2016 sampling, the vermifilter
16 system had been modified to include pre-filters between the solids separator and the vermifilter
17 for improved solids removal.

18 The vermifilter was a concrete enclosure (49 m x 11 m x 1.5 m) filled with a 1.2 m layer
19 of woodchips produced from heartwood of Douglas fir, white fir, and ponderosa pine, the top 30
20 cm of which were seeded with ~300 kg (live weight) of earthworms (*Eisenia fetida*) and a
21 microbial inoculant (Biofiltro) (Fig. 2**Figure 2**). Below the woodchips was a 30-cm layer of river
22 cobbles (10 cm x 20 cm) to improve drainage. Twenty peripheral PVC pipes (12 cm diameter)
23 extended from the surface of the vermifilter to the bottom layer of river gravel to allow air

1 exchange between the bottom layer and ambient air.

2 Lagoon water (LAG) was first pumped into the solids separator (i.e. Biofiltro) using a
3 rotary screen (200 µm hole size) to remove sand and solids (Fig. 2Figure 2). The resulting
4 influent (INF) was then stored in a storage tank until it was applied to the surface of the
5 vermifilter (TOP) using rotary head sprinklers. Every hour, a total of 2,100 L of INF was applied
6 over 10 min to the TOP, after which the sprinklers were shut off to prevent pooling on the
7 surface of the vermifilter. The INF percolated to the bottom of the filtration system where the
8 resulting effluent (EFF) exited from the BOT. The EFF was stored until it was recycled as flush
9 water or applied as fertilizer to cropland.

10 During vermifilter operation in December 2016, LAG was processed through the solids
11 separator and stored. Prior to sprinkling, this stored wastewater was then applied to 8 wood
12 shavings filters in parallel to improve solids removal and prevent congestion of sprinkler heads
13 downstream. The wood shavings filters were 3,937 L (1,040 gallon) intermediate bulk containers
14 (IBCs) filled with pine shavings. To apply wastewater to the wood shavings filters, wastewater
15 was channeled through PVC pipe into a dual-layer mesh box filter (inner shell: 80% sunshade
16 mesh, outer shell: wood block fabric) inset in the IBC level with the surface of the shavings.
17 After treatment with the wood shavings filters, 2,100 L of the resulting INF was applied to half
18 of the vermifilter using one of the two lines of sprinklers over 10 minutes. (INSERT NEW
19 SPRINKLER HEADS SPECIFICATIONS) The sprinkler line used to apply the INF to its
20 respective half of the vermifilter alternated with each hour to permit the INF more time to
21 percolate through the vermifilter between sprinkling periods.

22 **3.2 Summer gas sampling**

23 To estimate the gaseous emissions from the vermifilter system, gas concentrations were

1 measured from subsamples of the system, emission rates were calculated for each subsample,
2 and finally subsample emission rates were scaled up to the entire system.

3 The University of California, Davis, Mobile Agricultural Air Quality (MAAQ) Lab
4 containing the necessary gas analyzers was transported to the study site and used for the air
5 monitoring. After three days of stabilization, gas analyzers were calibrated and used to measure
6 gas concentrations first from the wastewater samples (LAG, INF, and EFF), and then from TOP
7 and BOT. Ammonia and ethanol concentrations were measured using an Innova 1412
8 photoacoustic multi-gas analyzer (AirTech Instruments, Ballerup, Denmark), which had
9 detection limits of $0.2 \mu\text{g L}^{-1}$ for NH_3 and $0.08 \mu\text{g L}^{-1}$ for EtOH. Nitrous oxide was measured
10 using a 46i N_2O analyzer (Thermo Environmental Instruments, Waltham, MA), which had a
11 detection limit of $0.02 \mu\text{g L}^{-1}$. Carbon dioxide was measured using a LI-6252 CO_2 analyzer (LI-
12 COR Biosciences, Lincoln, NE), which had a detection limit of 1 ppm. Methane was measured
13 using a 55C Direct CH_4 and NMHC analyzer (Thermo Environmental Instruments, Waltham,
14 MA), which had a limit of detection of 20 ng L^{-1} for CH_4 . All gas analyzers measured gas
15 concentrations every minute during the sampling period.

16 Gas concentrations from liquids (LAG, INF, EFF) were measured using flux chambers
17 containing a sample of their respective wastewaters. The LAG sample was collected from an
18 outlet on the solids separator pulling water from the surface of the lagoon. The INF sample was
19 collected from a spigot in the pipe between the first storage tank and the vermifilter. The EFF
20 sample was collected from the outlet pipe channeling the EFF into the second storage tank. Each
21 of the three flux chambers consisted of a 19 L container capped with a lid, equipped with an inlet
22 for emissions sampling, an outlet for equilibration with ambient air, and an opening for air pump
23 tubing. During gas sampling, the flux chambers were filled with 5 L of their respective

1 wastewater and ambient air was bubbled directly into the wastewater at a flow rate of 15 Lpm
2 (liters per minute) to force the gases out of solution at a standardized rate. Ambient air was
3 sampled from an inlet affixed below the MAAQ. Gas concentration measurements and bubbling
4 began simultaneously and continued for 48 h. Gas concentrations were sampled sequentially:
5 each liquid was sampled for 30 min followed by sampling ambient air for 10 min before
6 sampling switched to the next liquid.

7 To capture gases from TOP, a triangular wind tunnel (equilateral triangle with 1.2 m
8 sides) was constructed using a PVC pipe frame covered with tarp, leaving the bottom open. The
9 wind tunnel was placed on the surface of the filter and spanned the entire internal width of the
10 filter, excluding the exhaust pipes (Fig. 4Figure 4). A fan on the east side of the tunnel pushed air
11 from east to west to reduce background emissions from the lagoon directly south of the filter. An
12 ultrasonic anemometer (Model 81000, R. M. Young Company, Traverse City, Michigan) was
13 installed on the inside of the south side of the triangular sampling tunnel to measure wind speed
14 and wind direction (Fig. 4Figure 4). Ambient air entering the tunnel was sampled from air
15 entering the fan on the north side of the sampling tunnel.

