Research article

A method to estimate the direct nitrous oxide emissions of municipal wastewater treatment plants based on the degree of nitrogen removal

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A B S T R A C T

The greenhouse gas nitrous oxide (N_2O) is produced in activated sludge tanks as a byproduct of nitrification and heterotrophic denitrification. Insufficient knowledge on how microbiological N_2O generation and degradation pathways impact N_2O emissions in activated sludge tanks still hampers the development of effective mitigation strategies. Our research contributes to overcome this gap by quantifying N_2O emissions through extensive measurement campaigns at ten full-scale wastewater treatment plants and correlating them to relevant operating parameters by multivariate regression analysis. Measurements revealed that N_2O production depends mainly on the activity of nitrifying bacteria and is triggered by high ammonium concentrations. In contrast, well-performing heterotrophic denitrification plays a key role as a sink of N_2O in activated sludge tanks. Following these patterns, low loaded plants achieving high nitrogen removal (83–92%) exhibited the lowest N_2O emission intensity (0.0012 ± 0.001 kg N_2O–N emitted per kg TKN in the influent wastewater). The regression analysis corroborated these results by revealing a negative linear correlation between the N_2O emission factor and the total nitrogen removal degree of the plants. The regression model represents a novel estimation method that links N_2O emissions with plant performance and provides a significant improvement over approaches applying fixed N_2O emission factors.

1. Introduction

In a future scenario, when energy-optimized wastewater treatment plants (WWTPs) are expected to purchase green electricity from the grid, the carbon footprint of municipal wastewater treatment will be dominated by direct emissions of the greenhouse gases nitrous oxide (N_2O) and methane. In particular N_2O, which has a global warming potential 265 times greater than that of CO_2 (IPCC, 2013; GWP_{100a} excl. CCF), has already been reported to be a major contributor to greenhouse gas emissions of WWTPs (Lorenzo-Toja et al., 2016; Delre et al., 2019). The estimated N_2O load emitted directly by wastewater treatment is significant at the national level, and its mitigation is therefore a prerequisite to achieve sustainable urban water management. In Austria, for example, N_2O emissions from WWTPs contribute approximately 5% of the total N_2O emissions (National Inventory Report, 2019).

Intensive research over the past two decades has led to a better

understanding of the biological formation and depletion pathways of N_2O in activated sludge (AS) tanks. Nevertheless, further efforts are needed to establish effective N_2O mitigation strategies for full-scale WWTPs. According to the current state of the art, nitrogen removal at municipal WWTPs is carried out using a combination of two microbiological processes: autotrophic nitrification and heterotrophic denitrification. Although N_2O formation is known to take place during both processes, N_2O emissions to the atmosphere occur predominantly in aeration tanks during aerated phases due to the significantly high gas mass transfer from the liquid phase to the air.

It is generally accepted that N_2O can be released by ammonium oxidizing bacteria (AOB) as a byproduct of the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) (Wunderlin et al., 2013). Depending on the applied operating conditions, two different biological metabolic pathways have been proposed as sources of N_2O in activated sludge tanks: i) the nitrifier denitrification pathway (e.g., Goreau et al., 1980; Tallec

et al., 2006), which is especially favoured by dissolved oxygen deficiency, and ii) the hydroxylamine oxidation pathway (e.g., Stein et al. 2011; Law et al., 2012), which is promoted by high turnover rates of the AOB and by the accumulation of hydroxylamine. However, especially at elevated concentrations of hydroxylamine and NO_2^- (e.g., side-stream nitritation), N_2O can also be generated to some extent by chemical (abiotic) reactions (Soler-Jofra et al., 2016).

During heterotrophic denitrification, N_2O is produced as an obligatory intermediate in the reduction chain from nitrate (NO_3^-) to molecular nitrogen (N_2) over NO_2^-, nitric oxide (NO) and N_2O. The four consecutive steps are individually catalysed by four different denitrification reductases. When production and consumption rates are balanced, N_2O does not accumulate. However, several factors can affect the activity of N_2O reductase (NOS) and promote N_2O accumulation. These factors include the presence of dissolved oxygen or hydrogen sulfide as well as significant NO and NO_2^- accumulation (von Schulthess et al., 1995). A lack of biodegradable organic carbon in terms of a high nitrogen to COD (chemical oxygen demand) ratio can also trigger N_2O accumulation (Ribera-Guardia et al., 2014). While AOB are known to be net sources of N_2O in an AS tank, there is evidence that heterotrophic denitrification can act not only as a source but also as a sink of N_2O. Recently, Chonte et al. (2019) used lab-scale experiments to demonstrate that the capacity of denitrifying microbial communities generally exceeds their capacity to produce N_2O.

Most investigations of N_2O production and consumption pathways to date have involved the operation of lab-scale and pilot-scale reactors for prolonged periods of time. The extent to which experimental observations on 'manipulated' activated sludge correctly describe the pathway of N_2O production and consumption in full-scale AS tanks remains questionable. Although the number of monitoring surveys at full-scale based on continuous online measurements has been increasing over the past decade (e.g., Aboobakar et al., 2013; Daelman et al., 2015; Spinelli et al., 2018; Gruber et al., 2019), predicting and understanding the behaviour of N_2O emissions at full-scale plants remain challenging. The large number of parameters influencing the complex microbiological generation/reduction patterns of N_2O hinders the efforts to correlate the intensity of N_2O emissions to driving operating parameters, as highlighted e.g. by Vasilaki et al. (2019). Varying measurement strategies and quantification methods also deteriorate the comparability of results achieved at different plants. Currently, all these challenges hamper i) the development of effective N_2O mitigation strategies at full-scale, and ii) the formulation of an estimation method that eases the quantification of N_2O emissions by avoiding resource-consuming long-term measuring campaigns. The present research addresses both issues by providing and analysing the results of twenty-two N_2O emission measurement campaigns performed at ten Austrian municipal WWTPs. The main objective of this comprehensive survey was to explore the correlations between N_2O emissions and relevant operating parameters driving nitrification and heterotrophic denitrification in full-scale AS tanks. This was supported by the application of a sound and consistent methodological approach to identify the temporal and spatial fluctuations in N_2O emissions, to monitor relevant process parameters and to describe statistical relationships. The outstanding result of the research relies in the established correlation between the N_2O emission factor and the removal degree of total nitrogen (TN) of the WWTPs that contributed to the development of a novel method to estimate N_2O emission of WWTPs linked to the treatment performance of the plant.

2. Material and methods

This research is based on N_2O emission measurement campaigns at ten full-scale Austrian WWTPs. The focus of the measurements was to link direct N_2O emissions to operational parameters and to plant performance indicators. The diversity in selected WWTPs and the chosen measurement methods reflect these objectives.

2.1. Field sampling sites

For this study, direct N_2O emissions from ten full-scale WWTPs were surveyed (Table 1). The selected municipal WWTPs form a representative sample reflecting the treatment processes most frequently applied in Austria. The sample includes a wide variety of loading conditions and sewage characteristics in terms of influent TN-to-COD ratio and the share between municipal/industrial wastewater.