16 For the BOT, the inlet for emission sampling was secured in the pipe at the top of the
17 elbow joint. To prevent ambient air from flowing into the exhaust pipe, unidirectional airflow out
18 of the pipe was created by pumping pure air through tubing threaded to the bottom of the exhaust
19 pipe at 10 Lpm. Ambient air was sampled directly above the exhaust pipe. Emissions were
20 sampled cyclically as follows: 20 min for TOP, 10 min for ambient air outside the sampling
21 tunnel, 20 min for the BOT, and 10 min for ambient air outside the exhaust pipe.

1 **4.1 Winter gas sampling**

2 For the winter sampling, the same procedure was performed as for the summer sampling
3 with three exceptions: (1) INF was collected from the same INF spigot as in the summer;
4 however, by the winter sampling, pre-filtration steps were added between the LAG and the INF
5 sampling spigot to enhance solids removal in response to clogging sprinkler heads. Specifically,
6 the wood shavings filters, a cone filter in the INF holding tank, a mesh irrigation filter-lined pre-
7 booster pump, three parallel hydro-cyclone sand filters, and three banks of sand media filter
8 (garnet media) were added in that order between the rotary screen and the INF spigot; (2) EtOH
9 was not measured from the TOP and BOT due to negligible emission rates observed during the
10 summer sampling; and (3) gases were sampled for 24 hours at all locations.

11 **3.3 Emission rate calculation**

12 For both sampling periods, gas concentrations measured over time were used to
13 calculate emission rates on a bihourly (LAG, INF, and EFF) or hourly (TOP and BOT) basis and
14 scaled up to the daily turnover of wastewater over the entire area of the vermifilter. To account
15 for instrumental error of initial gas concentration measurements, the first 3 h for the liquid
16 samples and the first 4 h for the TOP and BOT samples were removed for the summer sampling
17 only. . To account for residual gas in the sampling system, the first 4 min and the last minute of
18 each sampling period were removed before data analysis. Net gas concentrations were calculated
19 as the difference between the inlet and outlet (i.e. sample site and ambient, respectively), gas
20 concentrations averaged over the 26-min sampling period for the liquids (LAG, INF, and EFF),
21 and the 16-min sampling period for TOP and BOT.

22 For the summer sampling, the averaged net gas concentrations were multiplied by
23 airflow rates respective to each location, adjusted for temperature, molecular weight and volume,

1 and the resulting average net emission rates were then scaled up to the whole system per day for
2 each sampling site. The wastewater emissions (LAG, INF, and EFF) were scaled up to the total
3 volume added to the vermifilter every day (50,000 L d⁻¹ during the time of the study). The TOP
4 emissions were scaled up to the total surface area of the vermifilter (520 m²), and BOT emissions
5 were multiplied by the total number of exhaust pipes around the vermifilter (20 exhaust pipes).
6 Subsequently, the daily gas output for the INF, EFF, TOP, and BOT (Q_{INF}, Q_{EFF}, Q_{TOP}, and
7 Q_{BOT}, respectively) were used in the following equations to calculate the daily net emissions
8 from within the vermifilter (Q_{Vermifilter}):

$$9 \quad Q_{EFF} + Q_{TOP} + Q_{BOT} + Q_{Vermifilter} = Q_{INF} \quad (1)$$

$$10 \quad Q_{Vermifilter} = Q_{INF} - (Q_{EFF} + Q_{TOP} + Q_{BOT}) \quad (2)$$

11 Because Q_{Vermifilter} was considered separate from Q_{TOP} and Q_{BOT}, the phrase “emissions
12 from the vermifilter” refers to emissions from *within* the vermifilter and does not include
13 emissions from the surface or the bottom of the vermifilter.

14 The gas removal efficiency was then calculated as follows:

$$15 \quad \text{Gas removal efficiency [\%]} = Q_{Vermifilter}/Q_{INF} \quad (3)$$

16 **3.4 Wastewater chemistry analysis**

17 For INF and EFF, ~500 mL wastewater samples were collected and sent to Denele
18 Analytical, Inc. (Turlock, CA), for water chemistry analysis to measure the concentration of
19 NH₃, nitrate-nitrogen (NO₃⁻-N), nitrite (NO₂⁻) and total Kjeldahl N. Samples were processed
20 within 48 hours of collection. Nitrite nitrogen (NO₂⁻-N) concentration was empirically calculated
21 from the concentration of NO₂⁻.

22 **4 RESULTS AND DISCUSSION**

23 Emissions from wastewater before and after treatment (i.e. LAG, INF, and EFF) were

1 measured to elucidate the effect of vermifiltration on wastewater emissions, whereas emissions
2 from the TOP and BOT of the vermifilter were measured to discern whether the TOP, BOT, and
3 the middle of the vermifilter itself was a significant source of emissions. Collectively, the INF,
4 EFF, TOP and BOT emissions encompass emissions from the entire wastewater treatment
5 system after solids separation, allowing us to calculate by difference the gas removal efficiency
6 of the vermifilter.

7 Among the liquids, LAG and INF exhibited similar emission profiles for all measured
8 gases, implying that the solids separator and subsequent storage in the INF storage tank did not
9 drastically affect emissions. However, the emission profiles of LAG and INF differed from that
10 of EFF, indicating that the vermifilter altered emissions. Even when scaled to the entire system,
11 the BOT consistently emitted less gas than the TOP for all measured gases, likely due to nutrient
12 adsorption and conversion, which reduced emissions as the wastewater percolated through the
13 vermifilter.