All plants rely on the AS process to remove organic carbon, nitrogen and phosphorous. According to the Austrian regulation (EVO, 1996), WWTPs with a design load > 5.000 PE_{COD120} have to remove at least 70% of the incoming TN on a yearly average. Surplus and primary sludge are anaerobically digested (AD) at seven WWTPs. At WWTPs B, C and G, simultaneous aerobic sludge stabilization in the AS tanks is applied. Denitrification is mainly achieved upstream in anoxic pre-denitrification tanks and/or by intermittent aeration in AS tanks. Notably, the biological treatment at WWTP B is performed in sequencing batch reactors (SBRs), whereas WWTPs D, I and J are designed as two-stage activated sludge Hybrid® systems (Wandl et al., 2006) with reject water treatment in side stream.

Operational data of the WWTPs (self-monitoring and SCADA system) was used to define the process conditions in AS tanks during the survey. The consistency of the data was verified using mass balances for water flow, COD, TN and total phosphorus. During the measuring campaigns, all AS tanks were in operation (no maintenance work).

2.2. N_2O measurement campaigns

A total of twenty-two campaigns were performed during the period of 2012–2018. Most WWTPs were surveyed at least twice, once in the cold season and once in the warm season, to evaluate seasonal variations in N_2O emissions. Because the focus of the monitoring campaigns lies in short-term variability in emissions and their relation to the dynamics of plant process conditions rather than capturing the average nitrous oxide emissions over a long-term period, continuous online N_2O measurements were applied (temporal resolution of 1 min). This method complies with the suggestions by Daelman et al. (2013).

Within a measurement campaign, all aerated AS tanks along the length of each WWTP were continuously monitored for at least one week, including weekends. Tanks in cascade were monitored in consecutive weeks. At WWTPs with two or more parallel AS lines, only one line was sampled. Significant operational differences between parallel lines with potential impacts on N_2O emissions (e.g. uneven pollutant load distribution) were identified on the basis of respirometric measurements and other indicative key performance indicators and accounted for in the total N_2O emissions. N_2O emissions were also measured in tanks dedicated to reject water side-stream treatment (nitrification/denitrification or partial nitritation) when present.

The flux of gaseous N_2O emitted from AS tanks (F_m-N_2O_{AS} as kg N_2O min^{-1}) was estimated according to the method described in the Supplementary Material (Chapters 1 to 3), relying on the floating-hood method for off-gas collection. During aerated phases, the F_m-N_2O_{AS} stripped by the aeration air provides information on the N_2O production rate in the respective AS tank. To describe N_2O accumulation and/or degradation in denitrification phases, the dissolved N_2O concentration was continuously measured online close to the floating hood (microsensor Unisense A/S, Denmark).

Gaseous N_2O emission to the atmosphere occurs predominantly in the aerated phases and/or zones because of the significantly high gas mass transfer coefficient (k_La). Foley et al. (2010) estimated k_La values for quiescent tank zones of 3–4 d^{-1}, which were an order of magnitude smaller than those calculated for aerated zones (measurements collected from a lab-scale bubble column and at full-scale AS tanks). Based on this evidence, in the present study, gaseous N_2O emissions from quiescent phases and/or zones were neglected during the calculation of the total gaseous N_2O emissions of the WWTPs. The load of dissolved N_2O leaving

Table 1
Surveyed wastewater treatment plants.

Name	Design capacity [PE]***	Plant utilization [PE]	Process description	Type of wastewater	Influent TN/COD [-]	Volumetric loading rate* [kg COD m^{-3} d^{-1}]	Sludge age* [d]
A	140,000	80,000	cascade of 6 AS tanks with circulating flow, 3 pre-denitrification, 2 intermittently aerated and 1 continuously aerated, two lines, mesophilic AD	mainly municipal	0.1	0.15	50
B	35,000	17,000	4 sequencing batch reactors, pre-denitrification, simultaneous aerobic sludge stabilization, aerobic sludge post-stabilization	mainly municipal, food manufacturing	0.065	0.25	20
C	27,000	14,500	cascade of 3 AS tanks with circulating flow, pre-denitrification and intermittent denitrification, simultaneous aerobic sludge stabilization	mainly municipal	0.08	0.23	52
D	55,000	30,000	2 stage AS Hybrid® process, AS with circulating flow in the 2nd stage, pre-denitrification and intermittent denitrification, two lines, mesophilic AD, reject water nitrification/denitrification	mainly municipal	0.08	-**	1.5/13
E	950,000	725,000	cascade of 3 AS tanks with circulating flow, pre-denitrification and intermittent denitrification, four lines, mesophilic AD	municipal with high share of industrial wastewater (steel, paper and chemical)	0.08	0.56	15
F	180,000	175,000	cascade of 2 aerobic selectors, 1 plug-flow AS tank, 2 AS tanks with circulating flow, intermittent denitrification, mesophilic AD	municipal, paper and food industry	0.065	0.31	15
G	25,000	17,000	cascade of 1 CSTR**** AS and 1 AS tank with circulating flow, pre-denitrification and intermittent denitrification, two lines, simultaneous aerobic sludge stabilization	municipal	0.1	0.22	37
H	45,000	25,000	cascade of 3 longitudinal AS tanks, pre-denitrification and intermittent denitrification, mesophilic AD	municipal, winter tourism	0.09	0.83 (tourism) 0.5 (no tourism)	13
I	120,000	61,500	2 stage AS Hybrid® process, one longitudinal tank in the 1st stage, one tank with circulating flow in the 2nd stage, with pre-denitrification and intermittent denitrification, two lines, mesophilic AD, reject water nitrification/denitrification	municipal, winter tourism	0.06	-**	1.5/21
J	100,000	77,000	2 stage AS process, 1 longitudinal tank in the 1st stage, 1 tank with circulating flow in the 2nd stage, pre-denitrification and intermittent denitrification, two lines, mesophilic AD, reject water partial nitritation	municipal, winter tourism	0.09	-**	1/13

* Yearly average value (for 2-stage WWTPs 1st stage/2nd stage); ** not computable because of the hybrid internal sludge circulating loops; *** PE: population equivalents.

the WWTPs with the treated wastewater was also not quantified due to the low reliability and stability of the microsensor signal at N$_2$O concentrations below 0.1 mg L^{-1} (observations under laboratory conditions, data not shown).

2.3. Analytical methods

An infrared gas analyser (Thermo Scientific™ Model 46i) was employed to measure the N$_2$O concentration of the off-gas flow online. Results from the infrared gas analyser were compared to those of gas chromatography-mass spectrometry (GC-MS) using off-gas grab samples collected in evacuated glass vials (20 mL). Additional information is provided in the Supplementary Material (Chapters 4 to 6).