14 **4.1 Summer sampling**

15 *4.1.1 Ammonia*

16 After accounting for losses from TOP and BOT emissions, the vermifilter reduced NH_3
17 emissions from the INF by 90.24% (Table 1) and soluble NH_3 from 361 mg/L in the INF to 56
18 mg/L in the EFF (Table 1). Among the liquids, NH_3 emissions from LAG and INF were nearly
19 identical, with an estimated emission of 17.57 and 17.21 kg d^{-1} , respectively (Table 1). The loss
20 of 0.36 kg d^{-1} from LAG to INF was likely volatilized during solids separation: the rotary screen
21 agitated the wastewater and exposed the wastewater to air, increasing the surface area exposed to
22 volatilization. Contrarily, NH_3 emission from EFF was 1.54 kg d^{-1} , considerably lower than that

1 of LAG and INF (Table 1). Ammonia emission from the TOP of the vermifilter was 0.14 kg d^{-1}
2 and negligible ($<0.001 \text{ kg}$) from the BOT. The emissions from the surface of the vermifilter were
3 likely due to increased volatilization when the INF was sprinkled over the filter. Similar to the
4 action of the solids separator, as wastewater was forced through the sprinklers into smaller
5 droplets, the increased surface area of the wastewater exposed to air increased NH_3
6 volatilization. The BOT of the vermifilter lacked NH_3 emission because the wastewater was not
7 agitated as it passed through the BOT. Li et al. (2008) also observed a large reduction in NH_3
8 emissions after vermifiltration of swine slurry, reporting a 50% reduction in NH_3 emission from
9 the whole swine facility. Unlike the present study, Li et al. (2008) did not discern the effects of
10 the vermifilter from the rest of the system, so the effects of the vermifilter may have been
11 masked by the continued emission of NH_3 from the barn floor. Nonetheless, Li et al. (2008)
12 hypothesized the reduction in NH_3 emission was due to the efficient adsorption of NH_3 by the
13 vermifilter and subsequent microbial transformation of NH_3 through nitrification, though they
14 did not assay for an increase in products of nitrification (NO_3^- and NO_2^-). The findings of the
15 present study supported this hypothesis. The reduction in volatile and soluble NH_3 was coupled
16 with an increase in NO_3^- -N and NO_2^- -N (Table 2), indicating that the NH_3 was undergoing
17 nitrification.

18 Regardless of treatment or sampling site, temperature and wind speed dictate NH_3
19 emissions (Leytem et al., 2011, Leytem et al., 2013). In the present study, however, wind speed
20 was standardized and was accounted for in the emission rate calculation. Consequently, NH_3
21 emissions were largely determined by temperature and thus followed a diurnal trend, increasing
22 during the day and decreasing at night as the temperature respectively rose and fell (Figure 5a
23 and Figure 6a). Although the amount of NH_3 emitted from the EFF was considerably less than

1 that of the LAG and INF, emission rates from all three liquids peaked during the afternoon, when
2 ambient temperatures were the highest during the sampling period. These observations agreed
3 with previous studies quantifying ammonia emissions from dairy lagoons (Leytem et al., 2011,
4 Leytem et al., 2013, Moore et al., 2014, Todd et al., 2015).

5 4.1.2 Nitrous oxide

6 Among LAG, INF, and EFF, N₂O emissions on a per hour basis (Figure 5b) and on a per
7 day basis (Table 1) were minor (< 10 g d⁻¹), preventing meaningful comparison among these
8 samples. The N₂O emissions from TOP and BOT were higher than that of the wastewaters, with
9 the TOP emissions remaining consistently higher than that of BOT (ranging from 2.7- to 92.6-
10 fold higher) throughout the sampling period (Figure 6b). The sporadic rise and fall of the
11 emission rate may be due to the intermittent application of the INF: the INF was applied for 10
12 min/hr, causing the TOP to fluctuate between the INF saturating of the media's pores and the
13 INF vacating the pores as gravity pulled the liquid down. As the INF was applied to the TOP, the
14 INF may have displaced the gases trapped in the pores of the media, releasing them into the air.

15 On a per day basis (Table 1), N₂O emissions from the TOP (134.99 g d⁻¹) and BOT
16 (12.65 g d⁻¹) of the vermifilter were higher, and emission from within the vermifilter was
17 calculated to be -147.26 g d⁻¹. These relatively minor emissions of N₂O coupled with a large
18 reduction in NH₃ emission from the vermifilter system has been observed previously during
19 vermifiltration of swine slurry (Li et al., 2008, Luth et al., 2011). The increase in soluble NO₂⁻
20 and NO₃⁻ from the INF to the EFF coupled with the reduction in total Khejldahl N and minor
21 N₂O emission from the EFF implies that nitrification creates NO₂⁻ and NO₃⁻ faster than
22 denitrification removes these compounds, causing them to accumulate in the EFF. Importantly,
23 whatever quantify of NO₂⁻ and NO₃⁻ that is denitrified favors complete denitrification to N₂ over

1 incomplete denitrification to N_2O , as implied by the minor increase in N_2O emission from the
2 EFF. Li et al. (2008) also detected a large reduction in NH_3 emission concurrent with minor N_2O
3 emission and also concluded that vermifiltration enhances nitrification and complete
4 denitrification to N_2 as opposed to incomplete denitrification to N_2O . These findings disagree
5 with that of Lubbers et al. (2013), whose meta-analysis showed that earthworms increase
6 emissions of N_2O from soil by 42%; however, Lubbers et al. (2013) focused on earthworms in
7 soil as opposed to in vermifilters, a comparatively more nutrient-rich environment. Luth et al.
8 (2011) proposed that if the N content of the media was lower than that of the earthworm gut,
9 such as in soil, earthworms enhance N_2O emission; however, if the N content of the media
10 exceeded that of the earthworm gut, which was common in vermifilters, the presence of
11 earthworms decreased N_2O emission. Thus, in the present study, the high N content in the
12 vermifilter media likely favored complete denitrification to N_2 as opposed to N_2O , resulting in
13 low N_2O emission.