2.4. Normalization of N$_2$O emission results and regression analysis

The calculation of the emitted gaseous N$_2$O was based on 1-min frames. To better correlate N$_2$O emissions with the influent pollutant load, the 24-h window for the calculation of the daily N$_2$O flux was the same as that for the daily composite influent samples from the WWTPs. By calculating the average N$_2$O emission factor for the entire WWTP (EF$_{N2O-WWTP}$), the total gaseous N$_2$O flux of all aerated AS tanks over the entire measurement period (F-N$_2$O-N$_{WWTP}$ as kg N$_2$O-N), was normalized against the (oxidable) nitrogen influent load of the WWTP (TKN$_{in-WWTP}$ as kg N). To calculate the average N$_2$O emission factor EF$_{N2O-AS}$ for the biological mainstream treatment only, the total N$_2$O flux of the aerated AS tanks over the entire measurement period (F-N$_2$O-N$_{AS}$ as kg N$_2$O-N) was referred to the influent of the biological stage TKN$_{in-AS}$ (kg

N):

$$EF_{N2O-WWTP} = F - N_2O - N_{WWTP}/TKN_{in-WWTP} * 100 \tag{1}$$

$$EF_{N2O-AS} = F - N_2O - N_{AS}/TKN_{in-AS} * 100 \tag{2}$$

At WWTPs with two or more AS tanks in cascade, the average emission factors of each cascade and eventual side-stream treatment tanks were summed to obtain the total emission factor of the WWTP.

The correlation between the WWTP operating parameters and the N$_2$O emission factors EF$_{N2O-WWTP}$ and EF$_{N2O-AS}$ was examined via multivariate linear regression analysis using least squares estimation in the R software platform (Chapter 8, Supplementary Material).

3. Results and discussion

In this chapter, the outcomes of the N$_2$O measurement survey are presented and discussed. Due to the large amount of data collected during the survey, only representative examples are shown in figures and tables.

Rain events occurred during some campaigns. In particular, one campaign at WWTP F and one at WWTP H were characterized by an intense and prolonged rain event leading to high hydraulic loading rates with low pollutant concentrations throughout the measurement period. Considering the low comparability of this exceptional operating condition with the other campaigns, these two measurement campaigns were not included in the final evaluation of this work.

3.1. Nitrification is a net source of N₂O in AS tanks

The results of the measurement campaigns confirm that AS tanks of municipal WWTPs are net sources of N_2O. N_2O emissions were detected in all aerated AS tanks across the ten WWTPs. Nitrification could be identified as the main N_2O generation and emission pathway. For example, in a AS tank with circulating flow of WWTP G (2nd cascade following a small pre-denitrification tank), N_2O was emitted with the off-gas during the aerated phase until sufficient NH_4^+ was available in the bulk liquid (Fig. 1). Increasing N_2O emissions at the beginning of the aeration phase indicate more intense N_2O production at the onset of nitrification. The stripped N_2O load decreased continuously when N_2O production decreased because nitrification was limited by the low concentration of NH_4^+ in the tank. Results from WWTP G clearly show that N_2O is formed during nitrification even in low loaded aerated tanks (average sludge age of 37 days for simultaneous aerobic sludge stabilization) with an NH_4^+ concentration of approximately 1 mg N L^{-1}. However, the emitted N_2O (expressed as N) at this WWTP was comparably low, representing only 0.005% $\pm$ 0.0016% of the average daily influent TKN load.

The temporal variation in WWTP influent (daily, weekly and seasonal patterns) has been shown to have a relevant impact on N_2O generation and emission from AS tanks (e.g., Daelman et al., 2015; Pan et al., 2016). Indeed, at all investigated WWTPs, very pronounced dynamics in both the daily and weekly N_2O emission patterns were observed, as shown for WWTP H (Figure S4, Supplementary Material). The pronounced temporal fluctuations emphasize once more the importance of continuous online measurements over at least one week to understand the response of N_2O emissions to changing loading conditions. On dry weather days, the N_2O flux of aerated AS tanks exhibited a repeatable, distinct diurnal trend that could be traced back to the daily pattern of the NH_4^+ loading rate of the AS tanks. The highest N_2O emissions occurred together with daily loading peaks.

Furthermore, measurements at some plants (e.g., WWTP E, 3rd AS cascade) revealed that the peaks of N_2O produced and emitted with the off-gas were coupled with the variation in the NH_4^+ concentration in the AS tank (Fig. 2). The high N_2O generation rate is reflected by the increased N_2O concentration in the bulk liquid (Figure S5, Supplementary Material). The higher N_2O production can be a consequence of an increase in AOB activity according to the correlation between the NH_4^+ concentration and the growth rate of AOB described by the Monod curve. The coupled trend in N_2O production with NH_4^+ turnover in activated sludge is well documented in the literature. Law et al. (2012) and Ribera-Guardia and Pijuan (2017) found a nearly exponential correlation between the ammonia-specific oxidation rate and the N_2O production rate in laboratory experiments with an enriched AOB culture. Ahn et al. (2010) conducted an extensive survey at twelve WWTPs across the United States and used multivariate regression data mining to determine that high NH_4^+ concentrations along with high NO_2^- and dissolved oxygen (DO) concentrations were positively correlated with N_2O fluxes from aerobic zones of AS tanks. Due to a lack of NO_2^- analysis during the NH_4^+ peaks at WWTP E, it could not be evaluated whether N_2O production was also triggered by an accumulation of NO_2^-.

3.2. Heterotrophic denitrification acts as a sink of N₂O in AS tanks

During anoxic phases, no enrichment of N_2O was observed in the investigated AS tanks. In upstream pre-denitrification tanks, N_2O concentrations in the liquid phase were measured to be near zero at all WWTPs. Moreover, in intermittently aerated AS tanks when aeration was switched on after denitrification phases, no increase in N_2O emission was detected in the off-gas, which would be expected when accumulated N_2O is removed through stripping. As shown in Fig. 3 for WWTP F, the N_2O exhaust air content under the exhaust hood decreased significantly after switching on the aeration (ambient air is poor in N_2O) and then increased again after the onset of nitrification. The N_2O measurements in the liquid phase using a N_2O microsensor showed a similar trend.

The measurement results indicate that during denitrification, NO_3^- was completely reduced to N_2 without accumulation of the intermediate N_2O, and the N_2O formed in the AS tanks during the aeration phase was also removed in the subsequent phase. This result implies that denitrifying bacteria can reduce more N_2O than the amount produced from the NO_3^- reduction during the denitrification steps. By using lab-scale
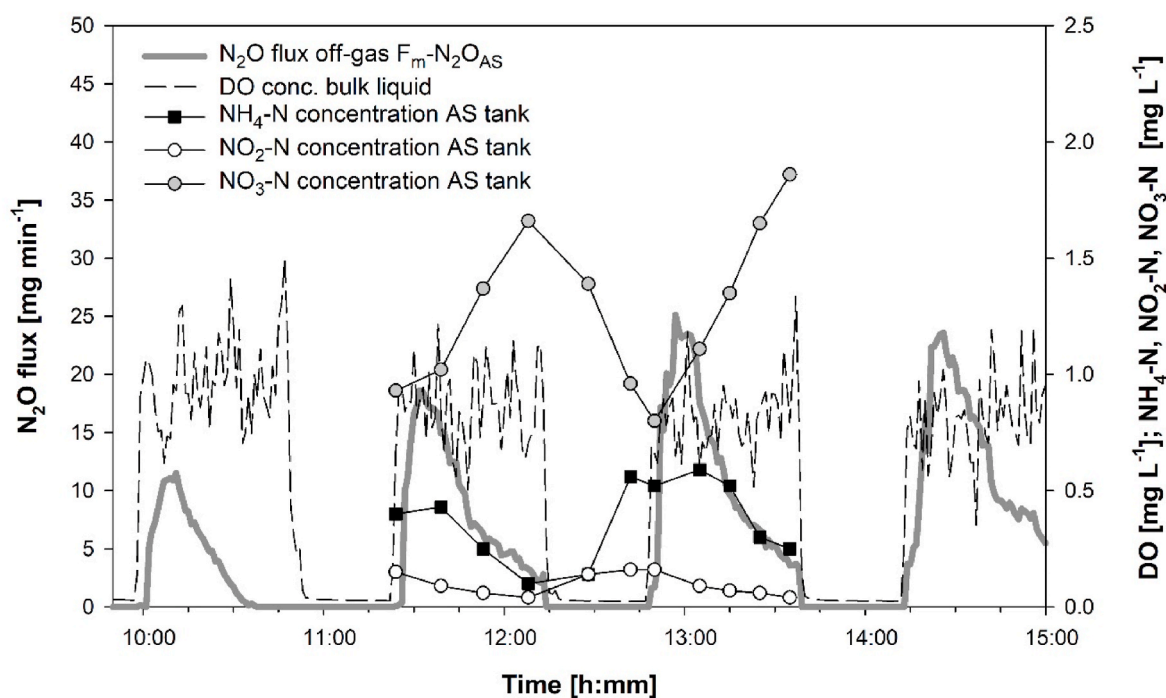
Fig. 1. N_2O emission patterns during nitrification: N_2O flux F_m–N_2O_{AS} measured online in the off-gas of an AS tank with intermittent fine-bubble aeration of WWTP G plotted against the NH_4–N, NO_2–N and NO_3–N concentrations and dissolved oxygen near the floating hood.
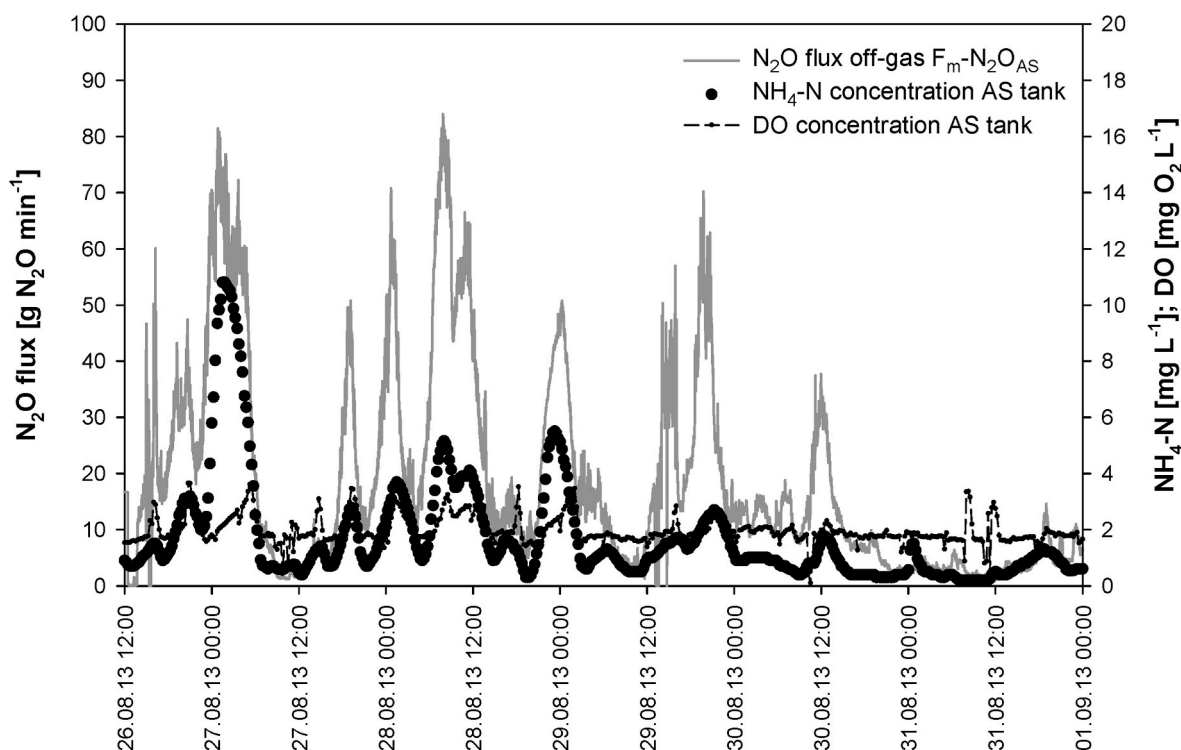
Fig. 2. N₂O flux in the off-gas F_m–N_2O_{AS} of the 3rd AS cascade of WWTP E, a circulating flow tank with continuous fine-bubble aeration: emission peaks of N₂O off-gas corresponded to increased NH_4^+ concentrations in the outlet of the AS.
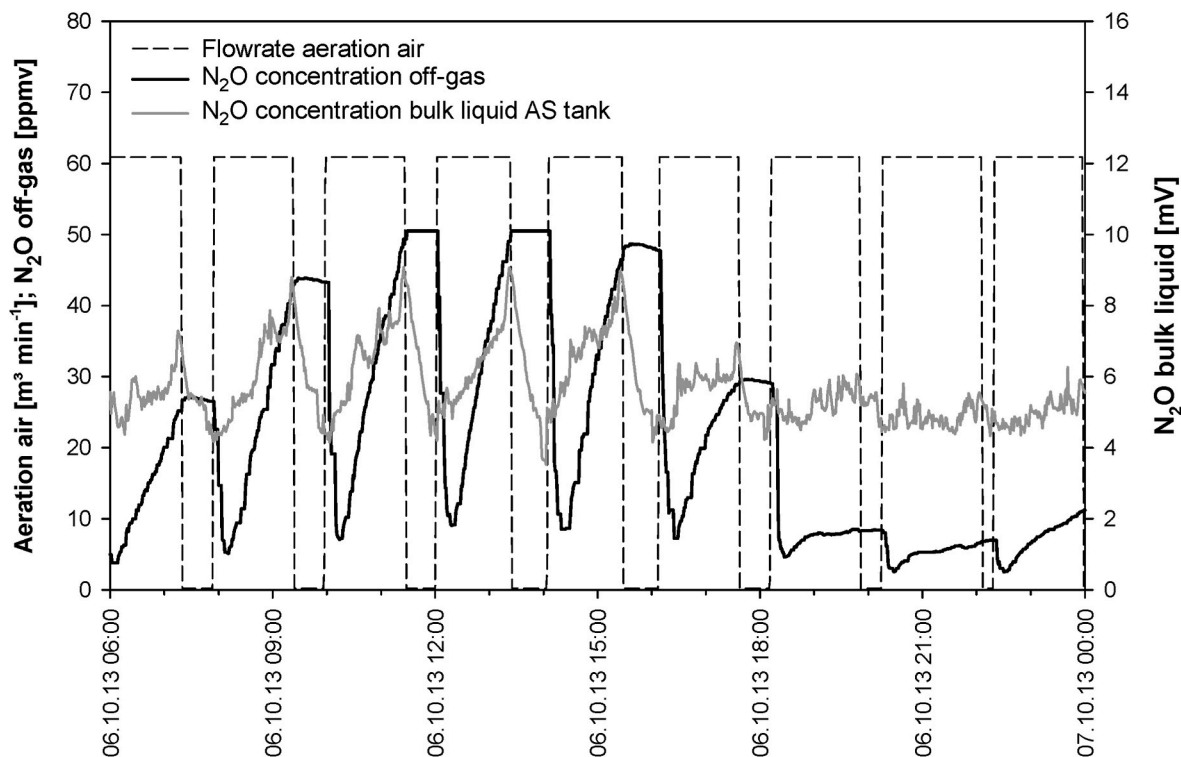
Fig. 3. N₂O concentration in the liquid and gas phases during intermittent aeration in a longitudinal AS tank of WWTP F (3rd cascade after two aerobic selectors, with fine-bubble aeration).