14 4.1.3 Carbon dioxide

15 Carbon dioxide was formed as a byproduct from respiration of both the earthworms and
16 the microbes that inhabit the vermifilter. On an hourly basis, CO_2 emission from the three
17 wastewaters (Figure 5c) and the TOP and BOT of the vermifilter were minute (Figure 6c).
18 Carbon dioxide emission from the three wastewaters (LAG, INF, and EFF) followed a similar
19 trend, beginning with the highest emission rate which rapidly dropped over time. Although the
20 starting emission rates differed among the three wastewaters, emission rates were negligible
21 overall, starting at $< 1,200 \text{ mg h}^{-1}$ then dropping precipitously to and remaining below $< 300 \text{ mg}$
22 h^{-1} 12h after the start of sampling. The TOP and BOT emissions were $< 300 \text{ mg h}^{-1}$ throughout
23 the sampling period, with TOP CO_2 emissions consistently higher than BOT emissions,

1 remaining up to 145-fold higher than that of BOT except when both TOP and BOT emissions
2 were zero. Similar to N₂O emission from the TOP, the erratic emission pattern of CO₂ from the
3 TOP likely resulted from the periodic application of INF.

4 Scaled up to an entire day, the EFF (43.73 kg d⁻¹) emitted less than half the amount of
5 CO₂ per day than the INF (97.15 kg d⁻¹), but the large difference was likely due to CO₂ loss via
6 the TOP emissions (54.49 kg d⁻¹) rather than adsorption by the vermifilter itself (Table 1).
7 Instead, the vermifilter itself contributed slightly to the CO₂ emissions (5.97 kg d⁻¹) in the EFF.
8 The vermifilter itself had little contribution to CO₂ emissions from the EFF because carbon
9 dioxide emission, a byproduct of decomposition largely driven by microbes (Lubbers et al.,
10 2013), depends on the application rate of organic matter as opposed to the presence of
11 earthworms in the vermifilter (Luth et al., 2011). Thus, the emissions from TOP were determined
12 by the application rate of the INF to the vermifilter, and were largely responsible for CO₂
13 removal from the INF. The emission of CO₂ from the TOP is not ideal because it is released into
14 the atmosphere instead of being absorbed by the vermifilter.

15 The vermifilter itself was responsible for contributing 6 kg of CO₂ to the EFF per day, or
16 14% of the 43.73 kg from EFF. This contribution from the vermifilter was less than half of the
17 estimate by Lubbers et al. (2013), who reported that earthworms have the potential to increase
18 CO₂ emissions by 33%; however their meta-analysis focused on the effect of earthworms on
19 GHGs in soil, which generally has a lower organic matter content than the livestock wastewater
20 applied to vermifilters. Luth et al. (2011) proposed that if C availability in the media exceeds that
21 of the earthworm gut, overall decomposition of the media will mask earthworm-enhanced CO₂
22 emission. Furthermore, earthworm-induced CO₂ production is not only minor, but is also
23 temporary: Lubbers et al. (2013) noted that earthworms increase CO₂ emissions for up to 200

1 days, after which their effect becomes negligible.

2 4.1.4 Methane

3 Because LAG originated from an anaerobic lagoon, which produces methane as a
4 byproduct of anaerobic decomposition (Casey et al., 2006), LAG was expected to have the
5 greatest rate of methane emission among the liquids. However, all three wastewaters exhibited
6 similar CH₄ emission rates throughout the day (Figure 5d). The unexpected similarity may be
7 attributed to the source from which the LAG was pumped: LAG was collected from a pump
8 floating on the surface of the water, where soluble CH₄ is extremely depleted. Additionally, the
9 emission rate calculations excluded the first 4 h of the sampling period, which may have
10 included a rapid initial spike in CH₄ emission. Irrespective of sample type, all emissions peaked
11 during the afternoon, when ambient temperatures were the highest. The observed influence of
12 temperature on CH₄ emission was similar to the findings of Leytem et al. (2011) and Grant et al.
13 (2015), who found that temperature was strongly correlated with CH₄ production from a dairy
14 lagoon in Idaho and dairy wastewater holding basins in Wisconsin, respectively.

15 Similar to the trend observed in other gases, CH₄ emission from TOP remained higher
16 than BOT emission during the duration of the sampling period except when both TOP and BOT
17 emissions dropped to zero (Figure 6d). Similar to the emission pattern of N₂O and CO₂, the
18 irregular emission from the TOP was likely due to the intermittent application of INF to the
19 surface of the vermifilter.

20 4.1.5 Ethanol

21 For the wastewaters (LAG, INF, and EFF), the EtOH gas concentrations were above the
22 limit of detection, but below the limit of quantification, and thus could not be accurately

1 reported. Ethanol emissions from the TOP were consistently higher than that of the BOT, but
2 even so, these emissions were relatively minor (Figure 6e).

3 **4.2 Winter sampling**

4 Because the majority of gases were expelled within the first 24 hours of sampling during
5 the summer, during the winter sampling, gas concentrations were measured for 24 hours.

6 *4.2.1 Ammonia*

7 Compared to the summer sampling, ammonia emissions from the LAG in the winter
8 sampling were 20-fold lower (Figure 5a and **Error! Reference source not found.a**). This
9 dramatic reduction in NH₃ was likely due to a combination of the seasonal effect (Bjerneberg et
10 al., 2009) and upstream management changes (i.e. the lagoon had been emptied and thus had a
11 lower soluble NH₃ to begin with). The lower NH₃ emission from LAG in turn predisposed all
12 downstream emissions to be lower as well compared to emissions in the summer sampling
13 period. Although emissions from the INF appear higher than that of the LAG, the difference is
14 negligible (~1 mg h⁻¹). The NH₃ emission from EFF consistently remains at ~0.3 mg h⁻¹, save for
15 the last hour (0.5 mg h⁻¹), while the emissions from the LAG and INF rise during the last 5 hours
16 of the sampling period, suggesting that the vermifilter is still effective at reducing NH₃ emission
17 from the INF. The lower INF NH₃ emission predisposed the TOP and BOT to similarly yield
18 lower emission rates. Overall, NH₃ emissions consistently increased throughout the 24-hour
19 sampling period, confounding whether we had captured the majority of emissions to accurately
20 extrapolate daily system-scale emissions. Thus, daily NH₃ emission removal efficiency was not
21 calculated.

22 *4.2.2 Nitrous oxide*

1 Nitrous oxide emissions were similar across the LAG, INF, and EFF, demonstrating that
2 the vermifilter did not increase the production of this GHG from treated wastewater despite the
3 reduction in NH₃ emission. Likewise, emissions from the TOP and BOT also remained low.

4 4.2.3 Carbon dioxide

5 Emission of CO₂ from the LAG and INF followed an almost identical trend and remained
6 consistently higher than that of the EFF.

7 4.2.4 Methane

8 Across the liquid samples, methane emissions remained low (<1 mg h⁻¹) after the initial
9 spike during the beginning of the sampling period. Notably, the LAG had the highest initial
10 emission of CH₄ among the liquids, followed by INF, and finally EFF. This ranking of emissions
11 was likely because the LAG was from the anaerobic lagoon, conditions that favor the growth of
12 methanogens, while the INF had been subjected to the partially aerobic pre-filters, preventing
13 methanogenic activity. The continued aeration of the wastewater similarly reduced CH₄ emission
14 from the EFF to levels below that of the INF. Methane emission from the BOT remained
15 negligible, ranging from <1 mg h⁻¹ to 3 mg h⁻¹, while emission from the TOP was too sporadic to
16 draw any meaningful conclusions.

17 5 CONCLUSION

18 Vermifiltration reduced ammonia emissions from dairy wastewater by 90.2% at the
19 expense of producing minor amounts of CO₂ in the summer, suggesting that this technology may
20 be a promising environmental solution for dairies in Central California; however, because
21 previous studies have reported seasonal effects, with summer exhibiting the highest emissions,
22 this study must be replicated across different seasons. The addition of wood shavings filters
23 upstream of the vermifilter did not drastically affect emissions from the resulting influent as

1 compared to the lagoon water, and gas emissions were lower from the effluent as compared to
2 the influent; however, it is unclear whether the reduction in gases is due to the action of the
3 vermifilter itself or emission from the surface or the bottom of the vermifilter. Furthermore,
4 because this vermifilter is currently the only vermifilter used at a commercial dairy, this study
5 must be repeated at future vermifilters built at other dairies for statistical verification of the
6 observed results.

1 **Tables**

2 **Table 1.** Daily net emissions (Q) from the wastewaters (LAG, INF, and EFF), the TOP and BOT
 3 of the vermifilter, and the vermifilter itself during July 2015.

	Daily net emission						Gas removal efficiency of the vermifilter* [%]
	Q _{LAG}	Q _{INF}	Q _{EFF}	Q _{TOP}	Q _{BOT}	Q _{Vermifilter} *	
NH ₃ [kg d ⁻¹]	17.57	17.21	1.54	0.14	0.00	15.53	90.24
N ₂ O [g d ⁻¹]	0.23	1.70	1.31	134.99	12.65	-147.26	-86.85
CO ₂ [kg d ⁻¹]	74.96	97.15	43.73	54.49	4.90	-5.97	-0.06
CH ₄ [kg d ⁻¹]	0.45	0.60	0.35	0.76	0.01	-0.51	-0.84

4 *Negative values indicate that the vermifilter contributed emissions to the wastewater.

5

6 **Table 2.** Water chemistry analysis of INF and EFF

Constituent	Units	Sample Location	
		INF	EFF
NH ₃	mg L ⁻¹	361	56
NO ₃ ⁻ -N	mg L ⁻¹	7.81	48.3
NO ₂ ⁻	mg L ⁻¹	*	79.4
NO ₂ ⁻ -N	mg L ⁻¹	N/A	24.1**
Total Kjeldahl N	mg L ⁻¹	498	129