experiments with fresh activated sludge as well as analysing literature results, Conthe et al. (2019) demonstrated that the maximum conversion rate of N₂O to N₂ of heterotrophic denitrifying microbial communities is in most cases 2 to 10 times higher than the maximum removal rate of NO_3^- to N₂. The similar abundance of nitrite reductase and N₂O reductase, both in terms of genes and proteins, indicates that the overcapacity observed in the sludge samples is a characteristic of denitrifier physiology (e.g., enzyme kinetics, electron affinity) and rather not derived

from the genetic composition of the microbial community.

Heterotrophic denitrification was also identified as a net sink of N_2O at a full-scale municipal WWTP by Bollon et al. (2016). In a long-term N_2O-monitoring campaign, they found that a denitrifying biofilter is able to consume a large amount of dissolved N_2O coming from the up-stream nitrification stage, provided that enough COD (in this case methanol) is available for denitrification. In contrast, other experiences at full-scale AS tanks indicate that anoxic zones can also represent a significant source of N_2O emissions of the same order of magnitude as in aerated compartments (Ahn et al., 2010; Bellandi et al., 2018).

In addition, Spinelli et al. (2018) observed that daily peaks of the NH_4^+-to-COD influent ratio at a full-scale municipal WWTP corresponded with higher N_2O fluxes from AS tanks. A similar pattern was also observed at some of the investigated WWTPs in this study. At WWTP E, increased N_2O fluxes occurred during the morning hours in the 2nd cascade (following a small pre-denitrification tank), which could be related to a decrease in denitrification activity (NO_3^- peaks in Fig. 4) because of an increased NH_4^+-to-COD ratio in the influent. This high loaded cascade is a circulating flow tank characterized by zones with low DO concentrations between the aeration fields and therefore offers a suitable environment for simultaneous denitrification. The lack of data on the NO_2^- concentration does not allow us to argue whether i) the influence of COD deficiency on N_2O reduction was indirect over the inhibition of NOS by NO_2^-/NO accumulation (von Schulthess et al., 1995; Itokawa et al., 2001) or ii) direct over electron competition among the different steps of denitrification, as suggested by Pan et al. (2013) and Ribera-Guardia et al. (2014). Considering that the N_2O peaks at WWTP E coincide with the time windows of low influent loading, the increased N_2O emissions and NO_3^- concentration cannot be ascribed to an increased incoming nitrogen load. Thus, considering that at a high NH_4^+-to-COD ratio, less nitrogen is removed through incorporation into the heterotrophic biomass, the load of N nitrified in the AS tank increases. This aspect certainly contributed to the high N_2O fluxes as well.

3.3. Linking N_2O emissions to process parameters

As depicted in Table 2, the N_2O emission factor normalized to the total nitrogen influent of the WWTP ($EF_{N2O-WWTP}$) varies significantly among the WWTPs, ranging from 0.002 to 1.52%. The emission factors gained in this survey are consistent in magnitude with those of previous studies at full-scale WWTPs (Kampschreur et al., 2009; Foley et al., 2010; Law et al., 2012). Dividing $EF_{N2O-WWTP}$ through the reported TN removal degree allows to relate N_2O emissions to the removed nitrogen load.

Variable N_2O emission factors were not only detected among the WWTPs featuring different process configurations and operating conditions. In some cases, the measurement results also exhibited high variability at a single WWTP from one campaign to the other, indicating how process and loading conditions can influence N_2O production and emission significantly. A high coefficient of variation in daily emissions also indicates high daily fluctuations in monitored N_2O fluxes (Table 2).

Among the surveyed plants, WWTPs with a long sludge age, which provides simultaneous aerobic sludge stabilization, showed the lowest average emission factors $EF_{N2O-WWTP}$ (WWTP B, C and G in Table 2). These plants exhibited high TN removal efficiency and low NH_4^+ concentrations in AS tanks. The high TN removal efficiency achieved at these plants is supported by a lack of primary sedimentation prior to biological treatment. Foley et al. (2010) observed that WWTPs designed and operated for near-complete TN removal had lower and less variable N_2O generation factors than plants achieving partial denitrification. According to the authors, this result relies on the typical design features – long sludge age and high internal recirculation rate – of this kind of plant. This finding is in line with the results of our research.

The emission patterns of WWTPs with anaerobic digestion of sewage sludge were more pronounced than those of plants featuring simultaneous aerobic sludge stabilization in most cases. This difference is likely due to i) a shorter sludge age and ii) a high TN-to-COD ratio in the influent of the biological stage due to the extraction of primary sludge and the return of ammonium-rich reject water from sludge dewatering.

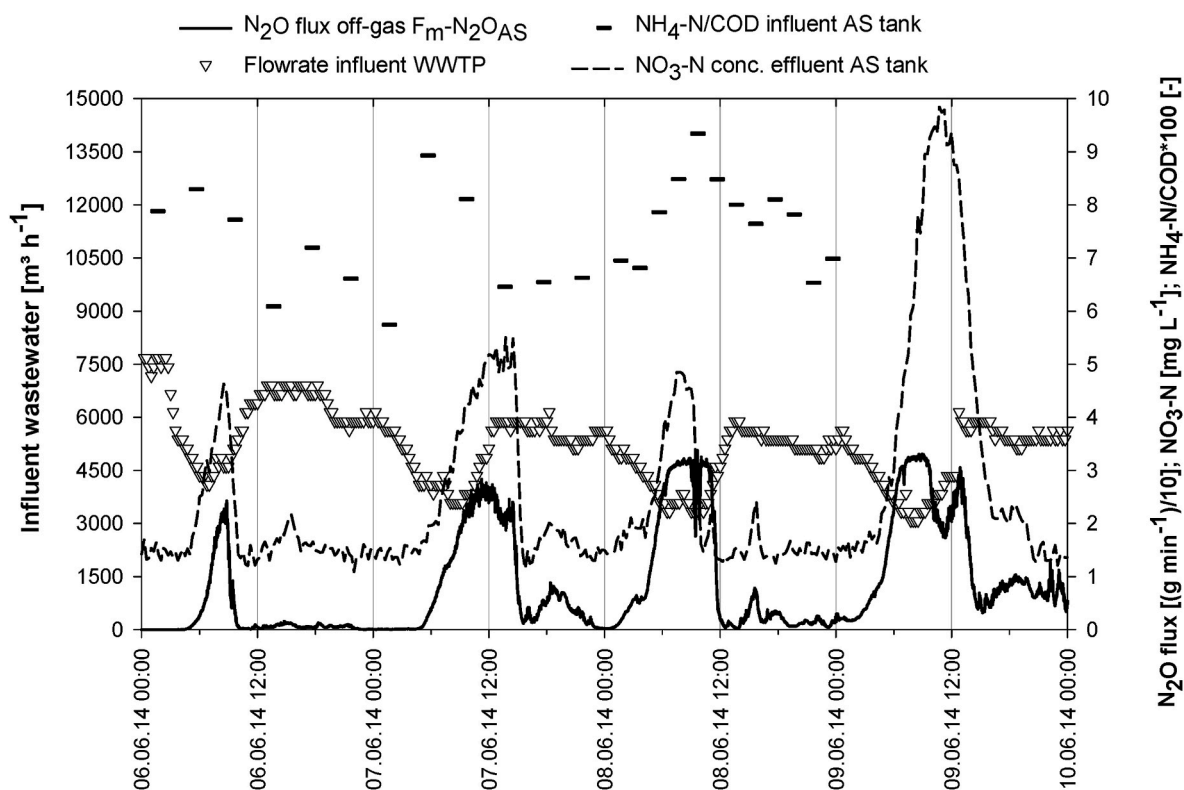**Fig. 4.** N_2O emission in the off-gas and NH_4-N-to-COD ratio in the wastewater entering the AS treatment in the 2nd cascade of WWTP E.

Table 2
Average N$_2$O–N emission factors measured during the measurement campaigns normalized to the TKN load in the influent to the WWTP (EF$_{N2O-WWTP}$) as well as to the influent to the AS tanks (EF$_{N2O-AS}$).

WWTP	Campaign	Duration	Wastewater temperature	TN/COD influent$_{AS}$	TN removal*	EF$_{N2O-WWTP}$	CV**	EF$_{N2O-AS}$	CV**
		[d]	[°C]	[-]	[%]	[%]	[-]	[%]	[-]
A	I	13	22.1	0.144	83.7	0.19	0.27	0.14	0.53
	II	15	10.9	0.161	82.6	0.006	0.07	0.007	1.05
B	I	2	21.3	0.043	90.1	0.13	0.40	0.13	0.40
	II	2	16.6	0.041	88.2	0.24	0.10	0.24	0.10
	III	3	15.7	0.061	86.5	0.35	0.80	0.35	0.80
	IV	5	22.8	0.057	91.2	0.07	0.33	0.07	0.33
	V	9	14.5	0.073	91.5	0.35	0.35	0.35	0.35
C	I	10	17.3	0.07	92.0	0.012	0.82	0.012	0.82
	II	8	15.2	0.08	92.5	0.002	0.73	0.002	0.73
D	I	11	19.4	0.148	68.7	1.52	0.27	1.02	0.98
	II	17	14.3	0.154	71.9	0.88	0.65	0.48	1.29
E	I	16	22.9	0.132	63.6	1.32	0.39	1.29	0.40
	II	14	22.6	0.123	75.8	0.80	0.39	0.76	0.39
F	I	33	16.4	0.094	84.4	0.05	0.70	0.05	0.76
G	I	10	19.4	0.100	92.5	0.005	0.65	0.005	0.65
	II	9	12.2	0.107	90.2	0.005	0.30	0.005	0.30
H	I	27	12.3	0.199	72.7	0.99	0.48	1.07	0.40
I	I	24	16.3	0.128	78.5	0.85	0.34	0.07	0.24
J	I	24	16.5	0.12	67.4	1.03	0.25	0.47	0.13
	II	40	17.	0.10	65.3	1.23	0.73	0.64	1.05

* Average TN removal of the WWTP; **coefficient of variation calculated as the quotient of the standard deviation and average value of the daily emission factors.

The first aspect could contribute to more pronounced daily fluctuations in the NH$_4^+$ concentration in AS tanks, while the latter could partially explain the low TN removal efficiency achieved at these plants. WWTP A and WWTP F were exceptions in this plant group, which exhibited low N$_2$O emission factors. The low N$_2$O emission factors in WWTP A probably occurred because of the low degree of capacity utilization of the plant, leading to a long sludge age and low NH$_4^+$ concentrations in the aerated AS tanks (<1 mg NH$_4$–N L^{-1}). At WWTP F, this was probably due i) to a comparatively low TN-to-COD ratio in the influent sewage (industrial contributions), which supported a more extensive TN removal, and ii) to a high dilution of the influent sewage by groundwater infiltration in the sewer system.

The N$_2$O emission intensity did not differ much between single-stage and two-stage plants. Although the emission factor was significantly higher in the side-stream treatment of the two-stage plants, this seemed to be compensated by a reduced emission factor in the mainstream AS tanks. Side-stream treatment of reject water over partial nitrification and denitrification contributed approximately 50% of the total N$_2$O emissions of WWTP D. On average, 1.6% of the NH$_4^+$ influent load was released as N$_2$O. In comparison, the 2nd stage AS tank exhibited an EF$_{N2O-AS}$ of 1.1%. At WWTP J, the side-stream treatment featuring partial nitritation led to an average emission of 1.8%. Referring to the oxidized NH$_4^+$, this corresponds to 3.6% N$_2$O–N per N$_{oxidized}$, which lies in the range of the values given in literature for partial nitritation on real reject water (Kampschreur et al., 2008; Schneider et al., 2013; Pijuan et al., 2014).

The correlation between the selected WWTP operating parameters and the N$_2$O emission factors EF$_{N2O-WWTP}$ and EF$_{N2O-AS}$ was examined via multivariate linear regression analysis. Based on the average values of the twenty measurement campaigns, the EF$_{N2O-WWTP}$ and EF$_{N2O-AS}$ values were correlated with the TN removal degree, TN-to-COD influent to AS tanks and water temperature. The nitrite concentration, DO and pH value were not examined in the multivariate analysis. The uneven

spatial distribution of these parameters in full-scale activated sludge tanks reduces the significance of the regression outcome.

The results of the multivariate linear regression analysis are illustrated in Tables S2 and S3 (Supplementary Material) for EF$_{N2O-WWTP}$ and EF$_{N2O-AS}$, respectively. The absolute values of the standardized regression coefficient β clearly indicate that the TN removal degree exhibits a much greater influence on the N$_2$O emission factors than the other variables. Due to the strong correlation with this variable, a bivariate regression was conducted (Tables S4 and S5, Supplementary Material). The output of the analysis shows that 86% of the variance (R^2) in EF$_{N2O-WWTP}$ can be explained by the TN removal degree achieved at the respective WWTP. For EF$_{N2O-AS}$, the regression result is 74.6%. The linear correlation is strongest when considering the entire WWTP, presumably because of the better quality of the analytical data and high sampling intensity of the influent WWTP compared to the influent in the biological treatment stage.