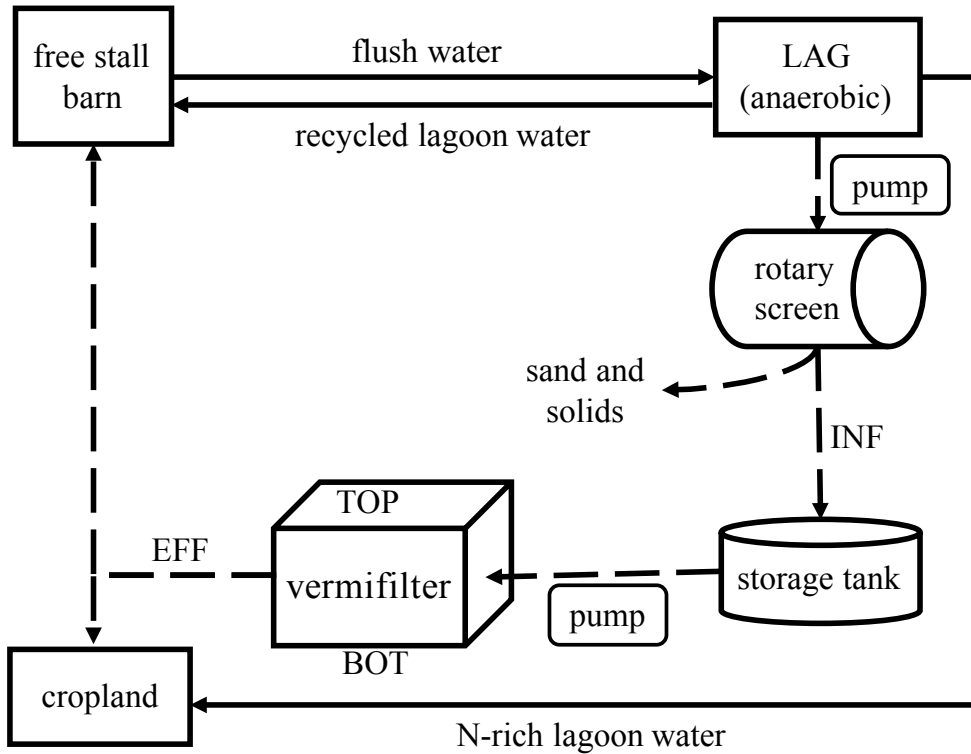
*Below limit of detection

**Empirically calculated from NO₂⁻ concentration

7

1 **Figures**

2



Wastewater management systems

- Conventional
- - - → Vermifilter

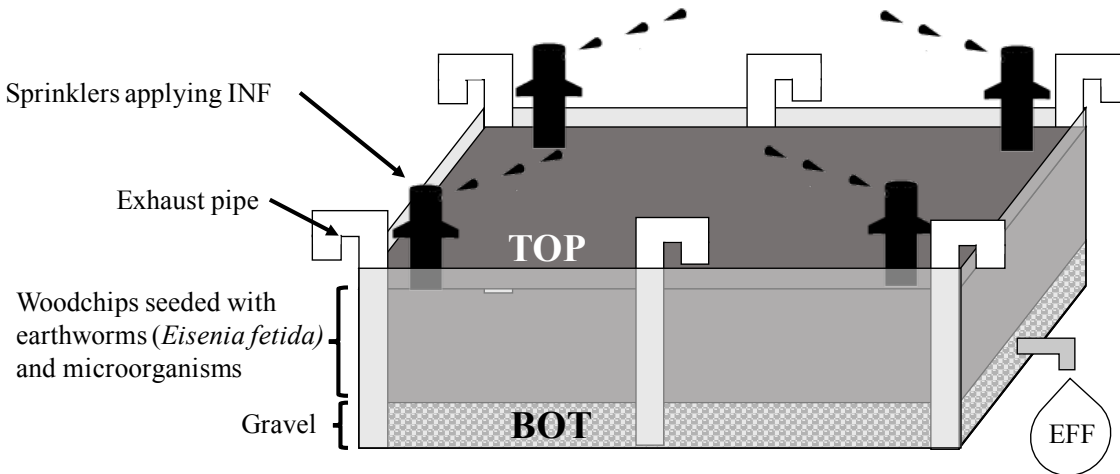
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4 **Figure 1.** Overview of conventional and vermifiltration wastewater management systems on the
5 dairy of the present study.

6 In the conventional system at the dairy of the present study, the free stall barn is flushed 2 or 3
7 times a day, and the flush water is sent to the anaerobic lagoon. The lagoon water (LAG) is then
8 recycled back to the free stalls as flush water, or applied to cropland. In the vermifiltration
9 system, LAG is pumped through a rotary screen to remove sands and solids, and the resulting
10 liquid, the influent (INF), is stored in the storage tank. For the first 15 minutes of every hour, the
11 INF is sprinkled over the top of the vermifilter (TOP). The INF percolates to the bottom of the
12 vermifilter (BOT) via gravity, and the resulting effluent (EFF) is stored in a storage tank until it
13 is used as flush water or applied to cropland.

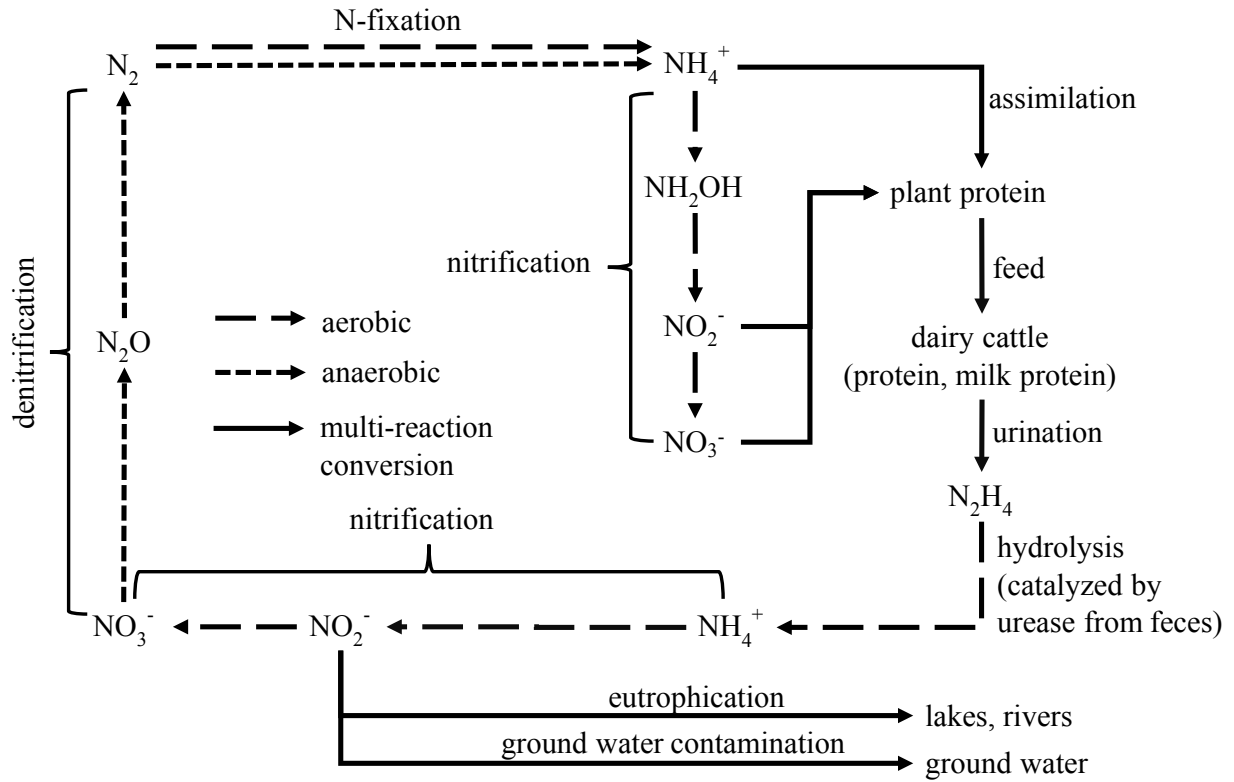
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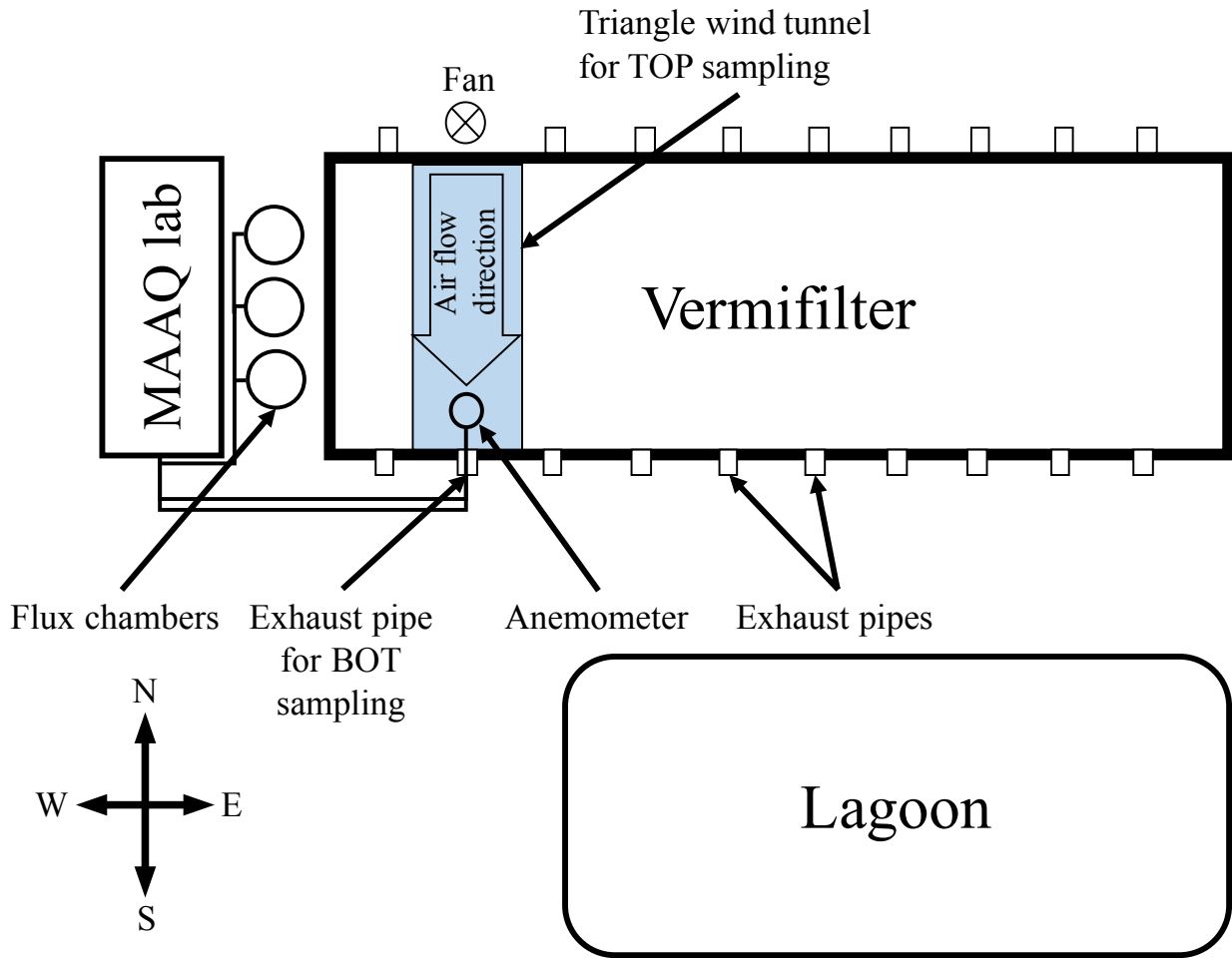
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2 **Figure 2.** Vermifilter design. The vermifilter is a concrete container (49 x 11 x 1.5 m) filled with
 3 woodchips seeded with earthworms and microbes to enhance solid and contaminant removal.
 4 The large particle size of the woodchips, the bottom layer of gravel, and the exhaust pipes that
 5 line the perimeter of the vermifilter enhance aeration of the vermifilters. Sprinklers apply the
 6 INF over the TOP of the vermifilter. The INF filters to the BOT of the vermifilter, resulting in
 7 the EFF. (Figure not to scale.)