The strong correlation between the N$_2$O emission factor and the TN removal degree is in line with observations obtained during the measurement survey. Regarding the hypothesis that N$_2$O is solely produced by the oxidation of NH$_4^+$ and that heterotrophic denitrification, when managed well, represents a sink for the produced N$_2$O, the intensity of N$_2$O emissions EF$_{N2O-AS}$ is expected to be negatively correlated with the denitrification degree of the oxidized TN load. The higher the fraction of oxidized nitrogen further reduced to N$_2$, the lower the N$_2$O emission factor is (Fig. 5). Notably, the operating conditions under which the nitrification process takes place can dramatically influence N$_2$O generation and hence emission factors. However, WWTPs achieving a high TN removal are usually characterized by generous aerobic tank volumes for nitrification and low effluent NH$_4^+$ concentrations, thus exhibiting low N$_2$O emission factors. The TN removal degree, as a key parameter to predict direct N$_2$O emissions, combines the effects of several parameters that influence N$_2$O production and emission in AS tanks. These parameters are i) the aerobic sludge age, indirectly depicting the loading
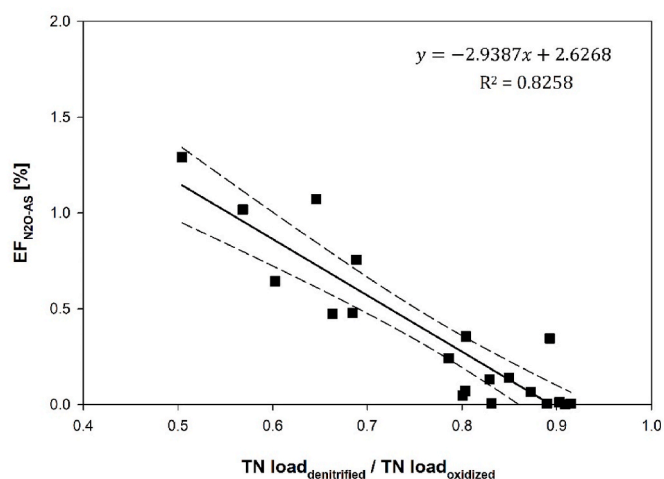
Fig. 5. Scatter plot of the N_2O emission factor EF_{N2O-AS} and the ratio denitrified to oxidized TN in the AS tanks of the surveyed WWTPs. The dashed lines represent the confident intervals of the regression analysis ($\alpha = 0.95$).

conditions of the AS tank and the capacity of the plant to absorb NH_4^+ loading peaks, and ii) the availability of COD for denitrification and the flexibility of the aeration control system in maximizing the anoxic volume for denitrification according to the actual loading conditions.

Results in Fig. 5 also indicated that the process configuration of the WWTPs can impact N_2O emissions provided it affects the TN removal performance of the plant. Higher N_2O emissions were revealed at plants with anaerobic digestion and two-stage configuration.

Based on these research findings, operation strategies to decrease N_2O emissions should focus not only on the minimization of N_2O production by AOB but also on how the N_2O scavenging potential of heterotrophic denitrification can be optimally exploited. This approach has been suggested by only a few researchers to date (e.g. Desloover et al., 2012; Conthe et al. 2019) and needs to be further addressed in future research. In line with this concern, the design approach applied at several Austrian WWTPs with a small pre-denitrification tank coupled with intermittent denitrification in the downstream AS cascades seems to provide high operational flexibility. The use of NH_4^+ online probes can also help optimize the duration of aeration phases. In addition, at WWTPs with anaerobic digestion of sewage sludge, the storage of the NH_4^+-rich reject water and controlled continuous feeding to the activated sludge tanks can be advantageous to avoid shock loads of NH_4^+ and unfavourable TN-to-COD ratios.

Similarly, as in other studies in temperate climates (Ahn et al. 2010; Daelman et al., 2015; Kosonen et al., 2016), we did not find any significant correlation between N_2O emissions and wastewater temperature (Tables S2 and S3, Supplementary Material). It can be argued that i) the influence of temperature was overlapped by the impact of other operating parameters, and ii) the investigated WWTPs had enough capacity reserve to address the low temperatures in winter and could expand the aerated tank volume without affecting denitrification and/or increasing NO_2^- concentrations.

Nitrite is considered one of the key factors affecting N_2O emissions during nitrification and denitrification. High N_2O generation has been associated with high NO_2^- concentrations in wastewater treatment systems (Foley et al., 2010; Daelman et al., 2015). In our study, the NO_2^- concentration was very low at most WWTPs (<1 mg NO_2–N L^{-1}), even during the winter months. Only plants D and H exhibited slightly higher values up to 3 mg NO_2–N L^{-1} in the first aerated cascades. However, the restricted number of collected grab samples does not allow for a proper statistical assessment of this parameter.

Dissolved oxygen concentrations of approximately 1 mg O_2 L^{-1} in aerobic zones (or phases by intermittent aeration) have been found to increase N_2O production by AOB (Tallec et al. 2006; Aboobakar et al.,

2013). The impact of low DO levels could not be investigated thoroughly at full-scale because of simultaneous fluctuations in several process parameters during operation. Low DO conditions appeared during loading peaks, when the higher NH_4^+ load could also trigger N_2O production.

3.4. A novel method for the estimation of direct N_2O emissions from WWTPs

The high variability in N_2O emissions measured at the WWTPs indicates clearly that the use of one single factor to estimate N_2O emissions for inventory purposes is inadequate. In contrast, current estimates for GHG inventories or life-cycle assessments (LCAs) usually use "fixed emission factor" approaches. In most cases, the default value of the 2006 IPCC guidelines is applied (IPCC 2006), which is 0.0032 kg N_2O inhabitant^{-1} year^{-1}, as derived from measurements by Czepiel et al. (1995) at a single WWTP. This value lies in the lower range of the emission spectrum observed in our survey, which ranged from 0.0002 to 0.16 kg N_2O inhabitant $^{-1}$ year^{-1}, with a median value of 0.027 (the unit conversion of $EF_{N2O-WWTP}$ in inhabitants per year was done assuming that one inhabitant produces two PE_{COD120}, as suggested for Austrian WWTPs by Zessner and Lindtner, 2005). The comparison emphasizes that the current international default estimation approach in most cases underestimates the N_2O emissions from municipal WWTPs. The revised version of the 2019 IPCC guidelines (IPCC 2019) addresses this gap by increasing the default emissions factor to 0.016 kg N_2O–N per kg $TN_{influent}$, which in contrast can be far too high for WWTPs with extensive TN removal. Unfortunately, the suggested new approach still does not consider the impact of the operating conditions and wastewater composition on N_2O emissions. A more suitable approach would be to differentiate between plants with high and low N_2O emission risk, according to their operating and configuration features, as suggested by Foley et al. (2010) and implemented by Porro et al. (2014) in their knowledge-based N_2O risk assessment model.

The site-specific prediction of N_2O production and emission during wastewater treatment based on mechanistic dynamic models is growing in maturity, but incomplete knowledge of the mechanisms involved in N_2O formation and release as well as the lack of data for calibration still hamper its application over a large scale (Mannina et al., 2016). Although there are no doubts about the advantages offered by dynamic mathematical modelling in developing N_2O mitigation strategies for WWTPs, it is questionable whether these highly complex tools will find extensive application for GHG emission inventories or for comparative LCA studies. Alternative steady-state approaches are urgently needed to improve estimation methods and provide a more accurate assessment of the order of magnitude of direct N_2O emissions.

Based on the results achieved in this study, a novel estimation method for direct N_2O emissions from WWTPs is proposed. Considering the established correlation between N_2O emissions ($EF_{N2O-WWTP}$) and plant performance in terms of TN removal, the yearly direct N_2O emissions can be estimated using the yearly average TN removal degree achieved by the plant and the regression model presented in Fig. 6. The choice to base the estimation model to the entire WWTP, instead of considering the AS tanks and an eventual side-stream treatment separately, is justified by a usually high data availability of influent and effluent nitrogen loads compared to internal nitrogen loads. Of course, a correlation model based on EF_{N2O-AS} (Figure S6, in Supplementary Material) can be applied as an alternative when supported by appropriate data availability.

As an alternative to the general models in Fig. 6 and S6, a site-specific regression model using data from an extensive monitoring campaign at a single WWTP can be built and used to predict average yearly N_2O emission loads (e.g., for inventory purposes) solely relying on the documented plant performance. This approach would prevent the need for continuous online monitoring measurements, which are time- and resource-consuming.
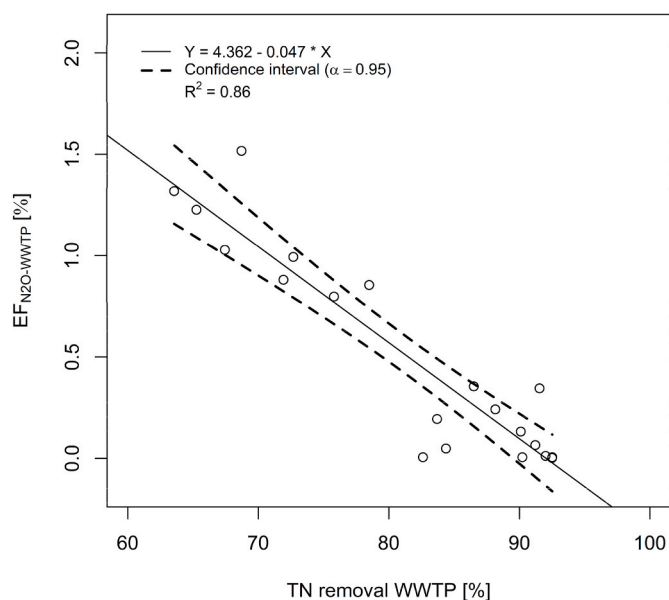
Fig. 6. Scatter plot of the average N_2O emission factor $EF_{N2O-WWTP}$ and the TN removal efficiency (%) of the surveyed WWTPs comprising 20 measurement campaigns fitted with a linear regression line. The dashed lines represent the confidence intervals of the regression analysis ($\alpha = 0.95$).

To the best of our knowledge, the proposed estimation model is the first in its kind that enables the adaptation of the N_2O emission intensity to the average treatment performance of a plant. In Austria, GHG inventories as of 2013 were based on this method to assess the direct N_2O emissions from domestic wastewater treatment at the national level. Using the input/output TN load data compiled by the Austrian Electronic Emission Register of Surface Water Bodies (EMREG-OW), the degree of yearly TN removal for each WWTP can be calculated. The corresponding $EF_{N2O-WWTP}$ values are then derived from the regression model (Fig. 6), and the N_2O emission fluxes are calculated and summed to a total national yearly emission load. For WWTPs with a TN removal degree lower than 70%, a maximum $EF_{N2O-WWTP}$ of 1.4% was applied; for TN removal degrees higher than 93%, the minimum $EF_{N2O-WWTP}$ was set at 0.03%.

The extent to which the regression model established in this study for Austrian WWTPs can be applied to other plants elsewhere still needs to be determined. Specific local conditions, such as plant design, treatment targets and wastewater composition, are expected to influence the outcome of the regression analyses. The negative correlation of the N_2O factor with the TN removal degree still applies, assuming that nitrogen removal is achieved biologically over nitrification and denitrification. The model should be applicable irrespective of the denitrification strategy pursued, being pre-denitrification, intermittent or simultaneous denitrification or a combination of these options. Whether the model can also accurately predict emissions of AS processes featuring step-feed denitrification or post-denitrification relying on an external carbon source remains an open question. Measurements at a large scale should determine whether the overall N_2O emissions are increased in these cases due to the spatial separation of nitrification and denitrification. Under these conditions, the N_2O reduction capacity of denitrification on the produced N_2O cannot be fully exploited. The regression does not apply for WWTPs without targeted nitrogen removal.

4. Conclusions

The present research corroborates the evidence that N_2O production and emission during nitrification in full-scale AS tanks can be reduced but not completely avoided, by optimizing process conditions. Among other parameters, an increased NH_4^+ concentration triggered N_2O

generation by AOB. Heterotrophic denitrification under favourable process conditions was shown to be a significant N_2O sink that promoted the reduction of N_2O to gaseous N_2. Hence, the level of the net N_2O flux emitted by an AS tank will mainly result from the combination of these generation and reduction pathways.

The results clearly suggest that N_2O emissions can be mitigated by avoiding nitrifier overloading and promoting denitrification, thus combining climate protection with water quality management goals. In addition to a well-thought-out plant design, providing sufficient nitrification reserve capacity, a proper operating and aeration strategy that dynamically maximizes the anoxic basin volume and COD availability for denitrification in response to influent fluctuations, can contribute to a low N_2O emission pattern.

The prediction of N_2O emissions in AS tanks is challenging because several operating parameters influence its formation, degradation and emission. The established correlation of the N_2O emission factor with the TN removal degree of the WWTP provides a novel estimation approach that links N_2O emissions with plant performance for the first time. This achievement represents a significant improvement over the 'fixed emission factor' approach applied by the 2006 IPCC guidelines as well as the 2019 refinement.

Future research in this field should focus on gaining a deeper understanding of the mechanisms behind the N_2O scavenging capacity of denitrifying microbial communities. Furthermore, the applicability of the N_2O emission estimation model suggested in this study needs to be verified at WWTPs relying on TN removal concepts other than pre-denitrification and/or intermittent denitrification.

Credit author statement

Tanya Valkova: Methodology, Investigation, Writing original draft. Vanessa Parravicini: Methodology, Investigation, Writing original draft. Ernis Saracevic: Methodology, Validation, Resources. Joseph Tauber: Investigation, Formal analysis. Karl Svardal: Conceptualization, Supervision, Writing review & editing. Jörg Krampe: Writing - review & editing. Tanya Valkova and Vanessa Parravicini contributed equally to the research work and to the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.111563.

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