1 **Figure 3.** The nitrogen cycle in dairy production

2



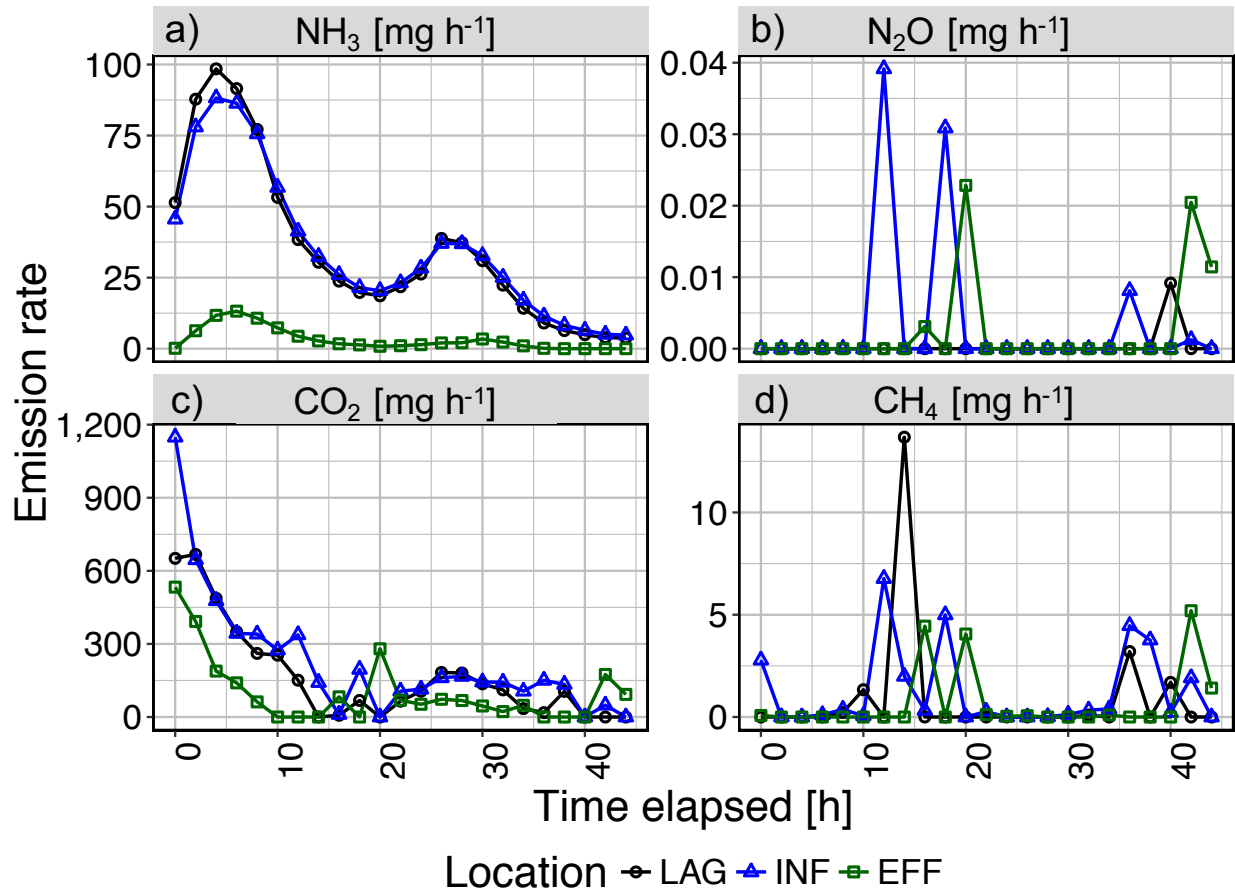
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2 **Figure 4.** Overview of gas sampling setup.

3 Flux chambers were used to measure gas concentrations from the liquids (LAG, INF, EFF). A
 4 triangle wind tunnel was used to measure gas concentrations from the TOP. A fan was placed on
 5 the north end of the triangle wind tunnel, creating a constant airflow from north to south to avoid
 6 contamination from the lagoon nearby. The anemometer placed on the south end measured wind
 7 speed and wind direction for subsequent emission rate calculations. BOT gas concentrations
 8 were measured from the indicated exhaust pipe. (Figure not to scale.)

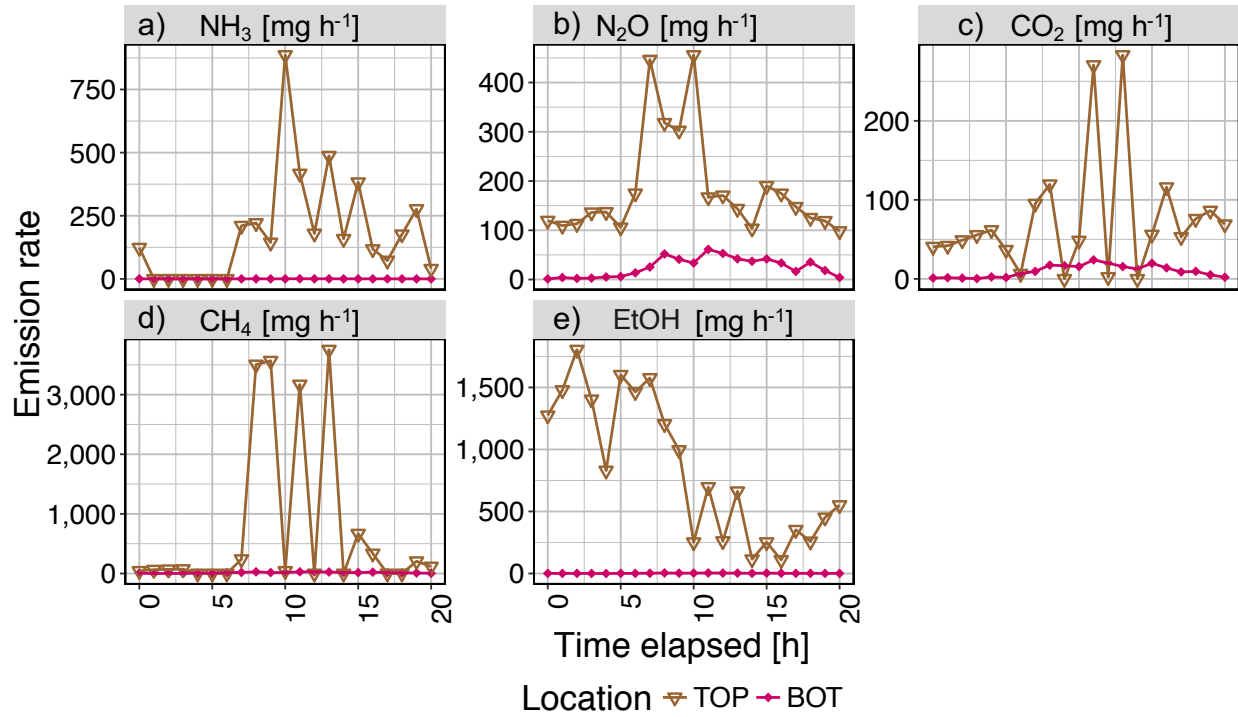
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2 **Figure 5.** Gaseous emissions on a bihourly basis of (a) NH₃, (b) N₂O, (c) CO₂, and (d) CH₄ from
 3 wastewater samples (LAG, INF, and EFF) in each flux chamber during July 2015. Hour 0
 4 corresponded to 12:00 PM.



1

2 **Figure 6.** Gaseous emissions on an hourly basis of (a) NH₃, (b) N₂O, (c) CO₂, (d) CH₄, and (e)
 3 EtOH from a subsection of the vermifilter surface (TOP) and an exhaust pipe (BOT) during July
 4 2015. Hour 0 corresponded to 1:30 PM.

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